Clarke, Modet & Cº

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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Doctor
GIANCARLO MARCENARO JIMÉNEZ
Superintendente Delegado Para la Propiedad Industrial

EXPEDIENTE: Expediente No. **04.001.331** Solicitud de Patente a nombre de **MAGNACHEM INTERNATIONAL LABORATORIOS, INC.**

Estimado Doctor:

En mi calidad de apoderada sustituta de la sociedad solicitante de la patente de la referencia manifiesto a usted lo siguiente:

1. Mediante memorial radicado en ese despacho el 27 de enero de 2006, el cual no ha sido resuelto aún, solicité que se realizara el examen de patentabilidad.

2. En vista de que han transcurrido casi **3 años** sin que se haya atendido dicha solicitud, ruego a usted se sirva dar las instrucciones del caso para que se proceda de inmediato a resolver este asunto.

Cordialmente,

DILIA RODRIGUEZ D'ALEMAN
C.C. 41.654.882 de Bogotá
T.P. 20824 del C.S.J.
p-530

US005962460A

United States Patent [19]

Tzeng et al.

[11] **Patent Number: 5,962,460**

[45] **Date of Patent: Oct. 5, 1999**

[54] **ANTINEOPLASTIC α-METHYLENE-γ-BUTYROLACTONES**

[75] Inventors: **Cherng-Chyi Tzeng; Kuan-Han Lee; Yeh-Long Chen; Bor-Ruey Huang,** all of Kaohsiung, Taiwan

[73] Assignee: **National Science Council (Taiwan),** Taipei, Taiwan

[21] Appl. No.: **08/495,212**

[22] Filed: **Jun. 27, 1995**

[51] **Int. Cl.**[6] **C07D 239/54**; C07D 239/553; A61K 31/505

[52] **U.S. Cl.** **514/274**; 514/242; 544/182; 544/313; 544/314; 544/312; 544/311

[58] **Field of Search** 544/313, 314, 544/311, 312, 182; 514/274, 242

[56] **References Cited**

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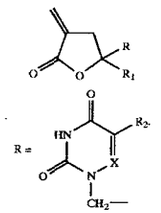
Primary Examiner—John M. Ford

Attorney, Agent, or Firm—Chi Ping Chang

[57] **ABSTRACT**

The present invention provides antineoplastic α-methylene-γ-butyrolactones represented by the general formula [I] wherein R_1 is a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4)alkyl, (C_1–C_4)alkoxy, phenyl, nitro and amino;$_2$ R represents hydrogen, halide, (C_1-C_4)alkyl or benzyl; X represents N; or wherein R_1 is a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4)alkyl, (C_1–C_4) alkoxy, nitro and amino; R_2 represents Cl, F, or benzyl; X represents CH. These α-methylene-γ-butyrolactones may be administered with an inert diluent or with a pharmaceutically acceptable carrier in controlling the growth of a neoplasm in a patient afflicted with a neoplasm disease.

[I]



4 Claims, No Drawings

ANTINEOPLASTIC α-METHYLENE-γ-BUTYROLACTONES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to the therapeutical application of α-methylene-γ-butyrolactone derivatives.

BACKGROUND OF THE INVENTION

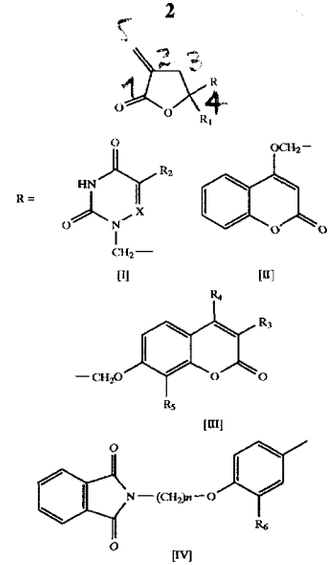
α-methylene-γ-butyrolactone constitute an important group of natural products which possess wide-ranging biological activities, including antineoplastic, bactericidal, fungicidal, antibiotic and anthelminthic properties (Hoffmann et al., Angew. Chem. Int. Ed. Engl., 1985, 24, 94). The present invention relates to the synthesis and measurement of novel antineoplastic α-methylene-γ-butyrolactones to be used in cancer therapy.

A number of sesquiterpenes bearing α-methylene-γ-butyrolactone functionality such as helenalin, and elephantopin have exhibited significant antitumor activities(Lee et al., *J. Med. Chem.,* 1972, 15, 609). It was soon characterized that the structural requirement for the cytotoxicity is O=C–C=CH_2 moiety which acts as an alkylating agent by a Michael-type reaction with biological cellular nucleophiles or sulphydryl-containing enzymes, leading to the breakage of DNA/RNA or the inhibition of enzyme activities(Kupchan et al., *Science,* 1970, 168, 376). Other clinically useful alkylating drugs against human maliganancy are nitrogen mustards: uracil mustard, chloroambucil and cyclophosphamide. Their utilization is, however, limited by the poor selectivity and the serious side effects such as bone marrow toxicity. The individual drugs also produce distinctive toxic effects on the intestine, kidneys, heart and other organs. Therefore, efforts have been made wordwide to discover more active and less toxic antineoplastic agents. Certain α-methylene-γ-butyrolactones had been prepared and screened (Lee et al., *J. Med. Chem.,* 1977, 20,911; Heindel et al., *J. Pharm. Sci.,* 1981, 70, 84; Sanyal et al., *J. Med. Chem.,* 1986, 29, 595) . Recently, we described the synthesis and cytotoxicity of certain uracil α-methylene-γ-butyrolactones and uncovered that an aryl substituent at 5-position of the α-methylene-γ-butyrolactone ring enhanced the antitumor potency (*Chin. Pharm. J.* 1991, 43, 447; Kaohsiung *J. Med. Sci.,* 1993, 9, 707). The present invention describes the preparation of certain novel α-methylene-γ-butyrolactones as antineoplastic agents. All compounds were evaluated in vitro against a total of 56 human tumor cell lines derived from nine cancer types.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have discovered four classes of novel compounds with excellent antineoplastic activity. As a result of intensive studies, it has been found that compounds represented by the formula [I–IV] have shown not only potent but also selective in inhibiting cancer cells.

The present invention provides antineoplastic α-methylene-γ-butyrolactones represented by the general formula [I–IV]:



For the formula [I], R_1 is a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4) alkyl,(C_1–C_4)alkoxy, phenyl, nitro and amino; R_2 represents hydrogen, halide, (C_1–C_4) alkyl or benzyl; X represents CH or N.

For the formula [II], R_2 is a methyl or a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro and amino.

For the formula [III], R_1 is a methyl or a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro and amino; R_3 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro or amino; R_4 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro or amino; R_5 represents hydrogen, (C_1–C_4)alkyl, (3-methylene-2-oxo-5-furanyl)methoxy, hydroxyl, or (C_1–C_7)alkoxy.

For the formula [IV], R_1 is a hydrogen; R6 represents hydrogen, halide, (C_1–C_4)alkyl or (C_1–C_4)alkoxy; n represents (C_3–C_8)alkyl.

The present invention also provides a cost-efficient method for the preparation of formula [I–IV].

Formula [I–IV] may be administered with an inert diluent or with a pharmaceutically acceptable carrier in controlling the growth of a neoplasm in a patient afflicted with a neoplasm disease.

This invention included the preparation and the antitumor evaluation of novel α-methylene-γ-butyrolactones which have been proved to be active against the growth of leukemia (CCRF-CEM, HL-60, MOLT-4, etc.), colon(COLO 205, HCT-116, HT-29, etc.) cancer cell lines, etc. These active compounds, as free type or their pharmaceutically acceptable salts, may be administered parenterally or orally

in a suitable pharmaceutical form. They also may be administered along or in conjunction with other antitumor agents, in combination with any pharmaceutically acceptable carrier.

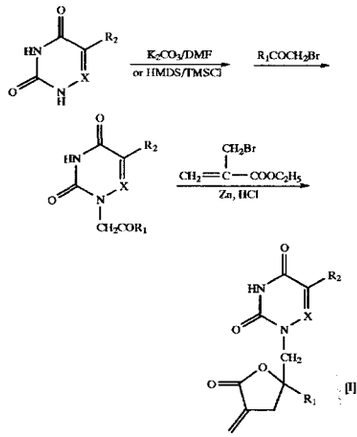
As used herein, the pharmaceutically acceptable salts include salts with inorganic acids, such as hydrochloride, hydrobromide, sulfate and phosphate; those organic acids, such as acetate, maleate, tartarate, methanesulfonate; and those with amino acids, such as arginine, aspartic acid and glutamic acid. Suitable pharmaceutical forms include sterile aqueous solutions or dispersions, sterile powders, tablets, troches, pills, capsules, and the like. In addition, the active compounds may be incorporated into sustained-release preparations and formulations. The pharmaceutically acceptable carrier includes any and all solvents, disintegrating agents, binders, excipients, lubricants, absorption delaying agents and the like. Although the compound of the present invention may also be present as a hydrate or as a stereoisomer, it is a matter of course that these hydrates and stereoisomers are also included in the scope of the present invention.

The new compounds can be prepared according to the following reaction schemes and protocols.

PART A

Preparation of 5-Aryl-3-methylene-2-oxo-5-(pyrimidin-1-ylmethyl)-tetrahydrofuran

(Scheme 1)



[I]

Uracil or its derivative was either silylated with hexamethyldisilazane(HMDS) and a catalytical amount of chlorotrimethylsilane(TMSCl)followed by the alkylation or was directly alkylated with a commercially available aryl bromomethyl ketone(2-bromoacetophenone, R_1=C_6H_5; 2-bromo-4'-fluoroacetophenone, R_1=C_6H_4F; 2-bromo-4'-chloroacetophenone, R_1=C_6H_4Cl; 2-bromo-4'-bromoacetophenone, R_1=C_6H_4Br; 2-bromo-4'-iodoacetophenone, R_1=C_6H_4I; 2-bromo-4'-methylacetophenone, R_1=$C_6H_4CH_3$; 2-bromo-4'-nitroacetophenone, R_1=$C_6H_4NO_2$; 2-bromo-4'-methoxyacetophenone, R_1=$C_6H_4OCH_3$; 2-bromo-4'-phenylacetophenone, R_1=$C_6H_4C_6H_5$) in the presence of potassium carbonate (K_2CO_3) and N,N-dimethylformamide (DMF) to afford the 1-ketonyl pyrimidines. Reformatsky-type reaction between ethyl 2-(bromomethyl)acrylate and the respective 1-ketonyl pyrimidines under nitrogen atmosphere produced the desired 5-aryl-3-methylene-2-oxo-5-(pyrimidin-1-ylmethyl)-tetrahydrofuran in good yield.

EXAMPLE 1

5-(5-Bromouracil-1-ylmethyl)-3-methylene-2-oxo-5-p-phenyl-phenyl)-tetrahydrofuran (1)

Bromouracil(1.91 g, 10 mmol), potassium carbonate (1.38 g, 10 mmol) and dry N,N-dimethylformamide (DMF) (40 ml) were stirred at room temperature under nitrogen atmosphere for 30 min. To this solution, 2-bromo-4'-phenylacetophenone(2.75 g, 10 mmol) in dry DMF(10 ml) was added at one portion. The resulting solution was stirred at room temperature for 48 h (monitored by TLC) and then poured into ice water (50 ml). The resulting pale yellow solid was filtered, washed with cold water(10 ml), and crystallized from methanol to afford 1-p-phenylphenacyluracil(1a) (1.94 g, 63%).

To the mixture of 1a(307 mg, 1 mmol), activated zinc powder(Aldrich® 32,493-0,-100 mesh, 99.998%) (85 mg, 1.3 mmol), and p-hydroquinone(2 mg) in dry tetrahydrofuran(THF) (20 ml) was added dropwise a solution of ethyl 2-(bromomethyl)acrylate(260 mg, 1.3 mmol) in dry THF(5 ml). The reaction mixture was refluxed under nitrogen atmosphere for 4 h. After cooling it was poured into an ice-cold 5% HCl solution (100 ml) and extracted with dichloromethane(50 ml×3). The dichloromethane extracts were combined and washed with saline, dried over anhydrous magnesium sulfate, and then evaporated to give a white solid which was crystallized from ethyl acetate. The white crystal was died in vaccuo at 80° C. over P_2O_5 afforded the title compound in a quantitative yield.

mp 238–241° C.; yield 69%;

UV λ max: 257.2(0.1 N HCl/CH_3OH), 257.0(CH_3OH), 254.2(0.1 N NaOH/CH_3OH);

^{1}H-NMR(DMSO-d_6): δ 3.40 (m, 2H, 4-CH_2), 4.26 & 4.31 (2d, 2H, NCH_2), 5.73 (t, 1H, vinylic H), 6.04 (t, 1H, vinylic H), 7.57 (m, 9H, Ar—H), 7.89 (s, 1H, 6-CH), 11.79 (brs, 1H, NH);

Anal. for $C_{22}H_{17}N_2O_4Br$: Calcd.: C, 58.29; H, 3.78; N, 6.18. Found : C, 58.43; H, 3.50; N, 6.01.

EXAMPLE 2

3-Methylene-2-oxo-5-(p-phenylphenyl)-5-(thymin-1-ylmethyl)-tetrahydrofuran (2)

The title compound was synthesized from thymine and 2-bromo-4'-phenylacetophenone according to the procedures described above for the preparation of 1:

mp 217–219° C.; yield 93%;

^{1}H-NMR($CDCl_3$): δ 1.71 (d, 3H, CH_3), 3.40 (m, 2H, 4-CH_2), 4.10 & 4.34 (d, 2H, NCH_2), 5.70 (t, 1H, vinylic H), 6.01 (t, 1H, vinylic H), 7.29 (d, 1H, 6-CH), 7.57 (m, 9H, Ar—H), 11.29 (brs, 1H, NH);

Anal. for $C_{23}H_{20}N_2O_4$: Calcd.: C, 71.12; H, 5.19; N, 7.21. Found : C, 71.11; H, 5.28; N, 7.26.

EXAMPLE 3

5-(p-Chlorophenyl)-3-methylene-2-oxo-5-(thymin-1-ylmethyl)-tetrahydrofuran (3)

Compound 3 was prepared from thymine and 2-bromo-4'-chloroacetophenone according to the procedure given for 1:

mp 188–189° C.; yield 78%;

UV λ max: 269.0(0.1 N HCl/CH_3OH), 269.0 (CH_3OH), 272.0(0.1 N NaOH/CH_3OH).

^{1}H-NMR($CDCl_3$): δ 1.90 (d, 3H, CH_3), 3.32 (m, 2H, 4-CH_2), 3.78 & 4.46 (d, 2H, NCH_2), 5.63 (t, 1H, vinylic H), 6.18 (t, 1H, vinylic H), 7.08 (d, 1H, 6-CH), 7.35 (m, 4H, Ar—H), 8.95 (br s, 1H, NH);

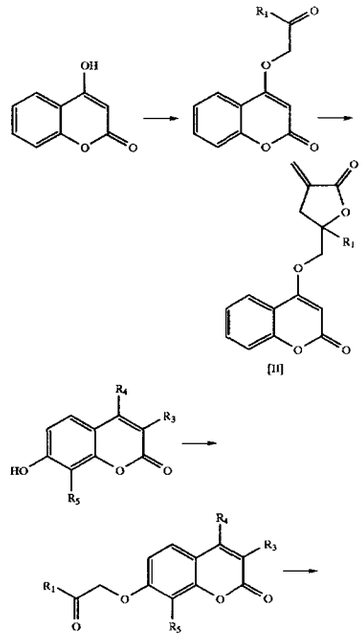
Anal. for $C_{17}H_{15}N_2O_4Cl$: Calcd.: C, 58.88; H, 4.36; N, 8.08. Found : C, 58.73; H, 4.57; N, 8.39.

PART B

Preparation of 4-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl)-methoxy]-2H-1-benzopyran-2-ones [II] and

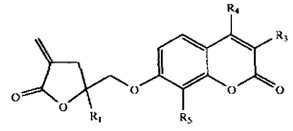
7-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl) methoxy]-2H-1-benzopyran-2-ones [III]

(Scheme 2)



[II]

-continued



[III]

Each of the commercially available 4-hydroxycoumarin, 7-hydroxycoumarin(R_3=R_4=R_5=H), 7-hydroxy-4-methylcoumarin(R_4=CH_3), 3-chloro-7-hydroxy-4-methylcoumarin(R_3=Cl, R_4=CH_3), and 7-hydroxy-3,4,8-trimethylcoumarin (R_3=R_4=R_5=CH_3) was treated with potassium carbonate and a haloketone (chloroacetone, R_1=CH_3; 2-bromoacetophenone, R_1=C_6H_5; 2-bromo-4'-fluoroacetophenone, R_1=C_6H_4F; 2-bromo-4'-chloroacetophenone, R_1=C_6H_4Cl; 2-bromo-4'-bromoacetophenone, R_1=C_6H_4Br; 2-bromo-4'-iodoacetophenone, R_1=C_6H_4I; 2-bromo-4'-methylacetophenone, R_1=$C_6H_4CH_3$; 2-bromo-4'-nitroacetophenone, R_1=$C_6H_4NO_2$; 2-bromo-4'-methoxyacetophenone, R_1=$C_6H_4OCH_3$; 2-bromo-4'-phenylacetophenone, R_1=$C_6H_4C_6H_5$) in acetone to provide (2-oxo-2-phenyl ethoxy)-2H-1-benzopyran-2-one which was reacted with ethyl 2-(bromomethyl)acrylate in tetrahydrofuran(THF) (Reformatsky reaction) to produce 4-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl) methoxy]-2H-1-benzopyran-2-ones and 7-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl)-methoxy]-2H-1-benzopyran-2-ones as shown in Scheme 2.

EXAMPLE 4

4-[(2,3,4,5-Tetrahydro-3-methylene-2-oxo-5-phenyl-5-furanyl)methoxy]-2H-1-benzopyran-2-one (4)

To a solution of 4-hydroxycoumarin(1.62 g, 10 mmol) in acetone(60 ml) were added 2-bromoacetophenone(1.99 g, 10 mmol) and potassium carbonate (5.53 g, 40 mmol) . The mixture was refluxed for 3 h. (monitored by TLC). Evaporation of the solvent gave a residue which was poured into ice water(50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford 4-(2-oxo-2-phenylethoxy)-2H-1-benzopyran-2-one(4a) (1.76 g, 62.9%) as a needle crystal. mp: 183–184° C.

To a solution of 4a(0.84 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.255 g, 3.9 mmol), hydroquinone(6 mg), and ethyl 2-(bromomethyl) acrylate(0.78 g, 4 mmol). The mixture was reflued under nitrogen atmosphere for 8 h. (monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2(75 ml×3). The dichloromethane extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from ethyl acetate to afford the title compound(0.90 g, 86.4%).

mp: 212–214° C.;

IR(KBr) V max: 1766, 1717, 1620;

UV($CHCl_3$) λ max(log ε): 306 (3.89), 277 (4.10), 266 (4.14);

^{1}H-NMR ($CDCl_3$): δ 3.33–3.66 (m, 2H, 4-CH_2), 4.26–4.32 (m, 2H, OCH_2), 5.60 (s, 1H, 3-H), 5.79 (t, 1H,

vinylic H), 6.42 (t, 1H, vinylic H), 7.20–7.61 (m, 9H, 5,6,7,8-H, and aromatic H);

Anal. for $C_{21}H_{16}O_5$ 0.25 H_2O: Calcd.: C, 71.48, H, 4.71. Found : C, 71.37, H, 4.67.

EXAMPLE 5

7-[(2,3,4,5-Tetrahydro-5-methyl-3-methylene-2-oxo-5-furanyl)methoxy]-2H-1-benzopyran-2-one (5)

Compound 5 was prepared from 7-hydroxycoumarin and chloroacetone according to the procedure given for 4: yield: 79.7%;

mp: 123–124° C.;

IR(KBr) V max: 1755, 1727, 1626;

UV($CHCl_3$) λ max(log ε): 312 (4,18), 243 (3.58);

^{1}H-NMR($CDCl_3$): δ 1.58 (s, 3H, 5-CH_3), 2.79–3.18 (m, 2H, 4-CH_2), 3.99–4.09 (m, 2H, OCH_2), 5.69 (t, 1H, vinylic H), 6.26 (d, 1H, 3-H), 6.29 (t, 1H, vinylic H), 6.76-7.65 (m, 4H, Ar—H);

Anal. for $C_{16}H_{14}O_5$: Calcd.: C, 67.13; H, 4.93. Found : C, 66.95; H, 5.10.

EXAMPLE 6

7-[(2,3,4,5-Tetrahydro-3-methylene-2-oxo-5-phenyl-5-furanyl)methoxy]-2H-1-benzopyran-2-one (6)

Compound 6 was prepared from 7-hydroxycoumarin and 2-bromoacetophenone according to the procedure given for 4: yield: 77.8%;

mp: 105–106° C.;

IR(KBr) V max: 1758, 1719, 1616;

UV($CHCl_3$) λ max(log ε): 321 (4.22), 243 (3.62);

^{1}H-NMR($CDCl_3$): δ 3.24–3.66 (m, 2H, 4-CH_2), 4.17–4.24 (m, 2H, OCH_2), 5.71 (t, 1H, vinylic H), 6.24 (d, 1H, 3-H), 6.31 (t, 1H, vinylic H), 6.72 (d, 1H, B—H), 6.78 (dd, 1H, 6-H), 7.35 (d, 1H, 5-H), 7.40–7.52 (m, 5H, Ar—H), 7.62 (d, 1H, 4-H);

Anal. for $C_{21}H_{16}O_5$ Calcd.: C, 72.41, H, 4.63. Found : C, 72.30; H, 4.67.

EXAMPLE 7

3-Chloro-7-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-phenyl-5-furanyl) methoxy]-4-methyl-2H-1-benzopyran-2-one (7)

Compound 7 was prepared from 3-chloro-7-hydroxy-4-methylcoumarin and 2-bromoacetophenone according to the procedure given for 4, yield: 71%;

mp: 149–150° C.;

IR(KBr) V max: 1762, 1724, 1618;

UV(($CHCl_3$) λ max(log ε): 328 (4.25), 244 (3.69);

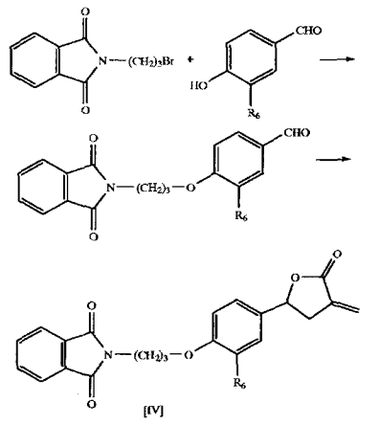
^{1}H-NMR($CDCl_3$): δ 2.52 (s, 3H, CH_3), 3.24–3.68 (m, 2H, 4-CH_2), 4.18–4.25 (m, 2H, OCH_2), 5.71 (t, 1H, vinylic H), 6.31 (t, 1H, vinylic H), 6.73 (d, 1H, 8-H), 6.84 (dd, 1H, 6-H), 7.38–7.52 (m, 6H, 5-H and Ar—H);

Anal. for $C_{22}H_{17}ClO_5$: Calcd.: C, 66.59, H, 4.32. Found: C, 66.23; H, 4.41.

PART C

Preparation of 3-Methylene-2-oxo-5-(p-N-phthalimidopropoxyphenyl)-tetrahydrofuran

(Scheme 3)



[IV]

4-Hydroxybenzldehyde (4-hydroxy-3-methoxybenzaldehyde, R_6=OCH_3) was treated with sodium ethoxide and then alkylated with N-(3-bromopropyl) phthalimide to give p-N-phthalimidopropoxybenzaldehyde which was reacted with ethyl 2-(bromomethyl)acrylate in THF to produce 3-methylene-2-oxo-5-(p-N-phthalimidopropoxyphenyl-tetrahydrofuran.

EXAMPLE 8

3-Methylene-2-oxo-5-(p-N-phthalimidopropoxyphenyl)-tetrahydrofuran (8)

To a solution of 4-hydroxybenzaldehyde(3.18 g, 25 mmol) in ethanol (20 ml) were added sodium ethoxide (0.58 g sodium in 30ml ethanol) and N-(3-bromopropyl) phthalimide(6.70 g, 25 mmol). The mixture was refluxed for 5 h. (monitored by TLC). The solvent was evaporated to give a residue which was disolved in $CHCl_3$(150 ml), washed with 5% aqueous NaOH (80 ml) and water(80 ml). Evaporation of the solvent provided a residual solid which was crystallized from ethyl acetate to afford p-N-phthalimidopropoxybenz aldehyde (8a) (4.57 g, 59 %) as a needle crystal.

To a solution of 8a(0.93 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder(0.255 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl) acrylate (0.78 g, 4 mmol). The mixture was reflued under nitrogen atmosphere for 30 h.(monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution(300 ml) and extracted with $CHCl_3$ (150 ml×2). The extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from methanol to afford the title compound (0.97 g, 85%).

