

Brigard & Castro

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Apartado 3692

Cra. 7 16-56
Bogotá - Colombia S.A.

Tel. Conm. 3802200
Tel./Fax: (57.1) 2827976
(57.1) 3802700

E-Mail:bricas@brigardycastro.com.co

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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E. S. D.

RESPUESTA A OFICIO 09286 DE OCTUBRE 07 2004

REF: Expediente No. 00 002.434 - Solicitud de Patente

a favor de **BRISTOL - MYERS SQUIBB COMPANY**

Titulada: COMPLEJO DE 1,4-BENZODIAZEPINAS SUSTITUIDAS Y SULFOBUTILETER-7-CICLODEXTRINA O 2-HIDROXIPROPIL-B-CICLODEXTRINA

HÉCTOR GÓMEZ MEJÍA, vecino de Bogotá, portador de la tarjeta profesional No. 19436, obrando como apoderado sustituto de la Sociedad **BRISTOL - MYERS SQUIBB COMPANY**, según lo acredita el documento de poder anexo, en respuesta a las observaciones de carácter técnico y jurídico relacionadas con la patentabilidad para la concesión de la Patente, de acuerdo con el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina, según concepto técnico No. 1176 notificado por fijación en lista de fecha 7 de Octubre de 2004, me permito allegar los siguientes documentos:

- 1) -STARKE
Vol. 23, No. 5, 1 mayo de 1971, WEINHEIM DE
Pags. 167 - 171
- 2) -AGRI. BIOLOG. CHEMISTRY
Vol. 34, No. 6, 1970,
Pags. 954 a 1956
KEN'ICHI TAKEO ET AL. "THIN-LAYER CHROMATOGRAPHY OF CYCLODEXTRINS",
pags. 954 - 956
- 3) -CHEMICAL PATENTS INDEX, BASIC ABSTRACTS JOURNAL
Week 8811, May 11, 1988
Derwent publication Ltd., London, GB;
AN 73862

De la señora jefe atentamente,



HÉCTOR GÓMEZ MEJÍA
C.C. No. 22720 de Bogotá
Cra 7a. No. 16-56 Piso 9

Bogotá, Diciembre 27, 2004
CHP/MEV/hsr

Inclusion compounds with cyclodextrins in aqueous solution are only known in case the included compound is a polar substance; with apolar substances only very slightly soluble inclusion compounds are formed.

In order to be able to make inclusion compounds with apolar substances in solution, hydrophilic cyclodextrin derivatives have been prepared, viz. the carboxymethylether Na-salt, the sulfopropylether Na-salt and a soluble epichlorohydrin resin of both α and β cyclodextrin. The molecular weight (apparent void concentration, respectively) of the derivatives is determined in a number of different ways.

(Zusammenfassung siehe Seite 171; Résumé à la page 171)

Introduction

Cyclodextrins (CD, cycloamyloses) form crystallised inclusion compounds, which are usually only slightly soluble in water [1], especially when apolar guest molecules are included. The inclusion compounds with polar substances are moderately soluble [2, 3]. In the latter case inclusion compounds in aqueous solution are formed to a much greater extent than in the case of apolar compounds.

Inclusion compounds in solution differ from the crystallised inclusion compounds in that the former are what we would call "molecular" inclusion compounds. In this case a guest molecule is included in the void of a CD host molecule, the whole complex being surrounded with water molecules. On the other hand, the crystallised inclusion compounds are "lattice" inclusion compounds in which a guest molecule is included in a void formed by a lattice, not by a single CD molecule. In principle molecular inclusion compounds should have a stoichiometric composition, whereas in a lattice inclusion compound the composition is determined by statistical factors.

Molecular inclusion compounds are not formed exclusively in solution. The inclusion compounds with cyclodextrin-epichlorohydrin resins (CD-E resins), described by Wiedenhof [4] also belong to the type of molecular inclusion compounds, because no lattice is formed.

In order to be able to study molecular inclusion compounds of CD with polar substances in aqueous solution, it was necessary to modify the CD molecules in such a way that they could not crystallise. The soluble CD-E resins [5] indeed do not crystallise and appear to form inclusion compounds in aqueous solution. CD derivatives in which polar substituents are introduced also appear to fulfil our purpose.

In this paper the preparation of two of these CD derivatives is described, viz. the carboxymethylether and the sulfopropylether of both α and β CD.

In subsequent papers inclusion compounds of these CD derivatives in aqueous solution will be described.

Preparations

Soluble CD-Epichlorohydrin Resin (CD-E resin)

The preparation has been described by Wiedenhof et al. [5]. This preparation is summarised in Table 1.

CD-Carboxymethylether Na-Salt (CD-CME)

The preparation is performed in analogy to the preparation of amylose-CME described by Husemann and Kafka [6].

Materials:

α and β CD, tested for purity [7, 8].

Monochloro-acetic acid: Merck, zur Synthese.

NaOH: Merck, p.a.

NaBH₄: Fluka, purum.

Hydrochloric acid: chem. pure.

Methanol: chem. pure.

Methanol (for second precipitation): Merck p.a.

Water: twice distilled.

α or β CD, water, a 30% NaOH solution in water and NaBH₄ are homogenised at 80 °C (see for quantities Table 2). While stirring a hot 50% (w/w) solution of monochloro-acetic acid in water is added. The mixture is kept at 80 °C during 45 min. Then it is cooled and by adding concentrated hydrochloric acid the pH is lowered to 9 (pH indicator paper). Methanol is added in such a quantity that on further addition no more precipitate is formed. The precipitate is filtered and dissolved in water (50% solution). Pure methanol is added until no more precipitate is formed. The precipitate is filtered and dried at 90 °C in vacuum.

CD-Sulfopropylether Na-Salt (CD-SPE)

CD-SPE is prepared analogously to amylose-SPE [6].

Materials:

Propanesultone: Shell, development chemicals.

Others: see preparation of CD-CME.

* Part V of this series: *Stärke* 22 (1971), 129.

** Part of the thesis of J. N. J. J. Lambers, Eindhoven University of Technology, Netherlands (October, 1970).

Table 1. Preparation of Soluble CD-E Resins.

	CD	H ₂ O	NaOH 16%	NaBH ₄	ECM*	reaction time	yield	code
	g	g	g	mg	g	min	$\frac{\text{g resin}}{\text{g CD}} \times 100$	
α CD	9	5	16	20	25	30	60	α -E25 solub
β CD	9	10	16	20	15	50	20	β -E15 solub

* epichlorohydrin.

Table 2. Preparation of CD-CME.

	CD	H ₂ O	NaOH 30%	chloro-acetic acid (50%)	yield	code
	g	g	g	g	$\frac{\text{g CME}}{\text{g CD}} \times 100$	
α CD	50	450	250	100	50%	α -CME*
β CD	50	450	250	150	50%	β -CME**

* α -CME 1/1-5 in *Leuninger's* thesis.** β -CME 2/3-2 in *Leuninger's* thesis.

The reaction is performed at 30 °C under nitrogen. CD, water, and a 40% NaOH solution are homogenised. While stirring, warm, molten propane-sulfone is slowly added during the reaction time of 3 h. Then, with concentrated hydrochloric acid, the pH is lowered to 9 (pH indicator paper). With methanol, the product is isolated as in the case of CD-CME.

Analysis

The aim of the analysis of the prepared CD derivatives is to find a mean value for the molecular weight or the AVC (Apparent Void Concentration: mole CD void/g CD-E resin) [5]. We only determine a mean value of M or AVC, remembering that undoubtedly mixtures of derivatives with different degrees of substitution are present. We have applied a number of different methods of analysis:

- Hydrolysis and subsequent oxidation.
- Interaction with m-chlorobenzoic acid.
- Determination of Na and Cl (in CD-CME and CD-SPE).
- Determination of sulfur (in CD-SPE).
- Titration of saltgroups with HClO₄ in HAc (in CD-CME).

Furthermore, an IR spectrum of β -CME and β CD has been made. It has also been shown that the derivatives are free from methanol. This has been done by analysing an aqueous solution gaschromatographically.

We shall now discuss the methods of analysis and give the results of each method. Finally, the results will be combined and a final value of the molecular weight (or AVC) be determined.

A. Hydrolysis and Subsequent Oxidation

The method consists of hydrolysis of the CD derivative followed by oxidation (after the method of Willstätter and Schudel [9], hypiodite oxidation) of the glucose-residue derivatives formed. The method had already been described by Wiedenhofer et al. [5].

It has been found that variation of neither the time of hydrolysis (5 h or longer) nor the oxidation time (20 min or longer) had any significant influence on the results (see thesis).

Furthermore, when applying this method to α and β CD it is found that the determination is about 10% too low with respect to the theoretical glucose content of α and β CD. This is not surprising because it is known that when the original concentration is low (0.01%), the sugar is fully hydrolysed, without appreciable side reactions [10], but too

Table 4. Results of the Hydrolysis and Oxidation of CD and its Derivatives*.

CD (derivative)	mean values		
	% found	molecular weight	AVC · 10 ⁴
α CD	90.4 ± 1.8%	872	5.84 ± 0.17 6.04 ± 0.14
β CD	89.9 ± 0.8%	1134	
α -E25 solub			
β -E15 solub			
α -CME		1138 ± 41	
β -CME		1537 ± 25	
α -SPE		1322 ± 50	
β -SPE		2282 ± 40	

* The uncertainties are determined for a level of significance of 95% in *Student's T* distribution. Each mean value is obtained from a series of 5 to 10 measurements.

Table 3. Preparation of CD-SPE.

	CD	H ₂ O	NaOH 40%	propane sulfone	yield	code
	g	g	g	g	$\frac{\text{g SPE}}{\text{g CD}} \times 100$	
α CD	30	30	30	30	73	α -SPE
β CD	30	35	45	30	140	β -SPE

little glucose is found [9], whereas when the original concentration is high (1%) the sugar is not fully hydrolysed [10] while the oxidation of the glucose is complete [9].

The results are given in Table 4.

B. Interaction with *m*-Chlorobenzoic Acid (MCB)

This method is founded on the linear increase of the solubility of MCB in water at increasing concentration of CD [11], by which an inclusion compound in solution is formed.

If the stability of the inclusion compound is independent of the nature of the derivative, then, at a given concentration of CD (mole/l) and the same concentration of a CD derivative, equal amounts of MCB will dissolve. The total concentration of MCB can be measured spectrophotometrically.

It appears that the wavelength of the absorbance maximum of MCB in solutions of CD (derivatives) is independent of the nature of the CD (derivative) when at least the solutions are buffered at pH = 1.2. MCB is then almost exclusively present in its undissociated form (pK = 3.82 at 23 °C).

However, the value of the absorbance at its maximum is dependent on the nature of the CD (derivative) (see Table 5).

Table 5. Values of the Absorbance at 283 nm of Saturated MCB Solutions (20 °C, pH 1.2) Containing 10⁻⁴ mol CD-oxide/l.

CD (derivative)	absorbance
none	1.03
α CD	1.11
α-CMB	1.10
α-SPE	1.10
α-E25 solub	1.06
β CD	0.99
β-CMB	1.05
β-SPE	1.05
β-E15 solub	1.05

Therefore, the results of this method should be interpreted with care, especially when the values of the absorbance in derivative solutions differ much from those of the corresponding CD (Table 5; α-E25 solub, β CD derivatives).

Experimental

Different amounts of CD (derivative) in the range of 25–50 mg and 20 g of buffer solution (hydrochloric acid/KCl; pH 1.2) are weighed into vials. About 50 mg MCB is added and the vials are sealed and equilibrated during

16–20 h at 20.0 °C. The absorbance of the supernatant is measured in a 1 mm cell against the same concentration of CD (derivative) in buffer solution at 283 nm on a Zeiss spectrophotometer PMQ II with double monochromator MMD 1.4.

The graphs of CD (derivative) concentration against absorbance show linear relationships, the slopes and accuracies of which can be calculated as has been done in the appendix of lit. 12.

Results

In Table 6 the calculated slopes of the interaction isotherms are shown. The molecular weight (AVC, respectively) has been calculated from the slope of the interaction isotherm of a CD (β CD, respectively) and those of the corresponding derivatives:

$$M = \frac{\text{slope } \alpha \text{ CD line}}{\text{slope } \alpha \text{-derivative line}} \times 972 \text{ (1134)}$$

C. Determination of Na and Cl

The chloride content measures directly the amount of NaCl still present in the CD derivative. When the total Na content is determined and corrected for Na, present as NaCl, the degree of substitution can be calculated if all substituents are present in the Na-salt form. Therefore, a solution of CD derivatives is also titrated with alkali to determine the content of substituent present in the acid form.

Experimental

Determination of chloride:

This is a spectrophotometric micro-determination. A CD derivative solution is added to a solution containing Fe³⁺ and HgSCN. The Cl⁻ ions react with HgSCN and the liberated SCN⁻ ions form a coloured complex with Fe³⁺ (see lit. 13, page 809). NaCl solutions of appropriate concentrations are used as standards.

