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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 02-108566- -00007-0000

Fecha 2007-05-10 17 07 35 Dep 2020 NUECREACIONE
Tra 11 PATENTEIYII Eve 379 FASENACIONALII
Act 444 RESPUESTAREQUE Folios 22

Ref: Expediente No. 02.108.566

Solicitud de patente de invención titulada: "COMPUESTOS CON UN GRUPO
SULFONAMIDA Y LAS COMPOSICIONES QUE CONTIENEN ESTOS
COMPUESTOS."

Solicitante: SCHERING AKTIENGESELLSHAFT.

RESPUESTA AL OFICIO No. 2373 NOTIFICADO POR FIJACIÓN EN LISTA

No. 54, DE FECHA 16 DE FEBRERO DE 2007.

(REQUERIMIENTO ARTÍCULO 46)

J. IAN RAISBECK, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 46 de la Decisión 486:

1. Presento copia completa de los siguientes documentos exigidos por el Examinador en el examen de búsqueda de anterioridades, según lo previsto en el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina:

1)

BARTH A ET AL: "INFLUENCE OF SUBCHRONIC
ADMINISTRATION OF OESTRADIOL,
ETHINYLOESTRADIOL AND OESTRADIOL
SULPHAMATE ON BILE FLOW, BILE
ACIDEXCRETION, AND LIVER AND BILIARY
GLUTATHIONE STATUS IN RATS"
ARCHIVES OF TOXICOLOGY, SPRINGER VERLAG,
DE,
vol. 71, no. 7, 1997, pages 443-449,
XP000986430
ISSN: 0340-5761

2)

ELGER W ET AL: "SULFAMATES OF VARIOUS
ESTROGENS ARE PRODRUGS WITH INCREASED
SYSTEMIC AND REDUCED HEPATIC ESTROGENICITY
AT ORAL APPLICATION"
JOURNAL OF STEROID BIOCHEMISTRY AND
MOLECULAR BIOLOGY, ELSEVIER SCIENCE LTD.,
OXFORD, GB,
vol. 55, no. 3/4, 1995, pages 395--403,
XP000618892
ISSN: 0960-0760

3)

MARYANOFF B E ET AL: "ANTICONVULSANT
SUGAR SULFAMATES. POTENT CYCLIC SULFATE
AND CYCLIC SULFITE ANALOGUES OF
TOPIRAMATE"
BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,
OXFORD, GB,
vol. 3, no. 12, 1993, pages 2653-2656,
XP000914306
ISSN: 0960-894X

Atentamente,



J. IAN RAISBECK
C.C. 79'376.996 de Bogotá D.C.
T.P. . 135.799 del C. S. de la J.

Anexo: - Artículos mencionados.

Received: 25 October 1996 / Accepted: 27 February 1997

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Female ovariectomized Mol: Wist rats were treated orally once daily for 7 days with different doses of the oestrogens. At 24 h after the last oestrogen administration, bile flow, bile acid excretion, and biliary and hepatic glutathione content were measured to determine whether (1) reduced canalicular glutathione transport contributes to the pathogenesis of cholestasis, and (2) the strong oestrogenic substance J95 has lower cholestatic side-effects than E and EE.

Adult female Wistar rats (strain, Mol: Wist; body wt. 180–200 g) were purchased from the Moellegaard breeding centre, Schön-

walde, Germany. The animals were kept in Makrolone cages type M4 in air conditioned rooms, kept at a temperature of $22 \pm 2^\circ\text{C}$ and humidity of 50–60%. Artificial light was used for 12 h daily. The animals were fed a standard pellet food (Altromin 1316) and had free access to tap water.

Experimental design

Two weeks before the first treatment, ovariectomy was performed under ether anaesthesia. At the same time the animals were allocated to treatment groups ($n = 8$) by randomization. The animals were treated orally with doses of EE (mol. wt. 296.4) and J995 (mol. wt. 351.4) molar equivalent to steroid moiety of 0.01, 0.1, 1 or 10 mg/kg E (mol. wt. 272.4), suspended in 2 ml/kg myrj [85 mg polyoxyethylene(50)-stearate in 100 ml saline] once daily over 7 days. The suspension was stabilized in an ultrasonic bath for 30 min at 60°C , during which formulation zirconium beads were added. Ovariectomized controls (cast, $n = 29$ –30) received the vehicle without the oestrogens; additionally 7 rats (C) without ovariectomy and without any steroid treatment were used to analyse the effects of castration.

At 24 h after the last oestrogen administration, eight rats per day (two vehicle treated controls, two E, two EE and two J995 treated rats) were anaesthetized by i.m. administration of 75 mg/kg body wt. ketamine (Ursotamin; Serumwerk, Bernburg, Germany) and 12 mg/kg body wt. xylazine (Rompun; Serumwerk, Bernburg, Germany) in 1 ml saline. The body temperature of the rats was maintained at 37°C by means of a heating table. The common bile duct was cannulated in its proximal third by stainless-steel needles of 0.8–0.9 mm in diameter. After an adaptation period of 5 min, bile was collected in pre-weighed tubes for the first 30 min, measured gravimetrically and used for measurement of bile acids. For a further 30 min, bile collected in pre-weighed tubes containing 0.1 ml perchloric acid (0.25 M) was used for estimation of total glutathione (tGSH). Perchloric acid is able to prevent autooxidation and degradation of reduced glutathione (GSH; Kretzschmar et al. 1989).

After measuring the bile/acid mixture gravimetrically, the samples were diluted with perchloric acid (0.25 M) to a final acid/bile ratio of 1:1 (v/v) and centrifuged. The acid supernatants were frozen and stored at -80°C . Bile flow was calculated from both of the periods of bile sampling. Following bile collection the rats were decapitated, the livers and uteri were excised and weighed. Portions of the liver were immediately processed for glutathione measurement; further biological material was stored at -80°C until required for analysis. The studies were revised by the official ethical committee for animal experiments and approved by the government of Thuringia.

Analytical procedures

Total bile acids in the bile were estimated using 3 α -hydroxysteroid dehydrogenase (Sigma Chemical Co., St. Louis Mo., USA) as described by Paumgartner et al. 1971. For tGSH analysis in bile the acid supernatants were neutralized with 0.25 M KOH. GSH and oxidized glutathione (GSSG) were measured as tGSH enzymatically using glutathione reductase (Sigma) according to Adams et al. (1983). Liver GSH concentration was estimated as described by Ellman (1959) and liver GSSG concentration according to Hissin and Hilf (1976).

Statistics

Data given are of mean $\pm$ SEM for $n = 7$ –8 (oestrogen treated rats), $n = 6$ –7 (C, non-ovariectomized controls), $n = 29$ –30 (cast, ovariectomized and vehicle treated controls). Student's *t*-test was used for statistical analysis, the 0.05 level of probability was accepted as the criterion of significance.

Results

Influence of castration

Using uterus weight as marker of oestrogenic activity, castration of female rats between the 9th and 10th weeks of age gave a lower uterus weight: i.e. one-third of that of normal females (Fig. 1). Moreover, ovariectomy enhanced body weight (C, 0.199 ± 0.004 kg; cast, 0.234 ± 0.004 kg) while bile flow, bile acid and biliary tGSH excretion, as well as liver GSH concentration were decreased significantly after 2 weeks in comparison to non-ovariectomized rats of the same age (Figs. 2–4, Table 1). Weight of the livers did not change during this experimental period.