^{1}H-NMR($CDCl_3$): δ 2.19 (m, 2H, CH_2) 2.89–3.34 (m, 2H, 4-CH_2), 3.91 (t, 2H, NCH_2), 4.03 (t, 2H, OCH_2), 5.45 (t, 1H, 5-H), 5.68 (t, 1H, vinylic H), 6.30 (t, 1H, vinylic H), 6.80 & 7.20 (m, 4H, phenyl), 7.70–7.86 (m, 4H, Ar—H);

Anal. for $C_{23}H_{19}NO_5$: Calcd.: C, 70.02, H, 5.07, N, 3.71. Found : C, 69.97, H, 5.07, N, 3.53.

In vitro antitumor assay

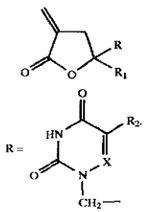
All compounds were evaluated in vitro against 55 human tumor cell lines derived from nine cancer cell types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer). For each compound, dose-response curves for each cell line were measured with five different drug concentrations, and the concentration causing 50% cell growth inhibition(GI_{50}), total cell growth inhibition(TGI, 0% growth) and 50% cell death(LC_{50}, −50% growth) compared with the control was calculated. The GI_{50} values of tested compounds are given in Table I. All of them proved to be active against the growth of leukemia(CCRF-CEM, HL-60, MOLT-4, etc.), colon (COLO 205, HCT-116, HT-29, etc.) cancer cell lines, etc.

The $\log_{10}GI_{50}$, $\log_{10}$TGI and $\log_{10}$LC50 are expressed in the form of mean graphs, however, only the mean graph midpoint values of $\log_{10}GI_{50}$, $\log_{10}$TGI and $\log_{10}$LC50 are listed in Table 2.

We claim:

1. A compound represented by the formula:

[I]



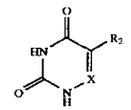
Formula [I], wherein R_1 is a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4)alkyl, (C_1–C_4) alkoxy, nitro and amino; R_2 represented Cl, F or benzyl; X represents CH.

2. A pharmaceutical composition for the treatment of leukemia cancer diseases comprises any compound of claim 1 or their salts in combination with any pharmaceutically acceptable carrier.

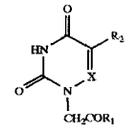
3. A method of treating leukemia cancerous cell growth in an animal, comprising administrating any compounds of claim 1 or their pharmaceutically acceptable salts in an amount effective to inhibit DNA or RNA replication in tumor cells.

4. A synthetic processes for preparing compounds of claim 1, comprising: silylating uracil or its derivative of formula [V] with hexamethyldisilazane (HMDS) and a catalytical amount of chlorotrimethylsilane (TMSCl) followed by alkylation or, in the alternative, alkylating said uracil or its derivative directly with aryl bromomethyl ketone R1COCH2Br in the presence of potassium carbonate (K2CO3) and DMF (N,N-dimethyl-formamide) to afford the 1-ketonyl pyrimidines of formula [VI]; performing Reformatsky-type reaction between ethyl-2-(bromomethyl) acrylate and the respective 1-ketonyl pyrimidines under nitrogen atmosphere to produce the desired 5-aryl-3-methylene-2-oxo-5-(pyrimidin-1-ylmethyl)-tetrahydrofuran of formula [VII], wherein R_1 is a phenyl group optionally substituted with one or two groups selected from the group consisting of halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, nitro and amino; R_2 represented Cl, F or benzyl; X represents CH.

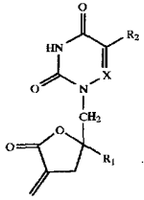
[V]



[VI]



[VII]



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Synthesis and Biological Activity of α-Methylene-γ-butyrolactones

By H. M. R. Hoffmann* and Jürgen Rabe

Dedicated to Dr. Wilhelm A. Schuler on the occasion of his 70th birthday

α-Methylene-γ-butyrolactones [dihydro-3-methylene-2(3*H*)furanones] constitute an important group of natural products and possess wide-ranging biological activities. Progress in the synthesis of the heterocycle and the classification of the synthetic methods are not only of practical interest, but also fundamentally important as a current example for the construction of an unusual 1,4-functionality distance and an α-substituted acrylic ester moiety which is susceptible to nucleophilic attack.

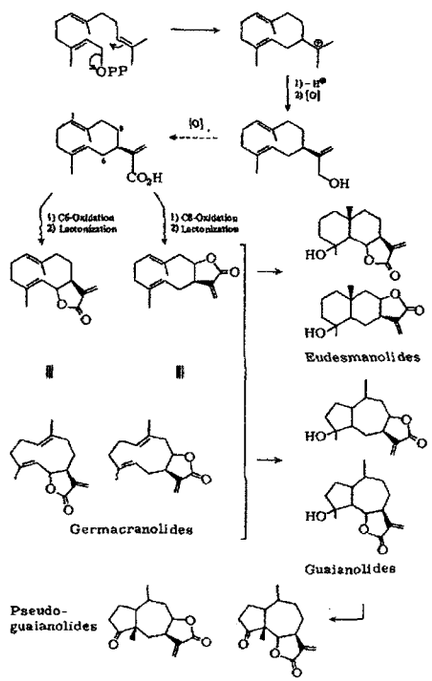
1. Introduction

The α-methylene-γ-butyrolactone ring is an integral building block of many natural products, especially the sesquiterpene lactones, which exhibit interesting biological properties. In a book[1a] published in 1973, ca. 200 representatives of this class of compounds together with their ^{1}H-NMR spectra and other physical data were listed; 6 years later there were already ca. 900[1b]. Since then the number of these compounds has risen further: a Chemical Abstracts Online Search with projection shows that at present at least 2000—3000 α-methylene-γ-butyrolactones, mainly of the sesquiterpene type, are known. Since the current number of structurally elucidated natural products has been estimated to be only between 25000 and 30000[2], α-methylenelactones represent (with approx. 10%) a major class of known natural products. α-Methylene-γ-butyrolactones have been dealt with in a series of articles[4a–d], which quickly appeared following the first review by *Grieco*[3] in 1975. After a description of the biological activities it is our purpose to present the various conceptions for the synthesis of the heterocycle and to describe recently developed syntheses.

2. Biosynthesis

The biosynthesis of the sesquiterpenoid α-methylene-γ-butyrolactones is fairly simple[1,5]. The compounds are derived from *trans,trans*-farnesyl pyrophosphate, which first cyclizes to give the strained cyclodecadiene skeleton of germacradiene (or germacra*triene*) and then reacts further intramolecularly to form a perhydroazulene system (e.g., guaiane and pseudoguaiane) or a decalin system (e.g., eudesmane). The sequence of oxidation steps is less well understood. In accord with Scheme 1, the lactone moiety, which is indicated by the suffix "olide", is *cis*- or *trans*-fused to a six-, seven-, or ten-membered ring. Further rearrangements and oxidative modifications give rise to the structural diversity of these compounds.

Many plants which belong to the large, species-rich family of the *Compositae*[1c] (composites) contain sesquiterpene lactones as characteristic constituents. These compounds are colorless, lipophilic, often bitter-tasting, and are mainly present in the leaf tissue, where they can constitute up to 5% of the dry weight. The closer two species are related to each other, the smaller is the difference between the distribution of these terpenes. In addition to morphology, i.e., the comparison of external plant form, chemotaxonomy, in which secondary plant metabolites are identified and compared, has established itself as a method for the botanical classification of plants and for the clarification of evolutionary problems (sesquiterpene lactones as chemotaxonomic markers)[1a,c].

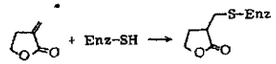


Scheme 1.

[*] Prof. H. M. R. Hoffmann, Dr. J. Rabe
Institut für Organische Chemie der Universität
Schneiderberg 1B, D-3000 Hannover (FRG)

3. Important Biological Activities

α-Methylene sesquiterpene lactones show cytotoxic, antitumoral, and bactericidal properties. Other representatives cause an allergenic contact dermatitis or affect plants by inhibition of growth. There are already several studies on the relationship between biological activity and structure[6–10]. As has been shown, α-methylene-γ-butyrolactones must be looked upon as alkylating agents which are biologically active because they undergo a Michael reaction with biological nucleophiles such as L-cysteine or thiol-containing enzymes (Enz-SH).

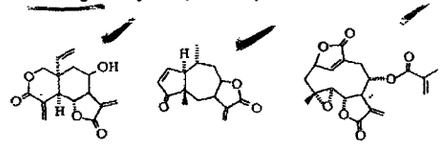


It is probable that these lactones inhibit the incorporation of selected amino acids into proteins, i.e., they inhibit the metabolism at the cellular level, but do not alkylate DNA[8, 11–15].

Presumably, the residual molecule and its lipophilicity also determine the specificity and the site of the activity.

3.1. A[redacted]

A large number of active sesquiterpene lactones, including vernolepin[18a], aromaticin[11], and elephantopin[18b], have been isolated from plant extracts which show tumor-inhibiting activity[16, 17] (Scheme 2).

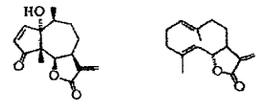


Scheme 2. From left to right: vernolepin, aromaticin, elephantopin.

It has been shown that almost all known cytotoxic sesquiterpene lactones possess an α,β-unsaturated lactone structure, and that the conjugated double bond must be exocyclic. A cyclopentenone or an additional α-methylene lactone moiety enhances the cytotoxic activity; hydroxy groups can also cause an enhancement. The high cytotoxicity of the sesquiterpene lactones is probably due to the inhibition of DNA synthesis and/or transcription[15]. However, protein synthesis, also, is partially impaired[13, 14]. It should be mentioned, however, that at present there is no sesquiterpene cytostatic which is used clinically, apparently because of the relatively high toxicity of the compounds. Attempts to increase the activity by chemical modification have, so far, been unsuccessful.

3.2. Allergenic Activity

Many people suffer from an allergic contact dermatitis (ACD) which is caused by contact with plants or their chemical constituents[19]. Sesquiterpene lactones, which are sometimes present in the pollen, can cause this allergic contact dermatitis—even when carried by the wind. The presence of an α-methylene butyrolactone moiety is a sufficient requirement for the allergenic activity. For example, parthenin, present in *Parthenium hysterophoros*, is a primary allergen of this wide-spread and unusually aggressive pioneer plant. The allergy thus caused represents a serious dermatological problem in India and her neighboring countries[20].

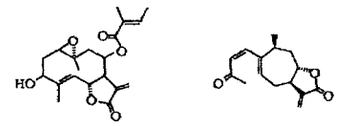


Scheme 3. Left: Parthenin; right: costunolide.

Perfume oils extracted from the costus root *(Saussurea lappa)*[21] or from the laurel *(Laurus nobilis)*[22], cultivated since antiquity, can also cause a dermatitis due to the germacranolide costunolide present in both mixtures. In order to obtain a perfume free from allergen, one passes the raw oil through a column containing a nucleophile on a solid support, e.g. β-aminoethyl-polystyrene. Thus, the allergen is bound to the nucleophile[19b]. Finally, even simple acrylic esters, such as those used in the printing trade and in other industries, can cause occupational dermatitis[19d]. With respect to the pathogenesis of ACD, it is assumed that the α-methylene lactone becomes bonded to a skin protein via a Michael reaction, thus forming an antigen which causes the sensitization of the lymphocytes[20b]. In view of the widespread occurrence of ACD, further research efforts can be expected in this area.

3.3. Phytotoxic and Antimicrobial Activities

In addition to the cytotoxic and allergenic activities described, phytotoxic activities are shown by a number of sesquiterpene lactones[6, 7]. Thus, heliangin, a germacranolide of the tuberous sunflower (*Helianthus tuberosus L.*, also called topinambur or Jerusalem artichoke), and vernolepin, from *Vernonia hymenolepsis*, cause plant growth inhibition[6, 23]. Xanthatin is also used in the regulation of plant growth[5] (Scheme 4).

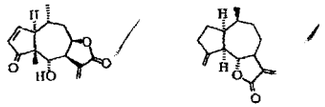


Scheme 4. Left: heliangin; right: xanthatin.

According to *Hager*[24a–c], α-methylene sesquiterpene lactones can attack an SH-group of the auxin-receptor complex; upon binding of the growth hormone auxin to its protein receptor the SH-group is set free. As a result of this

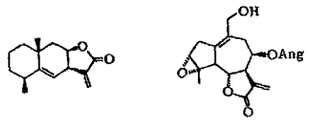
sulfur bond, the lactone effects the irreversible inhibition of plant growth. The α-methylene lactones present in the common sunflower *(Helianthus annuus L.)* appear to be *stress metabolites*, i.e. they are formed, e.g., during attack by pests, during periods of dryness or overexposure to sunlight and heat, and probably act mainly as chemical defenses against pests, especially microorganisms[24c]. This fascinating plant, originating in the USA and Mexico, is mainly cultivated at present in the USSR and in Eastern Europe. It is, after the soybean, the second most important oil-producing plant in the world (annual world production in 1982 more than 16 million metric tons of sunflower seeds)[24d].

Other α-methylene-γ-butyrolactones show bactericidal, fungicidal, and anthelminthic activities[25]. Helenalin[26a] and eremanthin[26b] are shown here as representatives of such compounds (Scheme 5).



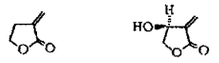
Scheme 5. Left: helenalin; right: eremanthin.

Recently, *Picman* et al. were able to show that sesquiterpene lactones such as parthenin and alantolactone, which are present in the sunflower, also play a role in the defense of plants against insects[27a–c] and even against herbivorous mammals[27d]. Similarly, euponin, which has been isolated from the leaves of *Eupatorium japonicum*, inhibits the development of the fruit fly *(Drosophila melanogaster)* at the egg stage and thus protects the plant from insect attack[27e] (Scheme 6).



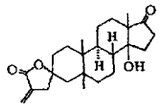
Scheme 6. Left: alantolactone; right: euponin.

Not only highly functionalized, complex sesquiterpene lactones but also simple representatives have been studied for their biological activity. Several years ago, two compounds with fungicidal properties were isolated from tulip bulbs and identified[28]. Tulipalin A is the simplest α-methylene-γ-butyrolactone possible (Chemical Abstracts Name: dihydro-3-methylene-2(3*H*)furanone). The 4-hydroxy derivative, i.e., tulipalin B, is present in nature as the (*S*)-enantiomer (Scheme 7). It appears certain that α-methylene-γ-butyrolactones, due to their high and selective antibiotic activities, afford important protection to plants. In the course of evolution, this fact, together with the outstanding adaptive and reproductive capabilities of the *Compositae*, has contributed to a selective advantage in the survival of this plant family and also to its broad distribution over all continents with the exception of *Antarctica*.

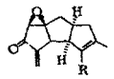


Scheme 7. Left: tulipalin A; right: tulipalin B.

In the future, the study of analogues[16] will become increasingly important. The steroidal spiro-α-methylene lactones[29], which are not found in nature, possess tumor-inhibiting activity (see also [10,66b]).

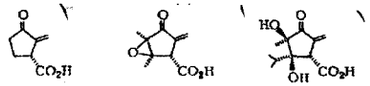


Thus, α-methylene-γ-butyrolactones can serve as a model in the search for biological activity. The structurally related five-membered-ring α-methylene ketones pleurotellol and pleurotellic acid have been found in fungi and show antibiotic activity[30] (Scheme 8).



Scheme 8. R = CH_2OH: pleurotellol; R = CO_2H: pleurotellinic acid.

Comparatively simple representatives of this type are the cyclopentanoid antitumor agents called pentenomycins and methylenomycins, e.g., sarkomycin, methylenomycin A, and xanthocidin[31] (Scheme 9).



Scheme 9. From left to right: sarkomycin, methylenomycin A, xanthocidin.

In view of the similarity of the ester group to the $P(O)(OR)_2$ group as π-acceptor (compare also the Horner-Wittig reaction), a corresponding modification of the lactone is of interest. As *Benezra* et al.[32] were able to demonstrate, the biological activity changes: cyclic phosphonates (Scheme 10) show no allergenic activity and only a very low cytotoxicity.

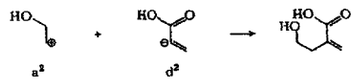
Scheme 10. 2-Methoxy-3-methylen-2-oxo-1,2λ⁵-oxaphospholane.

Because of their broad range of biological activities and their interesting structural features, α-methylene-γ-butyrolactones present a scientific challenge which is reflected in an increasing number of investigations and syntheses of these heterocycles.

4. Syntheses of α-Methylene-γ-butyrolactones

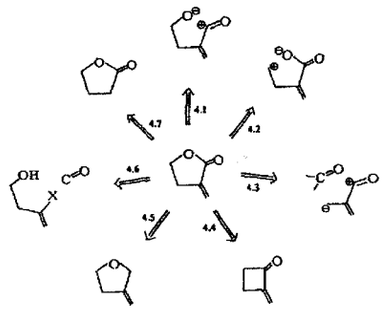
Two main problems dominate the synthesis of these lactones:

1. The construction of the unusual 1,4-functionality distance in the acrylic precursor of the heterocycle. This fundamental synthetic problem, which also appears in the synthesis of simpler five-membered heterocycles such as the furans, pyrroles, and thiophenes as well as their saturated derivatives, can be solved, among other methods, by "umpolung"[33], e.g., the combination a^2+d^2, where the d^2 component is frequently an enolate and the a^2 component an epoxide (umpolung). The combination a^1+d^3 is also feasible: in an efficient synthesis the d^3 building block (umpolung) is also an $a^1d^3d^{3'}$ component (cf. Section 4.3).



2. The construction of the nucleophile-sensitive α-acrylic ester moiety.

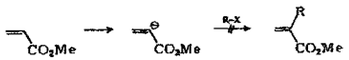
The presence of additional oxygen-containing functionality and the stereochemistry of the residual carbocyclic portion of the molecule increase the appeal of the naturally-occurring sesquiterpene lactones as a synthetic goal. However, the total syntheses of these complex compounds and the elegant work already carried out have recently been described comprehensively in a book by *Heathcock* et al.[34] and will remain largely neglected here. Instead, we will concentrate on the methods of synthesis of the simple, key five-membered-ring. Insofar as meaningful, we will use the retrosynthetic approach both for the classification and the mechanistic interpretation of the large number of synthetic methods. Thus, the organization of the article is predetermined (cf. Scheme 11).



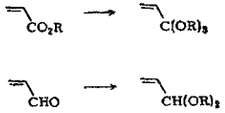
Scheme 11.

If the molecule is dissected as outlined in Sections 4.1–4.4, the educt can be regarded as a functionalized acrylic ester.

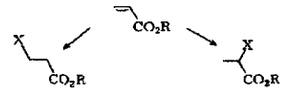
The direct α-functionalization of acrylic compounds via the α-anion is only of limited use due to the pronounced tendency of acrylic esters to polymerize under basic conditions[35]. For this reason, the detour via masked acrylic esters is often chosen, since these are generally easier to functionalize.



The problem of polymerization of α-acrylic esters can be circumvented in different ways. On the one hand, the reactivity of the carbonyl group can be masked by conversion of the ester group or the aldehyde group into an ortho ester or an acetal, respectively.



On the other hand, it is possible to mask the CC double bond, e.g., by adding groups in the α- and β-positions that are capable of undergoing elimination (Scheme 12). Masking is also possible by reversible cycloaddition (see Section 4.8).



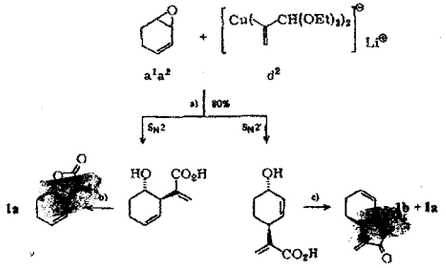
Scheme 12. X = groups capable of elimination.

4.1. Cyclization of 4-Hydroxy-2-methylenebutanoic Acids

Dissection:

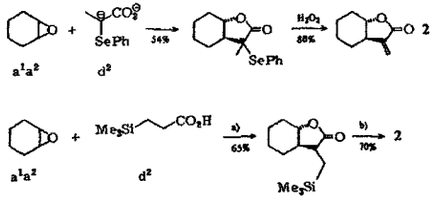
This dissection reduces the synthesis to the preparation of a homoallyl alcohol, the methylene group of which must also be part of an acrylic acid or a masked acrylic acid.

Marino et al.[36a,b] used the diethyl acetal of acrolein as acrylic acid equivalent and allowed it to react as the cuprate (d^2 building block) with epoxides (a^1a^2 building block) (Scheme 13). The two *isomeric alcohols formed* contain the desired 1,4-functionality distance or vinylogous 1,4-distance and can be cyclized to *trans*- and *cis*-fused α-methylene lactones **1a** and **1b**, respectively, following oxidation to their respective acids. As a d^2 component for the reaction with epoxides, dimethyl sodiomalonate has been used, also; subsequent decarboxylative methylenation (cf. Section 4.7) gives the α-methylene lactone[36c].



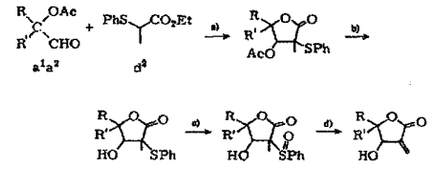
Scheme 13. (a) 1) Tetrahydrofuran (THF)/ −40°C, 2) Ag_2O/OH$^\ominus$. (b) Dicyclohexylcarbodiimide (DCC), $CHCl_3$; 5-*exo*-*trig*. (c) TosOH (*Tos* = tosyl), C_6H_6; 5-*exo*-*trig*.

After masking of the double bond, acrylic compounds can also be functionalized. This can be accomplished by introducing a phenylseleno group via the dianion of 2-phenylselenopropionic acid according to *Petragnani* and *Ferraz*[37] or of a trimethylsilyl group according to *Fleming* et al.[38a] (Scheme 14). After cyclization, both groups can be eliminated with regeneration of the double bond. However, in the case of the silicon compound a bromination is necessary; the selenide, which only needs to be oxidized to the selenoxide, is especially useful, because intramolecular elimination is spontaneous. In both reactions the *trans*-lactone **2** is formed.



Scheme 14. (a) 1) 2 Lithium diisopropylamide (LDA), 0 C, then reflux, 2) TosOH, toluene, reflux. (b) LDA, THF, −78°C, 2) Br_2, CCl_4, −78°C, 3) $(PhCH_2)Me_3N^\oplus F^\ominus$, THF.

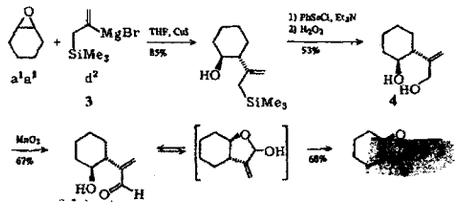
Benezra et al.[38a] used an α-phenylthiopropionic ester as a d^2 acrylic acid in the synthesis of β-hydroxy-α-methylenelactones (Scheme 15). Similarly to the selenide group, the sulfide group can be removed after oxidation to the sulfoxide. However, unlike the selenoxide, the sulfoxide requires heating for elimination to occur.



Scheme 15. (a) LDA, −78°C (acyl-shift). (b) 1) $Ba(OH)_2$, 2) $H^\oplus$. (c) $[O]$. (d) Δ, toluene.

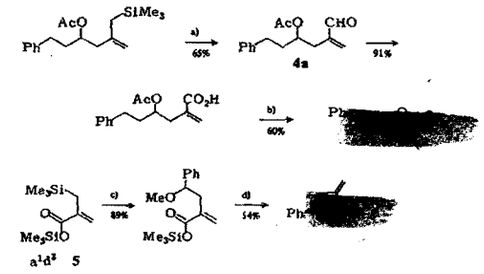
Homologues of α-phenylselenopropionic esters can generally be used in the synthesis of α-alkylidene-γ-butyrolactones also[39].

The allylsilane **3** was employed by *Itoh* et al.[40] as a masked d^2 acrylic acid and was allowed to react with an epoxide (a^2a^1) to give the functionalized homoallylic alcohol (Scheme 16). Reaction with PhSeCl and subsequently with H_2O_2 (desilylating oxidation via the allyl selenide and oxidative rearrangement) gave the allyl alcohol **4**, which was oxidized with MnO_2 to give selectively the α,β-unsaturated aldehyde. As also occurs in other examples (see below), the second hydroxyl function is not attacked by MnO_2; the aldehyde cyclizes to give the lactol, which is further oxidized in situ to the lactone. The starting material for the Grignard reagent **3** is 2-bromo-3-(trimethylsilyl)propene **21**, which was also used by *Trost* et al.[85], although in a different way, in the synthesis of α-methylene lactones (see Section 4.6).



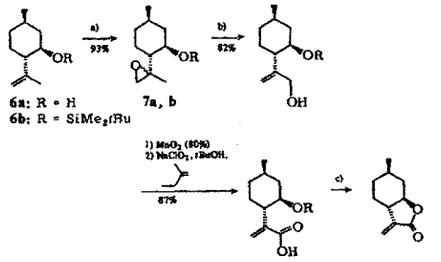
Scheme 16.