Determination of Na:

In a CD derivative solution of known concentration the Na content is measured with a flame photometer. NaCl solutions are used as standards.

Determination of substituents in acid form:

A solution of CD derivative is titrated with 0.1 N NaOH from a microburet against phenolphthalein.

Table 7 summarises the results.

D. Determination of Sulfur in CD-SPE

The sulfur content is determined by complete oxidation to sulfate, followed by a sulfate determination. From the

Table 6. Slopes of the Interaction Isotherms and Calculated Molecular Weights (AVC).

CD derivative	slope of interaction isotherm		M (AVC)
	CD derivative	corresponding CD	
α-CMB	0.0797 ± 4.8%	0.0948 ± 3.6%	1156 ± 70
α-SPE	0.0648 ± 1.6%	0.0948 ± 3.6%	1422 ± 57
α-E25 solub	0.0607 ± 5.1%	0.0948 ± 3.6%	(6.39 ± 0.41) · 10 ⁻⁴
β-CMB	0.0476 ± 5.0%	0.0703 ± 8.7%	1675 ± 168
β-SPE	0.0343 ± 4.4%	0.0669 ± 6.9%	2212 ± 182
β-E15 solub	0.0507 ± 11.0%	0.0703 ± 8.7%	(6.36 ± 0.89) · 10 ⁻⁴

Table 7. Determination of x (= part of substituent in acid form), S (= degree of substitution per CD molecule), M (= molecular weight) and NaCl Content for CD-CME and CD-SPE.

CD derivative	weight % NaCl	x	S	M
α -CME	4.73 ± 0.43	0.0025	2.90 ± 0.21	1204 ± 17
α -SPE	2.74 ± 0.2	—	3.19 ± 0.35	1431 ± 51
β -CME	0.00 ± 0.05	0.0028	3.29 ± 0.17	1395 ± 14
β -SPE	0.38 ± 0.08	—	6.46 ± 0.71	2064 ± 102

sulfur content the degree of substitution and the molecular weight have been calculated. The results are shown in Table 8.

Table 8. Determination of S and M for CD-SPE.

CD derivative	sulfur content (%)	S^a	M^a
α -SPE	5.63 ± 0.1	7.79 ± 0.16	1301 ± 22
β -SPE	10.22 ± 0.5	6.68 ± 0.44	2096 ± 63

^a See Table 7 for explanation.

B. Titration of Salt Groups in CD-CME with HClO_4 in HAc

By this method the substituents in CD-CME in the Na-salt form are titrated with HClO_4 . In acetic acid the CD-CME⁻ ion is a strong base and can be titrated with HClO_4 , which in this solution is a strong acid. It has been shown that addition of pure CD to the CD-CME solution has no influence on the result of the titration.

Experimental

A quantity of dry CD-CME is dissolved in 10 ml HAc and 1 ml water by heating. The solution is poured into a volumetric flask of 25 ml and diluted to the mark with HAc . A solution of HClO_4 (0.1 N) is standardised on a 0.02 N solution of Na_2CO_3 in HAc .

The titration is carried out in a Radiometer titration equipment provided with a titrator and a buret of 2.5 ml. 5 ml of the CD-CME solution has been titrated. The titration vessel is provided with a glass electrode and a calomel electrode. The equivalence point is found from the titration curve.

The amount of substituents in the Na-salt form can be calculated from the results of the titration. The substituents in the acid form are known from method C.

Table 9 shows the results.

Table 9. Determination of S and M for CD-CME.

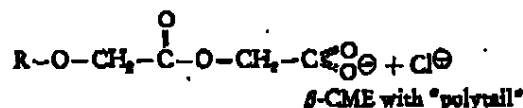
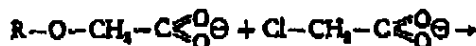
CD-derivative	S^a	M^a
α -CME	3.02 ± 0.03	1213 ± 3
β -CME	3.80 ± 0.02	1436 ± 2

^a See Table 7 for explanation.

IR-Spectrum of β CD and β -CME

From the results of the determinations of the molecular weights of the CD-derivatives it is clear that in the case

of β -CME the results do not agree within the limits of errors (Table 11). It is possible that a byproduct has been formed:



in which $\text{R} = \beta$ CD

When appreciable amounts of "polytails" are present in β -CME, they should show up in the IR-spectrum. Figure 1 shows the IR spectra of β CD and β -CME.

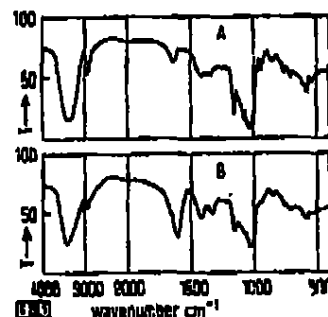


Figure 1. IR spectra of A: β CD; B: β -CME. KBr pellets.

In Table 10 some characteristic bands are enumerated.

Table 10. Some Bands of the IR Spectrum of Figure 1.

wave number (cm ⁻¹)	interpretation of bands ^a	
	β CD	β -CME
700; 760; 950	pyranose-ring vibration CO/CC str.; (C) str. glycosidic bond; OH bend. CH def.	
1000-1180		
1300-1380		
1530	—	CH def.
1410; 1600	—	CO ₂ str.
1620	OH bend. (in water)	—
3400	—	CH str. OH str.

^a See also lit. 5.

In the spectrum of β -CME the additional bands of CH def. and CO₂ str. originate, to all probability from its substituents. Furthermore, the characteristic bands of β CD have been retained in the β -CME spectrum.

Table 11. Survey of the Determinations of the Molecular Weights (AVC).

method	A	B	C	D	E
CD derivative					
α -CME	1138 $\pm$ 4	1156 $\pm$ 70	1204 $\pm$ 17		1213 $\pm$ 3
α -SPB	1322 $\pm$ 50	1422 $\pm$ 57	1431 $\pm$ 51	1301 $\pm$ 22	
α -E25 solub	(5.84 $\pm$ 0.17) $\cdot 10^{-4}$	(6.59 $\pm$ 0.41) $\cdot 10^{-4}$			
β -CME	1537 $\pm$ 25	1675 $\pm$ 168	1395 $\pm$ 14		1436 $\pm$ 2
β -SPE	2282 $\pm$ 40	2212 $\pm$ 182	2064 $\pm$ 102	2096 $\pm$ 63	
β -E15 solub	(6.04 $\pm$ 0.14) $\cdot 10^{-4}$	(6.36 $\pm$ 0.87) $\cdot 10^{-4}$			

However, no ester groups (which should be present in the "polytails") are detectable; otherwise additional bands would have been present in the β -CME spectrum at 1750–1800 cm^{-1} and at 1200–1300 cm^{-1} .

Because methods C and E give results which significantly differ from methods A and B, minor amounts of "polytails" should be present in β -CME. Thus not too much value should be attached to the results of methods C and E.

Results

In Table 11 a survey of the determinations is given. The mean molecular weight (AVC) has been calculated by counting method A twice, method B once and the mean value of methods C–E also once. This has been done because methods A and B give directly the amount of CD voids, while method B is less reliable, whereas methods C–E give the quantity of substituents and take no account of possible "polytails" (especially in the case of β -CME).

Table 12. Mean Values of the Molecular Weights and AVCs.

CD derivative	M (AVC)
α -CME	1160 $\pm$ 54
α -SPE	1358 $\pm$ 76
α -E25 solub	(6.09 $\pm$ 1.08) $\cdot 10^{-4}$
β -CME	1541 $\pm$ 169
β -SPE	2214 $\pm$ 152
β -E15 solub	(6.15 $\pm$ 0.47) $\cdot 10^{-4}$

In Table 12 the values of the molecular weights and AVCs thus calculated are shown. When calculating mean molecular weights using a different weighing procedure, the results are affected to only a very small extent.

Acknowledgement

The authors wish to thank Professor Dr. C. L. van Pantbaleon van Eck for his interest shown throughout this study and Ir. N. Wiedenbof for his stimulating discussions. We are grateful to Mr. W. Brekelmans for the preparation of the soluble CD-E resin and Mr. P. L. N. van den Bosch for carrying out the sulfur determination.

Zusammenfassung

Die Eigenschaften der Cyclodextrine. VI. Teil. Wasserlösliche Cyclodextrinderivate. Herstellung und Analyse. In wässriger Lösung sind nur solche Einschlussverbindungen mit Cyclodextrinen

bekannt, bei denen die eingeschlossene Verbindung eine polare Substanz ist. Mit unpolaren Substanzen werden nur sehr leicht lösliche Einschlussverbindungen gebildet.

Um in der Lage zu sein, Einschlussverbindungen mit unpolaren Substanzen in Lösung herzustellen, wurden hydrophile Cyclodextrinderivate hergestellt, nämlich die Natriumsalze des Carboxymethyläthers und des Sulfopropyläthers, sowie ein lösliches Epichlorhydrinäther des α - und des β -Cyclodextrins. Das Molekulargewicht, bzw. die scheinbare Hohlraumkonzentration der Derivate läßt sich auf verschiedenen Wegen bestimmen.

Résumé

Propriétés des cyclodextrines. Partie VI. Dérivés de cyclodextrines hydrophiles. Préparation et analyse. Les composés d'inclusion avec les cyclodextrines en solution aqueuse ne sont connus que lorsque le constituant inclus est une substance polaire; des composés d'inclusion très peu solubles sont formés à partir de substances apolaires.

Afin de pouvoir former des composés d'inclusion avec des substances apolaires en solution, des dérivés hydrophiles de cyclodextrines, à savoir les sels de sodium des carboxyméthyléthers, et des sulfopropyléthers ainsi que des résines solubles d'épichlorohydrine de α - et β -cyclodextrine ont été préparés. Le poids moléculaire de ces dérivés (concentration apparente de cavité respectivement) sont déterminés par différentes techniques.

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Address of Authors: Ir. J. N. J. J. Lammers, Ph. D., Ir. J. L. Koole, and J. Hurkmans. Technische Hogeschool Eindhoven, Laboratorium voor Algemene Chemie, Postbus 513, Eindhoven (Netherlands).

New Address of First Author: Koninklijke Shell-Laboratorium Amsterdam, Badhuirweg 3, Amsterdam-Noord (Netherlands).

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Thin-layer Chromatography of Cyclodextrins

By Ken'ichi TAKEO, Yôtarô KONDO* and Takashi KUGE

Department of Agricultural Chemistry
Kyoto Prefectural University, Kyoto

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The cyclodextrins are a series of homologous cyclic oligosaccharides obtained from starch by the action of *Bacillus macerans* amylase. In the dextrins α -, β -, and γ -dextrins are respectively composed of six, seven and eight D-glucopyranoses that are linked at α , 1 $\rightarrow$ 4 in a cyclic form. The dextrin exhibit a unique character to form inclusion compounds with a wide variety of organic and inorganic compounds, in which a single dextrin molecule provides hollow space available for the inclusion of guest molecules.

In the course of the studies on the inclusion complexes of the dextrins by means of gas chromatography,¹⁾ polarography²⁾ and X-ray diffractions,^{3,4)} a simple and rapid procedure has been required to characterize the dextrins produced during the *B. macerans* enzymolysis of starch and to check the purity of the individual dextrins.

Several chromatographic techniques have been described for the separation of dextrins. Cramer *et al.*^{5,6)} succeeded in separating α -,

β -, and γ -dextrins by circular paper chromatography. This method usually requires an irrigation period of about 20-25 hr to permit their isolation. On the other hand, Wiedenhorf⁷⁾ reported the separation of dextrins by using the technique of microchromatoplate developed by Peifer.⁸⁾ However, in this case, no description has been given for the separation of γ -dextrin.

Because of the long separation time in paper chromatography and of the lack of the method for the separation of γ -dextrin in microchromatoplate, both methods do not seem to satisfy our requirements. Accordingly, conventional thin-layer chromatography was examined for efficiency in separating dextrins. The thin-layers examined were Kieselguhr (Kieselguhr G, Merck), microcrystalline cellulose (Avicel SF, Funakoshi Chem. Co. Ltd.) and silicagel (Kieselgel G, Merck). All the plates were prepared in the usual manner to give a thickness of 250 μ . Both the plates of Kieselguhr and silicagel were allowed to stand at room temperature for 1 hr, and placed in an oven at 110°C for 30 min and then stored over silicagel in a dessicator. The plates of cellulose were dried overnight in the air and used without further conditioning. The following solvent systems which are commonly used for the separation of linear oligosaccharides were examined in various proportions, i. e., *n*-butyl alcohol-

* Present address: Department of Agricultural Chemistry, Kyoto University, Kyoto.

1) T. Kuge and K. Takeo, *Agr. Biol. Chem.*, **32**, 753 (1968).

2) T. Kuge and K. Takeo, Abstracts of Papers for the Annual Meeting of the Agricultural Chemical Society of Japan (in Japanese), Nagoya, April, 1968, p. 25.

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7) N. Wiedenhorf, *J. Chromatog.*, **15**, 100 (1964).