Influence of oestrogens on body, liver and uterus weights

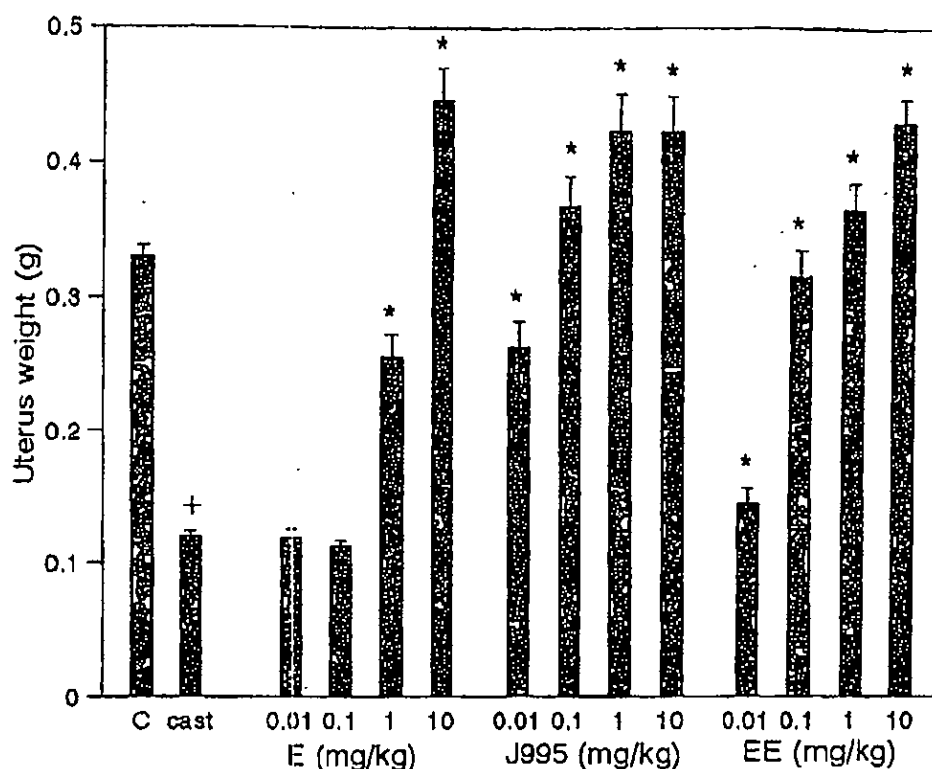
Body weight in castrated females was normalized after 7 days treatment with the highest dose of oestrogen used; the dose of 1 mg/kg oestradiol applied over 7 days was found to enhance uterus weight significantly (Fig. 1) although there was no change in liver weight. The high oestrogenic activity of J995 was demonstrated by the enhancement of uterus weight after 7 days with the lowest dose of 0.01 mg/kg (Fig. 1). Body weight was diminished after 0.1 mg/kg J995 and at higher doses, whereas liver weight was not influenced in comparison to ovariectomized controls (not shown). Ethinyloestradiol gave nearly the same high oestrogenic activity as J995: uterus weight was enhanced by the lowest dose (Fig. 1) and body weight was diminished at a tenfold higher dose of 0.1 mg/kg. The highest dose of 10 mg EE/kg increased liver weight significantly (cast, 8.16 ± 0.26 g; EE, 9.76 ± 0.33 g).

Because of growth reduction the liver/body weight ratio was significantly enhanced only after the 10 mg dose of E or J995, but after 1 as well as 10 mg/kg EE [cast, 37.99 ± 0.96 g liver per kg body wt.; E, 40.33 ± 1.53 ; J995, 41.54 ± 0.90 ; EE, (1 mg), 39.51 ± 0.83 and (10 mg) 46.91 ± 1.38]. All livers of controls and E and EE treated rats showed normal consistency and colour. Four out of eight livers of rats treated with 1 mg/kg body wt. J995 and two out of eight livers of rats treated with 10 mg/kg body wt. J995 showed altered consistency and colour. These livers were of tenser consistency, visibly fatty with net-like markings. The measured parameters did not fall outside of normal range.

Influence of oestrogens on bile flow and bile acid excretion

Oestradiol treatment did not give any change of bile flow in comparison to castrated controls. However the higher level of bile flow in non-ovariectomized rats could not be

Fig. 1 Uterus weights of untreated (C; $n = 7$), ovariectomized and vehicle treated (cast; $n = 30$) and ovariectomized rats treated for 7 days with oestradiol (E), oestradiol sulphamate (J995) or ethinyloestradiol (EE), $n = 8$. Data are of means $\pm$ SEM. Student's t -test, $P \leq 0.05$.
* Significantly different from C;
* significant differences between cast and oestrogen treated rats



achieved even with 10 mg E/kg because of a tendency to cholestatic action of E (Fig. 2). J995 was also not able to increase bile flow to levels observed in the controls without ovariectomy, but the highest dose used diminished bile flow significantly in comparison to castrated controls (Fig. 2). Ethinyloestradiol showed the strongest

cholestatic activity, with bile flow diminished to the same extent as J995 at a tenfold lower dose of 1 mg/kg (Fig. 2).

Bile acid excretion was significantly lower after 0.01 mg/kg E, whereas the three higher doses did not reveal any influence on bile acid excretion. Lower bile flow together with higher bile acid concentration after

Fig. 2 Bile flow in untreated (C; $n = 6$), ovariectomized and vehicle treated (cast; $n = 29$), and ovariectomized rats treated for 7 days with oestradiol (E), oestradiol sulphamate (J995) or ethinyloestradiol (EE), $n = 7-8$. Data of mean $\pm$ SEM. Student's t -test, $P \leq 0.05$.
* Significantly different from C;
* significant differences between cast and oestrogen treated rats

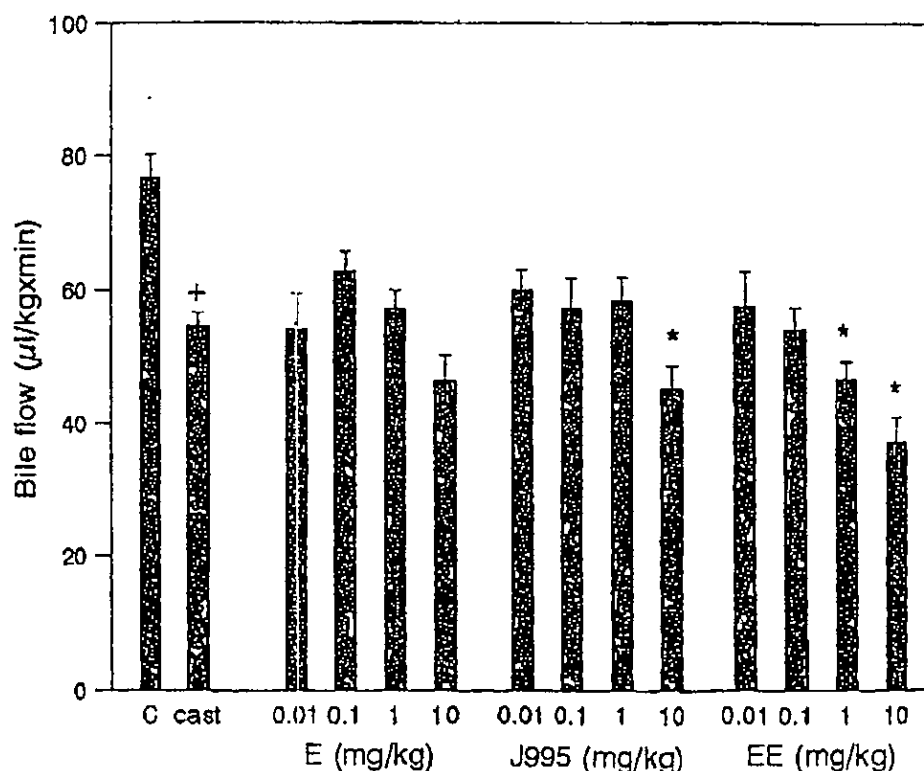
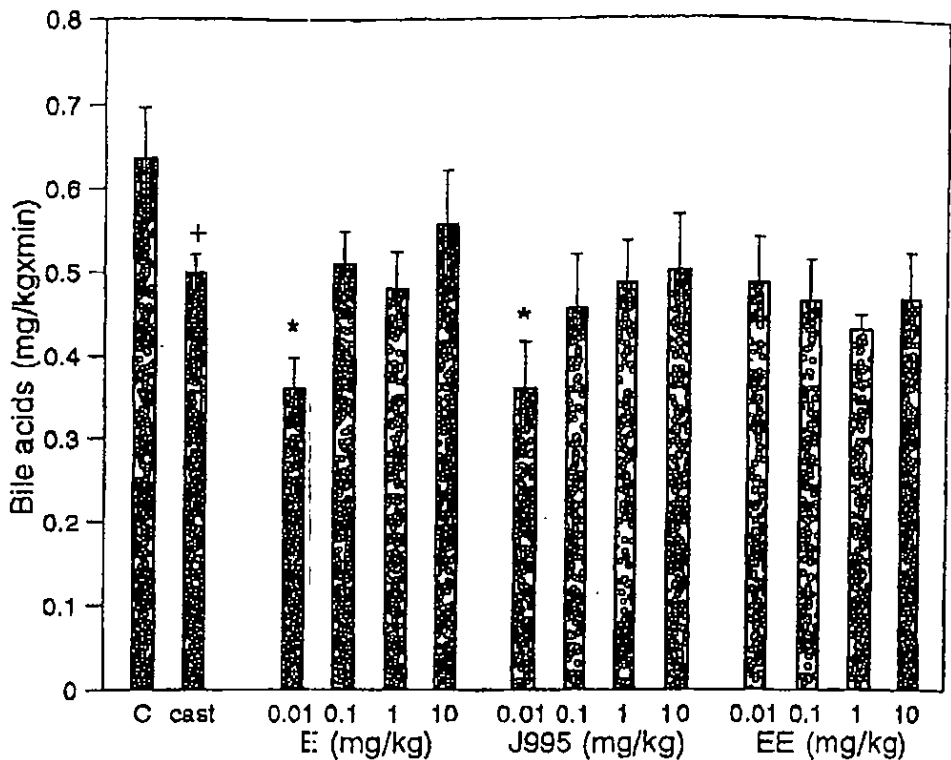


Fig. 3 Bile acid excretion in untreated (C; $n = 6$), ovariectomized and vehicle treated (cast; $n = 29$), and ovariectomized rats treated for 7 days with oestradiol (E), oestradiol sulphamate (J995) or ethinyl-oestradiol (EE), $n = 7-8$. Data of mean $\pm$ SEM. Student's t -test, $P \leq 0.05$. * Significantly different from C, * significant differences between cast and oestrogen treated rats



10 mg/kg E over 7 days resulted in unchanged bile acid excretion (Fig. 3). Decreased bile acid concentration is the likely cause of decreased bile acid excretion only after 0.01 mg/kg J995. With the highest and cholestatic J995 dose bile acid excretion was not affected. The high bile acid excretion rate of non-ovariectomized rats could not be reached with any of the J995 doses used (Fig. 3). As seen with E and J995, cholestatic doses of EE did not influence bile acid excretion because of increased bile acid concentration after the highest EE dose (Fig. 3).