Functionalized allylsilanes appear as intermediates in the syntheses described by *Fujita* et al.[41] and *Sakurai* et al.[14,42a] also. The key step in *Fujita*'s (Scheme 17) work is a desilylating oxidation of an allylsilane to give an α,β-unsaturated aldehyde. This was achieved in a novel way, using iodosylbenzene, which was activated by coordination with a Lewis acid. In contrast to the oxidation of an allylsilane with *m*-chloroperbenzoic acid, which proceeds with allyl inversion (intermediate formation of the epoxide), the formation of **4** and **4a** proceeds with allyl retention. In the case of oxidation giving **4** and **4a**, an S_E2' attack on the electron-rich allylsilane is followed by an intramolecular 3,2-shift. *Sakurai* et al.[42a] allowed the trimethylsilyl ester **5** to react with an acetal and obtained by dealkylation the α-methylene-γ-butyrolactone (Scheme 17).



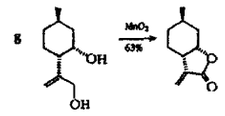
Scheme 17. (a) C_6H_5IO, BF_3·Et_2O. (b) DCC, pyridine. (c) $PhCH(OMe)_2$, BF_3·Et_2O. (d) 1) Me_3SiI, 2) MeOH.

The combined homoallylic alcohol-allylic alcohol such as **4**, formed in the reaction sequence of *Itoh*[40] (Scheme 16), is an intermediate in many other synthetic routes since the selective oxidation with MnO_2 yields a cyclizable compound and therefore offers convenient access to the desired lactones. Thus, in the work of *Pinnick* et al.[42b] (Scheme 18) the protected isopulegol **6b** is converted into the epoxide **7b**, which in the presence of the strong base $LiNEt_2$ undergoes a regioselective E2 reaction to give the allylic alcohol. The further oxidation of the α,β-unsaturated aldehyde, formed by reaction of the alcohol with MnO_2, to give the α,β-unsaturated carboxylic acid is not trivial, nor is the oxidation of other allylic alcohols to their carboxylic acids. Recently, inexpensive $NaClO_2$ has been recommended for this oxidation, also in the presence of sensitive functional groups.

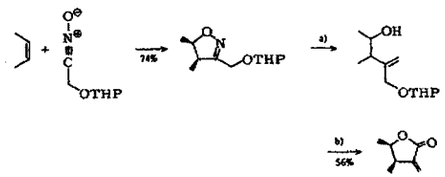


Scheme 18. (a) *m*-Chloroperoxybenzoic acid (CPBA). (b) $LiNEt_2$, −78°C→RT. (c) 1) Desilylation, 2) DCC, pyridine (77%); or 1) Me_3SiI, $CHCl_3$, Δ, 2) Distillation.

With MnO_2, the epimeric diol **8** is directly converted via the cyclic hemiacetal into *cis-p*-menthenolide[42b]. Thus, cyclization to the *cis*-fused lactone proceeds more easily than to the *trans*-fused isomer.

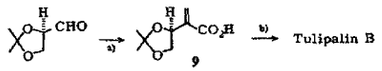


In the route described by *Kozikowski* et al.[43a] (Scheme 19) an isoxazoline, formed by cycloaddition, is reductively opened and the *exo*-methylene double bond is then formed by a Wittig reaction. Again, cleavage of the tetrahydropyranyl (THP) ether and oxidation with MnO_2 affords the α-methylene lactone.



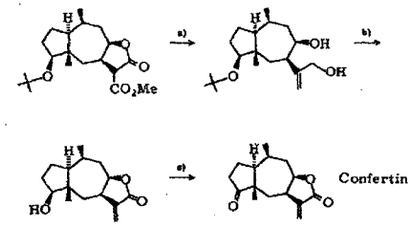
Scheme 19. (a) 1) H_2, Raney nickel, 2) $H_2C{=}PPh_3$. (b) 1) TosOH, MeOH (OTHP→OH), 2) MnO_2, CH_2Cl_2.

2,3-*O*-Isopropylideneglyceraldehyde—a chiral building block which has been used a great deal recently and has perhaps also become something like a vogue compound[43b]—was homologized in several steps to **9** and then cyclized to tulipalin B using acid catalysis[43c] (Scheme 20).



Scheme 20. (a) 5 steps. (b) 1 NHCl.

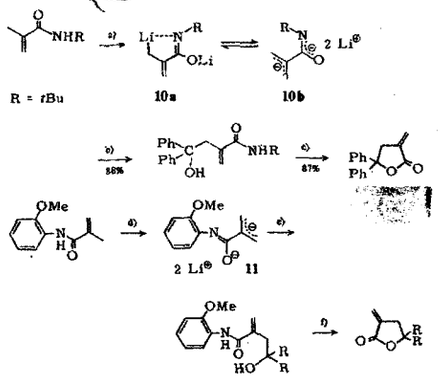
Another possibility for obtaining the desired "crossed" allylic-homoallylic alcohol was demonstrated by *Marshall* et al.[44] in the synthesis of confertin (Scheme 21). The enolate of a modified malonic ester, formed on using KH, is reduced by $LiAlH_4$ to the dialcohol stage. Oxidation with MnO_2 affords the α-methylene lactone, although in unreported yield. This method was already described in 1965 and is still used today[45,46].



Scheme 21. (a) 1) KH, 2) $LiAlH_4$. (b) 1) MnO_2, C_6H_6, 2) CF_3CO_2H, 3) *i*PrOH, NaOH. (c) CrO_3, pyridine, CH_2Cl_2 (Collins oxidation).

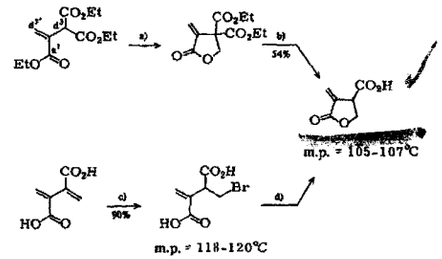
N-substituted methacrylamides can be used as synthetic equivalents of the hypothetical $a^1d^3d^{3'}$ methacrylic acid since they are more easily deprotonated and alkylated than acrylic esters. This method, developed in 1980, was used by three different research groups in the synthesis of α-substituted acrylamides[47] and of α-methylene lactones[48,49a] (Scheme 22). These syntheses proceed via the dianions **10b** and **11**, which polymerize less easily, probably because the amide nitrogen chelates a lithium ion and the reactivity of the carbonyl group is simultaneously reduced by electron delocalization. Trapping with ketones yields the cyclizable homoallylic alcohols (cf. also Section 4.3, i.e. the umpolung of (*Z*)-2-bromomethyl-2-alkenoic esters to give $a^1d^3d^{3'}$ components).

An interesting $a^1d^3d^{3'}$ building block is the readily accessible, doubly activated triethyl 2-propene-1,1,2-tricarboxylate, as shown recently by *Dowd* et al.[49b]. For this compound even catalytic amounts of potassium bicarbonate suffice for deprotonation and for bringing about the nucleophilic addition to formaldehyde. After decarboxylation of the crude lactone diester with hydrochloric acid, the crystalline β-carboxylic acid of α-methylene-γ-butyrolactone is obtained in 54% overall yield. Alternatively, butadiene-2,3-dicarboxylic acid has been converted smoothly into bromomethyl-itaconic acid, which in turn cyclizes



Scheme 22. (a) 2 BuLi/*N,N,N',N'*-tetramethylenediamine (TMEDA)/THF, −70 → −20°C, 1 h. (b) Ph_2CO. (c) Xylene, reflux, 3 d. (d) KOtBu/BuLi. (e) 1) R_2CO, 2) H_2O. (f) 10% HCl.

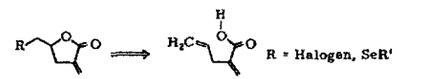
spontaneously to the same lactone in neutral or basic conditions[49b] (Scheme 23).



Scheme 23. a) 35% CH_2O, catalytic amounts $KHCO_3$, 65°C, 25 min. b) 20% HCl. c) HBr/AcOH (1 mol). d) neutral or basic solvent.

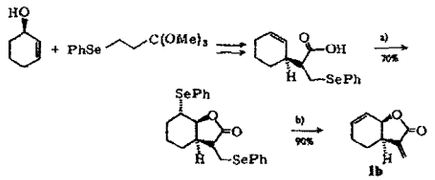
4.2. Cyclization of 2-Methylene-4-pentenoic Acids

Dissection:

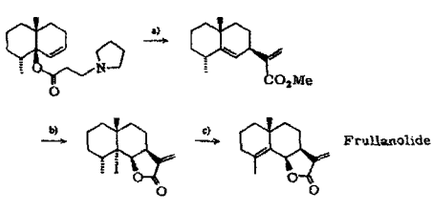


Raucher et al.[50] used the Claisen ortho ester rearrangement, which leads directly to the desired arrangement of carboxyl group and terminal CC double bond, i.e., to the γ,δ-unsaturated carboxylic acid or 4-pentenoic acid; cyclization was accomplished via seleno-lactonization. Oxidation of the selenium group and spontaneous elimination gave the α-methylene group and—thanks to the second phenylselenide group—another double bond (Scheme 24).

By analogy with the synthesis described by *Raucher* et al.[50], *Still* et al.[51] had previously started from an allylic alcohol and used a Claisen triethylsilylketene acetal rearrangement, i.e., the Ireland variant, for the synthesis of a functionalized acrylic ester, which was converted into the desired frullanolide in two steps in 30% total yield (Scheme 25).



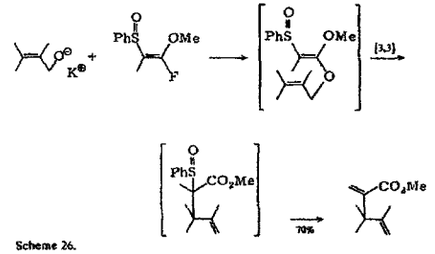
Scheme 24. (a) PhSeX. (b) 30% H_2O_2, THF. The two parallel arrows mean: 1) heating in mesitylene (160°C, 24 h) in the presence of trimethylacetic acid (0.1 equiv.); 2) demethylation with LiI in 2,6-dimethylpyridine, reflux, 2 h.



Scheme 25. (a) 1) LDA/THF, 2) Et_3SiCl, 3) Δ, 4) $(MeO)_2SO_2$, MeOH, K_2CO_3. (b) Iodolactonization; *5-exo-trig*. (c) 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU).

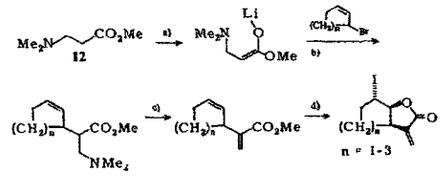
Mechanistically related is the one-pot reaction recently described by *Vatele*[52] for the synthesis of α-methylene-γ,δ-unsaturated esters. An allylic alcohol is again used as starting material, which is transformed in three steps into a cyclizable 2-methylene-4-pentenoic acid (Scheme 26):

1. nucleophilic substitution at the activated vinylic carbon atom,
2. Claisen ester enolate rearrangement, and
3. sulfoxide elimination to introduce the double bond.



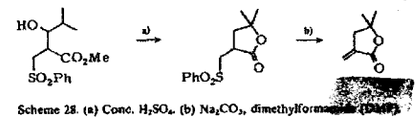
Scheme 26.

Helquist and *Yu*[53a] used methyl 3-(*N,N*-dimethylamino)propionate **12**, which is readily available via Michael addition of dimethylamine to acrylic ester, as d^2 acrylic building block. As in the case of the methacrylamides already described (Section 4.1), metalation of the ester is facilitated by complexation with nitrogen[53]. Reaction of the anion with allyl bromides gives the desired γ,δ-unsaturated ester, which, following elimination of the amino group, is subjected to iodolactonization.



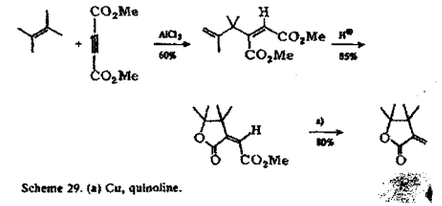
Scheme 27. (a) LDA, THF, −78°C. (b) THF, hexamethylphosphoric triamide (HMPT), −78°C→25°C. (c) 1) MeI, CH_3OH, 2) 1,5-Diazabicyclo[4.3.0]non-5-ene (DBN), C_9H_6. (d) 1) KOH, CH_3OH, H_2O, 2) HCl, H_2O, 3) KI, I_2, $NaHCO_3$.

The methods of *Shono* et al.[54] (Scheme 28) and *McCulloch* et al.[55] are mechanistically interesting but not generally applicable because of their drastic reaction conditions. *Shono* et al.[56] applied a method for lactone formation described by *Dobrev* and *Ivanov*[56] to the synthesis of α-methylene lactones.



Scheme 28. (a) Conc. H_2SO_4. (b) Na_2CO_3, dimethylformamide (DMF).

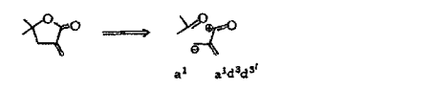
The synthesis of *McCulloch* et al.[55] (Scheme 29) begins with an $AlCl_3$-catalyzed ene reaction[57] of the strongly enophilic dimethyl acetylenedicarboxylate. After acid-catalyzed lactonization, the ester function is removed by reductive treatment with copper in quinoline. Since the reduction is only successful for highly substituted five-membered rings, the range of application of the reaction is limited.



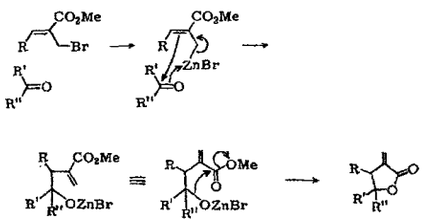
Scheme 29. (a) Cu, quinoline.

4.3. Metal-Promoted Reaction of Aldehydes and Ketones with $a^1a^3a^{3'}$ Components

Dissection:



In this method (Scheme 30), developed by *Dreiding* et al.[58,59] and *U. Schmidt* et al.[60], a (*Z*)-2-bromomethyl-2-alkenoic ester ($a^1a^3a^{3'}$ building block) is treated with zinc to give an $a^1d^3d^{3'}$ intermediate with umpolung. The combination with the a^1 aldehyde takes place with allylic shift and yields an intermediate alkoxide, which cyclizes spontaneously to the α-methylene lactone. The Dreiding-Schmidt reaction, which is also incorrectly called a Reformatsky synthesis (in which d^2 enolate esters, but not d^3 components are used), has been very useful in the synthesis of monocyclic and spiro-α-methylenelactones[61].

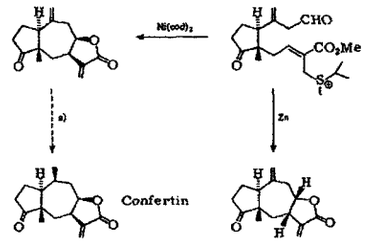


Scheme 30.

The intramolecularization of the reaction affords bicyclic α-methylenebutyrolactones from acyclic compounds in one pot[62] (Scheme 31).

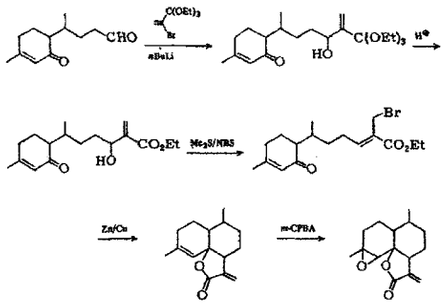
Scheme 31.

In this fashion, *Semmelhack* et al.[63] successfully synthesized (±)-confertin in 1978 (Scheme 32). They obtained the confertin isomer with the unnatural configuration by zinc-promoted cyclization, whereas nickel-promoted cyclization yielded the natural isomer. The selective hydrogenation of this precursor to confertin was unsuccessful; however, it was accomplished by a detour.



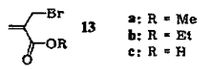
Scheme 32. (a) Low yield. cod = cyclooctadiene.

Dreiding et al.[64] synthesized compounds of the arteannuin type (Scheme 33).



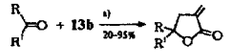
Scheme 33. NBS = *N*-bromosuccinimide.

Perhaps the most important building blocks in the synthesis of γ-monosubstituted and γ,γ-disubstituted α-methylene-γ-butyrolactones are the 2-(bromomethyl)acrylic ester **13a** and the ethyl ester **13b**.



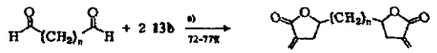
13 a: R = Me; b: R = Et; c: R = H

Recently, the synthesis of **13a** in three steps (50% yield) and that of **13b** in two steps (30% yield) have been described[65a]. 2-(Bromomethyl)acrylic acid **13c** is also available commercially[65b]. Starting from **13b**, *Benezra* et al.[10] reported the synthesis of more than thirty lactones, some of which are structurally quite complex (Scheme 34).



Scheme 34. (a) 1) Zn/THF, 2) dil. HCl.

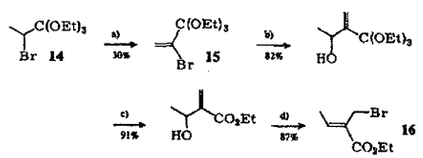
Heindel et al.[66a], *Lee* et al.[66b], and *Cassady* et al.[67a] (Scheme 35) proceeded similarly; *Cassady* et al. also obtained bislactones[67a], i.e., double haptens, from dialdehydes by using the corresponding one-pot reaction[67c].



Scheme 35. (a) 1) Zn/THF, 2) dil. H_2SO_4, 0°C.

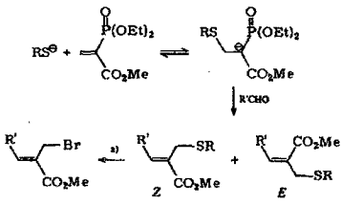
A synthetic difficulty in the metal-promoted cyclization to give bicyclic α-methylene lactones has been in the synthesis of the functionalized methacrylic acid as starting material.

Starting from the ortho ester **14**, *Dreiding* et al.[68a] synthesized the ortho ester **15** of 2-bromoacrylic acid in several steps. **15** was metalated with *n*-butyllithium to give the corresponding lithium compound, i.e., a d^2-acrylic ester. After trapping of the lithium compound with aldehydes, the hydroxy ester was rearranged to the substituted allyl bromides **16** (Scheme 36).



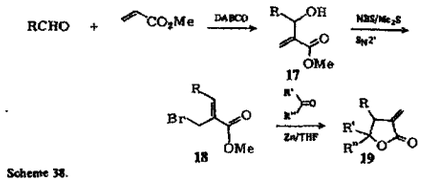
Scheme 36. (a) 1) KOrBu, 2) Br_2, CuO, 3) KOH, EtOH. (b) 1) BuLi, 2) CH_3CHO. (c) H_2SO_4. (d) NBS/Me_2S.

The synthesis of the allylic sulfur compound used by *Semmelhack* et al.[68b] as $a^1a^3a^{3'}$ precursor, also requires several steps and is not (*Z*)-selective. The last step, the conversion of the allylic sulfide into the bromide, is not very simple either (Scheme 37).



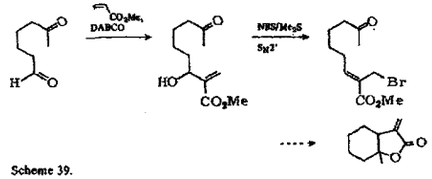
Scheme 37. (a) 1) [O], 2) $(MeO)_3P$, 3) PBr_3 (overall yield 20—25%); or 1) $MeOSO_2F$, 2) LiBr (overall yield 93%).

In connection with the synthesis of a wide range of allyl alcohols and their precursors, which are further functionalized on the central carbon atom[69], we have prepared the necessary $a^1a^3a^{3'}$ coupling reagents (the allylic bromides **18**) in excellent yields by DABCO (diazobicyclo[2.2.2]octane)-catalyzed coupling of acrylic esters and aldehydes to **17** and subsequent bromination accompanied by allylic rearrangement[70–72] (Scheme 38).



Scheme 38.

The reaction of 2-(hydroxyalkyl)acrylates **17** with *N*-bromosuccinimide/dimethyl sulfoxide yields regioselectively (S_N2' reaction) and stereoselectively (*Z*)-2-(bromomethyl)-2-alkenoic esters **18** irrespective of the group R[70–72]. Metal-induced coupling of **18** with carbonyl compounds allows ready access to β-substituted α-methylene lactones (compare **19**). Another advantage of this approach is that parent acrylic ester can be α-functionalized without loss of material, much additional functionality being tolerated. Esoteric acrylic acid building blocks and organometallic conditions are unnecessary. The selective coupling of a ketoaldehyde, e.g., 6-oxoheptanal, with an acrylic ester in the presence of DABCO is also possible; the less reactive ketone group need not be masked, e.g. by ketalization. After bromination with NBS/Me_2S, which entails clean allylic rearrangement, a cyclizable molecule is obtained[73] (Scheme 39).



Scheme 39.

In addition to these compounds, other a^1d^3 components—as already described in Section 4.1—have been used in the cyclization: the allylsilane **5** and the dianions **10** and **11**. The allylic bromoester method offers the advantages of a simple synthesis, a high degree of convergence, mild reaction conditions, and compatibility with other functionality. At least for many of the structurally more simple α-methylene-γ-butyrolactones, this is the method of choice.

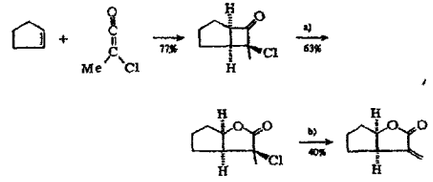
4.4. Insertion of the Oxygen via Baeyer-Villiger Oxidation of 2-Methylenecyclobutanones

Dissection:

There are only a few examples of the synthesis of α-methylene lactones via the Baeyer-Villiger oxidation.

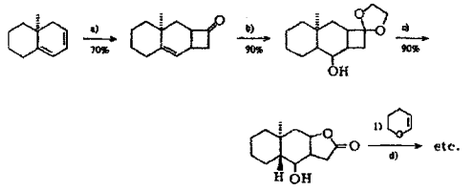
The syntheses of *Hassner* et al.[74] and *Roberts* et al.[75] will be presented here. As shown in Scheme 40, this approach begins with a [2+2]-cycloaddition of chloro- and bromoketenes to reactive double bonds to form a functionalized cyclobutanone. Baeyer-Villiger oxidation with exclusive migration of the bridgehead carbon atom and subsequent elimination of hydrogen halide yields the desired lactone.

In following the fate of the three carbon atoms of the chloroketene one may see that these are incorporated into the product as the desired acrylic moiety. In the example given here, the total yield of α-methylene lactone—starting from the haloketene precursor—is 20%. It remains to be seen whether this route can be developed preparatively. A limitation of the cycloaddition route is that only *cis*-fused lactones result (cf. also Section 4.8).



Scheme 40. (a) H_2O_2, CH_3CO_2H. (b) Diazabicyclo[2.2.2]octane (DABCO), NaI, dimethyl sulfoxide (DMSO).

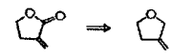
Grieco et al.[76] also used a Baeyer-Villiger oxidation to construct a γ-lactone and subsequently incorporated the α-methylene group by reaction of the enolate with formaldehyde (see Section 4.7) (Scheme 41).



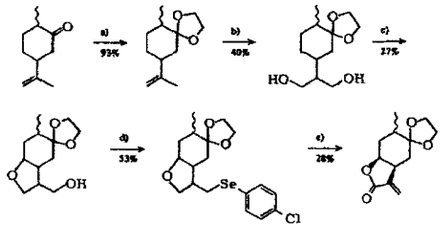
Scheme 41. (a) 1) $Cl_3CHCOCl$, 2) Zn/CH_3CO_2H. (b) 1) Ketal formation, 2) BH_3/THF, NaOH/H_2O_2. (c) 1) 10% HCl, 2) HOAc/30% H_2O_2. (d) α-Methylenation.

4.5. Introduction of the Carbonyl Oxygen by Oxidation of 3-Methylenetetrahydrofurans

Dissection:



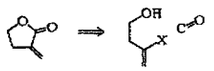
This very simple method, which could also be of biosynthetic interest, has only been described recently. Starting from dihydrocarvone, *Brocksom* and *Ferreira*[77] synthesized monoterpenoid α-methylene-γ-butyrolactones (Scheme 42). After an interesting oxidative cyclization using lead tetraacetate to give the furan derivative (compare also the Barton reaction), the *exo*-methylene group is formed by selenoxide elimination. Finally, the carbonyl oxygen is introduced, in analogy to the method of *Dauben* et al.[78], by Collins oxidation with $CrO_3 \cdot 2$ pyridine. It remains to be seen whether other oxidizing agents will bring about an improvement in yield.



Scheme 42. (a) TosOH, $HOCH_2CH_2OH$. (b) 1) *m*-CPBA, 2) LDA, 3) B_2H_6, H_2O_2, NaOH. (c) $Pb(OAc)_4$. (d) 1) TosCl, pyridine, 2) *p*-Cl–C_6H_4–$Se^{\ominus}$. (e) 1) H_2O_2, THF, 2) CrO_3, pyridine.