8) J. J. Peifer, *Mikrochim. Acta*, **352**, 529 (1962).

ethyl alcohol-water, *n*-butyl alcohol-pyridine-water, *n*-butyl alcohol-glacial acetic acid-water and *n*-butyl alcohol-isopropyl alcohol-water, etc. Three~five μg of a pure dextrin or about 20 μg of a mixture of the dextrins were applied to a streak on the plates and the plates were irrigated in an ascending manner in a glass vessel with fresh solvent without prior equilibrium. The irrigation was continued until the front of the solvent ascended about 10~13 cm from the original point. After the completion of the irrigation, the plates were dried on a hot plate and detection was made by spraying 1% methanolic iodine solution. Distinct colors developed instantaneously, that is, violet for α -dextrin, yellow for β -dextrin and brown for γ -dextrin. This method made simultaneous detection of the individual dextrins possible in a short time.

After trying various combinations of the thin-layers and the solvent systems mentioned above, the following two combinations were found to give good results in separating dextrins; (I) microcrystalline cellulose layer with *n*-butyl alcohol-ethyl alcohol-water

(4:3:3 by volume) and (II) Kieselguhr layer with *n*-butyl alcohol-ethyl alcohol-water (4:3:2 by volume). When the combination (I) was

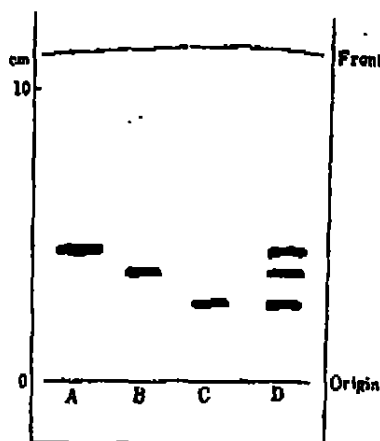


FIG. 2. Thin-layer Chromatogram of Cyclodextrins on Microcrystalline Cellulose Obtained after Twice-repeated Irrigations.

Solvent system, *n*-butyl alcohol-ethyl alcohol-water (4:3:3 by volume); (A) α -cyclodextrin, (B) β -cyclodextrin, (C) γ -cyclodextrin, (D) mixture of α -, β -, and γ -cyclodextrins.

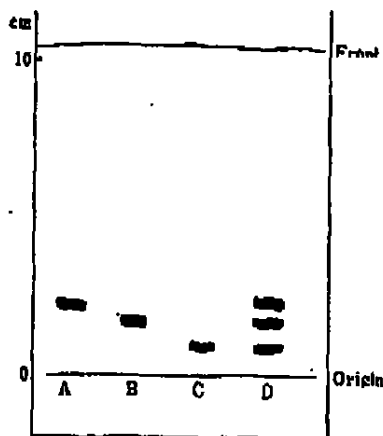


FIG. 1. Thin-layer Chromatogram of Cyclodextrins on Microcrystalline Cellulose.

Solvent system, *n*-butyl alcohol-ethyl alcohol-water (4:3:3 by volume); (A) α -cyclodextrin, (B) β -cyclodextrin, (C) γ -cyclodextrin, (D) mixture of α -, β -, and γ -cyclodextrins.

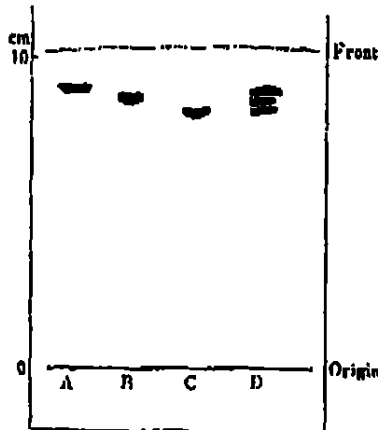


FIG. 3. Thin-layer Chromatogram of Cyclodextrins on Kieselguhr G.

Solvent system, *n*-butyl alcohol-ethyl alcohol-water (4:3:2 by volume); (A) α -cyclodextrin, (B) β -cyclodextrin, (C) γ -cyclodextrin, (D) mixture of α -, β -, and γ -cyclodextrins.

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used, the results obtained were satisfactory for the separation of the dextrans as shown in Fig. 1. The individual dextrans were resolved in sharply defined bands without tailing. The time of the irrigation was about 4 hr. Irrigations repeated twice with the same mixture of the solvents by a multiple ascending technique gave better resolution as shown in Fig. 2. Thus, the R_f values considerably increased. When the combination (II) was used, irrigation was completed in a short time of about 1.5 hr. The chromatogram obtained is shown in Fig. 3. In this case, separation was rather poor and the spots were less sharp than when the combination (I) was used. However, each dextrin can obviously be distinguished by inherent color formation with iodine spray. In both the cases of (I) and (II), there is no interference for the detection of the dextrans with linear oligosaccharides which

may also be produced during *B. macerans* enzymolysis, since the oligosaccharides only form a very faint color with iodine.

Attempts to separate the dextrans by the use of silicagel were unsuccessful due to appreciable tailings of the spots regardless of solvent systems.

The above experimental results now demonstrate the usefulness of thin-layer chromatography for the separation and identification of the dextrans. The present procedure has the advantage of sharp resolution as well as speed as compared with paper chromatography. It may be said that the combination (I) is most suitable for the checking of the purity of the individual dextrans, and that the combination (II) may be helpful for a quick check of the appearance of the dextrans during the enzymolysis of starch by *B. macerans* amylase.

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Ref # 3 124

Subject: [Fwd: Re: Do we have this...]
From: Jeannette S Manello <jeannette.manello@bms.com>
Date: Wed, 27 Oct 2004 14:25:02 -0400
To: Debra Wojcik <debra.wojcik@bms.com>
CC: Mary Laskow <mary.laskow@bms.com>

Debra,

Listed below are the abstract from Derwent WPI for the accession number you listed and also the abstract from Chemical Abstracts for the corresponding patent number.

If this is not what you were looking for, please call me to discuss.

Regards,
Jeannette Manello
ext 4846-LV

L1 ANSWER 1 OF 1 WPIX COPYRIGHT 2004 THE THOMSON CORP on STN
AN 1988-073862 [11] WPIX
TI New compen. with negligible toxicity - contg. water-insol. or slightly soluble pharmacologically active matter and glucosylated branched cyclodextrin.
PA (SAOC) SANRAKU OCEAN CO LTD
PI JP 63027440 A 19880205 (198811)* 7
PRAI JP 1986-168015 19860718
AB JP 63027440 A UPAB: 19950117
Compen. in which a water-insol. or slightly soluble pharmacologically active matter and glucosylated branched cyclodextrin coexist is new. Pref. water-insol. or -slightly soluble drugs are e.g. fat-soluble vitamins, e.g., vitamin E, K1, K3 and D3; steroid hormones; nonsteroid series antiinflammatory agents; central nervous system depressing agents, e.g. phenobarbital or nitraepam; cardioponic agents, e.g. digitoxin or digoxin; water-insol. antibiotics, e.g. griseorulvin; etc. The cyclodextrin can be produced from the cyclodextrin generating soln. by sepg. methods, e.g., solvent pptn. column chromatography with various resins, ultrafiltration, or reverse osmosis. The soln. can be prepd. by reacting cyclodextrin glucanotransferase with starch or its hydrolysed matter at 50-70 deg. C, pH 5-10 for 18-72 hrs.. The inclusion of thereby obtd. cyclodextrin with the drugs can be carried out as follows. 0.3-2.0 pts. of water are added to cyclodextrin to knead; an equimolar amt. of the drug is added to the kneaded cyclodextrin to knead by stirring for 0.5-several hrs.; and kneaded mixt. is freeze-dried and made into powder to obtain the compsn..
USE/ADVANTAGE - Glucosylated branched cyclodextrin is highly soluble in water and can improve the solubility of insol. or slightly soluble drugs in water. It has almost no toxicities, e.g. cythemolysis.

L4 ANSWER 1 OF 1 HCA COPYRIGHT 2004 ACS on STN
AN 110:121385 HCA
TI Pharmaceutical compositions containing glucosylated .alpha., .beta., or .gamma.-cyclodextrins to improve water-solubility of drugs
PA Sanraku Co., Ltd., Japan
IN Koizumi, Kyoko; Okada, Yasuyo; Kubota, Yoko; Utamura, Toshiko
PATENT NO. KIND DATE

PI JP 63027440 A2 19880205
PRAI JP 1986-168015 19860718
AB Glucosylated branched cyclodextrins are compounded with water-insol. or sparingly water-sol. pharmaceutical agents to improve their water-soly. and bioavailability. Hydrolyzates of potato starch were treated with cyclodextrin glucanotransferase and glucoamylase to give 6-O-.alpha.-D-glucosyl-.alpha.-cyclodextrin(I), 6-O-.alpha.-D-glucosyl-

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.beta.-cyclodextrin(II), and 6,6'-di-O-.alpha.-D-glucosyl-.beta.-cyclodextrin(III). The water required to dissolve 1 g of I, II, and III was 1.1, 1.0, and 0.7 mL, resp.; however, 5.7, 53.3, and 3.8 mL of water was needed to dissolve .alpha.-cyclodextrin, .beta.-cyclodextrin, and .gamma.-cyclodextrin, resp. III and estriol in a mole ratio of 1:1 were added to a physiol. saline soln. and freeze-dried. The freeze-dried powder and lactose were tableted and the tablet was dissolved in water; the water-sol. was detd. to be 2,606 .mu.g estriol/mL, compared to 32 .mu.g/mL for the control tablet comprising estriol and .alpha.-cyclodextrin.

----- Original Message -----

Subject: Re: Do we have this...

Date: Wed, 27 Oct 2004 14:09:04 -0400

From: Mary Laskow <mary.laskow@bms.com>

Organization: Bristol-Myers Squibb Company

To: Jeannette S Manello <jeannette.manello@bms.com>

References: <417FD0C1.9040202@bms.com> <417FDC75.4010206@bms.com>

She is looking for AN73892, Week 8811. If that is not enough information, yes, can you kindly contact her?

Appreciate it -

Mary

Jeannette S Manello wrote:

The client may be looking for a specific abstract. We have this available via the Derwent WPI online and also via Derpict. Do you want me to contact her to see what she is looking for specifically?

Mary Laskow wrote:

Hi Jeannette -

Do we have this reference electronically?

Thanks,
Mary

----- Original Message -----

Subject: Photocopy Request Form

Date: Wed, 27 Oct 2004 09:37:39 -0400 (EDT)

From: debra.wojcik@bms.com

To: lawrenceville.library@bms.com

CC: debra.wojcik@bms.com, mary.laskow@bms.com

Photocopy Request Form

Request Tracking Number: DOC20041027097729

Date Submitted: 10/27/2004

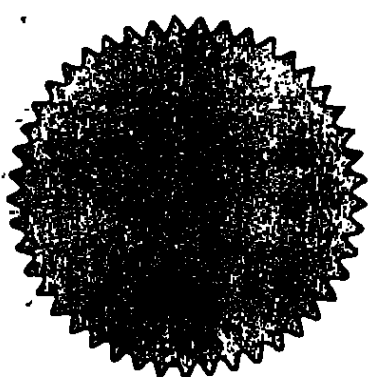
Requestor Information

E-Mail Address: debra.wojcik@bms.com
Name: Debra Wojcik
Cost Center Code: 9990523
Site: Lawrenceville
Location/Mailcode: D33-07
Telephone No.: 609-252-5520

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STATE OF NEW JERSEY, } ss.
County of Mercer



I, CATHERINE DiCOSTANZO, Clerk of the County of Mercer,

Do Hereby Certify that *Diane C. Barth*
Esq., whose name is subscribed to the acknowledgment, proof or affidavit of
the annexed instrument, and thereon written, is and was at the time of
taking the same, a Notary Public in and for said State,
dwelling in said County, commissioned and sworn and duly authorized by
the laws thereof to take the proofs and acknowledgments of deeds or other
instruments of writing, and affidavits to be recorded in said State, and that I
am well acquainted with the handwriting of said official, and verily believe
that the signature is genuine.
Further Certify that impression of Seal of Notaries Public not required by
law be filed in my office.

In Testimony Whereof, I have hereunto set my hand and affixed the seal of
said County and Courts, at Trenton this *15th* day of

April A.D. nineteen hundred and *twenty one*
Catherine DiCOSTANZO Mercer County Clerk.

En la Notaria Cuarenta de este Circuito se
notó la presente fotocopia que coincide con
el original que se ha tenido a la vista.
29 DIC. 2004
NOTARIO CUARENTA (E)
Nery Fernando More Sandoval
Bogotá, D.C., Colombia



Brigard & Castro

SANTA FE DE BOGOTÁ - COLOMBIA

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PODER

Yo (nosotros)

domiciliado(s) en

Nombre(amos) a: RAMIRO CASTRO DUQUE y/o HECTOR GOMEZ MEJIA, de Santafé de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, acciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(s) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistír a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el recibo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeren conducentes al resguardo de mis (nuestros intereses), con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistír, transigir, comprometer y/o sustituirlo en todo o en parte y revocar sus sustituciones. Asimismo nombro(amos) a: RAMIRO CASTRO DUQUE y/o HECTOR GOMEZ MEJIA, domiciliado(s) en Carrera 7 No. 16-58 Piso 9, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales.