Influence of oestrogens on biliary excretion of total glutathione (tGSH)

The dose of 0.1 mg/kg E was not active oestrogenically, as seen from measurement of uterus weight (Fig. 1). However there was significantly higher tGSH excretion into bile in comparison to ovariectomized and vehicle-treated rats, without reaching the high values of non-ovariectomized controls (Fig. 4). The highest dose of E significantly decreased biliary tGSH excretion whereas bile flow showed only a tendency to decreased values in comparison to ovariectomized controls (Figs. 2, 4).

J995 with the strongest oestrogenic activity did not enhance the lowered tGSH excretion caused by ovariectomy, but even 1 mg/kg significantly diminished biliary tGSH excretion without changing bile flow (Figs 2, 4). A tenfold higher dose of J995 nearly eliminated biliary tGSH excretion (Fig. 4). The strong and most cholestatic oestrogen EE also did not enhance biliary tGSH excretion compared to non-ovariectomized controls, but the decrease of biliary tGSH excretion after

1 mg and 10 mg/kg EE was much more pronounced than the same doses of J995 (Fig. 4).

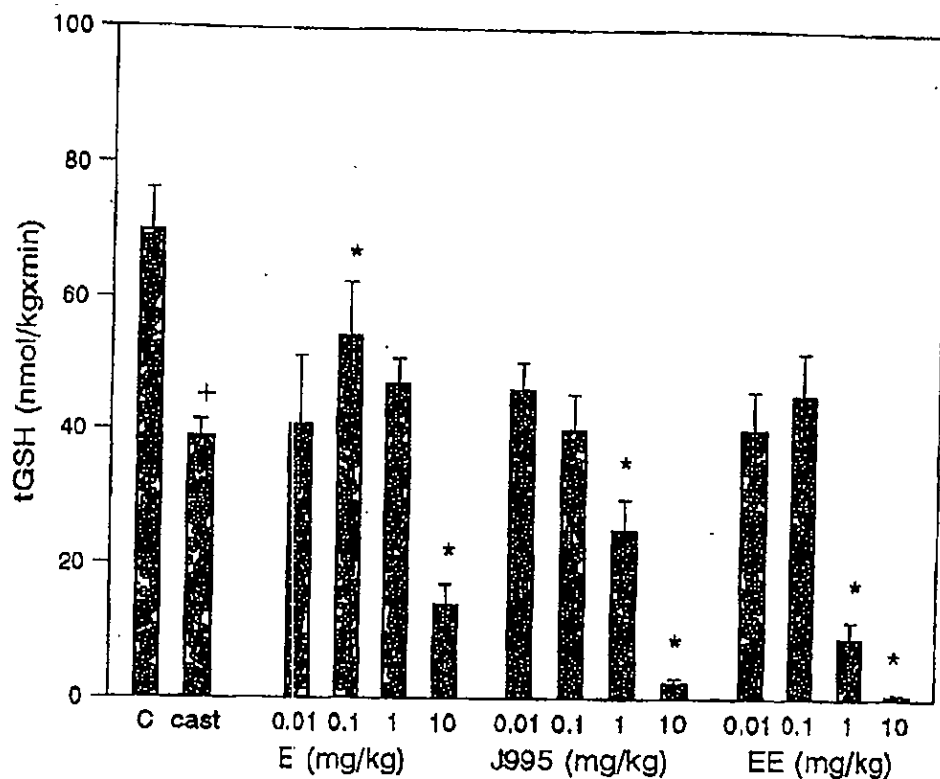
Influence of oestrogens on liver GSH and GSSG concentrations

Only treatment with 0.1 mg/kg E diminished liver GSH concentration, whereas the two higher and oestrogenically active doses did not significantly affect GSH. J995 also decreased GSH concentration at the same dose as E, while the two higher doses of J995 increased GSH dose dependently. However, EE did not change liver GSH concentration (Table 1). Neither of the three oestrogens in any of the doses used altered liver GSSG concentrations (not shown).

Discussion

Bile flow, biliary excretion of bile acids, and tGSH as well as liver concentrations of GSH and GSSG in non-ovariectomized control rats were found to be in the normal range (Fleck and Barth 1990; Kretzschmar et al. 1989; Kretzschmar and Klinger 1989). Growth inhibition is a well-known effect of oestrogens caused by reduced food intake (Heywood and Wadsworth 1980; Barth et al. 1986). The dose-related reduction of body weight was seen after treatment with all three oestrogens investigated, being more pronounced with J995. The higher body weights after ovariectomy also confirm the inhibitory effects of oestrogens on body weight. After the highest EE dose used, liver weight was significantly

Fig. 4 Total biliary glutathione (tGSH) excretion in untreated (C; $n = 6$), ovariectomized and vehicle treated (cast; $n = 29$), and ovariectomized rats treated for 7 days with oestradiol (E), oestradiol sulphamate (J995) or ethinyloestradiol (EE), $n = 7-8$. Data of mean $\pm$ SEM. Student's t -test, $P \leq 0.05$. + Significantly different from C, * significant differences between cast and oestrogen treated rats



enhanced. Hornstein et al. (1992) found a 65% increase in liver volume after EE treatment, which was caused by hypertrophy rather than by hyperplasia. Treatment with E and J995 did not give any changes in liver weight, the increased liver/body weight ratio was caused by body weight reduction only. The highest liver/body weight ratio observed after EE treatment may be caused by both growth reduction and increase of liver volume.

Oestrogens are biotransformed and excreted into bile as glucuronides, sulphates or sulphoglucuronides (Adlercreutz and Martin 1980). 17 β -Glucuronides of oestrogens induce cholestasis in rats and humans (Sano et al. 1993; Takikawa et al. 1996). Gosland et al. (1993) reported that the cholestatic oestradiol-17 β -glucuronide is a substrate for the multidrug resistance transporter,

P-glycoprotein, which is a canalicular carrier also for other organic anions and GSSG. GSH was not a substrate for ATP-dependent P-glycoprotein mediated transport (Takikawa et al. 1996; Leier et al. 1996). Landmann (1995) considered EE related cholestasis as a minimal lesion model, since neither morphological alterations nor distribution changes of membrane proteins were observed. EE was found to disturb the sorting machinery in endosomes followed by altered intracellular transport routes (Meier-Abt 1990; Bossard et al. 1993; Stieger and Landmann 1996). Bile acids in serum and bile are important indicators of hepatobiliary integrity (Thompson 1996). Active canalicular bile acid secretion and subsequent paracellular diffusion of primarily Na⁺ and water produce bile acid-dependent bile flow. GSH appears to be a major osmotic factor in the generation of bile acid-independent bile flow (Ballatori and Truong 1989; Bouchard et al. 1994).

In present investigations we found a significant cholestatic effect of ovariectomy. Administration of E, J995 and EE could not restore the bile flow of non-ovariectomized controls because of the specific cholestatic side-effects of the oestrogens. Compared to the ovariectomized controls, cholestatic activity was in the order E < J995 < EE. Whereas castration impaired both parts of bile flow as seen by unchanged bile acid concentration and decreased bile acid excretion, the oestrogens seemed to disturb the bile acid-independent fraction of bile flow, as seen with a dose dependent increase of bile acid concentrations resulting in unchanged bile acid excretion (Fig. 3). Chico et al. (1996) demonstrated a minor and transient enhancement of bile acid synthesis in rat hepatocyte monolayers after exposure to

Table 1 Liver glutathione (GSH) concentration in untreated controls (C; $n = 7-8$), ovariectomized and vehicle treated controls (cast; $n = 30$), and oestrogen treated ovariectomized rats ($n = 7-8$). The rats were administered 0.01, 0.1, 1, 10 mg/kg body weight oestradiol, oestradiol sulphamate (J995) and ethinyloestradiol orally once daily over 7 days in 2 ml/kg myrj. Data are of mean $\pm$ SEM. Student's t -test, $P \leq 0.05$. * Significant difference between C and cast, significantly different between cast and oestrogen treated rats (myrj Polyoxyethylene stearate in saline)

Dose (mg/kg)	Liver GSH concentration (μ g/g)		
	Oestradiol	J995	Ethinyloestradiol
	(Controls: C, 2180 $\pm$ 59; cast, 1957 $\pm$ 39*)		
0.01	1993 $\pm$ 45	1860 $\pm$ 121	1944 $\pm$ 93
0.1	1704 $\pm$ 69*	1740 $\pm$ 71*	1915 $\pm$ 133
1	2059 $\pm$ 113	2058 $\pm$ 126	1922 $\pm$ 101
10	2063 $\pm$ 155	2423 $\pm$ 114*	2051 $\pm$ 94

10 μ M oestradiol. Our results confirm those of Gumucio and Valdivieso (1971) and Vore (1987) that oestrogens mostly influence bile acid-independent bile flow.