4.6. Insertion of a Carbonyl Group into Functionalized Homoallylic Alcohols

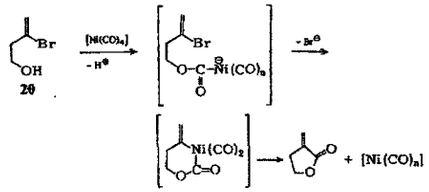
Dissection:



This synthesis can be realized using reactions taken from the organic chemist's "box of magic tricks", namely by

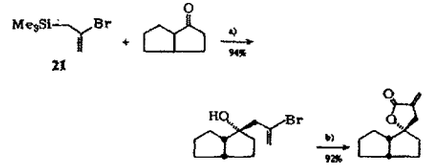
a) transition-metal-catalyzed carbonylation of a vinylically substituted homoallylic alcohol and
b) use of the Shapiro reaction.

a) In recent years the intramolecular version of the transition-metal-catalyzed carbonylation described by *Corey* and *Hegedus*[79] has been used increasingly for the synthesis of α-methylene lactones. Zero-valent transition metal compounds, usually of nickel or palladium, function as catalysts and reagents. Scheme 43 shows the proposed mechanism[80,81] of the nickel-induced reaction, in which 3-bromohomoallylic alcohols **20** have been employed as starting compounds. Recently, *Ban* et al. have also used the more reactive 3-iodohomoallylic alcohols[86b].



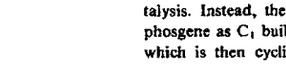
Scheme 43.

Representative of other syntheses[82–84] are those of *Trost* et al.[85] and *Stille* et al.[86], described here. *Trost* et al.[85] joined a functionalized allylsilane to a carbonyl compound to obtain the functionalized homoallylic alcohol which is necessary for the insertion of the carbonyl group. The tricyclic spiro-α-methylenelactone is formed stereoselectively and in high yield (Scheme 44).

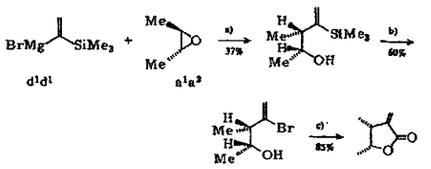


Scheme 44. (a) $TiCl_4$, CH_2Cl_2, $-70 \rightarrow 0$°C. (b) 1.5 $[(Ph_3P)_2Ni(CO)_2]$, Et_3N, THF, reflux.

The Grignard reagent **3** prepared from 2-bromo-3-(trimethylsilyl)propene **21** can also be combined with epoxides to establish the desired 1,4-distance of functional groups[40] (cf. Section 4.1) and added to α,β-unsaturated enones in Michael fashion using CuI catalysis, cyclopentane precursors being formed[85]. The possibility of storing the carbanion reactivity of 2-bromo-3-(trimethylsilyl)propene while selectively activating the allylsilane (with $TiCl_4$), and subsequently metalating the bromide (with magnesium) makes **21** a valuable equivalent for the hypothetical dianion **22** (cf. also the conversion of **3** into **4**).

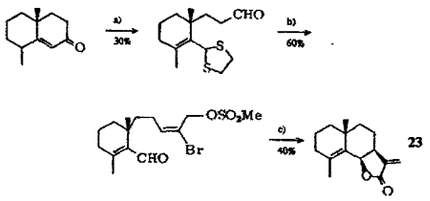


Stille et al.[86a] reacted a vinylic Grignard compound (a d^1d^1 ethylene building block) with epoxides (a^1a^2 components) and thus obtained the desired homoallylic alcohol. Electrophilic substitution of the trimethylsilyl group by bromine yielded the 3-bromohomoallyl alcohol required for the carbonylating cyclization[86a] (Scheme 45).



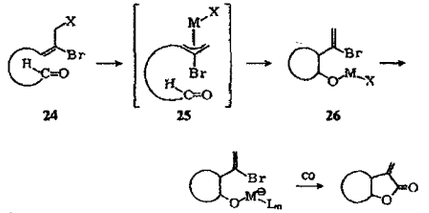
Scheme 45. (a) 1) CuI, THF, Et_2O, 2) $H^{\oplus}$. (b) 1) Br_2/CH_2Cl_2 −78°C, 2) NaOMe/MeOH. (c) 1) CO, $[Pd(PPh_3)_4]$, 2) CH_3CN, K_2CO_3.

Carbonylnickel plays an interesting double role in the synthesis of (±)-frullanolide (*Semmelhack* et al.[87], Scheme 46).



Scheme 46. (a) 4 steps. (b) 2 steps. (c) $[Ni(CO)_4]$, C_6H_6, 65°C.

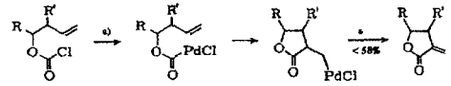
The allylic system in **24** can first be activated as π-allylnickel complex **25** (M = Ni)[88] and cyclized with the aldehyde function to give **26** (potentially a *cis/trans* mixture). In the next step, tetracarbonylnickel functions as carbonylating reagent to form the α-methylene lactone (Scheme 47).



Scheme 47.

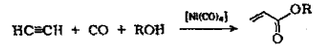
Experimentally, a very high selectivity is observed for the formation of a *cis*-fused lactone as well as for the *syn*-orientation of the lactone with respect to the angular methyl group (cf. **23**). The reason for these stereochemical results is not yet clear.

A variant of the transition-metal-catalyzed cyclization was recently presented by a French research group[92]. In this reaction, CO is not inserted and cyclized using Pd^0 catalysis. Instead, the homoallyl alcohol is esterified with phosgene as C_1 building block to give the chloroformate, which is then cyclized to give the α-methylene lactone (Scheme 48).



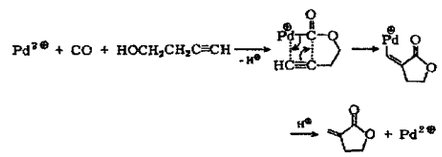
Scheme 48. (a) $[Pd(PPh_3)_4]$, Na_2CO_3.

A fundamental result of Reppe chemistry is the synthesis of acrylic esters by nickel-catalyzed carbonylation of acetylene[89] (Scheme 49).



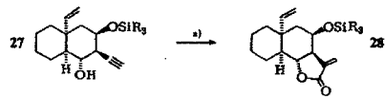
Scheme 49.

As *Norton* et al. were able to show, this reaction can also be intramolecularized by treating a homopropargyl alcohol with CO in the presence of catalytic $PdCl_2/SnCl_2$ and a tertiary phosphane[90] (Scheme 50).



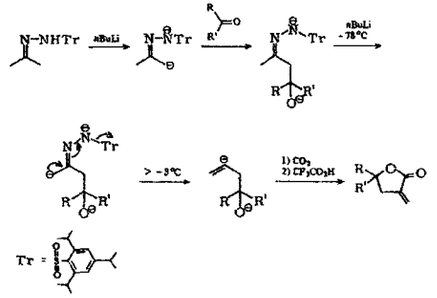
Scheme 50.

The reaction proceeds under comparatively mild conditions. In a model experiment for establishing the functionality in the BC ring, *Heathcock* et al.[91] converted the *trans*-homopropargyl alcohol **27** into the *trans*-fused lactone **28** (Scheme 51). Although the yield is low (21%), the α-methylene lactone is formed directly in this way, and the normally chosen α-methylenation of a γ-lactone precursor (cf. Section 4.7) is not necessary.



Scheme 51. (a) $PdCl_2$, CO, thiourea.

b) A further variant is the insertion of CO, according to *Barrett* et al., via the Shapiro reaction, in which CO_2 functions as CO building block[93] (Scheme 52).

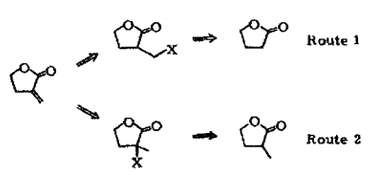


Scheme 52.

The dianion from the activated hydrazone of acetone is allowed to react with ketones. As usual, elimination of nitrogen yields a vinyl anion (cf. also **22**), which after trapping by carbon dioxide (carboxylation instead of carbonylation) is cyclized to the α-methylene lactone. This reaction sequence requires strongly basic conditions, which are not always tolerated by highly functionalized molecules.

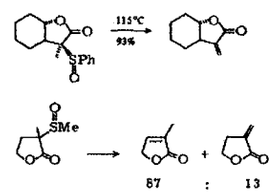
4.7. α-Methylenation of 2-Oxotetrahydrofurans

Dissection:



The later incorporation of the α-methylene group into a preformed lactone has been used for many years in the synthesis of α-methylene lactones. Since the various methods have also been reviewed, only variations and new methods will be mentioned here.

For the incorporation of the α-methylene moiety, a functionalized C_1 building block is usually introduced and the double bond is formed by subsequent elimination (Route 1). However, the introduction of a leaving group α to the carbonyl group, when the methyl group is already present, is also known (Route 2). In this case, elimination results in the exocyclic double bond, although the endocyclic double bond can also be formed[94–96] (Scheme 53).



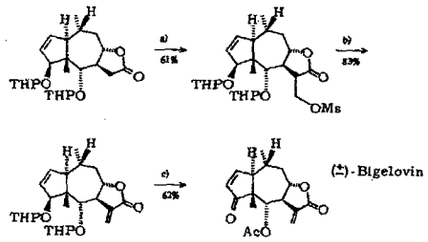
Scheme 53.

In order to avoid the formation of the endocyclic double bond, one introduces the leaving group advantageously β to the carbonyl group (Route 1).

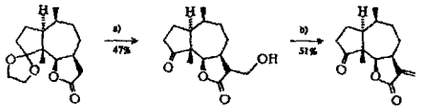
Two methods are used most frequently at present:

1. The lactone is converted into the enolate and subsequently trapped with formaldehyde to give the hydroxymethyl derivative. Alternatively, the anion is trapped with formic esters and reduced to give the hydroxymethyl derivative. After conversion of the hydroxy group into a tosylate or mesylate group, elimination is easily carried out with a base.

As almost arbitrary examples the synthesis of (±)-bigelovin by *Grieco* et al.[97] (Scheme 54) and of (±)-damsin by *Vanderwalle* et al.[98] and *Kretchmer* et al.[99] (Scheme 55) are shown here.



Scheme 54. (a) 1) LDA, 2) CH_2O, 3) MsCl (Ms = methanesulfonyl), pyridine. (b) DBU. (c) 1) Hydrolysis, 2) MnO_2, 3) Ac_2O, dimethylaminopyridine (DMAP).

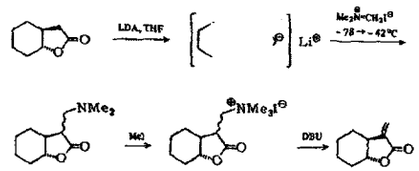


Scheme 55. (a) 1) NaH, HCO_2Et, 2) $NaBH_4$, MeOH, 3) HCl, MeOH. (b) 1) TosCl, 2) Δ, pyridine.

Other sesquiterpene lactones which have been obtained via α-methylenation are, e.g., (±)-ambrosin, (±)-psilostachyin C[100a], as well as (±)-eriolanin and (±)-eriolangin[100b]. This methodology, which was developed by *Grieco* et al.[101c], has also been applied to other representatives of the guaianolides (which have been investigated much less than the pseudoguaianolides), including, inter alia, (±)-compressanolide and (±)-estafiatin (*Vanderwalle* et al.[101a, b]).

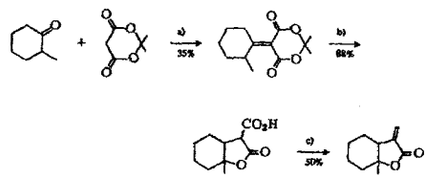
2. Another valuable method for introducing the α-methylene group uses the $-\overset{\oplus}{N}R_3$ group as leaving group. This can be introduced by trapping of the enolate of the lactone with dimethyl(methylene)ammonium salts. After quaternization of the nitrogen, elimination affords the double bond[102–105] (Scheme 56).

The synthesis of (±)-eriolanin by *Schlessinger* et al.[106a] and that of vernolepin by *Isobe* et al.[106b] were carried out similarly. The incorporation of the α-methylene group by reductive amination of an α-carboxy lactone with CH_2O/



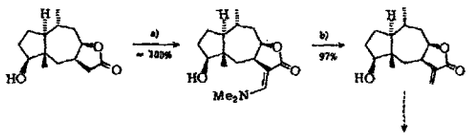
Scheme 56.

$HNEt_2$ has been known for ten years[107a] and was used again recently in the synthesis of bicyclic α-methylene lactones via Meldrum's acid[107b] (Scheme 57).



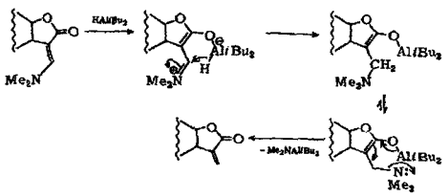
Scheme 57. (a) $TiCl_4$, THF, pyridine. (b) H_2SO_4. (c) 1) $H_2CO/HNEt_2$, 2) NaOAc/HOAc.

A disadvantage of many of the methods described until now is the use of strong bases for generating the lactone enolate. In this respect the introduction of the Bredereck reagent[108] by *Ziegler* et al.[109] has brought about an improvement (Scheme 58).



Scheme 58. (a) $(Me_2N)_2CHOMe$, Δ. (b) 1) $HAlBu_2$, THF, hexane, −78→25°C, 2) aq. NH_4Cl.

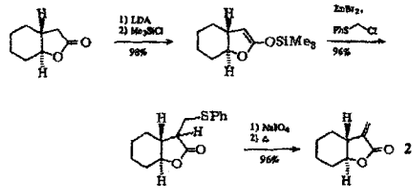
Using this reagent, one prepares the vinylogous carbamate in the first step. This is reduced to the α-methylene lactone selectively and in remarkably high yield with $HAlBu_2$ (Scheme 58). The use of strong bases, which are otherwise necessary for generating the lactone enolate, is thus avoided. The reaction mechanism formulated by us



Scheme 59.

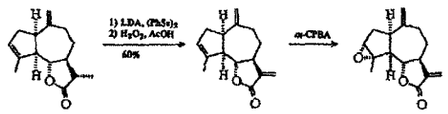
(Scheme 59) suggests that the specific deuteration of the methylene position[109a] should be feasible by reduction of the vinylogous carbamate with diisobutylaluminum deuteride (DAl/Bu_2).

Sulfur and especially selenium may also serve as leaving groups. The following synthesis, in which sulfur is incorporated exocyclically in the β-position (Route 1) was described by *Paterson* and *Fleming*[110a]. The sulfur, following its oxidation to the sulfoxide, is removed as usual in a pericyclic reaction by heating (Scheme 60).



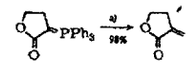
Scheme 60.

The incorporation of the phenylseleno group via electrophilic diphenyl diselenide and its elimination to give the double bond were steps in the synthesis of estafiatin[110b, c] (Scheme 61).



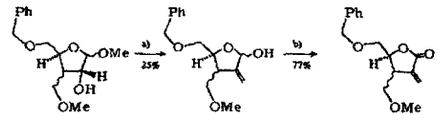
Scheme 61.

It has been known for several years that α-methylene lactones can be synthesized via a Wittig reaction[111, 112], but this reaction has only been employed in specific cases (Scheme 62).



Scheme 62. a) Paraformaldehyde.

Fraser-Reid et al.[113] and *Nair* et al.[114a] have shown that chiral α-methylene lactones can be obtained from the chiral pool using a Wittig reaction (Scheme 63).

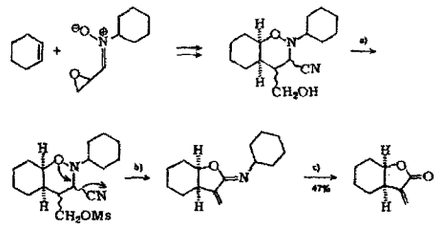


Scheme 63. a) 1) Pyridiniumchlorochromate (PCC), CH_2Cl_2, 2) $Ph_3P{=}CH_2$, 3) 1,2-Dimethoxyethane (DME)/H_2O. b) Ag_2CO_3-celite.

4.8. Other Methods

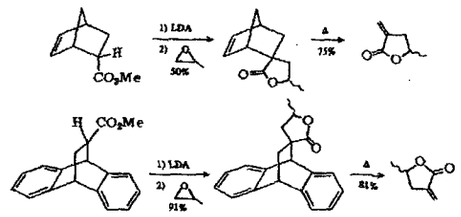
Synthetic methods which have not been considered in Scheme 2 will be dealt with in this section. These involve pericyclic reactions and rearrangements:

a) The cycloaddition of nitrones to olefins (Diels-Alder reaction with inverse electron demand) described by *Eschenmoser* et al.[115] was further studied by *Riediker* and *Graf*[116]. As already mentioned (see Section 4.4), the cycloaddition route is limited in its applicability insofar as only *cis*-fused lactone rings can be prepared (Scheme 64).



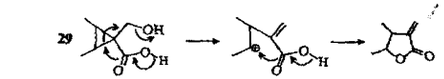
Scheme 64. a) MsCl/pyridine. b) KO*t*Bu/*t*BuOH. c) 0.25 N H_2SO_4, CCl_4.

b) The Diels-Alder reaction can be used for the protection of the CC double bond of acrylic ester as cycloadduct. After the deprotonation with lithium diisopropylamide (LDA), the S_N2 reaction with propylene oxide gives the desired 1,4-functionality distance and then the spirolactone is formed. In the final step, the protecting group is cleaved off by cycloreversion[117, 118] (Scheme 65).



Scheme 65.

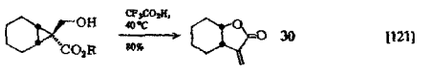
c) A third route to synthesize α-methylene lactones is the cyclopropane rearrangement developed by *Hudrlik* et al.[119]. All the atoms of the product are already present in the cyclopropane educt **29**. The cyclopropylmethyl-homoallyl rearrangement is facilitated by S_N1-like conditions (Scheme 66). In the case of the formation of the bicyclic lactone **30** the two rings are *cis*-fused (Scheme 67).



Scheme 66.

Recent examples have been described by *Hiyama* et al.[120] and *Hudrlik* et al.[121]. The remarkably sensitive tulipalin A is formed in 43% yield under almost neutral, anhydrous conditions[120].

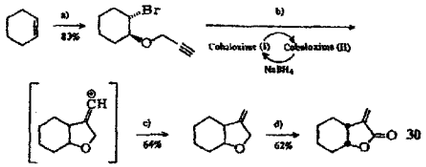
[120]



[121]

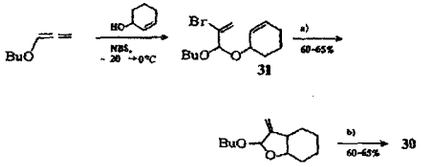
Scheme 67.

d) The formation of CC bonds can also be brought about by carbon radicals, and this approach has become a popular field of research in recent years. Carbon-centered radicals react with electron-rich and electron-deficient π-systems under neutral conditions. Thus, compounds with numerous further potential reaction centers are employable without the need for protecting groups. One example is the cyclization of a propargyl ether to a secondary carbon radical. This radical is formed by single electron transfer (SET) from catalytic amounts of cobaloxime(I), which is cyclically regenerated from cobaloxime(II)[123]. The last step, i.e. the oxidation of the 3-methylenetetrahydrofuran to the lactone, is still preparatively difficult (Scheme 68).



Scheme 68. (a) HC≡C–CH_2OH, NBS, −30°C→RT. (b) $e^{\ominus}$(−$Br^{\oplus}$). (c) Solvent-H. (d) CrO_3, pyridine, CH_2Cl_2.

One other route starts from functionalized bisallyl ether **31**, which, once again, is prepared with NBS and an excess of allyl alcohol at low temperature, probably via an ionic intermediate. The generation of the vinyl radical from **31** takes place under normal conditions. Although **31** carries different types of labile hydrogen atoms (allylic and one acetal hydrogen), the cyclization proceeds in respectable yield (60—65%). Jones oxidation furnishes the desired lactone **30**[124] (Scheme 69).



Scheme 69. (a) Bu_3SnH, 2,2'-azobis(isobutyronitrile) (AIBN), C_6H_6. (b) Jones oxidation.

Besides 2-cyclohexenol, other allylic alcohols can be used for the reaction with the allenyl ether.

The various radical reactions illustrate that new synthetic methods must go through the trials of practical application and total synthesis. Conversely, it is clear that such an important class of heterocycles as the α-methylene-γ-butyrolactones mobilizes the whole arsenal of synthetic methods which are at our disposal.

5. Outlook

Kupchan's discovery in 1970 of the cytotoxic activity of the sesquiterpene lactones was the green light for a rapid development, which is continuing today and proceeding in various directions. The isolation and identification of many additional representatives, especially by *Bohlmann* et al.[125], the total syntheses, and the discovery of a broad spectrum of biological activities followed. At the same time the lactones functioned as a lead in the search for analogues and attention was focussed also on completely different constituents of the *Compositae*, as well as on their pharmaceutical and economic uses. Of the many new and ingenious methods that have been published on the preparation of the α-methylene-γ-butyrolactone moiety, a large number are of academic interest only. The most commonly used methods include:

1. oxidative cyclization of crossed allylic-homoallylic alcohols (Section 4.1),
2. halogeno-lactonization and seleno-lactonization of 2-methylene-4-pentenoic acids (Section 4.2),
3. metal-promoted cyclization of $a^1a^3a^3$' components with carbonyl compounds (Section 4.3),
4. transition-metal-catalyzed carbonylation of 3-bromo- and 3-iodohomoallylic alcohols (Section 4.6);
5. α-methylenation of preformed γ-butyrolactones (Section 4.7).

The investigations carried out in this sector have enriched heterocyclic chemistry, terpene synthesis, and, at the same time, contributed to the development of new synthetic methods.

The future offers a wide choice for interdisciplinary efforts regarding the use of α-methylene-γ-butyrolactones in the fields of organic chemistry, biology, immunology, medicine, and pharmacology.

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Molecular recognition in allergic contact dermatitis to natural products

CLAUDE BENEZRA

Laboratoire de Dermatochimie, Clinique Dermatologique
Université Louis Pasteur, CHU, 67091 Strasbourg, France

Abstract- The chemical stuctures of natural skin allergens is discussed: the majority is electrophilic or "proelectrophilic" (i.e. they can be transformed in the skin into electrophiles. A number of natural and model skin allergens (or haptens), in particular optically active β-substituted-α-methylene-γ-butyrolactones such as tulipalins B, have been synthesized and their allergenic activity tested in the guinea pig. A remarkable enantiospecificity of the *in vivo* response is noted when the chiral center is close to the protein site of attack, the α-methylene group. Use of an *in vitro* model, reaction of selected haptens with a dipeptide, BOC-Cys (SH)-Ala-OMe, shows parallelism between the stereoselectivity of the Michael addition and enantiospecificity of the *in vivo* reaction, allowing some reasonable predictions to be made .

Allergic Contact Dermatitis (ACD) is a disease of the skin consecutive to the contact with some substances of natural or synthetic origin called allergens or haptens (ref. 1). The compounds responsible for this adverse skin reaction are generally electrophilic or "pro-electrophilic" and can react with nucleophilic groups in proteins. It is believed that haptens (or allergens) which are low molecular weight compounds penetrate into the skin, get bound to epidermal proteins and are taken up by the macrophage of the epidermis, the dendritic Langerhans cell (ref. 2) . The latter processes the hapten-protein adduct (called complete antigen) though pinocytosis and endocytosis and enzymatic hydrolysis leads to hapten-peptide conjugates which are reexpressed to the surface of the cell, along with elements of the major histocompatibility complex (MHC) called Ia or D_R antigen. The ternary hapten-peptide-MHC complex is presented to another cell which plays an important role, the T-lymphocyte , where it is recognized by receptors. A clonal selection and blastogenesis of those T-cells (helper cells) able to recognize the hapten then occurs in the node and the system is now ready ("hypersensitive") for another contact with the same or a related allergen. The whole process takes about 5-7 days. In the first stage (sensitization) nothing is visible on the skin and it is only if a second contact takes place. The process is then faster (12-24 hours) and results in skin inflammation (redness or erythema plus swelling or edema). Fortunately another subpopulation of T-cells also forms at the same time, suppressor cells, which are responsible for diminishing the inflammation reaction or even to suppress it. Not everybody will give an eczematous reaction when exposed to the same substance. The presence or absence of allergic contact dermatitis (ACD) is the result of a delicate balance between effector and suppressor T-cells. Hereditary factors seem to regulate this.

A molecular approach of the ACD problem addresses the following questions:

-What structural features make a compound an allergen (or a hapten)? (This should help in prevention of ACD).

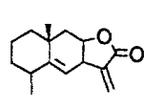
-How specific is the molecular recognition of a hapten?

-Is it possible to conceive compounds able to generate the formation of T-suppressor cells, in order to regulate this immunological phenomenon?