Otorgado y firmado en

nov de 19

Firma

Nombre

Cargo

POWER OF ATTORNEY

I (We) BRISTOL-MYERS SQUIBB COMPANY

domiciled in

Princeton, New Jersey, USA

Hereby appoint RAMIRO CASTRO DUQUE and/or HECTOR GOMEZ MEJIA, of Santafé de Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names and emblems, registration of copyright and contracts related thereto, certificates of obtainment of vegetable varieties, as well as extensions, renewals and recordal of assignments and changes of name and domicile related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or exploitation of licenses, action of unfair competition; penal demands, actions of indemnities for damages; claims or observations on patent applications, models, industrial designs and utility models; in the exercise of preventive measures; and in other actions or similar proceedings.

To that effect they hereby empowered to file applications, formulate descriptions, amendments, protests, declarations, oppositions, cancellations, nullities, appeals and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights granted or in the process of granting; to receive all kinds of documents and valuables, giving the respective release of receipt, and, in general, to take before said authorities the necessary steps to such effect, and any measures that they may deem pertinent to the safeguard of my (our) interest(s) with all the other necessary legal powers.

This power includes the faculty to ratify or confirm and compromise on my (our) behalf, and to exercise this power in whole or in part and to revoke substitutions. At the same time I (We) hereby appoint RAMIRO CASTRO DUQUE and/or HECTOR GOMEZ MEJIA, domiciled in Carrera 7 No. 16-58, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

Granted and signed in Princeton, New Jersey, USA

this 14th

Signature

Name

Title

day of April 1999

BRISTOL-MYERS SQUIBB COMPANY

Stephen B. Davis

Stephen B. Davis

Associate Counsel Patents

100/34

CERTIFICADO NOTARIAL (SOCIEDAD)

NOTARIAL CERTIFICATE (CORPORATION)

Hoy de de
personalmente compareció ante mí

a quien conozco y de quien me consta que firmó el
anterior documento debidamente juramentado declaró
y dijo que él es

ss

Sociedad legalmente constituida para un período de
duración que aún no ha terminado que el declarante
está autorizado por dicha compañía para conferir este
poder, que el sello estampado por él al pie de tal poder
en nombre y representación de la expresada compañía,
es el sello oficial de ella y que el objeto para el cual se
confiere este instrumento está comprendido dentro del
de la sociedad

El suscrito Notario hace constar que tuvo a la vista las
pruebas de la existencia de la sociedad

Estado de
Fecha

y que quien confiere el presente poder es su
representante

On this 14th day of April of 1998
personally appeared before me

Stephen B Davis

to me known and known to me to be the person who
signed the foregoing instrument who being by me duly
sworn did depose and say that he (she) is the

Associate Counsel Patents

of BRISTOL-MYERS SQUIBB COMPANY

Which is legally constituted for a term not yet expired
that deponent is authorized by said Company to confer
this Power of Attorney that the seal affixed hereunto
by the deponent in the name and on behalf of said
Corporation is the corporate seal thereof and that the
purpose for which this instrument is granted is included
within the corporate purpose of company

The undersigned Notary Public attests that he has seen
the documents that prove the existence of the
company

State of Delaware

under date of November 13, 1989

and that he (she) who grants the present power of
attorney is its Representative

Notario Público (sello)

**CERTIFICADO NOTARIAL
PERSONA NATURAL**

Hoy de de comparecieron)
ante mí

a quien (es) conozco y me consta que es(son) la(s)
persona(s) firmante(s) del documento que precede y
quien(es) declara(n) que otorgan el mismo para los
fines y propósitos que en él se expresan

Notario Público (sello)

**LEGALIZACION ANTE CONSUL COLOMBIANO ES
REQUERIDA**

Diane C. Bartram Public Notary (Seal)

Notary Public of New Jersey
My Commission Expires March 9, 2000
En la Notaría comparecieron)
cotejó a presenta fotocopias
al original que se ha tenido a la
29 DIC. 2004
NOTARIO CUARENTA (B)
Nerby Fernanda Mora Sanchez
Bogotá, D.C., Colombia

**NOTARIAL CERTIFICATE
NATURAL PERSON**

On the 14th day of April of 1999
personally appeared before me

Stephen B Davis

who is(are) known to me and who I attest executed the
foregoing document and he (they) declared he (they)
grant(s) the document for the uses and purposes therein
expressed

Notary Public (Seal)

Diane C. Bartram
Notary Public of New Jersey
My Commission Expires March 9, 2000

LEGALIZATION BEFORE COLOMBIAN CONSUL IS REQUIRED

EL SUSCRITO CONSUL DE COLOMBIA**VISTOS LOS DOCUMENTOS PRESENTADOS POR LOS INTERESADOS****CERTIFICA**

Que el Señor (a) **CATHERINE Dicostanzo** Clerck del condado de Mercer estado de New Jersey autentico la firma de la notano(a) publico(a) sr (a) **DIANE C BARTRAN** del estado de New Jersey, Estados Unidos de América y quien ejercia las funciones antes expresadas y que la firma y sello que en el documento aparecen como suyos son los que usa y acostumbra en sus actos oficiales

Que la notano(a) antes citada autentico la firma del señor **STEPHEN B DAVIS-** quien actua en calidad de Asesor de Patentes de la Compañia **BRISTOL-MEYERS SQUIBB COMPANY** sociedad que existe y esta legalmente organizada bajo las leyes del estado de Delaware U S A



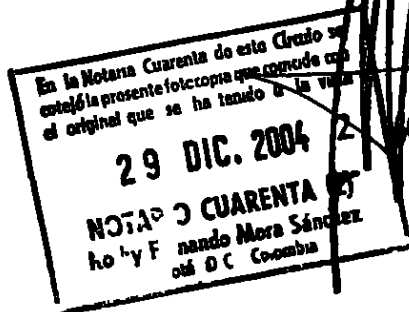
Que ha tenido a la vista los documentos probatorios de los hechos citados y constatados por el notano publico y el County Clerck del estado de New Jersey los cuales forman parte integral del presente documento

Se expide la presente certificación en cumplimiento de los articulos 259 del Codigo de Procedimiento Civil y 480 del Codigo de Comercio y a solicitud del interesado el 20 de Abril de 1999

NO SE ASUME RESPONSABILIDAD POR EL CONTENIDO DEL DOCUMENTO ADJUNTO

PAGADO IMPUESTO DE TIMBRE Y DERECHOS CONSULARES

VALOR CINCUENTA Y SIETE DOLARES US\$57.00 RECIBO No. 200283



Edérico Mejía Mejía
Cónsul de Colombia

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TRADUCCION OFICIAL No 260/99 de las Legalizaciones que se encuentran a continuación de un Poder otorgado por BRISTOL-MYERS SQUIBB COMPANY, una sociedad domiciliada en Princenton, New Jersey, Estados Unidos de América al doctor RAMIRO CASTRO DUQUE y/o HECTOR GOMEZ MEJIA, de Santafe de Bogotá, D C , Colombia, del inglés al español, las cuales para su identificación se sellan con el Sello del Traductor al mismo tiempo que la presente -

+++++

ANEXOS El Poder arriba mencionado, Certificaciones Notariales, Certificación de las Firmas Notariales, Certificación del Consulado General de Colombia en New York, Estado de New York, Estados Unidos de América, Autenticación por el Ministerio de Relaciones Exteriores de Colombia -

+++++

Diane C Bartram (firmado)

Notario Público

HAY UN SELLO QUE DICE

Diane C Bartram

Notario Público de New Jersey

Mi Comisión Expira el 9 de Marzo del
año 2000

+++++

Diane C Bartram (firmado)

Notario Público

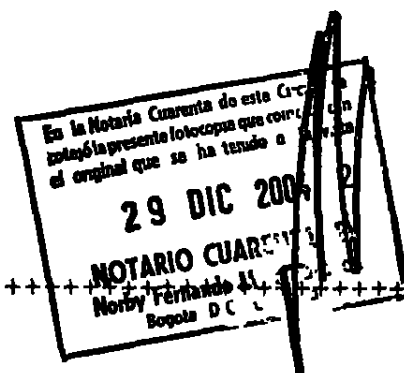
HAY UN SLLLO QUE DICE

Diane C Bartram

Notario Público de New Jersey

Mi Comisión Expira el 9 de Marzo del
año 2000

+++++



Diane C Bartram (firmado)

Notario Público

HAY UN SELLO QUE DICE

Diane C Bartram

Notario Público de New Jersey

Mi Comisión Expira el 9 de Marzo del
año 2000

[Handwritten signature]
Norby Fernando LL.
Bogotá D.C.

137/13

+++++

Diane C. Bartram (firmado)
Notario Público

HAY UN SELLO QUE DICE:

Diane C. Bartram
Notario Público de New Jersey
Mi Comisión Expira el 9 de Marzo del
año 2000

+++++

Diane C. Bartram (firmado)
Notario Público

HAY UN SELLO QUE DICE:

Diane C. Bartram
Notario Público de New Jersey
Mi Comisión Expira el 9 de Marzo del
año 2000.

+++++

Diane C. Bartram (firmado)
Notario Público

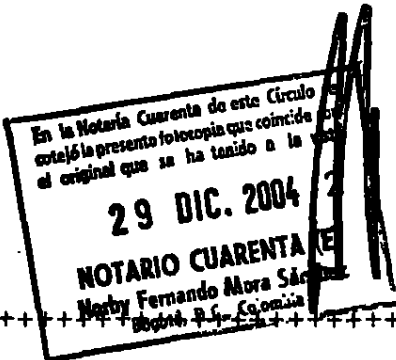
HAY UN SELLO QUE DICE:

Diane C. Bartram
Notario Público de New Jersey
Mi Comisión Expira el 9 de Marzo del
año 2000

+++++

ESTADO DE NEW JERSEY
Condado de Mercer

Yo, CATHERINE DiCOSTANZO, Escribano del Condado de Mercer,
Por Medio del Presente Certifico que Diane C. Bartram, cuyo nom-
bre está suscrito al reconocimiento, prueba o declaración jurada
del instrumento anexo, y en éste escrito, es y era en el momento
de tomar el mismo, un Notario Público en y para dicho Estado, re-
sidente en dicho Condado, comisionado y juramentado y debidamente
autorizado por las leyes de éste para tomar las pruebas y recono-



CONSEJO NOTARIAL ALVAÑEZ
REGISTRADO EN EL ESTADO DE NEW JERSEY

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cimientos de escrituras u otros instrumentos escritos, y declaraciones juradas para ser registrados en dicho Estado, y que estoy familiarizado con la caligrafía de dicho funcionario, y de veras creo que la firma es genuina.

Además Certifico que la impresión del Sello de Notarios Públicos no está requerida por la ley para ser presentada en mi oficina.

En Testimonio de lo Cual, he firmado el presente de mi puño y letra y fijado el sello de dichos Condado y Tribunales, en Tranton este día 15 de Abril del A.D. de mil novecientos noventa y nueve.

Catherine DiConstanzo (fdo.) Escribano del Condado de Mercer.

SELLO EN RELIEVE.

+++++
EN ESPAÑOL: La Certificación del Consulado General de Colombia en New York, Estado de New York, Estados Unidos de América bajo el número 200283 de fecha 20 de Abril de 1999; Autenticación hasta por el Ministerio de Relaciones Exteriores de Colombia bajo el número 044498 de fecha 29 de Abril de 1999.

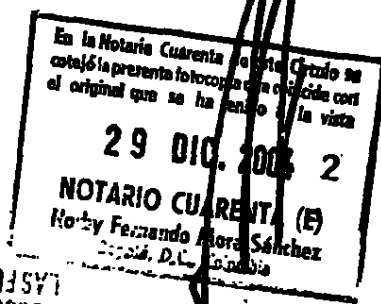
(Pagado Impuesto de Timbre y Derechos Consulares por valor de \$57.00 Dólares).

+++++
ES TRADUCCION FIEL Y COMPLETA DE LOS DOCUMENTOS ADJUNTOS.

ALVARO MARTINEZ ALVAREZ
TRADUCTOR E INTERPRETE OFICIAL
Reg. No. 177 de fecha 1997

Resolución número 1876 del Ministerio de Justicia.

Fecha: Mayo 3 de 1999.