To evaluate the contribution of glutathione to oestrogen induced cholestasis, we measured liver and biliary glutathione status. Surprisingly, ovariectomy diminished biliary tGSH concentration and excretion significantly. In parallel with cholestatic activity, the three oestrogens reduced biliary tGSH excretion in the order $E < J995 < EE$. The glutathione reducing effects apparently do not correlate with oestrogenic activity because the uterus weight-enhancing dose of 1 mg/kg E did not change biliary tGSH excretion, nor was this parameter changed by the two oestrogenically active doses of J995 and EE (0.01 and 0.01 mg/kg; Figs 1, 4). The highest dose of E showed only a tendency to reduced bile flow, but the biliary tGSH was diminished to one-third. The two other oestrogens J995 and EE at the highest doses used, however, diminished bile flow to 83 and 65%, respectively, but stopped biliary tGSH excretion almost completely (Figs 2, 4).

In adult rats, ductular bile flow can be neglected and active transport of bile acids is responsible for 30 to 40% of total bile flow (Paumgartner 1979; Barth et al. 1981; Klaassen and Watkins 1984). If tGSH was the only osmotically active substance responsible for the bile acid-independent fraction of bile flow, almost complete inhibition of biliary tGSH excretion by J995 and EE ought to inhibit the bile flow to a much greater extent. The present results support the hypothesis that GSH together with still unidentified solutes appears to be a major factor in the generation of bile acid-independent bile flow (Ballatori and Truong 1989; Erlinger 1992; Anwer 1993). Another explanation for high bile flow without GSH excretion may be the altered osmotic activity of bile acids dependent on their micelle-forming capacities, or bile acids may modulate transport systems of other osmotically active solutes (Klaassen and Watkins 1984; Oude Elferink and Ottenhoff 1991).

However, the oestrogens tested disturbed bile flow and biliary tGSH excretion severely. According to Ballatori and Dutczak (1994), at least two ATP-independent, electrogenic GSH transport mechanisms are present on the canalicular membrane: a high-affinity component for the delivery of GSH conjugates, gamma-glutamyl compounds and other anions and a low-affinity component with high capacity to excrete large amounts of GSH into the bile. We propose that the intrahepatic cholestasis produced by the three oestrogens E, J995 and EE, with effects more pronounced by the synthetic J995 and EE, may be caused by interference of the oestrogens with these GSH secretory processes. Bouchard et al. (1994) found a similar reduction of bile flow and biliary tGSH secretion in rats treated with EE for 5 days. Additionally they found a large increase in liver GSH and GSSG concentrations, suggesting that EE interfered with glutathione transport from the liver to bile. In our experiments E and EE failed to increase liver glutathione even at higher doses, but J995 administration resulted in

a dose dependent GSH accumulation in the liver (Table 1).

Bile is a rich source of GSH, protecting intestinal mucosa against luminal peroxidized lipids (Aw 1994). GSH may also be important for canalicular transport of organic anions as well as for maintaining the redox state of sulphhydryl groups of membrane proteins (Tiribelli 1994; Yoshida et al. 1994). Thus a disturbance of glutathione status in the liver and glutathione deficiency in bile and intestine due to oestrogens may contribute to or aggravate the cholestatic disease. Comparing the cholestatic and glutathione reducing activity of all three oestrogens, natural E revealed the lowest oestrogenic activity and side-effects. J995 showed stronger oestrogenic activity than EE but a markedly lower cholestatic effect with tGSH reduction in bile similar to that of the known cholestatic EE. Surprisingly, only J995 enhanced the liver GSH content without changing GSSG content. The importance of this latter effect as a sign of GSH accumulation due to impaired biliary GSH transport remains to be investigated. The present study demonstrates that oestrogen interference with canalicular glutathione transport may be partly the cause of cholestasis. A tenfold higher dose of J995 exhibited the same cholestatic effect as EE. Together with higher systemic oestrogenic activity, J995 may be used as a prodrug with reduced hepatic side-effects to replace EE in contraception strategies.

Acknowledgements We thank Mrs E. Franke and Mrs H. Stadler for excellent technical assistance.

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Sulfamates of Various Estrogens are Prodrugs with Increased Systemic and Reduced Hepatic Estrogenicity at Oral Application

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Oral therapy with natural or synthetic estrogens, like ethinylestradiol, suffers from low, suboptimally defined bioavailability and excess hepatic estrogen actions. *N,N*-alkylated and non-alkylated sulfamates of ethinylestradiol, estradiol and estrone overcome these deficiencies. Ovariectomized Wistar rats ($n = 6-7/\text{group}$) were orally treated for 7 days, and killed on day 8, plasma was gained on days 0, 4, and 8. Systemic estrogenicity was quantified by assessment of uterine weight, vaginal cornification, and measurement of gonadotropins by homologous RIA. Estrogenicity in the liver was analysed. Angiotensinogen was estimated by RIA of angiotensin-1 after incubation of EDTA-plasma with porcine renin. Total and high-density cholesterol were measured by enzymatic methods. Preliminary biotransformation studies were performed after oral administration of $10\text{ }\mu\text{g}$, $5\text{ }\mu\text{Ci}$ [$2,4,6,7\text{-}^3\text{H}$]estradiol sulfamate. Ethinylestradiol led to distinct elevation of angiotensin-1 and dramatic depression of cholesterol fractions, reflecting hepatic estrogen effects, already at doses with marginal systemic effects. Estradiol and estrone had systemic and hepatic estrogenic activity at much higher doses only. Estrogen sulfamates had systemic estrogen activity 10-90-fold above that of their parent estrogen. Non-alkylated sulfamates of given estrogens were more active than *N*-alkylated ones. Elevation of systemic estrogen activity was always combined with a dramatic reduction of hepatic estrogenicity. Estradiol sulfamate had a 90-fold elevated systemic estrogen activity vs estradiol, but lacked hepatic activity including the 30-fold dose inducing vaginal response. Three hours after administration no unchanged estradiol sulfamate was detectable in plasma. Rather peaks, probably representing estradiol and estrone, were found. Estrogen sulfamates are considered prodrugs of their parent estrogen, which do not interact with any liver function during the first-pass. They represent a new strategy of oral hormone administration. Their main potential seems to be the systemic generation of natural estrogens when used in oral contraceptives.

J. Steroid Biochem. Molec. Biol., Vol. 55, No. 3/4, pp. 395-403, 1995

INTRODUCTION

The main therapeutically used estrogens comprise natural estrogens and a few "synthetic" ones, ethinylestradiol, mestranol and the non-steroidal diethylstilbestrol. Natural as well as non-natural estrogens dominate certain therapeutic areas for good reasons. The use of synthetic estrogens is restricted to contraception and tumor therapy. Their preference for

these indications is certainly due to their superior and more reliable inhibitory effect on the hypothalamic-pituitary-gonadal axis. Their advantage versus natural estrogens is a much higher, i.e. better defined, oral bioavailability and a longer retention in target tissues [1, 2]. Successful attempts to develop hormonal contraceptives with natural estrogens are not known. Very careful pharmacokinetic studies with orally administered estradiol led to the conclusion that inter- and intra-individual variability of pharmacokinetic parameters is so substantial that it is *theoretically*

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Received 28 Apr. 1995; accepted 29 Aug. 1995.

impossible to develop estradiol-containing oral contraceptives [3].

The disadvantage of synthetic estrogens are effects on metabolic functions. Their physiological background are sexually dimorphic liver functions which are determined by male and female gonadal hormones. The pharmacology of these dimorphisms is complex due to interaction of pituitary hormones as growth hormone or prolactin [4, 5] and other hormones as insulin, glucocorticoids, thyroid hormones. The role of these factors for the transcription of the estrogen modulated angiotensinogen gene in the liver is well established [6, 7].

The synthesis of important factors of the blood clotting system is estrogen-modulated. This also applies to that of various lipoproteins, angiotensinogen, CBG, SHBG, thyroxin-binding globulin, coeruloplasmin, and various enzymes of the cytochrome P450-system [1, 8]. Ethinylestradiol or DES have much higher hepato-cellular than systemic estrogenicity: in relation to FSH-secretion inhibitory activity these estrogens are 4-18 times more active in the liver than estrone sulfate [8]. A similar ratio of systemic and hepatic effects was estimated comparing ethinylestradiol and estradiol [1].

The main identified risk arising from the use of combined oral contraceptives is an increased cardiovascular morbidity. The use of natural estrogens in hormone replacement therapy was the opposite effect [9, 10]. There is consensus that oral contraception related cardiovascular risks are not dependent on the duration of contraception and cease with the end of their use. This was considered as strong evidence of a thrombotic origin of complications like myocardial infarction. It was concluded that the estrogen component in contraceptives is to be blamed for this problem [11].