The following paper tries to give an answer to the first two questions.

NATURAL SKIN CONTACT ALLERGENS

There are thousands of substances responsible in nature for the development of ACD (ref. 3, 4) . Several families of plants are known for their agressivity on the skin. Thus, the Compositae family is one of the most offensive ones. The compounds responsible for ACD are sesquiterpene lactones such as, for instance alantolactone **1**. In India, a Compositae, inadvertently imported from the States in the 50's with wheat, grew to such an extent as to become a real national threat, this is *Parthenium hysterophorus* L. known as Congress Grass, particularly in the region of Poona. Another major problem, in Europe this time, is the benzoquinone, primine **2**, present in *Primula obconica* (Hance). Finally every American is aware of the adverse skin reaction to two species of Anacardiaceae plants, poison ivy (*Rhus radicans* L.= *Toxicodendron radicans* (L.) Kuntze, mostly in the East Coast) and poison oak (*Rhus diversiloba* Torr & Gray= *Toxicodendron diversilobum* Greene). Both contain urushiols, a mixture of 3-alkylcatechol derivatives, such as the pentadecycatechol **3** .



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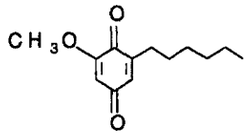

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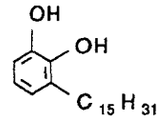

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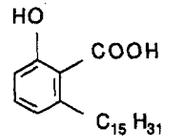
There are a number of other long chain phenols also responsible for allergic contact dermatitis. Beside examples taken from the Anacardiaceae family (such as cashew nut or *Anacardium occidentale* L., or mango or *Mangifera indica* L.) , one could mention salicylic acid derivatives present in ginkgo (*Ginkgo biloba* L.). We have studied recently Allergic Contact Dermatitis to ginkgo extracts in the guinea pig. Cross-reactions (i.e. a person allergic to a compound A can react to a compound B, structurally related) to ginkgo in poison ivy-sensitive patients have been described. It was believed that ginkgolic acid **4** could be metabolized into cardanol **5**, which could in turn be reoxidized into a catechol, present in *Rhus* plants.



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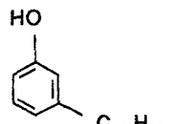

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In guinea pigs, however, no cross-reactions between ginkgo-sensitized animals and poison ivy-sensitized ones was observed (ref.5).

Famous other examples of skin contact allergens are α-methylene-γ-butyrolactone (also known as tulipalin A), present in tulips, quinones in tropical woods, aldehydes in cinnamon (cinnamaldehyde) or in perfumes (example, citral in lemongrass) etc.

MOLECULAR MECHANISMS OF ALLERGIC CONTACT DERMATITIS (ACD) INDUCTION

The few examples shown above show that the majority of natural skin haptens are electrophiles and in particular Michael acceptors. This is the case obviously for α-methylene-γ-butyrolactones, sesquiterpenic or not, quinones, α,β-conjugated aldehydes

such as cinnamaldehyde. Some are prohaptens, i.e. they must undergo a chemical transformation in order to become truly allergenic. Thus, catechols are known to be easily oxidized (either *in vivo* or *in vitro*) into reactive o-quinones. Aminoacid candidates for reaction with skin haptens are mostly cysteine with its SH group and lysine with its ε-NH_2 function.

By using a model skin allergen, ^{13}C-methyl alkylsulfonate:

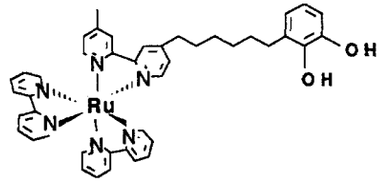
$$CH_3\text{-}(CH_2)_{11}\text{-}SO_3{}^{13}CH_3$$

and a model protein, human serum albumin, HSA, we have demonstrated by ^{13}C-NMR that lysines were involved in hapten-protein covalent bond formation. The only "free" cysteine present in HSA did not react, but there was some methionine-hapten bond formation (ref. 6).

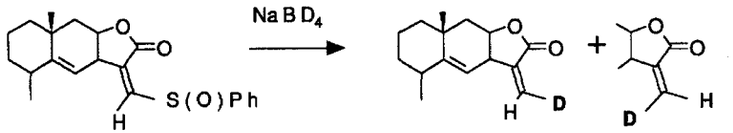
In order to try and establish some structure-activity relationships of the sensitizing (i.e. contact allergy inducing) substances, it seemed of interest: a) to label natural haptens in order to follow them in the skin or in skin extracts and b) to synthesize models of natural skin haptens.

LABELLING SKIN HAPTENS

We have attached a fluorescent probe to a derivative of the famous poison ivy allergen (ref. 7) This is the following tris-bipyrydyl Ruthenium complex.



Model labelling with deuterium was effected by reducing vinylsulfoxides derived from alantolactone and isoanlantolactone, two sesquiterpene α-methylene-γ-butyrolactones with deuterium borohydride (ref. 8). The reaction gave good yields of the deuterated lactones and always proceeded with predominant (2/3) retention of configuration (Scheme I).



Scheme I Deuteration of a vinyl sulfoxide derivative of alantolactone.

SYNTHESIS OF MODEL SKIN HAPTENS

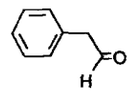
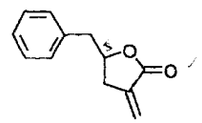
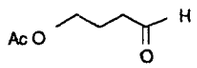
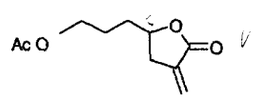
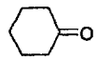
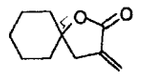
In order to try and understand structural factors associated with the mechanism of allergic contact dermatitis (ACD), we have synthesized a number of allergens and model allergens.

We have studied in particular α-methylene-γ-butyrolactones with different substituents. For many of the lactones, the main sythetic route used was Reformatsky reaction with methyl bromomethymethacrylate (ref.9). However, it is not always convenient to use the usual basic conditions and require in particular for good yields nonenolizable ketones.

We have therefore developed modified Reformatsky conditions in order to be able to use either methacrylic acid or methacrylates. Thus in a method (A) using the

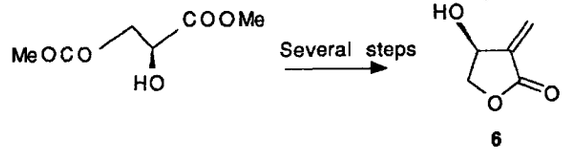
bromomethacrylate ester, a 5:2 THF/Water solvent mixture saturated with ammonium chloride was used. In a second method (B), bromomethacrylic acid could be used directly in a THF-water mixture saturated with ammonium chloride and containing triethylamine. The yields were superior to the ones described in the literature (Table 1).

Table 1. Reformatsky synthesis of α-methylene-γ-butyrolactone (Yields in isolated products)

Substrate	Product	Yields Methods A	B	lit.
		75	52	15
		70	--	12
		77	47	31

Recently, we have used stannous chloride and amberlyst 15 catalysis to prepare a number of α-methylene-γ-butyrolactones under acidic conditions; yields of α-methylene-γ-butyrolactones ranged from 65 to 92 % with aldehydes(ref. 10). None of these methods is suitable for the synthesis of optically active lactones. For this purpose, we used chirons from different sources.

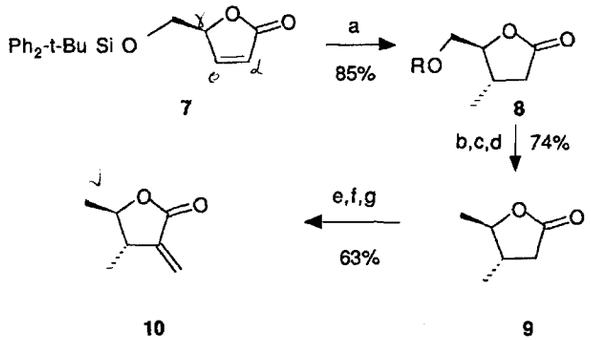
Thus, a number of β-substituted-α-methylene-γ-butyrolactones have been prepared. Both natural tulipalin B **6** and its unnatural enantiomer were synthesized from (-)- S- and (+)-R-methyl lactates respectively (Scheme II) (ref.11).



6

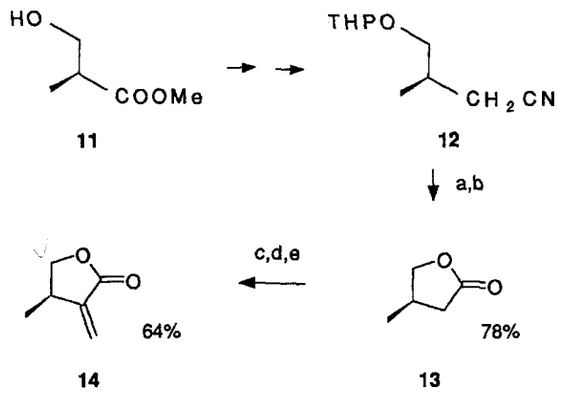
Scheme II Synthesis of (+)-Tulipalin B **6**

(+)-*trans*-β,γ-Dimethyl α-methylene-γ-butyrolactone **10** was readily prepared from γ-t-butyl-diphenylsilyloxymethyl-γ-butenolide **7** obtained in four steps from L-glutamic acid. Michael addition of lithium dimethyl cuprate resulted in high diastereofacial differenciation, resulting in 85% yield of pure enantiomer **8** with the silyl and methyl group *trans* to each other. Classical desilylation, followed by bromination of the γ-hydroxymethyl group and reduction by tributyltin hydride gave β,γ-dimethyl-γ-butyrolactone **9**. The α-methylene group was introduced by LDA treatment followed by reaction with Eschenmoser salt and DBU (Scheme III) (ref.12).



Scheme III Synthesis of *trans*-β, γ-dimethyl-α-methylene-γ-butyrolactone **10** a:a a: Me_2CuLi (R=Ph_2(t-Bu)Si); b:FNBu4; c: CBr_4, PPh3; d:Bu_3SnH, AIBN; e: LDA, H_2CNMe_2I; f: MeI; g: DBU

β–Methyl α-methylene-γ-butyrolactone **14** was synthesized in 5 steps (ref.12) from the known nitrile **12** obtained from methyl β-hydroxybutyrate **11** by Mori's procedure (ref.13) in an overall 82% yield. Hydrolysis of nitrile **12**, followed by deprotection of the alcohol and cyclization with HCl afforded lactone **13** in 78% yield. The α-methylene group was introduced as above with an overall 64% yield (Scheme IV),



a: KOH; b: HCl; c: LDA, $H_2C{=}NMe_2I$; d: MeI; e: $NaHCO_3$

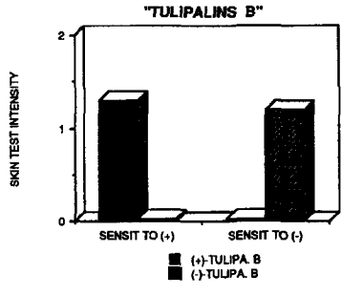
Scheme IV Synthesis of β-methyl–α–methylene-γ-butrolactone **14** from methyl β-hydroxybutyrate

BIOLOGICAL ACTIVITY OF α-METHYLENE-γ-BUTYROLACTONES

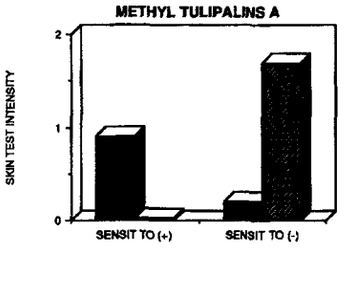
Determination The biological activity of the haptens was determined by *in vivo* methods, using classical sensitization methods in the guinea pig (ref.14) and in mice. In the guinea-pig, the induction of allergy (i.e.sensitization) was effected by injecting intradermally, in the nuchal region of the animal, a 1:1 mixture of Freund complete adjuvant (FCA) and of saline, containing the hapten in varying concentrations. This was repeated three times. Then, the animal, after a 2 weeks rest, was tested epicutaneously by depositing an olive oil:acetone dispersion of the hapten on a measured circular area of the back of the animal. After 24h (but also after 48 and 72 hours), the test was read and graded in a 0-3 scale according to the intensity of the skin reaction (erythema or redness and edema or swelling). The results presented in the different Tables are the averages of the reaction of groups containing 8 to 10 animals and are statistically significant at a $p<0.01$ level.

Results: β-substituted-α-methylene-γ-butyrolactones

These were found to be significant sensitizers (ref. 14) , contrary to reports of the literature (ref.16). In particular, β-hydroxy lactones such as tulipalin B **6**, were as good haptens as the unsubstituted methylenelactones . We sensitized two groups of animals to both natural and unnatural enantiomers of tulipalin B. There was no cross-reaction between the two groups: (+)-tulipalin B-sensitized animals did not react to (-)-tulipalin B and reciprocally (Graph 1). Thus, molecular recognition of these allergens is **enantiospecific**. The same was found for (+)- and (-)-β-methyl-α-methylene-γ-butyrolactones **14** (Graph 2) (ref. 12). This was also true for sesquiterpene lactones from a liverwort, *Frullania:* there is no cross-reactions between the two enantiomers which occur in two different species, namely (+)- and (-)-frullanolides (ref. 17).



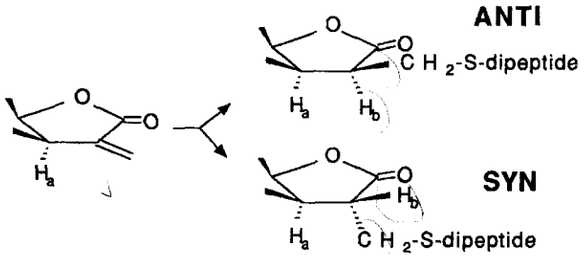
Graph.1 Skin test reactions to (+)- and tulipalins B. No cross-reaction was observed.



Graph 2. Skin test reactions(-)- to (+)- and (-)-β-methyl–α–methylene-γ-butyrolactones.No cross-reaction occurred.

AN *IN VITRO* MODEL OF THE ENANTIOSPECIFICITY OF MOLECULAR RECOGNITION OF β-SUBSTITUTED-α-METHYLENE-γ-BUTYRO-LACTONES

Is it possible to predict the specificity of the skin reaction to β-substituted-α-methylene-γ-butyrolactones? In order to address this question and to try and find a chemical model of protein-aminoacids-hapten reactions, we have prepared a dipeptide, BOC-L-Cys(SH)-Ala-OMe and reacted it with the different enantiomeric lactones according to Scheme V.



Scheme V Reaction of BOC-Cys(SH)-Ala-OMe with α-methylene-γ-butyrolactones.

The reaction conditions were 0.2 M sodium phosphate buffer at pH 7.4 containing absolute methanol to dissolve the dipeptide and the lactone. The results are shown in Table 2.

Thus, when the nucleophilic addition is stereoselective, with high anti/syn ratios, the reaction on the skin is specific and no cross-reaction is observed (i.e. a (+)-enantiomer sensitized animal does not recognize the (-)-enantiomer). When the dipeptide addition is not or poorly stereoselective, the reaction in the skin is not selective: (+)-enantiomers sensitized animals recognize the (-)-enantiomer and conversely. Selectivity of the skin reaction and of the Michael addition of the BOC-Cys(SH)-Ala-OMe peptide to methylenelactones are parallel (ref. 18). Therefore, this model dipeptide although a very rough image of what may happen in skin between nucleophilic proteins and incoming haptens, gives an idea about the selectivity of *in vivo* molecular recognition .

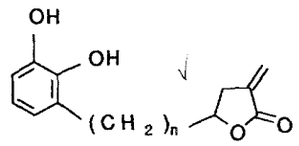
Table 2. Results of addition of BOC-Cys(SH)-Ala-OMe to α-methylene-γ-butyrolactones and of skin tests in guinea-pigs.

γ-LACTONE	Anti-Syn ratio	Average skin reaction to enantiomers[a] (+)	(-)
(+)-β-methyl-	nd	0.9	0
(-)-β-methyl-	9.5/1	0.2	1.7
(+)-γ-methyl	6/1	1.3	0.4
(-)-γ-methyl	2/1	0.8	0.9
(+)-Frullanolide	ANTI	2.0	0.2
(-)-Frullanolide	ANTI	0.3	1.8

[a] Average of skin test intensities varying from 0 to 3 (very strong)

CONCLUSION

The aim of this work was to try and predict adverse skin reactions due to natural and synthetic substances. Some conclusions can be drawn: 1) molecular recognition of haptens by the skin seems enantioselective 2) a chemical model of the supposed *in vivo* protein-hapten interaction gives satisfactory results. Finally, some compounds, not described in this paper seem to have promising properties for inducing specific skin tolerance to some chemicals, such as, for instance, the double-headed hapten below which induces ACD to the pyrocatechol ("poison ivy like") end and tolerance to the α-methylene-γ-butyrolactone ("tulipalin like") end (ref. 19).



Acknowledgement

The efficient and skillful contributions of my coworkers, whose name is mentioned in the references is gratefully acknowledged.

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United States Patent [19]

Tzeng et al.

[11] **Patent Number: 5,646,164**

[45] **Date of Patent: Jul. 8, 1997**

[54] **α-METHYLENE-γ-BUTYROLACTONES: NEW INHIBITORS OF PLATELET AGGREGATION**

[75] Inventors: **Cherng-Chyi Tzeng; Yeh-Long Chen; Tai-Chi Wang**, all of Kaohsiung; **Che-Ming Teng**, Taipei, all of Taiwan

[73] Assignee: **National Science Council**, Taipei, Taiwan

[21] Appl. No.: **557,268**

[22] Filed: **Nov. 14, 1995**

[51] **Int. Cl.**[6] **C07D 405/12**; C07D 407/12; A61K 31/47; A61K 31/37

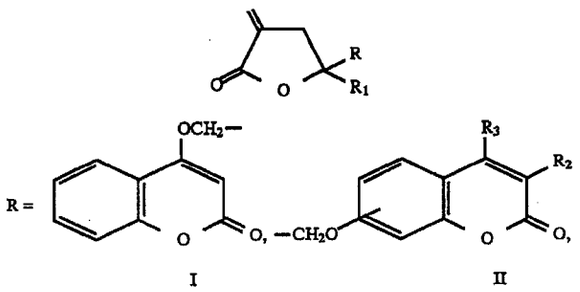
[52] **U.S. Cl.** **514/314**; 514/457; 546/178; 549/284

[58] **Field of Search** 546/178; 514/314, 514/457; 549/284

Primary Examiner—David B. Springer
Attorney, Agent, or Firm—W. Wayne Liauh

[57] **ABSTRACT**

The present inventors have discovered three classes of novel α-methylene-γ-butyrolactones with excellent antiplatelet activity. As a result of intensive studies, it has been found that compounds represented by the formula I–III are potent inhibitors of platelet aggregation.



I II

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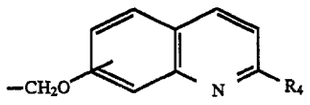


III

For the formula I, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, $(C_1–C_4)$ alkyl, $(C_1–C_4)$ alkoxy, phenyl, nitro, amino.

For the formula II, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, $(C_1–C_4)$ alkyl, $(C_1–C_4)$ alkoxy, phenyl, nitro, amino; R_2 represents hydrogen, halide, $(C_1–C_4)$ alkyl, phenyl, nitro, amino; R_3 represents hydrogen, halide, $(C_1–C_4)$ alkyl, phenyl, nitro, amino.

For the formula III, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, $(C_1–C_4)$ alkyl, $(C_1–C_4)$ alkoxy, phenyl, nitro, amino; R_4 represents hydrogen, hydroxy, $(C_1–C_4)$ alkyl.

The present invention also provides a cost-efficient method for the preparation of formula I–III.

Formula I–III may be administered orally or parenterly with an inert diluent or with a pharmaceutically acceptable carrier in the treatment or the prevention of cardiovascular disease.

5 Claims, No Drawings

α-METHYLENE-γ-BUTYROLACTONES: NEW INHIBITORS OF PLATELET AGGREGATION

TECHNICAL FIELD OF THE INVENTION

The present invention relates to the therapeutical application of antiplatelet α-methylene-γ-butyrolactones.

BACKGROUND OF THE INVENTION

Cardiovascular diseases, especially various forms of thrombosis, such as coronary, embolic, venous and traumatic thrombosis, account for a large number of death per year. In fact it is estimated by the American Heart Association that 54% of all deaths in the United States can be attributed to cardiovascular disease. It is therefore important for us to be familiar with physical, chemical and clinical aspects of drugs used to treat these form of thrombosis. Since it is believed that initiation of thrombus formation is dependent on platelet aggregation, the inhibitors of platelet aggregation could be prototypes for drugs that are more effectively combat thrombosis that leads to heart attacks and strokes. It was therefore prompted us to search for novel compounds possessing more potent inhibiting activity on platelet aggregation.

Coumarin derivatives such as bishydroxycoumarin and warfarin are the principal anticoagulants. Other clinically useful antiplatelet drugs are aspirin, eicosapentanoic acid (EPA), dipyridamole, dazoxiben, and ticlopidine. Their utilization is, however, limited by the potency and the side effects. This invention describes the preparation of α-methylene-γ-butyrolactone-containing coumarins, quinolines, and quinolinones from commercially available starting materials in an efficient route. The products describes herein are suitable for large-scale production and exhibit very strong and extensive antiplatelet activity.

DETAILED DESCRIPTION OF THE INVENTION

This invention included the preparation and the antiplatelet evaluation of novel α-methylene-γ-butyrolactones which have been proved to be potent inhibitors of platelet aggregation. These active compounds, as free type or their pharmaceutically acceptable salts, may be administered parenterally or orally in a suitable pharmaceutical form. They also may be administered along or in conjunction with other antiplatelet agents, in combination with any pharmaceutically acceptable carrier.

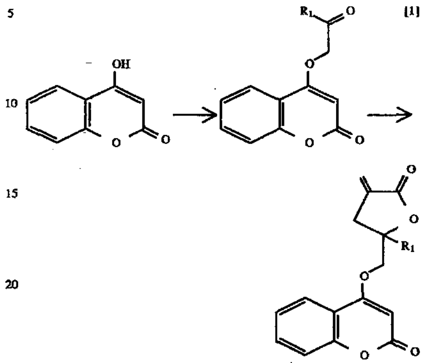
As used herein, the pharmaceutically acceptable salts include salts with inorganic acids, such as hydrochloride, hydrobromide, sulfate and phosphate; those organic acids, such as acetate, maleate, tartrate, methanesulfonate; and those with amino acids, such as arginine, aspartic acid and glutamic acid. Suitable pharmaceutical forms include sterile aquous solutions or dispersions, sterile powders, tablets, troches, pills, capsules, and the like. In addition, the active compounds may be incorporated into sustained-release preparations and formulations. The pharmaceutically acceptable carrier includes any and all solvents, disintegrating agents, binders, excipients, lubricants, absorption delaying agents and the like. Although the compound of the present invention may also be present as a hydrate or as a stereoisomer, it is a matter of course that these hydrates and stereoisomers are also included in the scope of the present invention.

The new compounds can be prepared according to the following reaction schemes and protocols.

PART A

Preparation of 4-[(2, 3, 4, 5-Tetrahydro-3-methylene-2-oxo-5-furanyl) methoxy]-2H-1-benzopyran-2-ones (Scheme 1)

(I)



The commercially available 4-hydroxycoumarin was treated with potassium carbonate and a haloketone (chloroacetone, R_1=CH_3; 2-bromoacetophenone, R_1=C_6H_5; 2-bromo-4'-fluoroacetophenone, R_1=C_6H_4F; 2-bromo-4'-chloroacetophenone, R_1=C_6H_4Cl; 2-bromo-4'-bromoacetophenone, R_1=C_6H_4Br; 2-bromo-4'-iodoacetophenone, R_1=C_6H_4I; 2-bromo-4'-methylacetophenone, R_1=$C_6H_4CH_3$; 2-bromo-4'-nitroacetophenone, R_1=$C_6H_4NO_2$; 2-bromo-4'-methoxyacetophenone, R_1=$C_6H_4OCH_3$; 2-bromo-4'-phenylacetophenone, R_1=$C_6H_4C_6H_5$) in acetone or N,N-dimethylformamide (DMF) to provide (2'-oxoethoxy)-2H-1-benzopyran-2-ones which were reacted with ethyl 2-(bromomethyl)acrylate in tetrahydrofuran (THF) (Reformatsky reaction) to produce 4-[(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl)methoxy]-2H-1-benzopyran-2-ones (I).