JEFE DE LA OFICINA DE
SANTAFE DE BOGOTA D.C.
99 MAY - 4 AM 11:09
RESPONSABILIDAD DEL TEXTO
NO SE ASUME

MINISTERIO DE RELACIONES
EXTERIORES
0446201
CUYA FIRMATA APARECE EN EL
DOCUMENTO DESEMPLEADO
LAS FUNCIONES INDICADAS



GAVIRI A

ORGANIZACION ELECTORAL
REGISTRADURÍA NACIONAL DEL ESTADO CIVIL

REGISTRO CIVIL DE DEFUNCIÓN

Indicativo
Serial

5562241

Datos de la oficina de registro									
Clase de oficina:	Registraduría	Notaría	☒	Consulado	Corregimiento	Insp. de Policía	Código	9795	
Pob. - Departamento - Municipio - Corregimiento o Inspección de Policía									
COLOMBIA CUNDINAMARCA BOGOTA D.C. NOTARIA 32.									
Datos del inscrito									
Apellidos y nombres completos									
CASTRO DUQUE RAMIRO									
Documento de identificación (Clase y número)					Sexo (en Letras)				
C.C.No. 170322 DE BOGOTA					MASCULINO				
Datos de la defunción									
Lugar de la Defunción: País - Departamento - Municipio - Corregimiento o Inspección de Policía									
COLOMBIA CUNDINAMARCA BOGOTA D.C.									
Fecha de la defunción				Hora		Número de certificado de defunción			
Año	2	0	0	Mes	1	Día	3	0	04:10 PM A- 2094375.
Presunción de muerte					Fecha de la sentencia				
Jurado que profiere la sentencia					Año Mes Día				
Documento presentado					Nombre y cargo del funcionario				
Acta de defunción judicial					ANDRES DIAZ CAMPOS.-				
Certificado Médico					R.M. No. 79982662				
Datos del denunciante									
Apellidos y nombres completos									
EDGAR DAVID REGINO II.									
Documento de identificación (Clase y número)					Firma				
C.C.No. 15.030.154 DE LORICA/ CORDOBA									
Primer testigo									
Apellidos y nombres completos									
Documento de identificación (Clase y número)					Firma				
Segundo testigo									
Apellidos y nombres completos									
Documento de identificación (Clase y número)					Firma				
Fecha de interposición					Nombre y firma del funcionario que actúa				
Año	2	0	0	Mes	1	Día	0	2	BLANCA CHOIJA GONZALEZ RESTREPO
ESPACIO PARA NOTAS									
PADRES: ALFONSO CASTRO									
MERCEDES DUQUE.									

ORIGINAL PARA LA OFICINA DE REGISTRO

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020

Bogotá, D.C., 18 de marzo de 2005

Apoderado: Héctor Gómez Mejía

Oficio:

12429

REFERENCIA: Radicación No. 0-2434

Tramite 002

Evento 001

Actuación 430

Folios

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:

- ◆Capítulo descriptivo folios 7 a 27;
- ◆Capítulo reivindicatorio, folios 108 a 112;
- ◆1° examen de forma folios 100 y 101;
- ◆Respuesta técnica al primer examen de forma, folio 103 a 114;
- ◆Solicitud de la cual se reivindica prioridad, folios 35 a 93;
- ◆2° examen de forma folio 115;
- ◆Examen de patentabilidad, Art-46, folios 118 a 120;
- ◆Respuesta al examen Art-46, folios 222 a 131.

Objeto de la Invención:

La presente solicitud cuyo título es "COMPLEJO DE 1,4-BENZODIAZEPINAS SUSTITUIDAS Y SULFOBUTILETER-7-CICLODEXTRINA O 2-HIDROXIPROPIL- β -CICLODEXTRINA"

El objeto los complejos de derivados de complejo de 1,4-benzodiazepinas sustituidas y sulfobutileter-7-ciclodextrina o 2-hidroxipropil- β -ciclodextrina, que tienen la propiedad de inhibir la ras-farnesiltransferasa y las composiciones que los comprenden.

El objeto de la invención se puede clasificar según la clasificación internacional como A61K 31/55, 31/415.

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Claridad de la solicitud:

En el artículo 30 de la decisión 486 de la Comisión de la Comunidad Andina se establece: *"Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción"*.

En el primer examen de forma se le había objetado la reivindicación 1 por falta de claridad, "aralquilo" y términos semejantes, son características químicas de los compuestos, no son estructuras específicas e inequívocas; además en la descripción no aparecen sustentadas todas las estructuras que cumplen con esas características, por lo cual se considera que no están sustentadas enteramente en la descripción y por demás no está delimitado el alcance de la invención.

Igualmente en las reivindicaciones es inaceptable que para definir y precisar el significado de un sustituyente se utilice expresiones tales como "por ejemplo".

El alcance de la reivindicación 1 se dice que es amplia y especulativa porque el alcance no está limitado y no son razonables las generalizaciones de acuerdo con lo descrito y sustentado en los ejemplos.

Determinación del estado de la técnica:

El artículo 16 de la Decisión 486 de la Comisión establece: *"El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida."*

Sólo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40."

Se realizó la búsqueda del estado de la técnica con fecha anterior a la fecha de presentación de la primera solicitud, de la cual se reivindica prioridad, 21 de enero 1999. Consultada la fuente de información con que cuenta este despacho se encontró los siguientes documentos:

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No.	Documento No.	Título	*Fecha de Publicación
D1	US5134127	Derivatives of cyclodextrins exhibiting enhanced aqueous solubility and the use thereof	28-jul-1982

Evaluación de los requisitos de patentabilidad:

El artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina establece: *"Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial."*

En cumplimiento con este artículo, se procedió a evaluar los requisitos de novedad, nivel inventivo y aplicación Industrial con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

Evaluación de la Novedad:

El art.16 de la Decisión 486 de la Comisión de la Comunidad Andina establece: *"Una invención se considerará nueva cuando no está comprendida en el estado de la técnica." "El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida."*

Sólo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40."

La solicitud en estudio reclama complejos formados de una β -ciclodextrina y un derivado 1,4-benzodiazepinas y sus sales farmacéuticamente aceptables.

-La anterioridad D1 enseña las ciclodextrinas y la forma como se obtienen diferentes derivados de ciclo dextrina para mejorar la solubilidad de los principios activos insolubles. Las ciclodextrinas son conocidas por mejorar las tasas de disolución de las drogas, y además mejorar la estabilidad en solución acuosa por formar complejos. Se encontro que las ciclodextrinas tienen aplicación en la industria farmacéutica por formar complejos de inclusión teniendo una mejorada solubilidad y estabilidad para las moléculas de baja solubilidad acuosa, gracias a que las ciclodextrinas tienen un arreglo y conformación de unidades de β -glucopiranosas, que tienen libre rotación en los ligandos glicosídicos y por lo tanto las ciclodextrinas existen como una forma cónica con los ligandos situados en un cono secundario internamente, donde se forma el complejo con la droga insoluble. Dentro de las moléculas insolubles con las que puede formar complejos y que están determinadas explícitamente están las diazepinas, no en forma explícitamente los derivados que se nombran en la solicitud en estudio, esa es la única diferencia, entre esta anterioridad y la solicitud en estudio, por lo que se considera nueva la invención reclamada.

Evaluación del Nivel Inventivo:

El art. 18 de la Decisión 486 de la Comisión de la Comunidad Andina establece: *"Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica."*

Observando el documento D1 y todo lo enseñado allí en todo el documento, se toma como la parte central las ciclodextrinas que son las que tienen las propiedades descritas para formar complejos solubles, con cualquier molécula que por su naturaleza química son insolubles. Independientemente de la droga que se trate, solo que deseemos aplicar lo enseñado en la anterioridad y vemos que tales complejos no dependen de la molécula a solubilizar, sino de las propiedades descritas de las β -ciclodextrinas, entonces la oficina considera que se deriva evidentemente del estado de la técnica, porque para una persona del nivel medio

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del arte, puede hacer múltiples modificación de los complejos a formar, solo tomando las β -ciclodextrinas y formando el complejo con la molécula de interés, como en el presente caso una benzodiazepina, y que no es la primera vez que se solubiliza de esta manera una molécula de estas características químicas, ni es la primera vez que se forma un complejo ciclo las β -ciclodextrinas -benzodiazepina y a demás es de esperar que conserve su efecto farmacológico como en todas las anterioridades y dicha modificación no sería una solución original, porque no se aparta del camino previsto por las anteriores, por lo tanto se considera que, la presente solicitud no posee nivel inventivo.

En conclusión los requisitos de Patentabilidad de la presente solicitud están afectadas así:

No.	Documento	Relvindicaciones	Requisito que afecta
D1.	US4338453	1 a 10	Nivel inventivo

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 la 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 conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo 5° del título 1° de la Circular Única n°.10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL.

Jefe de la División de Nuevas Creaciones

31 MAR. 2005

El anterior requerimiento fue notificado por fijación en lista de fecha_____.

CONCEPTO TÉCNICO No. 419	EXAMINADOR TÉCNICO Código: 202012
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US005134127A

United States Patent [19]

Stella et al.

[11] Patent Number: 5,134,127

[45] Date of Patent: Jul. 28, 1992

[54] DERIVATIVES OF CYCLODEXTRINS
EXHIBITING ENHANCED AQUEOUS
SOLUBILITY AND THE USE THEREOF

[75] Inventors: Valentino Stella; Roger Rajewski,
both of Lawrence, Kans.

[73] Assignee: University of Kansas, Lawrence,
Kans.

[21] Appl. No.: 469,087.

[22] Filed: Jan. 23, 1990

[51] Int. Cl.³ C08B 37/16; A61K 31/71;
A61K 31/715

[52] U.S. Cl. 514/058; 536/103;
514/778; 514/964; 514/965

[58] Field of Search 536/103; 514/58, 778,
514/964, 965

[56] References Cited

U.S. PATENT DOCUMENTS

3,426,011	2/1969	Parmerter et al.	536/103
3,816,393	6/1974	Hayashi et al.	536/103
4,497,803	2/1985	Harada et al.	536/103
4,533,152	8/1985	Szejtli et al.	536/103
4,535,504	11/1985	Jones	536/103
4,582,900	4/1986	Brandt et al.	536/103
4,596,795	6/1986	Pitha	536/103

4,638,058	1/1987	Brandt et al.	536/103
4,727,064	2/1988	Pitha	536/103
4,746,734	5/1988	Tsuchiyama et al.	536/103
4,764,604	8/1988	Mueller	536/103
4,774,329	9/1988	Friedman	536/103
4,808,232	2/1989	Beasley	536/103
4,869,904	9/1989	Uekama et al.	514/58

OTHER PUBLICATIONS

Proceedings of the Fourth International Symposium on Cyclodextrins, (1988) pp. 369-382, "Cyclodextrin Derivatives for Solubilization, Stabilization, and Absorption of Drugs" B. W. Mueller et al.

Third International Symposium on Recent Advances in Drug Delivery Systems, (1987), pp. 1-12, "Amorphous Water Soluble Derivatives of Cyclodextrins: From Test Tube to Patient", Josef Pitha.

Primary Examiner—Ronald W. Griffin

Attorney, Agent, or Firm—Birch, Stewart, Kolasch & Birch

[57]

ABSTRACT

Sulfoalkyl ether cyclodextrin derivatives and their use as solubilizing agents for water insoluble drugs for oral, intranasal, or parenteral administration are disclosed.

45 Claims, 7 Drawing Sheets

DERIVATIVES OF CYCLODEXTRINS EXHIBITING ENHANCED AQUEOUS SOLUBILITY AND THE USE THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

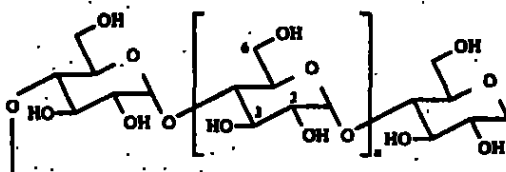
The present invention relates to cyclodextrin derivatives and to their pharmaceutical application as clathrating agents.

2. Discussion of the Background

Cyclodextrins (CD) are a group of cyclic homologous oligosaccharides that are obtained from the degradation of starch by the action of the enzyme cyclodextrin transglycosylase elaborated by the bacterium *Bacillus macerans*. Published methods exist for the production of cyclodextrin transglycosylase as well as making and isolating the cyclodextrins.

Cyclodextrins are cyclic molecules containing six or more α -D-glucopyranose units linked at the 1,4 positions by α linkages as in amylose. As a consequence of this cyclic arrangement, the molecule is characterized as having neither a reducing end group nor a non-reducing end group.

The molecule is represented below by schematic formula (1) where the hydroxyl groups are shown in the 2, 3, and 6-positions of the glucopyranose units.



Variable n may be a number from 4 to 6, or higher.

When $n=4$ the molecule is commonly known as α -cyclodextrin or cyclohexaamylose, when $n=5$ the molecule is commonly known as β -cyclodextrin or cycloheptaamylose and when $n=6$ the molecule is commonly known as γ -cyclodextrin or cyclooctaamylose. When reference is made here to "cyclodextrin", it is intended to include the foregoing forms of cyclodextrin as well as molecules where $n>6$.