The discussed problems of oral estrogen therapy were the main object of our research. The conventional oral treatment with natural estrogens overcomes the barrier represented by the liver by mere mass of the administered hormone. The pharmacodynamic implication of this strategy are distinct alterations of estrogen regulated liver functions. These can be avoided, when an equivalent systemic estrogen action is achieved by parenteral administration [10, 12].

Natural estrogens are extensively metabolized at first liver passage [13]. The only established means to obtain high oral bioavailability of steroidal estrogens is the prevention of their biotransformation in the liver at first passage. Oxidation of the tertiary 17 β -hydroxy group of ethinylestradiol is not possible. Impaired oxidation of 17 β -hydroxyl group compared to estradiol-17 β was seen in case of a 14 α ,15 α -methylene estradiol (J 824) using human placental 17 β -OH-steroid dehydrogenase (G. Kaufmann, Jenapharm, unpublished data). This compound is orally active, with pronounced effects on hepatic functions [14]. It thus seems that impairment of 17-hydroxy oxidation,

independent of its mechanism, leads to increased oral activity, but implies profound alteration of pharmacodynamic and pharmacokinetic properties of corresponding molecules compared to systemically generated estradiol.

There is a much prolonged retention of ethinylestradiol at the cellular level [1, 15]. Prolonged retention of ethinylestradiol in target cells, together with high concentrations in the portal vein blood during resorption from the gastro-intestinal tract are the likely cause of excessive hepatic estrogenicity. The contribution of first liver passage versus inherent properties of ethinylestradiol are not undisputed. Studies comparing oral and vaginal administration of ethinylestradiol [16] demonstrated excessive hepatic estrogen effects with both routes of administration.

The question has to be raised, whether elevated hepatic estrogenicity is an unavoidable problem of good oral activity. This paper demonstrates that chemically modified, *per se* inactive derivatives of sulfates of natural and artificial estrogens, have an as yet unrecognized potential to overcome the liver barrier without compromising important liver functions. Estrogen sulfamates (see Fig. 1) may be used for a new treatment strategy to achieve an efficient estrogen treatment which avoids the above described liver-hormone and hormone-liver interactions. The presented material from studies with 3-sulfamates of estradiol, estrone, and ethinylestradiol is selected from results of ongoing research on novel estrogens for oral contraceptives and hormone replacement therapy. In particular, various steroidal sulfamates have been investigated since *N,N*-dialkylated sulfamates of ethinylestradiol, the first steroid sulfamates described, were found to be strong estrogens with a prolonged effect and antifertility activity upon oral administration [17, 18].

For electronic reasons the sulfamate moiety was suggested to act as a hydrogen acceptor, thus fulfilling prerequisites for a considerable binding with blood proteins. In fact erythrocytes were found to bind a substantial amount of administered *N,N*-alkylated ethinylestradiol sulfamates or their metabolites, respectively [17]. Pharmacological evaluation and synthesis of new compounds was resumed when it was found that one compound (J 271) had dissociated hepatocellular and systemic estrogenic activity [19].

Manifestation of hepatic estrogenicity varies determined by systemic or oral route of administration and by the chemical constitution of estrogen. Humans and rats show analogous responses in this respect. Strong evidence for direct hepato-cellular effects with respect to a given estrogen responsive parameter is a pronounced decrease of the ratio of systemic to hepatic activity at oral administration. The induction of angiotensinogen secretion represents a proven hepatocellular effect of estrogens [7, 20, 21]. The decrease of total and HDL cholesterol in the blood of estrogen treated ovariectomized rats is

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oral treatment suggesting a direct estrogen effect on lipoproteins in the liver [18, 19].

MATERIALS AND METHODS

Chemistry

The *N,N*-dimethyl and *N,N*-diethyl sulfamates of estrone (J 922, J 804) and the 3-*N,N*-dimethyl, 3-*N,N*-diethyl and 3-*N,N*-tetramethylene sulfamates of ethinylestradiol (J 665, J 271, J 272) were synthesized from the parent steroids and the corresponding sulfamoyl chlorides by phase transfer technique (X). Thus, at 60–80°C, a solution of the steroid and the *N,N*-disubstituted sulfamoyl chloride (1.5 equiv.) in toluene was intensively stirred with an aqueous solution containing triethyl benzylammonium chloride (0.1 equiv.) and sodium hydroxide (0.75 equiv.). Additional aqueous sodium hydroxide solution (1.5 equiv.) was added within a range of 2 h. Conventional work-up after TLC control and crystallization gave the desired esters.

Estrone *N,N*-dimethyl sulfamate (J 992): mp. 190–194°C (AcOEt); $[\alpha]_D^{20} + 116^\circ$ ($c = 1$, Chf).

Estrone *N,N*-diethyl sulfamate (J 804): mp. 176–180°C (MeOH); $[\alpha]_D^{20} + 109^\circ$ ($c = 1$, Chf).

Ethinylestradiol 3-*N,N*-dimethyl sulfamate (J 665): mp. 156–158°C (MeOH); $[\alpha]_D^{20} + 5^\circ$ ($c = 1$, Chf).

Ethinylestradiol 3-*N,N*-diethyl sulfamate (J 271): mp. 113–115°C (*i*-Pr₂O); $[\alpha]_D^{20} + 3^\circ$ ($c = 1$, Chf).

Ethinylestradiol 3-*N,N*-tetramethylene sulfamate (J 272): mp. 121–122°C (Et₂O); $[\alpha]_D^{20} + 10^\circ$ ($c = 1$, Chf).

Estrone sulfamate (J 994) was prepared by treating a solution of estrone in dimethyl formamide with sulfamoyl chloride (5 equiv.) at 20–22°C for 4 h. Upon addition of water the compound was isolated by filtration and crystallized from methanol.

Estrone sulfamate (J 994): mp. 199–202°C (Ac/n-Hx); $[\alpha]_D^{20} + 128^\circ$ ($c = 1$, Dx).

The 3-sulfamate as well as the 3-*N,N*-dimethyl and 3-*N,N*-diethylsulfamates of estradiol (J 995, J 991, J 981) were obtained by sodium borohydride reduction of respectively J 994, J 992 and J 804 in a mixture of methanol and tetrahydrofuran at +5°C.

Estradiol-3-sulfamate (J 995): mp. 204–206°C (Ac); $[\alpha]_D^{20} + 61^\circ$ ($c = 1$, Dx).

Estradiol 3-*N,N*-dimethyl sulfamate (J 991): mp. 204–208°C (Chf/MeOH); $[\alpha]_D^{20} + 61^\circ$ ($c = 1$, Chf).

Estradiol 3-*N,N*-diethyl sulfamate (J 981): mp. 176–178°C; $[\alpha]_D^{20} + 57^\circ$ ($c = 1$, Chf).

Ethinylestradiol-3-sulfamate (J 1028) was synthesized from ethinylestradiol-17-trimethyl silylether. Treatment of the silylether at 20–22°C with sulfamoyl chloride (5 equiv.) in dichloromethane solution in the presence of 2,6-tert-butyl-4-methylpyridine (3.5 equiv.) gave the esterified silylether which in turn was cleaved by stirring the reaction mixture with aqueous hydrochloric acid (18%) overnight. Upon work-up J 1028 was purified by flash chromatography on silica (eluent chloroform/ethyl acetate) and crystallization.

Ethinylestradiol-3-sulfamate (J 1028): mp. 209–211°C (Ac/n-Hx); $[\alpha]_D^{20} - 15^\circ$ ($c = 1.02$, Py).

Satisfactory spectral data (¹H-NMR, ¹³C-NMR, IR, UV, high resolution MS) and elemental analyses were obtained for all compounds. The purity of the sulfamates was determined by HPLC and was found to be 99.5–99.95%.

Animal studies

Ovariectomized adult female Wistar rats, 2 weeks after ovariectomy were used in experiments. The systemic and hepatic estrogenicity was evaluated. Animals

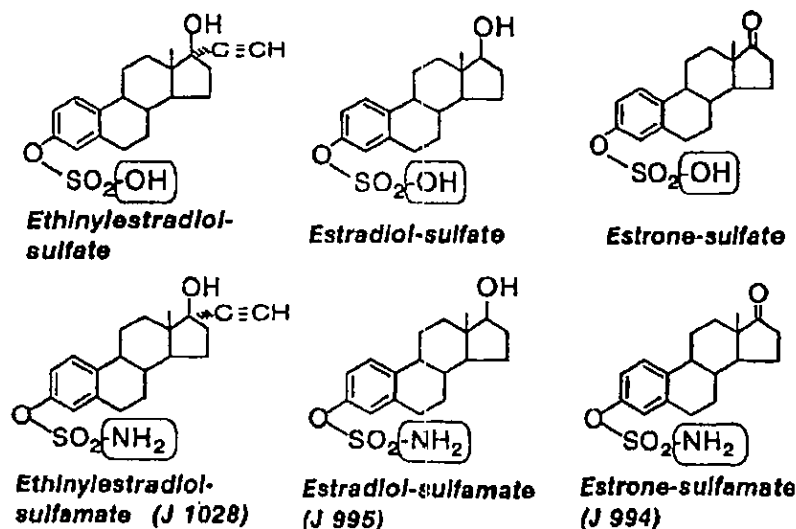


Fig. 1. Chemical structure of sulfates and sulfamates of estradiol, estrone and ethinylestradiol.