EXAMPLE 1

4-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2H-1-benzopyran-2-one (1)

To a solution of 4-hydroxycoumarin (1.62 g, 10 mmol) in acetone (20 ml) were added potassium carbonate (5.53 g, 40 mmol) and chloroacetone (1.38 g, 15 mmol). The resulting mixture was refluxed for 4 h. (monitored by TLC). Evaporation of the solvent gave a residue which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford 4-(2-Oxopropoxy)-2H-1-benzopyran-2-one (1a) (1.28 g, 55.1%) as a white needle crystal. mp: 163°–165° C.; IR(KBr) ν_{max}: 1716, 1625; UV($CHCl_3$) λ_{max}(log ε): 305 (3.83), 266 (4.05); ^{1}H-NMR ($CDCl_3$): δ2.36 (s, 3H, CH_3), 4.77 (s, 2H, OCH_2), 5.57 (s, 1H, 3-H), 7.28–7.36 (m, 2H, 6- and 8-H), 7.54–7.63 (m, 1H, 7-H), 7.88–7.94 (m, 1H, 5-H). Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 66.01; H, 4.64.

To a solution of 1a (0.655 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.255 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.78 g, 4 mmol). The mixture was reflued under nitrogen atmosphere for 36 h. (monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml×3). The dichloromethane extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from ethyl acetate to afford the title compound (0.656 g, 76.4%) as a pale yellow crystal. mp: 161°–162° C.; IR(KBr) ν_{max}: 1766, 1703, 1627; UV ($CHCl_3$) λ_{max}(log ε): 306 (3.79), 276 (4.01), 266 (4.05); ^{1}H-NMR ($CDCl_3$): δ1.64 (s, 3H, 5'-CH_3), 2.88 (dt, 1H, 4'-H), 3.19 (dt, 1H, 4'-H), 4.08, 4.20 (two d, 2H, OCH_2), 5.67 (s, 1H, 3-H), 5.75 (t, 1H, vinylic H), 6.38 (t, 1H, vinylic H), 7.12–7.33 (m, 2H, 6 and 8-H), 7.51–7.65 (m, 2H, 5 and 7-H). Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 67.14; H, 5.01.

EXAMPLE 2

4-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2H-1-benzopyran-2-one (2)

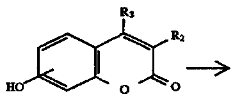
To a solution of 4-hydroxycoumarin (1.62 g, 10 mmol) in acetone (60 ml) were added 2-bromoacetophenone (1.99 g, 10 mmol) and potassium carbonate (5.53 g, 40 mmol). The mixture was refluxed for 3 h. (monitored by TLC). Evaporation of the solvent gave a residue which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford 4-(2-Oxo-2-phenylethoxy)-2H-1 -benzopyran-2-one (2a) (1.76 g, 62.9%) as a needle crystal. mp: 183°–184° C.; IR(KBr) ν_{max}: 1721, 1703, 1626; UV($CHCl_3$) λ_{max}(log ε): 306 (3.79), 253 (4.28); ^{1}H-NMR ($CDCl_3$): δ5.50 (s, 2H, OCH_2), 5.61 (s, 1H, 3-H), 7.26–7.35 (m, 2H, 6- and 8-H), 7.50–7.71 (m, 4H, 5-, 7-H and aromatic H), 7.94–8.02 (m, 3H, aromatic H). Anal. Calcd for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.85; H, 4.72.

To a solution of 2a (0.84 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.255 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.78 g, 4 mmol). The mixture was refluxed under nitrogen atmosphere for 18 h. (monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml×3). The dichloromethane extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from ethyl acetate to afford the title compound (0.90 g, 86.4%). mp: 212°–214° C.; IR(KBr) ν_{max}: 1766, 1717, 1620; UV($CHCl_3$) ν_{max}(log ε): 306 (3.89), 277 (4.10), 266 (4.14); ^{1}H-NMR ($CDCl_3$): δ3.33 (dt, 1H, 4'-H), 3.66 (dt, 1H, 4'-H), 4.26, 4.32 (two d, 2H, OCH_2), 5.60 (s, 1H, 3-H), 5.79 (t, 1H, vinylic H), 6.42 (t, 1H, vinylic H), 7.20–7.61 (m, 9H, 5,6,7,8-H, and aromatic H). Anal. Calcd for $C_{21}H_{16}O_5 \cdot 0.25H_2O$: C, 71.48; H, 4.71. Found: C, 71.37; H, 4.67.

PART B

Preparation of [(2,3,4,5-Tetrahydro-3-methylene-2-oxo-5-furanyl) methoxy]-2H-1-benzopyran-2-ones (Scheme 2)

(II)



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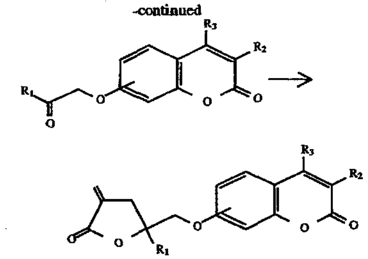


Each of the hydroxycoumarins (R_2=H, Cl, CH_3; R_3=H, Cl, CH_3) was treated with potassium carbonate and a haloketone (chloroacetone, R_1=CH_3; 2-bromoacetophenone, R_1=C_6H_5; 2-bromo-4'-fluoroacetophenone, R_1=C_6H_4F; 2-bromo-4'-chloroacetophenone, R_1=C_6H_4Cl; 2-bromo-4'-bromoacetophenone, R_1=C_6H_4Br; 2-bromo-4'-iodoacetophenone, R_1=C_6H_4I; 2-bromo-4'-methylacetophenone, R_1=$C_6H_4CH_3$; 2-bromo-4'-nitroacetophenone, R_1=$C_6H_4NO_2$; 2-bromo-4'-methoxyacetophenone, R_1=$C_6H_4OCH_3$; 2-bromo-4'-phenylacetophenone, R_1=$C_6H_4C_6H_5$) in acetone or DMF to provide (2'-oxoethoxy)-2H-1-benzopyran-2-ones which were reacted with ethyl 2-(bromomethyl)acrylate in tetrahydrofuran (THF) (Reformatsky reaction) to produce [(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl)methoxy]-2H-1-benzopyran-2-ones (II).

EXAMPLE 3

7-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2H-1-benzopyran-2-one (3)

To a solution of 7-hydroxycoumarin (1.62 g, 10 mmol) in acetone (20 ml) were added potassium carbonate (5.53 g, 40 mmol) and chloroacetone (1.38 g, 15 mmol). The resulting mixture was refluxed for 4 h. (monitored by TLC). Evaporation of the solvent gave a residue which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford 7-(2-oxopropoxy)-2H-1-benzopyran-2-one (3a) (2.10 g, 96.1%) as a white needle crystal. mp: 165°–167° C.; IR(KBr) ν_{max}: 1709, 1620; UV($CHCl_3$) λ_{max}(log ε): 308 (4.14), 244 (3.52); ^{1}H-NMR ($CDCl_3$): δ2.31 (s, 3H, CH_3), 4.65 (s, 2H, OCH_2), 6.29 (d, 1H, 3-H), 6.76 (d, 1H, 8-H), 6.88 (dd, 1H, 6-H), 7.42 (d, 1H, 5-H), 7.65 (d, 1H, 4-H). Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.98; H, 4.61.

To a solution of 3a (0.655 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.255 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.78 g, 4 mmol). The mixture was reflued under nitrogen atmosphere for 36 h. (monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml×3). The dichloromethane extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from ethyl acetate to afford the title compound; Yield: 79.7%; mp: 123°–124° C.; IR(KBr) ν_{max}: 1755, 1727, 1626; UV($CHCl_3$) λ_{max}(log ε): 312 (4.18), 243 (3.58); ^{1}H-NMR ($CDCl_3$): δ1.58 (s, 3H, 5'-CH_3), 2.79 (dt, 1H, 4'-H), 3.18 (dt, 1H, 4'-H), 3.99, 4.09 (two d, 2H, OCH_2), 5.69 (t, 1H, vinylic H), 6.26 (d, 1H, 3-H), 6.29 (t, 1H, vinylic H), 6.76–6.84 (m, 2H, 6 and 8-H).

7.38 (d, 1H, 5-H), 7.65 (d, 1H, 4-H). Anal Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 66.95; H, 5.10.

EXAMPLE 4

7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-Phenyl-2-furanyl)methoxy]-2H-1-benzopyran-2-one (4)

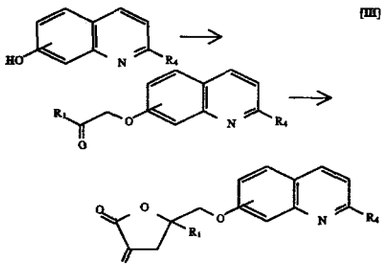
To a solution of 7-hydroxycoumarin (1.62 g, 10 mmol) in acetone (60 ml) were added 2-bromoacetophenone (1.99 g, 10 mmol) and potassium carbonate (5.53 g, 40 mmol). The mixture was refluxed for 3 h. (monitored by TLC). Evaporation of the solvent gave a residue which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford 7-(2-oxo-2-phenylethoxy)-2H-1-benzopyran-2-one (4a) (1.53 g, 73.1%) as a needle crystal. mp: 163°–165° C.; IR(KBr) ν_{max}: 1728, 1702, 1627; UV($CHCl_3$) λ_{max}(log ε): 320 (4.16), 249 (4.13); ^{1}H-NMR ($CDCl_3$): δ5.39 (s, 2H, OCH_2), 6.27 (d, 1H, 3-H), 6.80 (d, 1H, 8-H), 6.93 (dd, 1H, 6-H), 7.40 (d, 1H, 5-H), 7.49–7.68 (m, 4H, 4-H and aromatic H). Anal Calcd for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.98; H, 4.35.

To a solution of 4a (0.84 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.255 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.78 g, 4 mmol). The mixture was refluxed under nitrogen atmosphere for 18 h. (monitored by TLC). After cooling it was poured into an ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml×3). The dichloromethane extracts were combined and washed with saline, dried over Na_2SO_4, and then evaporated to give a residual solid which was crystallized from ethyl acetate to afford the title compound; Yield: 77.8%; mp: 105°–106° C.; IR(KBr) ν_{max}: 1758, 1719, 1616; UV($CHCl_3$) λ_{max}(log ε): 321 (4.22), 243 (3.62); ^{1}H-NMR ($CDCl_3$): δ3.24 (dt, 1H, 4'-H), 3.66(dt, 1H, 4'-H), 4.17, 4.24 (two d, 2H, OCH_2), 5.71 (t, 1H, vinylic H), 6.24 (d, 1H, 3-H), 6.31 (t, 1H, vinylic H), 6.72 (d, 1H, 8-H), 6.78 (dd, 1H, 6-H), 7.35 (d, 1H, 5-H), 7.40–7.52 (m, 5H, aromatic H), 7.62 (d, 1H, 4-H). Anal Calcd for $C_{21}H_{16}O_5$: C, 72.41; H, 4.63. Found: C, 72.30; H, 4.67.

PART C

Preparation of 2-Substituted [(2,3,4,5-Tetrahydro-3-methylene-2-oxo-5-furanyl)methoxy]quinolines (Scheme 3)

[III]



Each of the hydroxyquinolines (R_4=H, OH, CH_3) was treated with potassium carbonate and a haloketone (chloroacetone, R_1=CH_3; 2-bromoacetophenone, R_1=C_6H_5; 2-bromo-4'-fluoroacetophenone, R_1=C_6H_4F; 2-bromo-4'-chloroacetophenone, R_1=C_6H_4Cl; 2-bromo-4'-bromoacetophenone, R_1=C_6H_4Br; 2-bromo-4'-iodoacetophenone, R_1=C_6H_4I; 2-bromo-4'-methylacetophenone, R_1=$C_6H_4CH_3$; 2-bromo-4'nitroacetophenone, R_1=$C_6H_4NO_2$; 2-bromo-4'-methoxyacetophenone, R_1=$C_6H_4OCH_3$; 2-bromo-4'-phenylacetophenone, R_1=$C_6H_4C_6H_5$) in DMF or acetone to provide 2-substituted (2'-oxoethoxy)quinolines which were reacted with ethyl 2-(bromomethyl)acrylate in THF to produce 2-substituted [(2,3,4,5-tetrahydro-3-methylene-2-oxo-5-furanyl)methoxy]quinolines (III).

EXAMPLE 5

8-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2(1H)-quinolinone (5)

To a solution of 8-hydroxyquinoline (1.45 g, 10 mmol) in dichloromethane (50 ml) was added 3-chloroperbenzoic acid (MCPBA) (1.24 g, 13 mmol). The mixture was stirred at room temperature for 10 min, poured into 1.0N sodium bicarbonate (100 ml), and then extracted with dichloromethane (3×50 ml). The extract was washed with water, dried over magnesium sulfate, and evaporated to give a brown solid which was crystallized from dichloromethane and diethyl ether affording 8-Hydroxyquinoline 1-oxide (5a) (1.34 g, 83%) as yellow needle crystals. mp: 132°–133° C.; ^{1}H-NMR ($CDCl_3$): δ7.04–8.28 (m, 6H, Ar—H), 15.02 (br s, 1H, OH).

A mixture of 5a (0.81 g, 5 mmol) in acetic anhydride (20 ml) was heated at reflux for 2 h (monitored by TLC). After cooling, it was poured into ice water (100 ml). The resulting solid was collected and crystallized from dichloromethane to give 2-Acetoxy-8-hydroxyquinoline (5b) (0.85 g, 84%) as white crystals. mp: 240°–241° C.; ^{1}H-NMR (DMSO-d_6): δ2.55; (s, 3H, CH_3), 6.66 (d, 3-H), 7.15–7.23 (m, 3H, Ar—H), 7.78 (d, 4-H), 11.31 (br s, 1H, OH).

A mixture of 5b (1.02 g, 5 mmol), potassium carbonate (0.69 g, 5 mmol) and dry N,N-dimethylformamide (DMF) (40 ml) was stirred at room temperature for 30 min and then chloroacetone (0.46 g, 5 mmol) in dry THF (10 ml) was added in one portion. The resulting mixture was continued to stir at room temperature for 24 h (monitored by TLC). After this period, it was poured into ice water (100 ml) and the pale yellow solid thus obtained was crystallized from dichloromethane and ether to afford 2-Acetoxy-8-(2-oxopropoxy)quinoline (5c) (0.85 g, 66%). mp: 79°–80° C.; ^{1}H-NMR ($CDCl_3$): δ2.16 (s, 3H, OAc), 2.42 (s, 3H, 3'-CH_3), 4.85 (s, 2H, OCH_2), 7.06 (d, 3-H), 7.26–7.67 (m, 3H, Ar—H), 8.08 (d, 4-H).

To a solution of 5c (0.78 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.26 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl) acrylate (0.78 g, 4 mmol). The mixture was refluxed under nitrogen atmosphere for 6 h (monitored by TLC). After cooling, it was poured into an ice-cold 5% HCl solution (300 ml), neutralized with 1.0N $NaHCO_3$, and extracted with CH_2Cl_2 (75 ml×3). The dichloromethane extracts were combined and washed with water, dried over Na_2SO_4, and then evaporated to give a residual oil which was purified by column chromatography on silica gel using CH_2Cl_2 as the eluent to afford the title compound (0.59 g, 60%). UV λ_{max}(log ε): 258 (4.70) (0.1N HCl in MeOH), 246 (4.69) (MeOH), 261 (4.59) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ1.61 (s, 3H, 2'-CH_3), 2.78 (dt, 1H, 3'-H), 3.17 (dt, 1H, 3'-H), 4.50 (s, 2H, OCH_2), 5.65 (t, 1H, vinylic H), 6.29 (t, 1H, vinylic H), 6.91 (d, 1H, 3-H), 7.18–7.41 (m, 3H, Ar—H), 8.02 (d, 1H, 4-H). Anal Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.38; H, 5.32; N, 5.00.

EXAMPLE 6

8-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2(1H)-quinolinone (6)

2-Acetoxy-8-(2-oxo-2-phenylethoxy)quinoline (6a) was prepared from 2-acetoxy-8-hydroxyquinoline (5b) and

2-bromoacetophenone by the same procedure as 5c in 74% yield. mp: 141°–142° C.; ^{1}H-NMR ($CDCl_3$): δ1.92 (s, 3H, CH_3), 5.66 (s, 2H, OCH_2), 7.11 (d, 3-H), 7.25–8.04 (m, 8H, Ar—H), 8.05 (d, 4-H).

The title compound was prepared from 6a by the same procedure as 5 in 57% yield. UV λ_{max}(log ε): 260 (4.69) (0.1N HCl in MeOH), 247 (4.73) (MeOH), 261 (4.61) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ3.23 (dt, 1H, 3'-H), 3.63 (dt, 1H, 3'-H), 4.63 (d, 1H, OCH), 4.71 (d, 1H, OCH), 5.66 (t, 1H, vinylic H), 6.30 (t, 1H, vinylic H), 6.88 (d, 1H, 3-H), 7.15–7.52 (m, 8H, Ar—H), 8.00 (d, 1H, 4-H). Anal. Calcd for $C_{21}H_{17}NO_4$: C, 72.61; H, 4.93; N, 4.03. Found: C, 72.69; H, 4.94; N, 4.11.

EXAMPLE 7

8-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]quinoline (7)

8-Hydroxyquinoline (0.73 g, 5 mmol), potassium carbonate (0.69 g, 5 mmol) and dry N,N-dimethylformamide (DMF) (40 ml) were stirred at room temperature for 30 min. To this solution was added chloroacetone (0.46 g, 5 mmol) in dry THF (10 ml) in one portion. The resulting mixture was stirred at room temperature for 24 h. (monitored by TLC) and then poured into ice water (100 ml). The pale yellow solid thus obtained was collected and crystallized from dichloromethane and ether to afford 8-(2-oxopropoxy) quinoline (7a) (0.68 g, 67%) as a pale yellow needle crystal. mp: 58°–59° C.; ^{1}H-NMR ($CDCl_3$): δ2.32 (s, 3H, CH_3), 4.88 (s, 2H, OCH_2), 6.88–8.97 (m, 6H, Ar—H).

To a solution of 7a (0.60 g, 3 mmol) in dry tetrahydrofuran (60 ml) were added activated zinc powder (0.26 g, 3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl) acrylate (0.78 g, 4 mmol). The mixture was refluxed under nitrogen atmosphere for 6 h (monitored by TLC). After cooling, it was poured into an ice-cold 5% HCl solution (300 ml), neutralized with 1.0N $NaHCO_3$, and extracted with CH_2Cl_2 (60 ml×3). The dichloromethane extracts were combined and washed with water, dried over Na_2SO_4, and then evaporated to give a residual oil which was purified by column chromatography on silica gel using CH_2Cl_2 as the eluent to afford the title compound (0.66 g, 82%). UV λ_{max}(log ε): 250 (4.69) (0.1N HCl in MeOH), 237 (4.60) (MeOH), 238 (4.64) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ1.61 (s, 3H, 2'-CH_3), 2.84 (dt, 1H, 3'-H), 3.48 (dt, 1H, 3'-H), 4.25 (d, 1H, OCH), 4.32 (d, 1H, OCH), 5.66 (t 1H, vinylic H), 6.26 (t, 1H, vinylic H), 7.13–8.91 (m, 6H, Ar—H). Anal. Calcd for $C_{16}H_{15}NO_3 \cdot \frac{1}{2}H_2O$: C, 70.77; H, 5.65; N, 5.16. Found: C, 70.80; H, 5.75; N, 5.08.

EXAMPLE 8

8-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]quinoline (8)

8-(2-Oxo-2-phenylethoxy)quinoline (8a) was prepared from 2-bromoacetophenone by the same procedure as 7a in 69% yield. mp: 124°–125° C.; ^{1}H-NMR ($CDCl_3$): δ5.65 (s, 2H, OCH_2), 6.95–8.97 (m, 11H, Ar—H).

The title compound was prepared from 8a by the same procedure as 7 in 75% yield. mp: 101°–102° C.; UV λ_{max}(log ε): 250 (4.69) (0.1N HCl in MeOH), 237 (4.62) (MeOH), 237 (4.67) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ3.25 (dt, 1H, 3'-H), 4.09 (dt, 1H, 3'-H), 4.46 (d, 1H, OCH), 4.62 (d, 1H, OCH), 5.68 (t, 1H, vinylic H), 6.25 (t, 1H, vinylic H), 7.16–8.90 (m, 11H, Ar—H). Anal. Calcd for $C_{21}H_{17}NO_3$: C, 76.11; H, 5.17; N, 4.23. Found: C, 76.10; H, 5.19; N, 4.27.

EXAMPLE 9

8-[(2-(p-Chlorophenyl)-2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]quinoline (9)

8-(2-(p-Chlorophenyl)-2-oxoethoxy)quinoline (9a) was prepared from 2-bromo-4'-chloroacetophenone by the same procedure as 7a in 76% yield. mp: 111°–112° C.; ^{1}H -NMR ($CDCl_3$): δ5.56 (s, 2H, OCH_2), 6.96–8.96 (m, 10 H, Ar—H).

The title compound was prepared from 9a by the same procedure as 7 in 69% yield. mp: 107°–108° C.; UV λ_{max}(log ε): 249 (4.75) (0.1N HCl in MeOH), 236 (4.69) (MeOH), 238 (4.28) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ3.20 (dt, 1H, 3'-H), 4.06 (dt, 1H, 3'-H), 4.42 (d, 1H, OCH), 4.56 (d, 1H, OCH), 5.70 (t, 1H, vinylic H), 6.28 (t, 1H, vinylic H), 7.13–8.91 (m, 10H, Ar—H). Anal. Calcd for $C_{21}H_{16}ClNO_3$: C, 68.95; H, 4.41; N, 3.83. Found: C, 68.91; H, 4.44; N, 3.87.

EXAMPLE 10

8-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2-methylquinoline (10)

2-Methyl-8-hydroxyquinoline (0.80 g, 5 mmol), potassium carbonate (0.69 g, 5 mmol) and dry N,N-dimethylformamide (DMF) (40 ml) were stirred at room temperature for 30 min. To this solution was added chloroacetone (0.46 g, 5 mmol) in dry THF (10 ml) in one portion. The resulting mixture was stirred at room temperature for 24 h. (monitored by TLC) and then poured into ice water (100 ml). The pale yellow solid thus obtained was collected and crystallized from dichloromethane and ether to afford 2-methyl-8-(2-oxopropoxy)quinoline (10a) (0.77 g, 72%). mp: 99°–100° C.; ^{1}H-NMR ($CDCl_3$): δ2.35 (s, 3H, 2'-CH_3), 2.79 (s, 3H, 2-CH_3), 4.86 (s, 2H, OCH_2), 6.88–7.44 (m, 3H, Ar—H), 7.33 (d, 1H, 3-H), 8.03 (d, 1H, 4-H).

The title compound was prepared from 10a by the same procedure as 7 in 70% yield. UV λ_{max}(log ε): 253 (4.42) (0.1N HCl in MeOH), 238 (4.45) (MeOH), 239 (4.46) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ1.61 (s, 3H, 2'-CH_3), 2.71 (s, 3H, 2-CH_3), 2.80 (dt, 1H, 3'-H), 3.53 (dt, 1H, 3'-H), 4.24 (d, 1H, OCH), 4.30 (d, 1H, OCH), 5.67 (t, 1H, vinylic H), 6.28 (t, 1H, vinylic H), 7.08–7.42 (m, 3H, Ar—H), 7.26 (d, 1H, 3-H), 7.97 (d, 1H, 4-H). Anal. Calcd for $C_{17}H_{17}NO_3 \cdot \frac{1}{4}H_2O$: C, 71.50; H, 6.09; N, 4.90. Found: C, 71.26; H, 6.13; N, 4.73.

EXAMPLE 11

8-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2-methylquinoline (11)

2-Methyl-8-(2-oxo-2-phenylethoxy)quinoline (11a) was prepared from 2-bromoacetophenone by the same procedure as 10a in 72% yield. mp: 70°–71° C.; ^{1}H-NMR ($CDCl_3$): δ2.77 (s, 3H, 2-CH_3), 5.63 (s, 2H, OCH_2), 6.94–8.10 (m, 8H, Ar—H), 7.29 (d, 1H, 3-H), 7.99 (d, 1H, 4-H).

The title compound was prepared from 11a by the same procedure as 7 in 61% yield. mp: 108°–109° C.; UV λ_{max}(log ε): 253 (4.53) (0.1N HCl in MeOH), 239 (4.46) (MeOH), 240 (4.51) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ2.74 (s, 3H, CH_3), 3.25 (dt, 1H, 3'-H), 4.12 (dt, 1H, 3'-H), 4.41 (d, 1H, OCH), 4.56 (d, 1H, OCH), 5.71 (t, 1H, vinylic H), 6.31 (t, 1H, vinylic H), 7.06–7.60 (m, 8H, Ar—H), 7.27 (d, 1H, 3-H), 7.98 (d, 1H, 4-H). Anal. Calcd for $C_{22}H_{19}NO_3$: C, 76.51; H, 5.54; N, 4.06. Found: C, 76.52; H, 5.55; N, 4.15.

EXAMPLE 12

8-[(2-(p-Chlorophenyl)-2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2-methylquinoline (12)

8-(2-(p-Chlorophenyl)-2-oxoethoxy)-2-methylquinoline (12a) was prepared from 2-bromo-4'-chloroacetophenone by

the same procedure as 10a in 64% yield. mp: 112°–113° C.; ^{1}H-NMR ($CDCl_3$): δ2.77 (s, 3H, 2-CH_3), 5.56 (s, 2H, OCH_2), 6.94–8.12 (m, 8H, Ar—H), 7.31 (d, 1H, 3-H), 8.00 (d, 1H, 4-H).