It is believed that as a consequence of the cyclic arrangement and the conformation of the β -D-glucopyranose units, there is limited free rotation about the glycosidic bonds, and the cyclodextrins exist as conical shaped molecules with the primary hydroxyls situated at the small end of the cone and the secondary hydroxyls situated at the large opening to the cone. The cavity is lined by hydrogen atoms from C3 and C5 along with the glycosidic oxygen atoms resulting in a relatively lipophilic cavity but hydrophilic outer surface.

As a result of the two separate polar regions and the changes in solvent structure that occur upon complexation, cyclodextrins have the ability to form complexes with a variety of organic and inorganic molecules. The formation of cyclodextrin inclusion complexes with molecules is referred to as the host-guest phenomenon.

These unique properties of cyclodextrins have resulted in their commercial application in agriculture, water treatment, as surfactants and in drug delivery systems. The application of cyclodextrins in the pharmaceutical field has resulted in time release micro en-

capsulation, improved stability, and increased aqueous solubility of various drugs.

Cyclodextrins are known generally to improve the dissolution rate of drugs. The complexes formed are, however, also stable in aqueous solution, so that the improvement in dissolution is accompanied by an increase in the saturation solubility of the drug. Unfortunately the very β -cyclodextrin that forms the most stable complexes with most drugs has the lowest water solubility, so that drugs that are complexed with it cannot be brought into solution at therapeutic concentrations. The reason for this appears to be due to the crystalline structure of β -cyclodextrin itself.

Chemical modification of cyclodextrins is known to modulate their properties. Electroneutral cyclodextrins have been described by Parmerter et al (U.S. Pat. No. 3,453,259), and Gramera et al (U.S. Pat. No. 3,459,731). These are obtained by the condensation reaction of cyclodextrins with various epoxides or organic halides.

Other derivatives include cyclodextrins with cationic properties (Parmerter (I); U.S. Pat. No. 3,453,257), insoluble crosslinked cyclodextrins (Solms; U.S. Pat. No. 3,420,788), and cyclodextrins with anionic properties (Parmerter (II); U.S. Pat. No. 3,426,011). Among the cyclodextrin derivatives with anionic properties, carboxylic acids, phosphorus acids, phosphinous acids, phosphonic acids, phosphoric acids, thiophosphonic acids, thiophosphinic acids, and sulfonic acids (see Parmerter (II), supra), have been appended to the parent cyclodextrin.

Cyclodextrins have found applications in pharmaceutical delivery systems. As a "host" for "guest" drug molecules, these inclusion (clathrate) complexes have shown increased aqueous solubility for pharmaceuticals with intrinsically low aqueous solubility (Jones; U.S. Pat. No. 4,555,504).

This solubilization results in the improved bioavailability for some drugs. As a clathrate complex some drugs have shown improved chemical stability in aqueous solution (Harada et al; U.S. Pat. No. 4,497,803 and Hayashi et al; U.S. Pat. No. 3,816,394). In addition, cyclodextrins have proved effective in controlling the release of highly water soluble pharmaceuticals (Friedman; U.S. Pat. No. 4,774,329).

Despite this pharmaceutical utility, cyclodextrins are not without their limitations. The use of cyclodextrins in the clinical setting is limited to oral and topical dosage forms as the cyclodextrins exhibit nephrotoxicity upon entering the body unmetabolized. Since mammalian enzymes are specific for the degradation of linear starch molecules, the cyclodextrins remain largely unmetabolized and accumulate, due to their recirculation and reabsorption, in the proximal tubule cells.

Cyclodextrins and their derivatives are mostly crystalline solids and concentration in the renal tissue is followed by crystal formation causing necrotic damage to the cells. Despite forming water soluble clathrate complexes, the crystalline cyclodextrin drug complexes have been limited in their utility to sublingual administration.

Efforts have been made to inhibit crystal formation in cyclodextrin drug complexes by derivatizing parent cyclodextrins in a non-specific manner to obtain amorphous mixtures containing many cyclodextrin derivative components (cf. Pitha; U.S. Pat. Nos. 4,596,795 and 4,727,064). These mixtures prevent the crystallization processes seen with single compounds, providing a lowering of toxicity.

SUMMARY OF THE INVENTION

The present invention provides purified cyclodextrin derivatives present both as single derivatives and as mixtures of derivatives. These are obtained by heating a cyclodextrin starting material with a reagent(s) which introduces a specific anionic-type substituent, i.e., a (C₂₋₆ alkylene)-SO₃⁻ anionic substituent, onto the cyclodextrin molecule. These have been discovered to possess notably enhanced aqueous solubility and an advantageously low degree of toxicity. The more highly substituted cyclodextrin derivatives have further been found to advantageously cause essentially no membrane disruption. These derivatized cyclodextrins are useful as clathrating agents in parenteral pharmaceutical formulations and other related uses.

BRIEF DESCRIPTION OF THE FIGURES

A more complete appreciation of this invention and many of its attendant advantages will be readily obtained as the same becomes better understood by the reference to the following detailed description when considered in connection with the accompanying figures, wherein:

FIG. 1 sets out cumulative urinary cyclodextrin excretion in mice for underivatized cyclodextrin, hydroxy propyl-derivatized cyclodextrin, and two sulfoalkyl cyclodextrin derivatives of the present invention;

FIGS. 2 and 3 provide data showing that the more highly substituted alkylsulfonic acids of the present invention cause less membrane disruption, as determined by red blood cell hemolysis studies, as compared to the mono-substituted alkylsulfonic acid derivatives, with the underivatized cyclodextrin causing the most membrane disruption, and that the mono-substituted alkylsulfonic acid derivatives of the present invention cause about the same amount of membrane disruption as does the hydroxypropyl cyclodextrin derivative, as also determined by red blood cell hemolysis study;

FIGS. 4, 5 and 6 show that the association constants for the equilibrium between the sulfoalkyl cyclodextrin derivatives of the present invention and digoxin or progesterone are considerably larger than the association constant for the equilibrium between a hydroxypropyl cyclodextrin derivative and digoxin or progesterone, respectively; and

FIGS. 7, 8, 9 and 10 similarly show that with phenytoin and testosterone the sulfoalkyl cyclodextrin derivatives of the present invention possess notably greater association constants as compared to the hydroxypropyl cyclodextrin derivative.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Thus this invention provides cyclodextrin derivatives suitable for pharmaceutical use. These derivatives are suitable for use as clathrating agents with drugs to provide clathrate complexes which are useful in parenteral and other pharmaceutical formulations. Procedures for making and isolating the cyclodextrin derivatives are also provided.

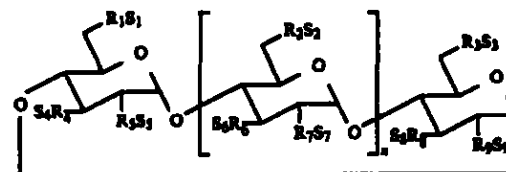
The cyclodextrin derivatives of the present invention are functionalized with (C₂₋₆alkylene)-SO₃⁻ groups, and are thus charged species. The fact that these compounds have been discovered to possess a very low level of toxicity is surprising in light of the prior art's belief that cyclodextrin derivatives must retain electroneutrality to sustain lack of toxicity (cf. Pitha,

"Amorphous Water-Soluble" "Third Int'l Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, Utah, Feb. 23-27, 1987).

The high aqueous solubility of the cyclodextrin derivatives of the present invention, and their resulting lowered nephrotoxicity, is further surprising in light of U.S. Pat. No. 4,782,064's disclosure that to maintain a high level of solubility for cyclodextrin derivatives, a mixture of derivatives should be used.

The aqueous solubility exhibited by the present sulfoalkyl cyclodextrin derivatives appears to be obtained through solvation of the sulfonic acid moieties. Thus heterogeneous mixture of the present cyclodextrin derivatives is not a requirement for the observed enhanced solvation to occur. Although a mixture of sulfoalkyl ether derivatives can be used in accordance with the present invention, such a mixture is not required for enhanced solubility.

In a preferred embodiment (1), the cyclodextrin derivatives of this invention have structures represented by formula (2):



wherein:

n is 4, 5 or 6;

R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are each, independently, O⁻ or a O-(C₂₋₆ alkylene)-SO₃⁻ group, wherein at least one of R₁ and R₂ is independently a O-(C₂₋₆ alkylene)-SO₃⁻ group, preferably a O-(CH₂)_m-SO₃⁻ group, wherein m is 2 to 6, preferably 2 to 4, (e.g. OCH₂CH₂CH₂CH₂SO₃⁻ or OCH₂CH₂CH₂CH₂SO₃⁻); and

S₁, S₂, S₃, S₄, S₅, S₆, S₇, S₈ and S₉ are each, independently, a pharmaceutically acceptable cation which includes, for example, H⁺, alkali metals (e.g. Li⁺, Na⁺, K⁺), alkaline earth metals (e.g. Ca⁺², Mg⁺²), ammonium ions and amines cations such as the cations C₁₋₆ alkylamines, piperidine, pyrazine, C₁₋₆ alkanolamine and C₄₋₆ cycloalkanolamine.

In another preferred embodiment (2):

R₁ is a O-(C₂₋₆ alkylene)-SO₃⁻ group, preferably a O-(CH₂)_m-SO₃⁻ group, (e.g. OCH₂CH₂CH₂CH₂SO₃⁻ or OCH₂CH₂CH₂CH₂SO₃⁻);

R₂ to R₉ are O⁻;

S₁ to S₉ are as defined in embodiment (1) supra.

In another preferred embodiment (3):

R₁, R₂ and R₃ are each, independently, a O-(C₂₋₆ alkylene)-SO₃⁻ group, preferably a O-(CH₂)_m-SO₃⁻ group, (e.g. OCH₂CH₂CH₂CH₂SO₃⁻ or OCH₂CH₂CH₂CH₂SO₃⁻);

R₄ to R₉ are O⁻; and

S₁ to S₉ are as defined in embodiment (1) supra

In another preferred embodiment (4):

R₁ to R₃ are as defined in embodiments (2) or (3); supra;

at least one of R₄, R₅ and R₆ is a O-C₂₋₆ alkylene)-SO₃⁻ group, preferably a O-(CH₂)_m-SO₃⁻ group (e.g. OCH₂CH₂CH₂CH₂SO₃⁻ or OCH₂CH₂CH₂CH₂SO₃⁻).

R₇, R₈ and R₉ are O⁻; and

S₁ to S₉ are as defined in embodiment (1) supra.

In another preferred embodiment (6):

R₁, R₂, R₃, R₄, R₅ and R₆ are each, independently, a O-(C₂₋₆-alkylene)-SO₃⁻ group, preferably a O-(CH₂)_n-SO₃⁻ group (eg. OCH₂CH₂CH₂SO₃⁻ or OCH₂CH₂CH₂CH₂SO₃⁻);

R₇, R₈ and R₉ are O⁻; and

S₁ to S₉ are as defined in embodiment (1) supra.

The terms "alkylene" and "alkyl" in this text (e.g., in the O-(C₂₋₆-alkylene)SO₃⁻ group or in the alkylamines) include both linear and branched, saturated and unsaturated (i.e., containing one double bond) divalent alkylene groups and monovalent alkyl groups, respectively. The term "alkanol" in this text likewise includes both linear and branched, saturated and unsaturated alkyl components of the alkanol groups, in which the hydroxyl groups may be situated at any position on the alkyl moiety. The term "cycloalkanol" includes unsubstituted or substituted (e.g., by methyl or ethyl) cyclic alcohols.

The present invention provides compositions containing a mixture of cyclodextrin derivatives having the structure set out in formula (2), where the composition overall contains on the average at least 1 and up to 3n+6 alkylsulfonic acid moieties per cyclodextrin molecule. The present invention also provides compositions containing essentially only one single type of cyclodextrin derivative.

The present cyclodextrin derivatives are either substituted at least at one of the primary hydroxyl group (i.e., at least one of R₁ to R₃ is a substituent), or they are substituted at both the primary hydroxyl group and at the 3-position hydroxyl group (i.e., both at least one of R₁ to R₃ and at least one of R₄, R₅ and R₆ are a substituent). Substitution at the 2-position hydroxyl group, while theoretically possible, on the basis of the inventors' studies, does not appear to appear to be substantial in the products of the invention.

The cyclodextrin derivatives of the present invention are obtained (as discussed below) as purified compositions, i.e., compositions containing at least 95 wt. % of cyclodextrin derivative(s) with the substitution occurring at least on the primary hydroxyl group of the cyclodextrin molecule (i.e. R₁, R₂ or R₃ of formula (2)), as determined by 300 MHz ¹H NMR). In a preferred embodiment, purified compositions containing at least 98 wt. % cyclodextrin derivative(s) can be obtained.

This is to be contrasted with the U.S. Pat. No. 3,426,011 disclosure which reports obtaining only reaction products of the reaction of a cyclodextrin with a sulfone reactant. These reaction products contain considerable quantities of unsubstituted cyclodextrin starting material.

In all of the compositions of the invention unreacted cyclodextrin has been substantially removed, with the remaining impurities (i.e., <5 wt. % of composition) being inconsequential to the performance of the cyclodextrin derivative-containing composition.