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Table 1. Comparison of ethinylestradiol, estrone and estradiol and their 3-sulfamates with respect to systemic and hepatic estrogen parameters at oral administration

Parent steroids and tested sulfamates	Code	Equipotent oral dose (µg/animal/day)		
		Uterus Δ100%†	Angiotensin I Δ50%†	HDL-cholesterol Δ50%‡
Ethinylestradiol*	EE	7.0-17	3.0-14	2.0-6.0
~ S	J 1028	0.2	7.0	3.0
~ DME-S	J 665	<10	> 10	> 10
~ DET-S	J 271	0.7	55	32
Estrone	E1	115	100	250
~ S	J 994	3.8	300	40
~ DME-S	J 992	7	> 100	> 100
~ DET-S	J 804	11	> 100	> 100
Estradiol	E2	150	190	210
~ S	J 995	1.7	> 30	> 30
~ DME-S	J 991	9.5	> 30	> 30
~ DET-S	J 981	11	> 100	> 100

S, 3-sulfamate; DME-S, 3-dimethylsulfamate; DET-S, 3-diethylsulfamate.
*ET 94.003; ET 94.048; ET 94.059; ET 95.015; ET 95 = file number of experiment.

are treated from day 1-7, and killed on day 8 of the study. The animal number per group was 7 as a rule. Ethinylestradiol was tested as standard substance in all studies.

Assessment of systemic estrogenicity. The vaginal response was microscopically scored (stage 1-3) checking unstained smears days 1-8. The lowest dose inducing cornification by day 8 (stage 3) in 100% of animals was determined. The dose per day, inducing a 100% increase of uterine weights compared to ovariectomized vehicle-treated controls, was calculated on the basis of dose-response curve. In some of the studies the lowest dose which prompted a drop of plasma gonadotropin levels was determined by RIA for rat LH and FSH (Amersham).

Assessment of hepatic estrogenicity. Blood was collected on days 0, 4, and 8 of the study under light ether anesthesia. Maximum amount of blood was collected when the animals were bled on morning of day 8.

TA plasma was prepared, shock frozen and kept in refrigerator at -70°C until analysis.

Changes of angiotensinogen were estimated by RIA of angiotensin-1 (RIA RENCTK, Sorin/Biomedica) after treatment of plasma with renin. Steps: EDTA-plasma was diluted with phosphate buffer pH 6.5, 0.1 M and kept at a temperature of 0°C. To 100 µl of this dilution 2.5 mU porcine renin in 25 µl solution (R2761, Sigma), and 2.5 µl PMSF (a converting enzyme inhibitor, Sorin/Biomedica kit) and 12.5 µl reaction buffer (kit) were added. This preparation was then incubated at 37°C for 3 h. For one determination 50 µl of medium were used in the above angiotensin-1 RIA.

Cholesterol fractions, HDL and total cholesterol, were determined by enzymatic colorimetric method (Dr Lange tests LNC 026, LNC 526, HDL-cholesterol precipitation with BCZ 526). The dose-level which led to a 50% increase of angiotensin-1 and a 50% decrease of HDL or total cholesterol was determined.

The presented material is selected from several studies in the course of compound finding as compounds became available. All evaluated parameters cannot be reported in the volume of this article. The presented data focus on studies with the non-alkylated 3-sulfamates of each of the above parent estrogens and their *N,N*-dimethyl- and *N,N*-diethyl-derivatives (see Table 1). In a separate controlled study three sulfamates of ethinylestradiol were compared at a molar equivalent dose of 10 µg steroid/animal/day vs 10 µg ethinylestradiol/animal/day at oral administration. This study included the *N,N*-dimethyl- (J 665), *N,N*-diethyl- (J 271) and the *N,N*-tetramethylene-sulfamates (J 272) of ethinylestradiol. The file numbers (ET) identify individual experiments.

Formulation and administration of test compounds

Saline, containing 85 mg polyoxyethylen(50)-stearat (MYRJ, ICI Speciality Chemicals Essen, Germany), a surface active agent per 100 ml, was used as vehicle. A suspension of compounds was prepared in a 25 ml Erlermayer flask by addition of Zirconium beads during formulation and ultrasound treatment in a water bath for 30 min. The daily dose was orally administered in 0.2 ml of vehicle. Control animals received the same volume of vehicle orally.

Preliminary studies on the metabolism of estradiol-3-sulfamate (J 995) after oral administration in female rats

Estrone- and estradiol-3-sulfamates were synthesized using [2,4,6,7-³H]estrone (103 Ci/mMol, Amersham) diluted with cold estrone as starting material. Specific activity of the final material (J 995) was 182 µCi/mMol. Two ovariectomized female rats were orally treated with 10 µg J 995 (= 5 µCi ³H) and completely bled in anesthesia 3 h later. A total volume of approx. 3 ml Heparin plasma per animal was gained. Each plasma was extracted twice with ether.

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Approximately 30% of plasma radioactivity was ether extractible. Labeled J 995 added to control plasma was found to be more than 90% extractible. The non-extractible activity in plasma of treated rats may represent native estrogen conjugates. These were not further analysed as yet. Chromatographic methods were used to analyse the extracted activity. These allow the complete separation of J 995 from estradiol and estrone which are expected products of J 995 hydrolysis. Column chromatography using Lipidex 5000 (Canberra/Packard). Conditioning and elution medium was trichloro-methane:n-hexane:methanol (50:50:1; v/v/v). 5 ml pipettes (inner diameter 6 mm) were filled with Lipidex 5000, length 8.5 cm. For separation of steroids a total volume of 40 ml eluent was used. Estradiol is eluted in fraction 5-13, J995 in fraction 20-40. Estradiol and J 995 are eluted with a recovery of more than 80%.

For the further differentiation of estradiol—and J 995—fraction TLC on silica gel [Polygram-Sil G/UV 254, Macharay-Nagel DC-system. Trichloro-methane (Roth)/acetic acid methyl ester (Merck) (7:3)] was used: System 200 imaging scanner (Packard Canberra) was used in the evaluation of studies with ³H-labeled substance J 995.

All reported studies were revised by the official ethical committee for animal experiments and approved by the government of Thuringen.

RESULTS

Evaluation of systemic estrogen activity

Estrone and estradiol had low oral activity. Oral doses of approx. 115 and 150 µg/day, respectively, were needed to increase the uterine weight by 100% compared to the vehicle controls (see Table 1). Ethinylestradiol was much more active than these estrogens. A corresponding elevation of uterine weights was already seen at doses between 7 and 17 µg/day in 4 studies using this estrogen as reference substance. Various sulfamates of these estrogens exceed the oral activity of their parent steroid by far. The most active compound was the non-alkylated sulfamate of a given steroid in all investigated cases. With respect of uterine growth ethinylestradiol sulfamate (J 1028) was approx. 40 times more potent than ethinylestradiol. Estrone sulfamate (J 994) was approx. 30 times more potent than estrone, estradiol sulfamate (J 995) was approx. 90 times more potent than estradiol. This increase of systemic oral estrogenic activity was also seen using vaginal cornification or depression of LH and FSH as indicators of activity. Vaginal cornification was seen near the threshold dose with respect to uterine growth inducing activity [see Fig. 2(A)]. A controlled study was performed comparing J 994 and J 995 versus estrone and estradiol with respect to antigonadotropic activity. This was seen at a dose of 10 µg/day of the sulfamate estrogens. Estradiol and estrone had

corresponding effects at doses of 100-1000 µg/day only (see Fig. 3). Lower doses of estrogen sulfamates (<10 µg/day) went along with FSH- and LH-levels above those of ovariectomized control animals. The latter findings need to be confirmed and analysed in more depth.