The title compound was prepared from 12a by the same procedure as 7 in 68% yield. mp: 129°–130° C.; UV λ_{max}(log ε): 253 (4.62) (0.1N HCl in MeOH), 238 (4.60) (MeOH), 240 (4.61) (0.1N NaOH in MeOH); ^{1}H-NMR ($CDCl_3$): δ2.74 (s, 3H, CH_3), 3.20 (dt, 1H, 3'-H), 4.08 (dt, 1H, 3'-H), 4.37 (d, 1H, OCH), 4.50 (d, 1H, OCH), 5.72 (t, 1H, vinylic H), 6.32 (t, 1H, vinylic H), 7.04–7.58 (m, 7H, Ar—H), 7.27 (d, 1H, 3H), 7.98 (d, 1H, 4-H). Anal. Calcd for $C_{22}H_{18}ClNO_3$: C, 69.56; H, 4.78; N, 3.69. Found: C, 69.51; H, 4.79; N, 3.75.

PART D: Antiplatelet activity

The pharmacological activity of these compounds were determined according to G.V.R. Born by turbidimetry (*J. Physiol.*, 1963, 168, 178). Based on the method, samples were suspended in rabbit platelets which were washed with platelet-rich plasma, the aggregation was then counted by the Lumi-aggregometer (Model 1020, Paytoon, Canada). The results are shown in Table 1. Formula (I–III) at the concentration of 100 μg/ml are found to inhibit the platelet aggregation perfectly which was induced by arachidonic acid (AA), collagen, ADP, and PAF. Since the structures of these compounds are different from those of known antiplatelet agents, the present invention have the potential for further development.

Compounds of this invention may be administered parenterally or orally in a suitable pharmaceutical form. They also may be administered along or in conjunction with other antiplatelet agents, in combination with any pharmaceutically acceptable carrier.

As used herein, suitable pharmaceutical forms include sterile aqueous solutions or dispersions, sterile powders, tablets, troches, pills, capsules, and the like. In addition, the active compounds may be incorporated into sustained-release preparations and formulations. The pharmaceutically acceptable carrier for oral dosage form are, in particular, fillers, such as sugars, for example, lactose, sucrose, mannitol, and furthermore binders, such as starch mucilage using, for example, wheat, rice or potato starch, and/or, if desired, disintegrating or adjuncts. Those carriers for parenteral dosage form are, in particular, aqueous solutions and furthermore lipophilic solvents or vehicles such as fatty oils, and/or, if desired, viscosity-increasing substance, for example sodium carboxymethyl cellulose, sorbitol.

Although the compound of the present invention may also be present as a hydrate or as a stereoisomer, it is a matter of course that these hydrates and stereoisomers are also included in the scope of the present invention. The prefer individual dose is 50 to 300 mg for oral administration and 2 to 15 mg for intravenous administration and can be administered up to 3 times daily.

(A) Preparation of platelet aggregation inducers:

1. Bovine thrombin, from the Parke Davis Co., was dissolved in 50% (v/v) glycerol for a stock solution of 100 NIH units/mL.

2. Collagen (type I: bovine Achilles tendon), from the Sigma Chemical Co., was homogenized in 25 mM acetic acid and stored (1 mg/mL) at –70° C.

3. PAF (1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine), purchased from Sigma, was dissolved in chloroform and diluted into 0.1% BSA-saline solution immediately prior to use.

4. AA (arachidonic acid), purchased from Sigma, was dissolved in deionized water.

(B) Preparation of platelets:

Platelet suspension was prepared from EDTA-anticoagulated Platelet-rich plasma according to washing procedures described previously (Teng, C. M. et. al., *Thromb Haemost* 59, 304, 1991). Platelets were counted by Hemalaser 2 (Sebia, France) and adjusted to a concentration of 4.5×10^8 platelets/mL. Platelet pellets were finally suspended in Tyrode's buffer (pH 7.4) of the following composition: NaCl (136.8 mM), KCl (2.8 mM), $NaHCO_3$ (11.9 mM), $MgCl_2$ (2.1 mM), NaH_2PO4 (0.33 mM), $CaCl_2$ (1 mM), glucose (11.2 mM) containing 0.35% bovine serum albumin.

(C) Platelet aggregation and ATP release reaction:

Aggregation was measured by the turbidimetry method as described by O'Brien (*J Clin Pathol* 15, 452, 1962). ATP released from platelets was detected by the bioluminescence method of DeLuca and McElroy (*Methods Enzymol* 57, 3, 1978). Both aggregation and ATP release were measured simultaneously in a Lumi-aggregometer (model 1020B, Payton, Canada) connected to two dt/al-channel recorders. Platelet preparations were stirred at 900 rpm. When DMSO was used as solvent, its final concentration was fixed at 0.5% (v/v) to eliminate the effect of the solvent. For the calculation of percentage aggregation, the absorbance of platelet suspension was designated as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation. The antiplatelet effects were shown in Table 1 and the inhibitory concentrations for 50% aggregation (IC_{50}) were expressed in Table 2.

BRIEF DESCRIPTION OF THE DRAWING

Table 1. Effect of α-methylene-γ-butyrolactones on the platelet aggregation (%) induced by thrombin (Thr), arachidonic acid (AA), collagen (Col) and platelet-activating factor (PAF) in washed rabbit platelets[a]

Table 2. IC_{50} values of α-methylene-γ-butyrolactones on the platelet aggregation induced by Thr (0.1 U/ml), AA (100 μg/ml), Col (10 μg/ml), and PAF (2 nM)

TABLE 1

Effect of α-methylene-γ-butyrolactones on the platelet aggregation (%) induced by thrombin (Thr), arachidonic acid (AA), collagen (Col) and platelet-activating factor (PAF) in washed rabbit platelets[a]

	Inducer			
Compds	Thr 0.1 U/ml	AA 100 μM	Col 10 μg/ml	PAF 2 nM
Control	91.7 ± 1.0(5)	86.4 ± 1.0(5)	89.2 ± 1.4(5)	88.2 ± 0.8(4)
1	74.4 ± 9.6(4)	23.3 ± 12.6(4)[b]	8.5 ± 6.9(3)[b]	33.6 ± 16.6(5)[b]
2	0.0 ± 0.0(4)	0.0 ± 0.0(3)	0.0 ± 0.0(3)	0.0 ± 0.0(4)[b]
3	22.8 ± 6.6(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]

TABLE 1-continued

Effect of α-methylene-γ-butyrolactones on the platelet aggregation (%) induced by thrombin (Thr), arachidonic acid (AA), collagen (Col) and platelet-activating factor (PAF) in washed rabbit platelets[a]

	Inducer			
Compds	Thr 0.1 U/ml	AA 100 μM	Col 10 μg/ml	PAF 2 nM
Control	91.7 ± 1.0(5)	86.4 ± 1.0(5)	89.2 ± 1.4(5)	88.2 ± 0.8(4)
4	0.0 ± 0.0(3)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(3)	0.0 ± 0.0(3)[b]
5	25.9 ± 0.7(3)[b]	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]	7.0 ± 1.1(3)[b]
6	54.3 ± 1.3(3)[b]	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]	42.2 ± 1.3(3)[b]
7	17.4 ± 7.9(3)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]
8	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]
9	0.0 ± 0.0(3)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(3)[b]
10	0.0 ± 0.0(3)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(3)[b]
11	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]	0.0 ± 0.0(4)[b]
12	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]	0.0 ± 0.0(3)[b]

[a]Platelets were preincubated with DMSO (0.5%, control) or (α-methylene-γ-butyrolactones (100 μg/ml) at 37° for 3 min, and the inducer was then added. Percentages of aggregation are presented as means ± standard errors of the mean (n).
[b]Significantly different from control value at $p < 0.001$.

TABLE 2

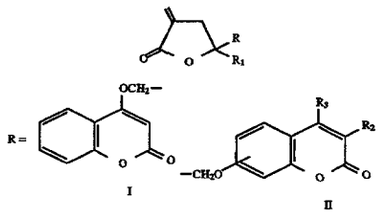
IC_{50} values of α-methylene-γ-butyrolactones on the platelet aggregation induced by Thr (0.1 U/ml), AA (100 μg/ml), Col (10 μg/ml), and PAF (2 nM)

	IC_{50} (μg/ml)			
Compds	Thr	AA	Col	PAF
1	>50	>50	>50	>50
2	ND[a]	2.86	ND	36.01
3	>50	26.08	ND	>50
4	ND	1.27	ND	5.70
5	ND	10.53	ND	ND
6	>50	8.30	ND	>50
7	>50	23.91	27.25	47.86
8	15.78	4.70	4.76	11.13
9	13.69	6.31	5.17	12.37
10	>50	17.51	16.24	39.56
11	14.88	4.44	5.76	10.54
12	ND	4.19	ND	10.82

[a]Not Determined

We claim:

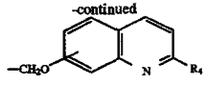
1. A compound represented by the general formula I–III as follow:



I

II

-continued



III

For the formula I, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino;

For the formula II, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino; R_2 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro, amino; R_3 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro, amino;

For the formula III, R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino; R_4 represents hydrogen, hydroxy, (C_1–C_4) alkyl.

2. A compound as claim 1, formula I, wherein R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino.

3. A compound as claim 1, formula II, wherein R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino; R_2 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro, amino; R_3 represents hydrogen, halide, (C_1–C_4) alkyl, phenyl, nitro, amino.

4. A compound as claim 1, formula III, wherein R_1 is a methyl, a phenyl group optionally substituted with one or two group selected from halide, (C_1–C_4) alkyl, (C_1–C_4) alkoxy, phenyl, nitro, amino; R_4 represents hydrogen, hydroxy, (C_1–C_4) alkyl.

5. A method of treating platelet aggregation in an animal comprising the steps of administrating any compound of claim 1 or their pharmaceutically acceptable salts in an amount effective to inhibit platelet aggregation.

* * * * *

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(71) Applicant *(for all designated States except US)*: THE JOHNS HOPKINS UNIVERSITY [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: KUHAJDA, Francis, P. [US/US]; 1211 Broadway Road, Lutherville, MD 21209 (US). PASTERNACK, Gary, R. [US/US]; 311 Edgevale Road, Baltimore, MD 21212 (US). TOWNSEND, Craig, A. [US/US]; 116 Midhurst Road, Baltimore, MD 21212 (US). MANI, Neelakandha, S. [IN/US]; Apartment C, 6203 Pimlico Road, Baltimore, MD 21209 (US).

(74) Agents: POSORSKE, Laurence, H. et al.; Baker & Botts, L.L.P., The Warner, 1299 Pennsylvania Avenue N.W., Washington, DC 20004 (US).

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(54) Title: INHIBITION OF FATTY ACID SYNTHASE AS A MEANS TO REDUCE ADIPOCYTE MASS

(57) Abstract

Weight loss was noted in nude mice treated with cerulenin, a non-competitive inhibitor of FAS. Sustained reduction of adipocyte mass in humans without toxicity would significantly impact disease prevention worldwide. Aside from psychological and self-esteem improvement, weight loss via reduction of adipocyte mass may: (1) ameliorate hyperglycemia associated with non-insulin-dependent diabetes mellitus thereby reducing diabetic complications such as arterial disease, blindness, cataracts, etc., (2) reduce hypertension, (3) reduce risk of coronary artery vascular disease and stroke, and (4) reduce the risk of other complications of massive obesity such as osteoarthritis, surgical complications, etc. There is also potential use in livestock and poultry to reduce the saturated fat content of meat products. Therefore FAS inhibitors are disclosed herein as novel agents for weight reduction. A family of compounds (γ-substituted-α-methylene-β-carboxy-γ-butyrolactones) whose synthesis was based on the cerulenin motif is shown herein to inhibit fatty acid synthesis, inhibit growth in certain susceptible tumor cells, and induce weight loss.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows induction of weight loss in nude mice by cerulenin.

Figure 2 shows cerulenin-induced weight loss in nude mice inoculated with OVCAR-3 cells.

Figure 3 shows C-75 inhibition of acetate incorporation into acylglycerides by HL60 cells.

Figure 4 shows C-75-induced weight loss in nude mice inoculated with OVCAR-3 cells and monitored for 40 days.

Figure 5 shows rescue of HL60 cells from C-75 inhibition by exogenous fatty acid.

Figure 6 shows C-75-induced weight loss in nude mice inoculated with OVCAR-3 cells and monitored for 19 days.

Figure 7 shows Kaplan-Meier Survival Curves for nude mice inoculated with OVCAR-3 cells with or without C-75 treatment.

Figure 8 shows maximum weight loss vs. survival for individual mice.

Figure 9 shows Kaplan-Meier Survival Curves for nude mice inoculated with OVCAR-3 cells with or without a single C-75 treatment.

DETAILED DISCLOSURE OF THE INVENTION

This invention describes: [1] the use of inhibitors of fatty acid synthase (E.C. 2.3.1.85, FAS), such as the inhibitor cerlenin, as a means to reduce body weight; [2] the synthesis of a family of compounds (α-methylene-β-carboxy-γ-butyrolactones) which are fatty acid synthesis inhibitors; [3] the use of α-methylene-β-carboxy-γ-butyrolactone fatty acid synthesis inhibitors as a means to treat tumor cells expressing FAS; [4] the use of α-methylene-β-carboxy-γ-butyrolactone fatty acid synthesis inhibitors as a means to reduce body weight; and [5] the use of any fatty acid synthase inhibitors to systematically reduce adipocyte mass (adipocyte cell number or size) as a means to reduce body weight.

Table 2. Effects of decreased intracellular fat storage and reduction in adipocyte mass

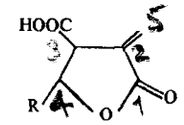
- Weight loss without muscle loss
- Reduction in hepatic fat
- Increased insulin responsiveness (especially in Type II diabetes mellitus)
- Decreased blood pressure
- Decreased arterial vascular disease
- Decreased susceptibility to liver injury associated with fatty change, including endotoxin mediated liver injury

The method of the present invention for inducing weight loss is applicable to animals, including vertebrates, especially mammals. Animals particularly contemplated include food animals such as poultry, swine, cattle, sheep, and other animals where reduction in fat accumulation without reduction in muscle mass may be desirable for veterinary health or economic reasons. Similarly, FAS inhibitors may be administered according to the method of this invention to dogs, cats, horses and other animals for veterinary health reasons, particularly reasons analogous to the reasons given herein for medical therapeutic use of this invention. Dosing protocols for the FAS inhibitors according to this method may be adapted to various animals from the medical procedures and the *in vitro* and *in vivo* data provided herein, in view of standard veterinary pharmacological principles. Generally, this method will not be applied to lactating animals.

Treatment according to this invention involves administering a compound according to this invention (an FAS inhibitor and/or an α-methylene-β-carboxy-γ-butyrolactone) to the subject of treatment. The pharmaceutical compositions containing any of the compounds of this invention may be administered by parenteral (subcutaneously,

intramuscularly, intravenously, intraperitoneally, intrapleurally, intravesicularly or intrathecally), topical, oral, rectal, or nasal route, as necessitated by choice of drug and disease.

Therapeutic compounds according to this invention are preferably formulated in pharmaceutical compositions containing the compound and a pharmaceutically acceptable carrier. The concentrations of the active agent in pharmaceutically acceptable carriers will depend on solubilities. The dose used in a particular formulation or application will be determined by the requirements of the particular type of disease and the constraints imposed by the characteristics and capacities of the carrier materials. The pharmaceutical composition may contain other components so long as the other components do not reduce the effectiveness of the compound according to this invention so much that the therapy is negated. Pharmaceutically acceptable carriers are well known, and one skilled in the pharmaceutical art can easily select carriers suitable for particular routes of administration (see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, 1985). Dose and duration of therapy will depend on a variety of factors, including the therapeutic index of the drugs, disease type, patient age, patient weight, and tolerance of toxicity. Dose will generally be chosen to achieve serum concentrations from about 1 ng to about 100 μg/ml, typically 0.1 μg/ml to 10 μg/ml. Preferably, initial dose levels will be selected based on their ability to achieve ambient concentrations shown to be effective in *in-vitro* models, such as that used to determine therapeutic index, and *in-vivo* models and in clinical trials, up to maximum tolerated levels. Standard clinical procedure prefers that chemotherapy be tailored to the individual patient and the systemic concentration of the chemotherapeutic agent be monitored regularly. The dose of a particular drug and duration of therapy for a particular patient can be determined by the skilled clinician using standard pharmacological approaches in view of the above factors. The response to treatment may be



S2

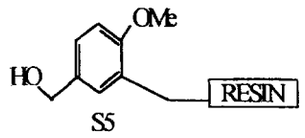
Simple molecular modeling exercises and structure-based searches based on the mechanism outlined above and in the key chemical elements noted above have yielded a small number of lead compounds, some known to possess FAS inhibitory properties specifically or antimicrobial/antitumor activities more generally. Among these was a group of α-methylene lactones of the general structure S2: protolichesteric acid (S2, R = $C_{13}H_{27}$) (Berdy, J., 1982, "Anonymous Handbook of Antibiotic Compounds", *CRC Press*, pp. 39-53), *allo*-perusaric acid (S2, R = $C_{13}H_{26}$ $COCH_3$) (Huneck et al., 1986, *Phytochem.*, **25**:543-549), nephromopsinic acid (S2, R = $C_{11}H_{23}$) (Berdy, 1982) and methylenolactocin (S2, R = C_3H_7) (Park et al., 1988, *Antibiotics*, **41**:751-758). The last of these compounds, for example, has been isolated from a *Penicillium* species and found to possess antibacterial and antitumor activity ((Park et al., 1988), but the mechanism(s) of action is/are not understood. A series of derivatives of S2 containing side chains (R) of various lengths has been synthesized by the present inventors in recognition of the carboxylic acid and the suitably-placed electrophilic α-methylene lactone as potential inactivators of the ketosynthase of FAS.

A considerable body of synthetic information exists to prepare compounds of this structural type (Murta et al., 1993, *J. Org. Chem.*, **58**:7537-7541; Azevedo et al., 1992, *J. Org. Chem.*, **57**:4567-4569). Prominent among these was an impressively efficient route to the specific class represented by S2 reported by Carlson and Oyler (Carlson et al., 1976, *J. Org. Chem.*, **41**:4065-4069). In this synthesis the dianion of 4-

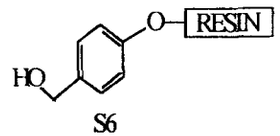
methoxybenzyl itaconate (S3) can be condensed with aldehydes (S4) of various chain description (R) to give in *one* step, after rapid exposure to strong acid, the desired α-methylene lactone carboxylic acid S2. Both the *trans*- and *cis*-isomers are generated slightly favoring the former. These diastereomers may be separated by flash silica gel chromatography using ethyl acetate:hexanes:acetic acid (30:70:1) as eluent and individually crystallized from boiling hexanes.

A series of derivatives of S2 has been prepared containing saturated alkyl, unsaturated alkyl and aryl-containing side chains (R)(e.g., $-C_6H_{23}$ - C_7H_{15} $-C_8H_{17}$, $-C_9H_{19}$, $-C_{11}H_{23}$ and $-C_{13}H_{27}$). Based on their *in vitro* biological activity against HL60 cells, human breast cancer cell lines, and normal fibroblasts, the S2 compound with R = $-C_8H_{17}$ was chosen as a representative compound for further study. This compound, designated C-75 herein, coincidentally has an alkyl side chain approximately the length of cerulenin. C-75 has been prepared in crystalline form. For biochemical studies, radiolabeled S2 is readily accessible from the tritiated aldehyde S4 (H* = ^3H), which is in turn easily available by reduction with sodium borohydride(^3H) and reoxidation to the aldehyde. A substantial kinetic isotope effect in the latter reaction is well known and leads to high retention of the heavy isotope as demonstrated in earlier work from this laboratory (Townsend et al., 1986, *J. Chem. Soc. Chem. Commun.*, 638-639). If higher specific radioactivity is required, more elaborate catalytic tritiation of a carbon-carbon double bond could be carried out to give the aldehyde at very high specific activity.

The preparation of these compounds and survey of various structural types may be amenable to combinatorial methods by use of the resin supports S5 and S6.



S5



S6

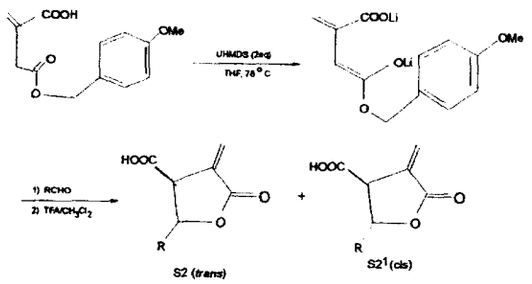
Esterification with itaconic anhydride would yield the ester corresponding to S3 ready for dianion formation and reaction with aldehydes S4. Unreacted aldehyde can be washed from the resin and the product obtained by acidification to cause cyclization to the lactone S2 and regeneration of the resins S5 and S6. Structural types of aldehydes represented above and by others should be assayed as described below to exclude inactive forms.

Alpha-methylene-β-carboxy-γ-butyrolactones, including tetrahydro-3-methylene-2-oxo-5-n-octyl-4-furancarboxylic acid (C-75), are easily and inexpensively synthesized and can be radiolabeled to high specific activity for *in vivo* pharmaokinetic analysis. Like cerulenin, C-75, and similar α-methylene-β-carboxy-γ-butyrolactones, will effectively inhibit the growth of tumor cells *in vivo*, particularly those such as OVCAR-3 cells which express high levels of FAS, and these compounds can be used in treating cancers containing such cells (see U.S. patent application No. 08/188,425, incorporated herein by reference).

EXAMPLES

In order to facilitate a more complete understanding of the invention, a number of Examples are provided below. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only.

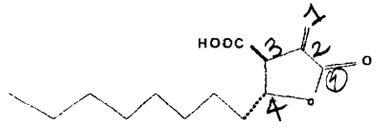
Example 1B. Preparation of α-methylene lactone derivatives—typical procedure



Lithiumhexamethyldisilyl amide (40 ML, 1M, 40 mmol) was added to a solution of 20 mmol of the itaconate half ester in 200 ML of anydrous THF cooled at -78 °C. After stirring for 1h, 20 mmol of the aldehyde (RCHO dissolved in 10 mL of anhydrous THF) was introduced to the reaction mixture through a cooled (-78 °C) cannula. After the addition, the solution was stirred at -78 °C for 3-4 h, and then the reaction was quenched with 20 mL of 6N H_2SO_4 and immediately extracted into either (250 mL). The ethereal solution was dried over anhydrous $MgSO_4$. The solution was filtered and evaporated under reduced pressure yielding a gummy solid. This crude product was taken up in CH_2C1_2 (100-125 mL) and treated with 1.5 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 10-12 h. Aqueous $NaHCO_3$ (100 mL) was then added to the reaction and the layers were separated. The bicarbonate layer was acidified to pH 1 with conc. HCl and extracted with ether (2 x 100 mL). The organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to yield the α-methylene lactones as a mixture of *cis*- and *trans*-diastereoisomers as a white solid in overall yields ranging from 58-67%.

The predominant *trans*-isomer (55-60%) was separated by flash column chromatography using silica gel (ethyl acetate:hexanes:acetic acid 30:70:1). The products were further purified by recrystallization from boiling heaxanes.

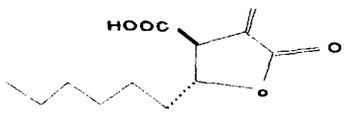
Example 1C. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-octyl-4-furancarboxylic acid (C-75)



mp 76-77 °C (hexane); IR (film) 3000-3400, 2924, 2852, 1743, 1717, 1660, 1621, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6.8 Hz), 1.2-1.8 (m, 14H), 3.59 (dt, 1H, J - 2.8, 5.6, 12.8 Hz), 4.77 (q, 1H, J = 6, 12.8 Hz), 6.0 (d, 1H, J = 2.8 Hz), 6.4 (d, 1H, J = 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 29.1, 29.14, 31.7, 35.1, 49.4, 78.7, 125.9, 132.2, 168.1, 174.5; exact mass: calculated = 254.1518, found = 254.1514.

***cis*-isomer**: mp 74-75.5 °C (hexane); IR (film) 3000-3400, 2922, 2855, 1748, 1713, 1663 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7 (b, 3H), 1.2-1.8 (b, 14H), 4.0 (dt, 1H, J = 2, 7.6 Hz), 4.6 (q, 1H, J = 5.6, 7.6 Hz), 5.9 (d, J = 2.4 Hz), 6.4 (d, 1H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 25.5, 29.1, 29.14, 29.3, 31.3, 31.7, 48.8, 78.0, 125.7, 133.1, 168.8, 174.4.