The more highly substituted alkyl sulfonic acid cyclodextrin derivatives of the present invention have been discovered to possess, in addition to notably enhanced solubility characteristics and low toxicity, the advantageous property of causing less membrane disruption. In red blood cell hemolysis studies, the more highly substituted cyclodextrin derivatives demonstrated negligible membrane disruption. The mono-substituted cyclodextrin derivatives caused about the same

amount of membrane disruption as the hydroxy propyl derivative.

Preparation of the Cyclodextrin (CD) Derivatives

The cyclodextrin derivatives described may be generally prepared by dissolving the cyclodextrin in aqueous base at an appropriate temperature, e.g., 70° to 80° C., at the highest concentration possible. For example, to prepare the cyclodextrin derivatives of embodiment (4), an amount of an appropriate alkyl sulfone, corresponding to the number of moles of primary CD hydroxyl group present, is added with vigorous stirring to ensure maximal contact of the heterogeneous phase.

To prepare the cyclodextrin derivatives of the embodiment (2) a molar amount of the alkyl sulfone, corresponding to the number of moles of CD used, is used. As would be readily determinable by one of skill in this art, to prepare cyclodextrin derivatives of embodiment (1), which encompasses both cyclodextrin derivatives embodiments (4) and (2), an amount of alkyl sulfone between that stated above is used. Other cyclodextrin derivatives provided by the present invention are prepared *Mutatis Mutandis*.

The mixtures are allowed to react until one phase results which is indicative of depletion of the alkyl sulfone. The reaction mixture is diluted with an equal volume of water and neutralized with an acid such as hydrochloric acid. The solution is then dialyzed to remove impurities followed by concentration of the solution by ultrafiltration.

The concentrated solution is then subjected to ion-exchange chromatography to remove unreacted cyclodextrin, and then freeze-dried to yield the desired product.

The CD used in this invention may be any CD obtained by known methods, e.g., by the action of cyclodextrin-glucanotransferase (CGTase, E.C., 2.4.1.19.) upon starch. Thus CD herein means α-CD in which six glucose units are linked together through α-1,4 bond, β-CD in which seven glucose units are linked together, or γ-CD in which eight glucose units are linked together, or a mixture thereof. Of these, use of β-CD is most preferred for production of partially derivatized products of broad utility.

As noted above and depending on the cyclodextrin derivative sought, the amount of alkyl sulfone used as the derivatizing agent should be not more than about one molar equivalent, based on the number of primary hydroxyl groups present in the CD, although the optimum amount may be somewhat dependent on the reactant concentration. Lithium hydroxide, sodium hydroxide and potassium hydroxide may be used as the accelerator. Of these, sodium hydroxide is preferable because of its low cost. Its amount must be more than about 30 molar equivalents, and should preferably be in the range of 80 to 200 molar equivalents, with the reactant concentration being set at a level higher than 10% (wt/wt), preferably in the range of 40 to 60% (wt/wt).

Any solvent which is substantially inert to the partial alkylation may be used as reaction medium. Typical examples are water, DMF, DMSO, and mixtures thereof, but use of water alone is most preferred for ease of after-treatment.

The type and concentration of alkylsulfone and alkali are not critical to the reaction. However, the reaction is normally carried out with stirring at 10° to 80° C. for one hour, preferably at 20° to 50° C. for 5 to 20 hours.

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Techniques commonly used in this field may be employed to isolate and purify the objective compounds from reaction mixtures. These include extraction with organic solvents, dialysis, adsorption chromatography with activated charcoal, silica gel, alumina and other adsorbents, chromatography using, as carrier, cross-linked dextrin, styrene/divinylbenzene copolymers and other cross-linked polymers, and combinations thereof.

Preparation of the Clathrate Complexes

The clathrate complexes of the invention may be prepared by any method known in the art for the preparation of complexes of cyclodextrins.

For example, to prepare the clathrate complexes, a cyclodextrin derivative dissolved in water or in an organic solvent miscible with water may be added to a physiologically active compound (drug) dissolved in an organic solvent which is miscible with water. After the mixture is heated, the desired product is obtained by concentrating the mixture under reduced pressure or leaving it to be cooled. In this case, the mixing ratio of organic solvent with water may be suitably varied according to the solubilities of the starting materials and products.

Examples of Drugs which may be complexed with the cyclodextrin derivatives include but are not limited to: diphenylhydantoin, adiphenine, allobarbitol, aminobenzoic acid, amobarbital, ampicillin, anethole, aspirin, azopropazone, azulene, barbituric acid, beclomethasone, beclomethasone dipropionate, bencyclane, benzaldehyde, benzocaine, benzodiazepines, benzothiazide, betamethasone, betamethasone 17-valerate, bromobenzoic acid, bromoisovalerylurea, butyl-p-aminobenzoate, chloralhydrate, chlorambucil, chloramphenicol, chlorobenzoic acid, chlorpromazine, cinnamic acid, clofibrate, coenzyme A, cortisone, cortisone acetate, cyclobarbitol, cyclohexyl anthranilate, deoxycholic acid, dexanmethasone, dexanmethasone acetate, diazepam, digitoxin, digoxin, estradiol, flufenamic acid, flucinolone acetate, 5-fluorouracil, flurbiprofen, griseofulvin, guaiazulene, hydrocortisone, hydrocortisone acetate, ibuprofen, iodoquin, landomethacin, iodine, ketoprofen, lankacidin-group antibiotics, mefenamic acid, menadiolone, mephobarbital, methobarbital, methicillin, metronidazole, mitomycin, nitrazepam (nitroglycerin), nitrosourea, paramethasone, penicillin, pentobarbital, phenobarbital, phenobarbitone, phenylbutyric acid, phenylvaleric acid, phenytoin, prednisolone, prednisolone acetate, progesterone, propylparaben, procicillarin, prostaglandin A series, prostaglandin B series, prostaglandin E series, prostaglandin F series, quinalone anti microbials reserpine, spironolactone, sulfacetamide sodium, sulfonamide, testosterone, thalidomide, thiamine dilaurylsulphate, thiamphenicolpalmitate, thio-pental, trisacrinolone, vitamin A, vitamin D3, vitamin E, vitamin K3, and warfarin.

The drug may be dissolved in water or an organic solvent (either miscible or immiscible with water). Convenient solvents include for example diethylether, tetrahydrofuran, dioxane, acetone, dimethylsulfoxide, dimethylformamide and lower aliphatic alcohols. Preferably the drug is dissolved in either water or a mixture of water and a water-miscible solvent such as methanol or ethanol. The drug may also be suspended in water.

After equilibrium is reached, the complex may be isolated by any suitable technique for example lyophilization, evaporation of the solvent, precipitation, low temperature crystallization, or spray-drying. Cyclodextrin

inclusion complexes may also be produced by physically grinding or kneading the cyclodextrin and the guest molecule with or without a small amount of solvent.

The ratio of cyclodextrin derivative to drug used to prepare the clathrate complexes of the invention may be any convenient ratio but conveniently the cyclodextrin derivative is used in a molar excess.

The benefits derived from the invention may be obtained by having the molar ratio of cyclodextrin derivative to drug in the range of 10:1 to 1:10 preferably 2:1 to 5:1 for example 3:1 and by using the methods and ratios described above. Complexes are conveniently obtained containing up to 20% w/w of the drug. However in view of the low doses of the drug normally administered and the difficulty of preparing homogenous mixtures of active ingredient and excipients it may be desirable to prepare the complex with an excess of the cyclodextrin derivative present, for example complexes containing in the order of 0.1 to 10% by weight of the drug, particularly in the range 0.5 to 0.2% by weight.

The clathrate complexes of the invention provide a more convenient way of administering the drugs, the cyclodextrin acting merely as a solubilizing agent without altering the therapeutic behavior of the drug in any way.

Composition Containing the Clathrate Complexes of the Invention

The invention thus also provides an inclusion complex as defined herein for use in human or veterinary medicine. The complex, for use as a pharmaceutical, may be presented as a pharmaceutical formulation.

The invention therefore provides in a further aspect a pharmaceutical formulation comprising an inclusion complex of a drug with a cyclodextrin derivative together with a pharmaceutically acceptable carrier therefor and optionally other therapeutic and/or prophylactic ingredients. The carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof. Suitably the pharmaceutical formulation will be in unit dosage form. Each unit dose will conveniently contain that amount of drug normally incorporated into a unit dose of such drug in the absence of a cyclodextrin.

The pharmaceutical formulations may be any formulation in which the inclusion complexes may be administered and include those suitable for oral, intranasal, intraocular or parenteral (including intramuscular and intravenous) administration. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration wherein the carrier is a solid are most preferably presented as unit dose formulations such as boluses, capsules, cachets or tablets each containing a predetermined amount of the active ingredient. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules optionally mixed with a binder, lubri-

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cant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding an inert liquid diluent. Tablets may be optionally coated and, if uncoated, may optionally be scored. Capsules may be prepared by filling the active compound, either alone or in admixture with one or more accessory ingredients, into the capsule cases and then sealing them in the usual manner. Cachets are analogous to capsules wherein the active ingredient together with any accessory ingredient(s) is sealed in a rice paper envelope.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example maize starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

The present invention also provides the complexes of the present invention in pharmaceutical formulations exhibiting sustained release of a drug. Pharmaceutical formulations exhibiting sustained release of a drug are generally known. Such formulations include devices made of inert polymers or biodegradable polyacids in which the active ingredient (the present complex), is either dispersed, covalently linked via labile bonds, or stored as a reservoir between polymer membranes. Sustained release is achieved through diffusion of the active ingredient through the polymer matrix or hydrolysis of any covalent linkages present.

Sustained release may also be presented by delivery of the active ingredient via osmotic pumps. Osmotic pumps consist of a reservoir of solution or suspension of active ingredient (i.e., the present complex) surrounded by a semipermeable membrane containing a drug portal. As water penetrates through the semipermeable membrane into the complex reservoir, the complex solution is pushed through the portal and released.

The cyclodextrin derivatives of the invention act as drug solubilizing agents in these systems. The present cyclodextrin derivatives can also act as osmotic driving agents providing potential for the influx of water in such systems.

Pharmaceutical formulations suitable for oral administration wherein the carrier is liquid may conveniently be presented as a solution in an aqueous liquid or a non-aqueous liquid, i.e., as an oil-in-water or water-in-oil liquid emulsion. Pharmaceutical formulations suitable for parenteral administration are conveniently presented in unit dose or multi-dose containers which are sealed after introduction of the formulation unit required for use.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example arachis oil, peanut oil, liquid paraffin or olive oil.

It should be understood that in addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

For these purposes the compounds of the present invention may be administered orally, topically, intranasally, intraocularly, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example of polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxy benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oil suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may

be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soya bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan mono-oleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc. containing the active ingredient are employed.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 1.0 to 750 mg. of active agent compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 weight percent of the total composition. Unit dosage forms will generally contain between from about 1 to about 500 mg. of active ingredient.

Administration of the Clathrate Complexes to a Patient

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Pharmaceutical formulations containing inclusion complexes may be administered at dosage levels and dosage intervals required to achieve the desired pharmacologic response normally associated with the drug and the disease state in absence of the cyclodextrin.

The other features of the invention will become apparent in the course of the following descriptions of

exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

The hydroxypropyl cyclodextrin derivative used in the experiments reported below was purchased from Pharmatec, Inc., Alachua, Fla.

Preparation of the Cyclodextrin Derivative of the Invention

EXAMPLE 1

Mono-sulfobutyl ether of β -cyclodextrin

In a 100 mL round-bottom flask, 10 grams β -cyclodextrin (8.81×10^{-3} mole) was added with stirring to an aqueous solution composed of 30 mL water and 5.0 grams sodium hydroxide maintained at 70° C. To this solution 1.8 mL (2.40 gm, 1.76×10^{-3} mole) butane sultone was slowly added with vigorous stirring to insure maximal contact of the heterogeneous phases.

After a single phase was observed indicating depletion of the alkyl sultone, the solution was cooled to room temperature and diluted with 20 mL water. The resulting solution was neutralized with 1 N hydrochloric acid and dialyzed against 3×700 mL water to remove salts and hydroxyalkyl sulfonic acids formed as side products.

The diastylate was concentrated by ultrafiltration and placed on an ion-exchange column composed of 50 grams A-25 DEAE-Sephadex packed in a 1.25 inch i.d. glass column. Unreacted β -cyclodextrin was removed by elution with distilled water. The mono-substituted sulfobutyl ether of β -cyclodextrin was isolated by elution with 0.1 N sodium hydroxide. The effluent fraction containing the mono-substituted derivative was ultrafiltered to remove any residual salts. The pH of the retentate was adjusted to neutrality and lyophilized to obtain 2.17 grams of the monosubstituted sulfobutyl ether of β -cyclodextrin as a white amorphous solid. Elemental analysis of the product showed a carbon to sulfur ratio of 13.7 which corresponds to Ca. 1.2 substitutions per molecule.