Evaluation of hepatic estrogenic activity

Effects on hepatic functions as defined in the description of methods can be induced with doses of estrone and estradiol above those which double the uterine weight vs controls. Estrone was found gradually more active in the liver than estradiol (see Table 1). Under estrone the threshold for changes of angiotensin-1 was below that of uterine weight changes as defined in this paper. In case of estradiol the reverse situation was found. The dose of ethinylestradiol which elevated

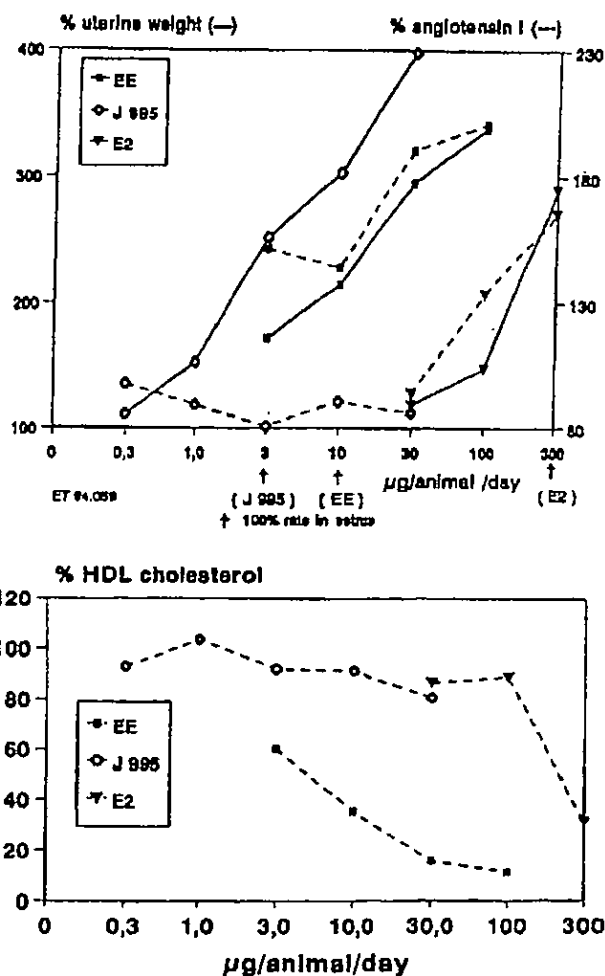


Fig. 2. Comparison of systemic and hepatic estrogen action of ethinylestradiol (EE), estradiol (E2), and estradiol sulfamate (J 995) in ovariectomized rats; oral treatment days 1-7, measurements on day 8 (autopsy). (A) Mean uterotrophic effect, vehicle controls set 100%. Arrow heads indicate lowest dose at which 100% of rats had vaginal estrus. Angiotensin-1 concentration: mean increase versus pretreatment value, which is set 100%. (B) Effects on HDL-cholesterol levels in the same study. Mean values, vehicle controls day 8 set 100%. ET # = file number of experiment.

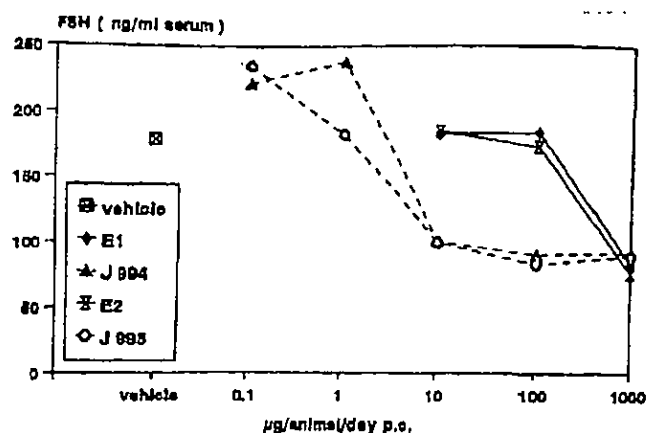


Fig. 3. Depression of FSH in serum of ovariectomized rats by estradiol sulfamate and estrone sulfamate and their parent estrogens at oral administration. Treatment day 1-7, blood collection on morning of day 8. ET # = file number of experiment.

uterine weight by 100% had substantial effects on angiotensin-1 and cholesterol fractions. Even lower doses still had distinct effects on these parameters (see Table 1). There is a different behaviour of hepatic parameters under sulfamate estrogens. Ethinylestradiol sulfamate (J 1028), which has 40 times higher systemic estrogen activity than ethinylestradiol had similar or lower hepatic estrogenicity than this estrogen. An even more pronounced dissociation of estrogen actions in the liver versus systemic estrogen parameters was found for the diethylsulfamate of ethinylestradiol (J 271) versus ethinylestradiol itself. This compound combines a nearly 10-fold increase of systemic with a nearly 10-fold reduction of hepatic estrogenicity.

The reduction of hepatic estrogenicity is even more impressive in the case of sulfamates of natural estrogens versus their parent estrogens. Distinct changes of hepatic parameters could not be detected at doses that led to maximum stimulation of uterine growth [see Fig. 2(A, B) and Table 1].

Comparison of sulfamates of ethinylestradiol (J 271, J 272, J 665) at a molar dose of 10 µg steroid/day at oral administration with ethinylestradiol

Ranking the compounds according to their effects on uterine growth the following can be seen (see Fig. 4). All sulfamates had much higher uterotrophic activity than ethinylestradiol, however, less effects on total cholesterol and angiotensin-1 levels on day 8. These differences were statistically significant. The higher the induced uterine growth, the lower were the effects on liver parameters. The systemically most active J 665 had no significant effect on total cholesterol and angiotensin-1 compared to vehicle treated controls.

Preliminary studies on biotransformation of estradiol sulfamate (J 995)

The recorded profiles in Fig. 5(A, B) demonstrate that labelled estradiol and J 995 yield distinct peaks in

their fractions. Findings in plasma 3 h after administration of [2,4,6,7-³H]estradiol sulfamate (J 995) suggest a very rapid decomposition of this compound *in vivo*. Only 30% of radioactivity were found to be ether extractable as the administered steroid itself. No peak was detectable in the J 995 fraction of plasma extracts at this time point, whereas distinct peaks appeared in the estradiol/estrone fraction [see Fig. 5(C and D)].

DISCUSSION

The evaluated *N,N*-alkylated and non-alkylated sulfamate substituents in C-3 have a profound effect on the pharmacology of their parent estrogen. The non-alkylated sulfamates of estrone, estradiol and ethinylestradiol had much higher systemic estrogen activity than the alkylated derivatives tested. Ethinylestradiol standard is hampered by an enormous inter-assay variation of effects of ethinylestradiol. The lowest and the highest value

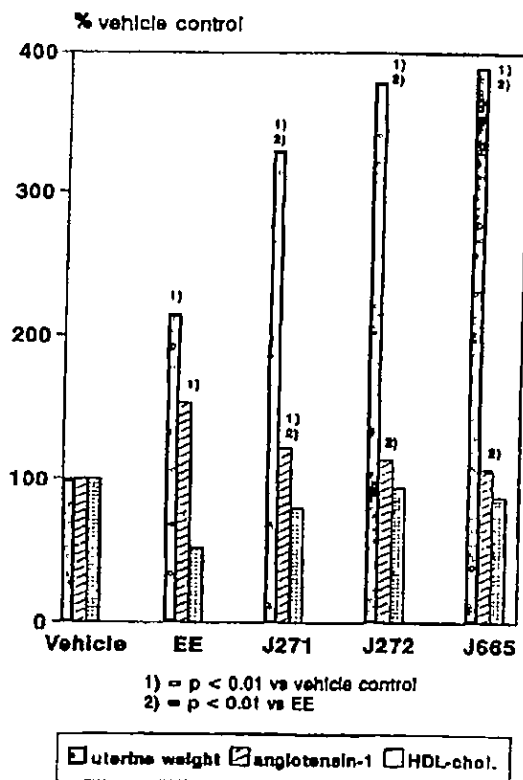


Fig. 4. Relative change of uterine weight, plasma angiotensin-1 and total cholesterol in ovariectomized rats as measured on day 8 after oral treatment days 1-7 with 10 µg ethinylestradiol (EE) per animal per day or molar equivalent dose of ethinylestradiol sulfamates J 271, J 272 and J 665. Mean values, values in vehicle treated controls on day 8 set 100%. All differences between controls vs EE were statistically significant ($P < 0.01$). J 271, J 272 and J 665 induced significantly more uterine growth than EE ($P < 0.01$). Cholesterol and angiotensin-1 levels under J 665 were significantly different vs EE but not from those in controls. ET # = file number of experiment.