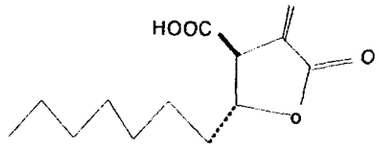
Example 1D. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-hexyl-4-furancarboxylic acid



IR (neat) 3000-3400, 2953, 2852, 1743, 1717 1660, 1256, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.8 (bt, 3H) 1.20-1.81 (m, 10H), 3.59 (dt, 1H, J = 2.8, 5.6 Hz), 4.76 (q, 1H, J = 6.0, 2.8 Hz), 5.91 (d, 1H, J = 2.8 Hz), 6.42 (d, 1H, J = 2.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.4, 24.6, 28.7, 31.5, 35.6, 49.4, 78.9, 126.0, 132.2, 168.4, 174.5.

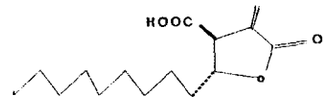
Cis isomer: IR (neat) 3000-3400, 2922, 2855, 1748, 1713, 1663, 1466 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7 (b, 3H), 1.2-1.8 (b, 10H), 4.0 (bd, 1H, J = 7.6 Hz), 4.6 (q, 1H, J = 5.6, 7.6 Hz), 5.9 (d, J = 2.4 Hz), 6.4 (d, 1H, J = 2.4 Hz).

Example 1E. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-heptyl-4-furancarboxylic acid



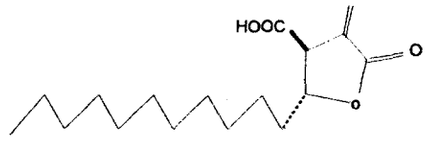
IR (neat) 3000-3400, 2960, 2840, 1747, 1654, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7 (b, 3H), 1.3-1.8 (m, 12H), 3.6 (dt, 1H, J = 2.8, 5.6 Hz), 4.79 (q, 1H, J = 5.6, 12.8 Hz), 6.0 (d, 1H, J = 2.4 Hz), 6.45 (d, 1H, J = 2.8 Hz).

Example 1F. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-nonyl-4-furancarboxylic acid



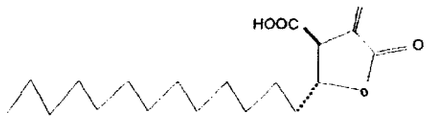
IR (film) 3000-3400, 2924, 2852, 1743, 1717, 1660, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6.8 Hz), 1.2-1.8 (m, 16H), 3.59 (dt, 1H, J = 2.8, 5.6 Hz), 4.77 (q, 1H, J = 6, 12.8 Hz), 6.0 (d, 1H, J = 2.8 Hz), 6.4 (d, 1H, J = 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 24.7, 29.1, 29.2, 29.23, 29.3, 29.4, 31.8, 35.7, 49.4, 78.8, 126.0, 132.2, 168.1, 174.7.

Example 1G. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-undecyl-4-furancarboxylic acid



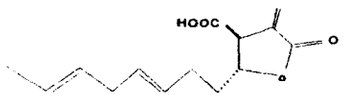
IR (film) 3000-3400, 2924, 2852, 1745, 1717, 1660, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6.8 Hz), 1.2-1.8 (m, 20H), 3.6 (dt, 1H, J = 2.8 Hz, 5.6 Hz), 4.77 (q, 1H, J = 2.8, 5.6, 12.8 Hz), 6.0 (d, 1H, J = 2.8 Hz), 6.4 (d, 1H, J = 2.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 29.1, 29.19, 29.3, 29.4, 29.47, 29.5, 31.8, 35.7 49.4, 78.8, 126.0, 132.2, 168.3, 174.8.

Example 1H. *trans*-Tetrahydro-3-methylene-2-oxo-5-*n*-tridecyl-4-furancarboxylic acid



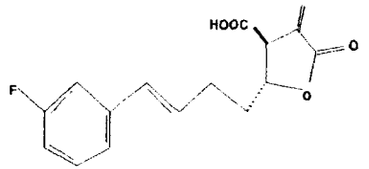
IR (film) 3000-3400, 2919, 2850, 1749, 1719, 1661, 1467 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6.8 Hz), 1.2-1.8 (m, 20H), 3.60 (dt, 1H, J = 2.8 Hz, 5.6 Hz), 4.77 (q, 1H, J = 5.6, 12.8 Hz), 6.10 (d, 1H, J = 2.8 Hz), 6.4 (d, 1H, J = 2.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 29.1, 29.2, 29.3, 29.38, 29.4, 29.48, 29.5, 29.58, 29.62, 31.8, 35.7, 49.4, 78.8, 125.9, 132.3, 168.3, 174.8.

Example 1I. *trans*-Tetrahydro-3-methylene-2-oxo-5-(3E, 6E-octadieny)-4-furancarboxylic acid



IR (neat) 3000-3400, 2924, 1743, 1717, 1660, 1610, 1465 cm-^{1}; ^1H NMR ($CDCl_3$) d 1.61 (d, 3H), 1.70-2.25 (m, 4H), 2.65 (m, 2H), 3.61 (dt, 1H, J = 2.8, 5.6 Hz), 4.80 (q, 1H, J = 5.5, 12.6 Hz), 5.2-5.4 (m, 4H), 6.05 (d, 1H, J = 2.8 Hz), 6.38 (d, 1H, J o= 2.8 Hz).

Example 1J. *trans*-Tetrahydro-3-methylene-2-oxo-5-(3*E*-4-(3-fluorophenyl))but-3-enyl-4-furancarboxylic acid



IR (neat) 3000-3400, 2917, 2877, 1749, 1717, 1660, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80-2.65 (m, 2H), 3.60 (dt, 1H, J = 2.8, 5.6 Hz), 4.80 (q, 1H, J = 5.5, 12.6 Hz), 6.04-6.42 (m, 4H), 6.75-7.38 (m, 4H).

Example 2. Cerulenin induces weight loss in athymic nude mice.

Athymic mice received 60 mg/kg cerulenin i.p. in 25% DMSO/saline or vehicle alone each day for 1 week. The animal weights were recorded every two days up to day 15. Average animal weight for both groups of mice is plotted against treatment day in Figure 1. Error bars represent standard error of the mean. Intraperitoneal administration of cerulenin for seven days to nude mice resulted in an average 28% loss of weight by day eight, with mice returning to 96% of their original weight by day 14 (Figure 1). In addition there was a 28% reduction of feces mass in the cerulenin-treated group consistent with deceased food intake. Water intake was not appreciable altered. Observation of the mice showed normal activity even during the period of maximal weight loss.

Necropsy of treated animals from a parallel experiment showed markedly decreased subcutaneous fat and abdominal fat deposits, and reduced liver volume consistent with reduction in hepatic fat accumulation. Microscopically, the liver was unremarkable without necrosis or inflammation but cerulenin-treated animals had an absence of the fat droplets

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCION DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
DILIA MARIA RODRIGUEZ D'ALEMAN

ASUNTO	Radicación	04-001331
	Trámite	011
	Evento	379
	Actuación	411
	Oficio	0 8 2 7 3
	Folios	007

Patente de Invención	■	Patente de Modelo de Utilidad		☐
Vía Nacional	☐			
Vía PCT (fase nacional)	■	Cap.I	☐	Cap. II ■
No. Sol. Internacional		PCT/US2002/18602		
Fecha de Presentación Internal.		12-06-2002		

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

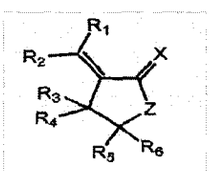
Descripción:	Folios 45-90 y 108	como se radicó inicialmente
	Folios 150-194	modificada en fase Nacional
Reivindicaciones:	Folios 91 a 107	como se radicó inicialmente
	Folios 195-200	modificadas en fase Nacional
Prioridad:	Folio 1	13-06-2001, US, 60/297875
Respuesta del solicitante	Folios 144-147	radicada en fase Nacional

2. Objeto de la invención

Título de la solicitud: Lactonas y composiciones farmacéuticas de las mismas (folio 145)

El problema técnico planteado en esta solicitud es la necesidad de encontrar nuevos compuestos derivados de lactonas, que sean útiles como agentes anti-infecciosos (antibacteriales, antifúngicos) y/o antiproliferativos para ser usados en composiciones farmacéuticas en el tratamiento de diversas condiciones como ulcera péptica, gingivitis, periodontitis y diferentes tipos de cáncer (folios 45-47; pág. 1-3).

Para solucionar este problema técnico la solicitud en estudio proporciona una composición farmacéutica que contiene una alfametilen-gamalactona de formula **Ia**.



Ia

El objeto de la presente invención se relaciona con la Clasificación internacional de Patentes (IPC): A61K 31/365, C07D305/12, A61P31/04, A61P31/10, A61P35/00.

3. De la solicitud de Patente (Art 14 D486)

Reivindicaciones *(artículo 30 D 486)*

Se solicita en las reivindicaciones 1-21, una composición farmacéutica que comprende una alfametilen-gamalactona de formula **Ia,** con radicales R_1 a R_6 y variables X y Z; sin embargo, se encuentra que existe falta de claridad, concisión y soporte en tal materia reclamada, puesto que:

- Términos generales y ambiguos como "alquilo, alquenilo, alquinilo, ciclos, arilo, heteroarilo, alcoxi, aroxi, alquiltio, ariltio, poliarilo, heterociclilos, aminoácidos, péptidos y polipéptidos" y diversos grupos "sustituidos" sin definir por cuáles sustituyentes, no permiten dar claridad, concisión y alcance definido a la materia reclamada; siendo entonces necesario que tales términos sean definidos de acuerdo con el compuesto de formula **Ia** que se encuentra en las composiciones que soportan la invención.

  Asimismo, se encuentra que el compuesto  : α-metilen-γ-butirolactona denominado LSMV-6 (folio 160; pág. 11), es el único compuesto probado en ensayos de actividad antibacterial, antineoplásica y antifúngica (folios 176,178, 181, 185,186, 191; pág. 27,29, 32, 36, 37,42).

- Se define cada una de las variables Z y X como un heteroátomo de O, S o N; pero, no es clara la expresión "agrupados en formas estructurales lineales, ramificadas o cíclicas"; es necesario revisar y corregir de acuerdo el compuesto de formula **Ia** que se encuentra en las composiciones que soportan la invención.

- En las reivindicaciones 3-9, 18 y 21 no es adecuada la caracterización de una formulación por ser "para tratar" diversos trastornos allí señalados; ya que una composición farmacéutica sólo debe caracterizarse por los elementos que la comprenden, como son el agente activo, excipientes y proporciones si es necesario. Por tanto, se recomienda eliminar estas reivindicaciones.

- En las reivindicaciones 10-11, se define inadecuadamente el portador farmacéuticamente aceptable por la forma farmacéutica (tableta, capsula, solución, crema, loción, etc.), ya que un portador o vehículo está referido es a los excipientes que permiten lograr una forma farmacéutica dada; es necesario revisar y corregir.

- En las reivindicaciones 12-13 y 17 se define inadecuadamente la composición farmacéutica por los métodos de aislar o de producir la lactona; ya que una composición farmacéutica sólo debe caracterizarse por los elementos que la comprenden, como son el agente activo, excipientes y proporciones si es necesario. Por tanto, se recomienda eliminar estas reivindicaciones.

Se requiere al Solicitante hacer las aclaraciones necesarias.

4. Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente es 21-01-2005, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a ésta.

N°	Documento	Título	Fecha de Publicación
D1	US5962460	Antineoplastic α-methylene-γ-butirolactones	05-10-1999
D2	Bibliografía 1	Synthesis and biological activity of α-methylene-γ-butirolactones	1985
D3	Bibliografía 2	Molecular recognition in allergic contact dermatitis to natural products	1990
D4	US5646164	α-methylene-γ-butirolactones: new inhibitors of platelet aggregation	08-07-1997
D5	WO9718806	Inhibition of fatty acids synthase as a means to reduce adipocyte mass	29-05-1997

Anterioridad **D1** en folios 203-205, **D2** en folios 206-214, **D3** en folios 215-218, **D4** en folios 219-222 y **D5** en folios 223-229.

Bibliografía 1: H. M. R. Hoffmann and Jürgen Rabe; "Synthesis and Biological Activity of α-Methylene-γ-butyrolactones"; Angewandte Chemie International Edition in English, Volume 24, Issue 2, pages 94–110, February 1985.

Bibliografía 2: Claude Benezra; "Molecular recognition in allergic contact dermatitis to natural products"; Pure & Appl. Chem., Vol. 62, No. 7, pp. 1251-1258, 1990.

D1 se refiere a α-metilen-γ-butirolactonas de formula I con actividad antineoplásica las cuales pueden ser administradas con un diluente inerte o un vehículo farmacéuticamente aceptable, en el tratamiento de enfermedades neoplásicas (folio 203; portada).

D2 se refiere a la síntesis y actividad biológica de α-metilen-γ-butirolactonas [dihidro-3-metilene-2(3H)furanonas] las cuales constituyen un importante grupo de productos naturales y que poseen un amplio rango de actividades biológicas (folio 206; pág. 94).

D3 se refiere a las estructuras químicas de alérgenos (o haptenos) naturales de la piel, particularmente β-sustituida-α-metilen-γ-butirolactonas, tales como tulipalinas B, las cuales han sido sintetizadas y ensayada su actividad alergénica (folio 215; pág. 1251).

D4 se refiere a tres clases de α-metilen-γ-butirolactonas, de formula I, II y III, como inhibidores de la agregación plaquetaria, sus métodos de preparación y su administración oral o parenteral en vehículos farmacéuticamente aceptables en el tratamiento de enfermedades cardiovasculares (folio 219; portada).

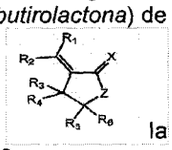
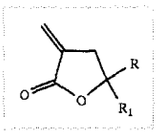
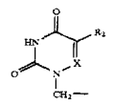
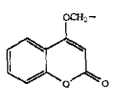
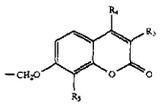
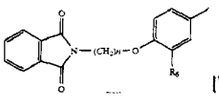
D5 se refiere a una familia de compuestos de γ-sustituidas-α-metilen-β-carboxi-γ-butirolactonas, como inhibidores de la síntesis de ácidos grasos y su uso en el tratamiento de células tumorales (folio 223; portada).

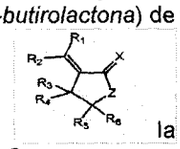
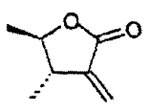
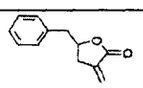
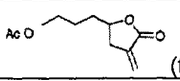
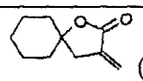
5. Análisis de patentabilidad (artículo 14 D486)

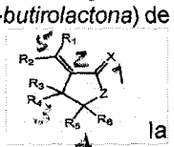
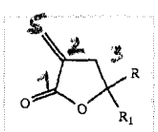
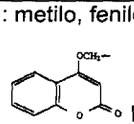
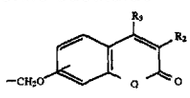
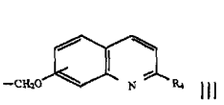
Novedad (artículo 16 D486)

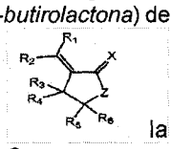
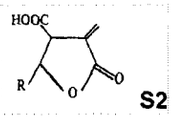
Se solicita en las reivindicaciones 1-21, una composición farmacéutica que comprende una alfametilen-gamalactona de formula **Ia**.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

SOLICITUD	D1
Composición farmacéutica que comprende	Composición farmacéutica que comprende (folios 203-204; col. 2-3)
una alfametilen-gamalactona (*α-metilen-γ-butirolactona*) de formula **Ia**  Ia	una α-metilen-γ-butirolactona de formula I, II, III o IV de acuerdo con el radical R.  (folios 203; col. 2)
X: O Z: O R_1 a R_4: H cada uno	
R_5: H, metilo, fenilo o fenilo sustituido	R_1: H, metilo, fenilo o fenilo sustituido
R_6: alquilo sustituido fenilo sustituido	R:  I ;  II ;  III  IV (folios 203; col. 2)

SOLICITUD	D3
Composición farmacéutica que comprende	Composición farmacéutica que comprende (folio 217; 1256)
una alfametilen-gamalactona (*α-metilen-γ-butirolactona*) de formula **Ia**  Ia	una α-metilen-γ-butirolactona de formulas:  10 (folio 217; 1255)
X: O Z: O R_3 y R_5: alquilo cada uno R_1, R_2, R_4, R_6, : H cada uno	
X: O Z: O R_1 a R_5: H cada uno R_6: alquilo sustituido	 ;  (folio 216; 1254)
X: O Z: O R_1 a R_4: H cada uno R_5 y R_6: forman cicloC_{1-20}	 (folio 216; 1254)
X: O Z: O R_1 a R_5: H cada uno R_6: alquilo sustituido	 6 (folio 216; 1254)
X: O Z: O R_3: alquilo R_1, R_2, R_4, R_5 y R_6, : H cada uno	 14 (folio 217; 1255)

SOLICITUD	D4
Composición farmacéutica que comprende	Composición farmacéutica que comprende (folios 220; col 2)
una alfametilen-gamalactona (*α-metilen-γ-butirolactona*) de formula **Ia**  Ia	una α-metilen-γ-butirolactona de formula I, II o III, de acuerdo con el radical R.  (folios 222; col 12)
X: O Z: O R_1 a R_4: H cada uno	
R_5: H, metilo, fenilo o fenilo sustituido	R_1: metilo, fenilo o fenilo sustituido
R_6: alquilo sustituido	R:  I ;  II ;  III (folios 222; col. 12)

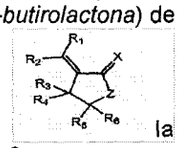
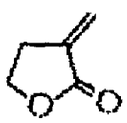
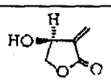
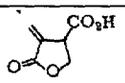
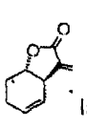
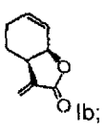
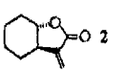
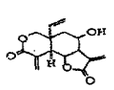
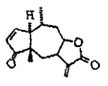
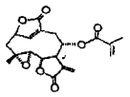
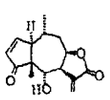
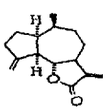
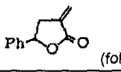
SOLICITUD	D5
Composición farmacéutica que comprende	Composición farmacéutica que comprende (folio 225; pág. 11)
una alfametilen-gamalactona (*α-metilen-γ-butirolactona*) de formula **Ia**  Ia X: O Z: O R_4: carboxi (-COOH) R_1 a R_3, y R_5: H cada uno	una α-metilen-β-carboxi-γ-butirolactona de formula S2 (folio 225; pág. 16)  **S2**
R_6: alquilo alquilo sustituido alquenilo alquilo sustituido	R: alquilo alquilo sustituido por arilo alquenilo sustituido por fenilo (folios 226, 227-229; pág. 17, 20-25, ej. 1B-1J)

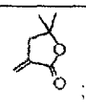
Se encuentra que una composición como la reclamada que comprende una α-metilen-γ-butirolactona de formula **Ia,** ya había sido divulgada en D1, D3, D4 y D5, puesto que éstos documentos enseñan composiciones que comprenden una α-metilen-γ-butirolactona con las características esenciales indicadas en la solicitud, tal como se muestra arriba en los cuadros comparativos.

En consecuencia, de acuerdo con lo divulgado por D1, D3, D4 y D5, la composición reclamada en las reivindicaciones 1-21, no cumple el requisito de novedad.

Nivel inventivo (artículo18 D486)

Para las otras composiciones solicitadas (no afectadas por novedad) en las reivindicaciones 1-21, que comprenden otras α-metilen-γ-butirolactonas de formula **Ia,** se encuentra:

SOLICITUD	D2
Composición farmacéutica que comprende	---
una alfametilen-gamalactona (*α-metilen-γ-butirolactona*) de formula **Ia**  Ia X: O Z: O R_1 a R_6: H cada uno	α-metilen-γ-butirolactonas  tulipalina A (folio 207; pág. 96)
X: O Z: O R_1 a R_3 y R_5 a R_6: H cada uno R_4: OH o -COOH	 tulipalina B (folio 207; pág. 96)  (folio 209; pág. 100)
X: O Z: O R_1 a R_3 y R_5: H cada uno R_4 y R6: forman ciclos hasta C_{20} sin sustituir o sustituidos	 Ia; (folio 208; pág. 98)  Ib;  2  Vernolepina  Aromaticina  Elefantopina (folio 206; pág. 95)  Helenalina  Eremantina (folio 207; pág. 96)
X: O Z: O R_1 a R_5: H cada uno R6: alquilo sustituido o fenilo	 ;  (folio 208; pág. 98)

X: O Z: O R_1 a R_4: H o alquilo cada uno R_5 y R_6: alquilo o fenilo	 ;  ;  (folio 209; pág. 101) (folio 209; pág. 100)
EFECTO: Composiciones con agentes activos antibacteriales, antifúngicos y antiproliferativos (folio	Agentes activos antibacteriales, antifúngicos y antiproliferativos (folios 206-207; pág. 94-96), que pueden tener un uso farmacéutico en la medicina (213; pág. 108)

El estado de la técnica más cercano es D2 que divulga que diversos compuestos de estructura química basada en el núcleo α-metilen-γ-butirolactona, incluyendo compuestos de formula Ia contendidos en la composición reclamada, tal como se muestra arriba en el cuadro comparativo; indicando D2 que tales compuestos son agentes activos antibacteriales, antifúngicos y antiproliferativos que pueden tener un uso farmacéutico en la medicina.

La diferencia de la solicitud con D2 es que señala específicamente una composición farmacéutica que comprende una α-metilen-γ-butirolactona de formula **Ia** (diferente a las contendidas en las composiciones afectadas por novedad).

El efecto técnico que logra tal diferencia es que se obtiene una composición farmacéutica de una α-metilen-γ-butirolactona de formula **Ia**, adecuada para usarse como antibaterial, antifúngica y antiproliferativa en el tratamiento de condiciones relacionadas con infecciones bacteriales, fúngicas y cáncer.

Así, el Problema Técnico Objetivo esta relacionado con la necesidad de obtener composiciones farmacéuticas de una α-metilen-γ-butirolactona de formula **Ia**, adecuada para usarse como antibaterial, antifúngica y antiproliferativa en el tratamiento de condiciones relacionadas con infecciones bacteriales, fúngicas y cáncer.

> Sin embargo, D1 enseña que α-metilen-γ-butirolactonas son un grupo de compuestos que poseen actividades antibaterial, antifúngica, antineoplásica (o antiproliferativa) y antihelmíntica; e indica la formación de composiciones farmacéuticas que comprenden una α-metilen-γ-butirolactona de formula I, las cuales pueden ser formuladas con un diluente inerte o un vehículo farmacéuticamente aceptable, para ser usadas en el tratamiento de enfermedades neoplásicas (folio 203; portada, col. 1 y 2; y tal como se muestra en el aparte de novedad, folio 233).

En consecuencia, la materia reclamada es obvia, porque el Experto en la Materia enfrentado al problema de obtener composiciones farmacéuticas de una α-metilen-γ-butirolactona de formula **Ia**, adecuadas para usarse como antibaterial, antifúngica y antiproliferativa, obtendría composiciones farmacéuticas de una α-metilen-γ-butirolactona como lo señala D1 usando otras α-metilen-γ-butirolactonas que posean tales actividades antibaterial, antifúngica y antiproliferativa como las enseñadas en D2, para lograr de esta forma la composición objeto de esta solicitud; careciendo, por tanto, la materia reclamada de nivel inventivo.

6. Respuesta al solicitante

El solicitante anexa un nuevo capitulo reivindicatorio (folios 195-200) enfocado en una composición farmacéutica, eliminando las reivindicaciones relacionadas con compuestos (aislados y sintetizados), sus proceso de aislamiento y síntesis, y los métodos de tratamiento con el fin de superar las objeciones presentadas a tal materia reclamada; capitulo reivindicatorio que es aceptado por esta Oficina.

Señala el solicitante que ha eliminado las reivindicaciones cuestionadas y ha limitado el alcance de la invención a una composición farmacéutica de un compuesto de formula IA (folio 147; pág. 4 de respuesta).

Al respecto se observa que en efecto, la materia antes reclamada (compuestos aislados y sintetizados, sus métodos de aislamiento y síntesis, y los métodos de tratamiento) ha sido eliminada, reclamando ahora una composición farmacéutica que comprende una alfametilen-gamalactona de formula **Ia**, materia a la que se le realiza el presente análisis de patentabilidad.

7. Conclusión

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones Afectadas	Requisito que Afecta
D1	US5962460	1-21	Novedad y Nivel inventivo
D2	Bibliografía 1	1-21	Nivel inventivo
D3	Bibliografía 2	1-21	Novedad
D4	US5646164	1-21	Novedad
D5	WO9718806	1-21	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el artículo 8 de la resolución reglamentaria No. 00210 del 15 de enero de 2001, advirtiendo que contra él no procede recurso alguno en la vía gubernativa.

JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 20 MAYO 2011

CONCEPTO TECNICO	EXAMINADOR TECNICO
No. 1240	Código No. 202079