EXAMPLE 2

Mono-sulfopropyl ether of β -cyclodextrin

The processes of Example 1 were repeated substituting 1.54 mL (2.15 grams, 1.76×10^{-3} mole) propane sultone for butane sultone to yield 1.97 grams mono-sulfobutyl ether of β -cyclodextrin as a white amorphous solid. Elemental analysis of the product showed a carbon to sulfur ratio of 12.1 which corresponds to Ca. 1.4 substitutions per molecule.

Example 3

Sulfobutyl ethers of β -cyclodextrin

In a 50 mL round-bottom flask, 5 grams β -cyclodextrin (4.41×10^{-3} mole) was added with stirring to an aqueous solution composed of 10 mL water and 2.0 grams sodium hydroxide maintained at 70° C. To this solution 4.5 mL (6.0 gm, 4.41×10^{-3} mole) butane solution 4.5 mL (6.0 gm, 4.41×10^{-3} mole) sultone was slowly added with vigorous stirring to insure maximal contact of the heterogeneous phases. After a single phase was observed indicating depletion of the alkyl sultone, the solution was cooled to room temperature and diluted with 20 mL water. The resulting solution was neutralized with

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1 N hydrochloric acid and dialyzed against 3×700 mL water to remove salts and hydroxyalkyl sulfonic acids formed as side products. The dialysate was concentrated by ultrafiltration and the pH of the retentate was adjusted to neutrality and lyophilized to obtain the sulfobutyl ether of β -cyclodextrin as a white amorphous solid. Elemental analysis of the product showed a carbon to sulfur ratio of 3.73 which corresponds to Ca. 7 substitutions per molecule. Thin layer chromatography of the product (2-butanone:methanol:water 4:4:2) shows the absence of unreacted β -cyclodextrin.

Example 4

Additional sulfoalkylethers of cyclodextrin

The processes of Example 3 were varied with regard to reactants and molar ratios to obtain cyclodextrin derivatives with varying degrees of substitution. Representative results follow

Cyclodextrin	Moles	Saltone	Moles	Carbon/Sulfur Ratio	Substitutions
β -	4.41×10^{-3}	propane	4.4×10^{-3}	3.28	7.2
β -	4.41×10^{-3}	propane	2.2×10^{-3}	5.53	3.6
β -	4.41×10^{-3}	propane	2.2×10^{-3}	4.83	4.7
γ -	1.54×10^{-3}	propane	7.71×10^{-3}		3.5
γ -	1.54×10^{-3}	butane	7.71×10^{-3}		3.2

*Substitution determined by peak areas from $^1\text{H-NMR}$ spectra.

Cumulative Urinary Cyclodextrin Excretion

The data provided in the group set out in FIG 1 indicates that although the sulfoalkyl cyclodextrin derivatives of the invention as well as the hydroxypropyl derivative are excreted faster and to a greater extent in mice than the parent compound, the present derivatives are excreted fastest. FIG 1 provides data for the underivatized cyclodextrin compound, the hydroxypropyl derivative, the sulfobutyl derivative of the invention, and the sulfopropyl derivative of the invention.

Acute Parenteral Toxicity

The sulfoalkyl cyclodextrin derivatives of the invention exhibited no observable toxic effects in male mice over a 30 day period following intraperitoneal of 5.49×10^{-3} mol/Kg.

This dose is equivalent to 7.1 gm/Kg for the mono-sulfoalkyl derivatives, 12.3 gm/Kg for the sulfobutyl derivative w/7 degrees of substitution, and 11.8 gm/Kg for the sulfopropyl derivative w/7 degrees of substitution.

Plasma Urea Nitrogen

Plasma urea nitrogen levels are an indicator of kidney function with higher levels indicating renal damage. The data in Table 1 indicates that the sulfoalkyl cyclodextrin derivatives of the invention do not cause increased plasma urea nitrogen levels in mice as compared to the underivatized parent compound (control). There is however no statistical difference between our derivatives and the hydroxypropyl derivative.

TABLE 1

Plasma Urea Nitrogen ⁽¹⁾		
	Sample time (hrs)	PUN + S.D. (mg/dL) ⁽²⁾
Control (normal saline)	24	15.88 $\pm$ 1.21
β -Cyclodextrin	24	160.10 $\pm$ 26.16
Molecular TM (hydroxypropyl)	24	15.43 $\pm$ 1.50

TABLE 1-continued

Plasma Urea Nitrogen ⁽¹⁾		
	Sample time (hrs)	PUN + S.D. (mg/dL) ⁽²⁾
derivative)		
Sulfopropyl ether of β -Cyclodextrin (3.6 substitution per CD molecule)	24	15.27 $\pm$ 0.71
Sulfobutyl ether of β -Cyclodextrin (4.7 substitution per CD molecule)	24	14.42 $\pm$ 0.46

⁽¹⁾ISOIMA Urea Nitrogen Procedure No. 640-A
⁽²⁾ $\sigma_{10} = 4$

Hemolysis of Red Blood Cells

As can be seen from the data in FIGS. 2 and 3 the more highly substituted alkylsulfonic acid derivatives of the invention caused less membrane disruption as indicated by the percent hemolysis than the mono substituted derivatives. The mono substituted derivatives caused about the same amount of membrane disruption as the hydroxypropyl derivative.

Phase Solubility Behavior

As can be seen from Table 2 below and the data provided in FIGS 4a and 4b the association constants for the equilibrium between the sulfobutyl derivatives of the invention and Digoxin are 5 times larger than that of the hydroxypropyl derivative.

TABLE 2

DIOXIN ASSOCIATION CONSTANTS	
	$K_{1:1}(M^{-1})$
β -Cyclodextrin	2.82×10^4
Molecular (hydroxypropyl- β -CD)	4.90×10^3
Sulfobutyl ether of β -cyclodextrin	
(1 sub) ⁽¹⁾	2.76×10^4
(4.8 subs)	1.71×10^5
(7 subs)	6.88×10^5
Sulfopropyl ether of β -cyclodextrin	
(1 sub)	2.74×10^4
(3.6 subs)	1.41×10^5
(7 subs)	5.29×10^5

⁽¹⁾No. of substitutions per CD molecule

TABLE 3

PROGESTERONE ASSOCIATION CONSTANTS	
	$K_{1:1}(M^{-1})$
β -cyclodextrin	—
Molecular (hydroxypropyl β -CD)	1.12×10^4
Sulfobutyl ether β -cyclodextrin	
(1 sub) ⁽¹⁾	1.72×10^4
(4.7 subs)	1.57×10^5
(7 subs)	1.83×10^5
Sulfopropyl ether β -cyclodextrin	
(1 sub)	1.66×10^4
(3.6 subs)	1.19×10^5
(7 subs)	7.44×10^5

⁽¹⁾No. of substitutions per CD molecule

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It should be noted that the x axis for the graphs of FIGS. 4a and 4b have a maximum of ~1.8% w/w cyclodextrin. If the relative solubilizing ability of the present derivatives is considered relative to that for the hydroxypropyl derivative (at 50% solutions as is done in U.S. Pat. No. 4,727,064, Table 1) the apparent solubility of digoxin is ~216 mg/mL for the present sulfoethyl derivatives as compared to ~80 mg/mL for the hydroxypropyl derivative. The value of 45.0 mg/mL reported in U.S. Pat. No. 4,727,064 was for a hydroxypropyl derivative with a different degree of substitution than the hydroxypropyl derivative used herein for comparison.

Similar results can be seen for progesterone (see Table 3 and FIGS. 5 and 6), phenytoin (see Table 4 and FIGS. 7 and 8), and testosterone (see Table 5 and FIGS. 9 and 10).

TABLE 5

TESTOSTERONE ASSOCIATION CONSTANTS	
	$K_{1:1}(M^{-1})$
β -cyclodextrin	1.78×10^4
Molecular (hydroxypropyl β -CD)	1.14×10^4
(1 sub) ⁽¹⁾	1.44×10^4
<u>Sulfoethyl ether β-cyclodextrin</u>	
(4.7 subs)	1.82×10^4
(7 subs)	2.25×10^4
<u>Sulfopropyl ether β-cyclodextrin</u>	
(1 sub)	1.87×10^4
(3.6 subs)	1.43×10^4
(7 subs)	9.63×10^3

⁽¹⁾No. of substituents per CD molecule

TABLE 4

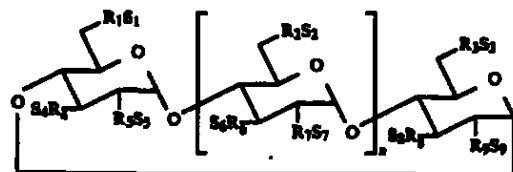
PHENYTOIN ASSOCIATION CONSTANTS	
	$K_{1:1}(M^{-1})$
β -cyclodextrin	1.51×10^3
Molecular (hydroxypropyl β -CD)	1.07×10^3
(1 sub) ⁽¹⁾	1.22×10^3
<u>Sulfoethyl ether β-cyclodextrin</u>	
(4.7 subs)	1.26×10^3
(7 subs)	7.56×10^2
<u>Sulfopropyl ether β-cyclodextrin</u>	
(1 sub)	1.03×10^3
(3.6 subs)	1.31×10^3
(7 subs)	8.24×10^2

⁽¹⁾No. of substituents per CD molecule

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

What is claimed as new and desired to be secured by Letters Patent of the United States is:

1. A purified cyclodextrin derivative composition which comprises a cyclodextrin derivative of formula



wherein:
n is 4, 5 or 6;

R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are each, independently, O- or a O-(C₂₋₆ alkylene)-SO₃⁻ group, and at least one of R₁ and R₂ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and

S₁, S₂, S₃, S₄, S₅, S₆, S₇, S₈ and S₉ are each, independently, a pharmaceutically acceptable cation, and wherein said composition shows an absence of underivatized cyclodextrin as measured by thin-layer chromatography.

2. The composition of claim 1, wherein R₁, R₂ and R₃ are each, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group.

3. The composition of claim 1, wherein at least one of R₁, R₂ and R₃ is, independently, a O-(CH₂)_m-SO₃⁻ group, wherein m is 2, 3, 4, 5 or 6.

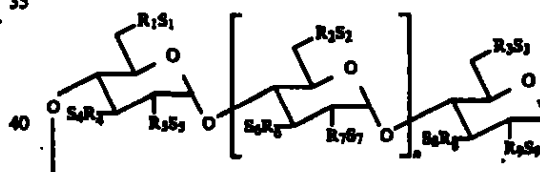
4. The composition of claim 1, wherein R₁, R₂ and R₃ are each, independently, a O-(CH₂)_m-SO₃⁻ group, wherein m is 3 or 4.

5. The composition of claim 1, wherein: at least one of R₄, R₅ and R₆ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and R₇, R₈ and R₉ are all O-.

6. The composition of claim 2, wherein at least one of R₄, R₅ and R₆ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and R₇, R₈ and R₉ are all O-.

7. The composition of claim 2, wherein: R₄, R₅ and R₆ are each a O-(C₂₋₆ alkylene)-SO₃⁻ group; and R₇, R₈ and R₉ are all O-.

8. A composition comprising a drug complexed to a cyclodextrin derivative of formula (2):



wherein:

n = 4, 5 or 6;

R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are each at least one of R₁ and R₂ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and

S₁ to S₉ are each, independently, a pharmaceutically acceptable cation; wherein said composition contains not more than 5 wt. % of underivatized cyclodextrin.

9. The composition of claim 8, wherein R₁, R₂ and R₃ are each, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group.

10. The composition of claim 8, wherein: at least one of R₄, R₅ and R₆ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and R₇, R₈ and R₉ are all O-.

11. The composition of claim 9, wherein: at least one of R₄, R₅ and R₆ is, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group; and R₇, R₈ and R₉ are all O-.

12. The composition of claim 11, wherein R₄, R₅ and R₆ are each, independently, said O-(C₂₋₆ alkylene)-SO₃⁻ group.

13. The composition of claim 8, wherein said drug is one member selected from the group consisting of amo-

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 0-2434

EDICTO No. 10065

LA SECRETARÍA GENERAL AD-HOC HACE SABER:

Que esta Superintendencia profirió Resolución No. 17968 de 27-JUL-2005. Que el contenido de la parte resolutive es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

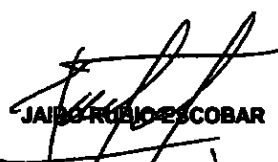
ARTICULO PRIMERO: Negar el privilegio de patente de invención para la solicitud correspondiente a "COMPLEJO DE 1,4-BENZODIAZEPINAS SUSTITUIDAS Y SULFOBUTILETER-7-CICLODEXTRINA O 2-HIDROXIPROPIL-8-CICLODEXTRINA".

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor Héctor Gómez Mejía, o a quien haga sus veces, en su calidad de apoderado de Bristol-Myers Squibb Company., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los

27 JUL 2005

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,


JAIME RUBIO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc 19-AGO-2005  Este edicto se desfija hoy 01-SEP-2005 a las cinco (5:00 p.m.) 
Secretaría General Ad-Hoc
EDICTO No. 10065