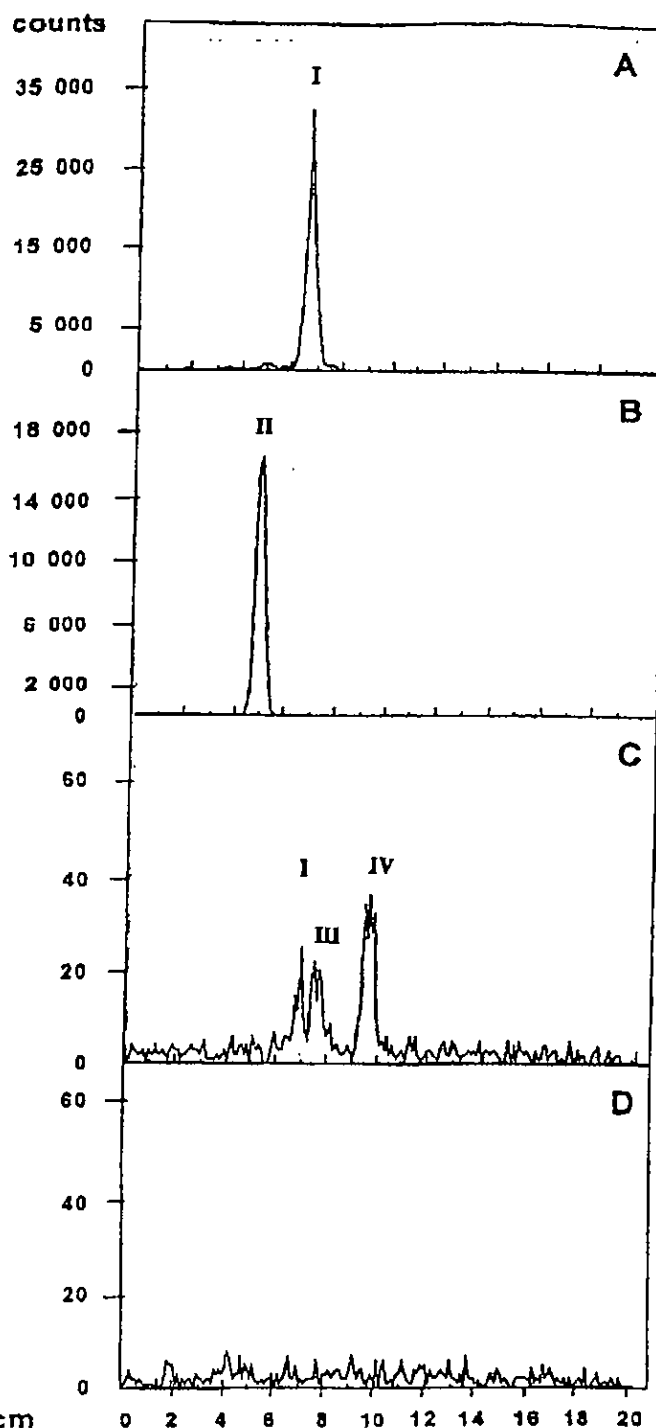


Fig. 5. Silica gel TLC of [2,4,6,7- ^3H]estradiol and [2,4,6,7- ^3H]estradiol sulfamate. For location of estradiol (I) and estradiol sulfamate (II), see peaks in (A) and (B). Peaks in (C) and (D) show activity in ml 7-14 ("estradiol fraction") and ml 20-40 ("estradiol sulfamate fraction") of lipidex 5000 column chromatography of ether extractable activity from plasma 3 h after oral administration of 10 $\mu\text{g}/5 \mu\text{Ci}$ of [2,4,6,7- ^3H]estradiol sulfamate to an ovariectomized rat. Note that three discernible peaks were only present in C: Peak I is very likely estradiol, peak III and IV are in the position of 17 α -OH-estradiol and estrone, respectively. There was no peak in position of estradiol sulfamate in D.

found in four assays using ethinyl-estradiol as reference compound differ by a factor of 2.4 (see Table 1). Varying rates of responding and non-responding

animals were found at a defined dose level. Due to the erratic behaviour of the reference compound relative activity of test compounds versus this estrogen was not calculated. An extraordinary inter- and intraindividual variation of pharmacokinetic parameters as bioavailability (more than factor 3 between lowest and highest values), C_{max} , T_{max} and half-life at oral application which prevails in women [2] may exist in the rat as well. In spite of the described biostatistical problem, there can be no doubt that all tested sulfamate derivatives of ethinylestradiol, and the "free" sulfamates of estrone and estradiol (i.e. J 994 and J 995) exceed ethinylestradiol with respect to systemic estrogen activity by a factor up to 40 in case of ethinylestradiol sulfamates or by factor 3-10 in case of the latter.

Surprisingly, there was no correlation of systemic effects with those on estrogen modulated liver functions. Alterations of these factors in the blood at *parenteral* administration of estrogens can only be seen at dose levels several times above those which induce substantial uterine growth [14, 19, 21]. The presented studies with *oral* administration of ethinylestradiol or the tested natural estrogens show effects on liver function as defined in this paper close to the dose which doubles uterine weights. This is evidence that the selected parameters indeed reflect hepatic estrogen actions. Gradual differences with respect to the ratio of systemic and hepatic estrogenicity among estrone and estradiol were seen in the study but were not analyzed in detail.

Orally administered estrogen sulfamates behave to both the systemic and the hepatic estrogen parameters as parenterally administered natural estrogens. As far as evaluated with respect to a broader spectrum of reproductive functions, they exert powerful effects on uterus, vagina and gonadotropin secretion. However, very consistently, they have no or very moderate effects on the liver over a wide range of doses. The threshold for hepatic effects as defined in this paper may be 100 times above a 100% uterine growth inducing dose. It could not be identified for some analogues in spite of the fact that a broad range of doses was tested.

The observed dissociation of effects is evidence of an as yet unknown mode of passage of estrogen through the liver. It is generally agreed that steroid sulfates are not active hormones [22]. It seems very likely, that this applies to estrogen sulfamates as well. Studies with the dextran charcoal assay of estrogen receptors in MCF-7 cytosol (donated by Abbott) revealed very little displacement of specific [^3H]estradiol (0.5-3 nM) binding with a 200-fold excess of unlabeled J 271, J 994, J 995 (personal communication, A. Hedden and E. V. Jensen, IHF Hamburg). A hydrolysis of the estrogen sulfamate releasing the parent estrogen has to take place *systemically* in a very efficient manner, otherwise the high systemic activity cannot be explained. The low level of

estrogen activity in the liver indicates that this organ is unlikely to play a major role in this activation process. The site or sites of prodrug activation and its mechanism are not yet known.

The virtual absence of J 995 in plasma 3 h after administration and the appearance of ether extractible metabolites, which probably represent estradiol and estrone at this time point are consistent with this notion. However, the chemical identity of identified peaks remains to be studied. Incubation of [2,4,6,7-³H] J 995 in plasma at 37°C over 24 h failed to generate corresponding peaks in the estradiol fraction (data not shown). This finding indicates that the observed peaks are not due to spontaneous degradation of J 995 in plasma and that the relevant activating enzymes are not present there. The substantial level of non-extractible radioactivity in plasma is unlikely to represent unchanged J 995, since this compound itself is easily ether extractible. This fraction of metabolites might consist of sulfates and glucuronides formed as secondary metabolites.

Lacking extraction of administered non-activated sulfamate steroids during the first passage through liver is probably the explanation of both, high systemic activity and the absence of hepatic estrogen action. The investigated sulfamates of ethinylestradiol, which are likely to act estrogenic via the release of ethinylestradiol, show a reverse correlation of systemic and hepatic activity. This indicates a profound effect on ethinylestradiol concentrations in the liver and systemic estrogen target tissues depending on administration as sulfamate or in a direct form.

The significance of the presented findings for the human is unknown. The oral bioavailability of ethinylestradiol is much lower in rats than in humans (4 vs 40%) [3, 23]. Sulfamates of this steroid could lead to an improved oral bioavailability of the parent steroid close to 100% and overcome its awkward variability of pharmacokinetic parameters in humans. These prevent precise definition of estrogen dosing [2]. An increase of activity by the same factor as found in the rat is not very likely in humans since only the much lower first pass loss could be avoided. It goes without saying that a reduction of hepatic estrogen effects of ethinylestradiol, including those on factors of the clotting system and on angiotensinogen secretion would contribute to the safety of hormonal contraception at a most crucial point.

An even higher therapeutic potential of estrogen sulfamates lies in the option to replace the ethinylestradiol in contraceptives, but also estrogens in hormone replacement therapy by more efficient prodrugs of natural estrogens. The presented data suggest that sulfamates of estradiol and estrone generate natural estrogens in the system without the well known [8, 10] hepatic impact or orally administered estradiol. Their systemic activity in the human may reach or even exceed that of ethinylestradiol. The exclusion of hep-

atic estrogen effects beyond the physiological sex dimorphism would be an obvious advantage. The lack of side effects might also help to optimize the dose of estrogen in contraceptives with respect to the control of bleeding pattern. Today's reductions of ethinylestradiol doses may be seen as dubious attempts to reduce metabolic side effects at the expense of a well controlled endometrium.

Sulfamates of estrogens and other steroids were synthesized and evaluated by various groups with different pharmacological intentions [24-27]. The latter quoted group [27, 28] found that steroid sulfamates including estrone sulfamate are potent sulfatase inhibitors. A therapeutic strategy was discussed to prevent the generation of estrogen from estrogen sulfates at the tissue level in hormone-dependent tumor disease. The powerful estrogen activity of the given estrogen sulfamates demonstrated is certainly unfavourable for estrogen ablative strategies. It remains to be evaluated whether sulfamate estrogens interfere with sulfatases and the generation of estrogens from endogenous precursors, i.e. estrone sulfate. Another aspect is their potential hydrolysis of sulfamates by these enzymes. Tissues might be exposed to locally released estrogens depending on their enzyme activity modulating the response of receptive tissues. There is as yet no evidence for or against tissue specific effects, these however have to be taken into account.

Acknowledgements—We thank Mrs Franke, Mrs Freund, Mrs Gran, Mrs Matz and Mrs Triller for excellent experimental work performing studies in rats; Mrs Krah, Mrs Müller and Mrs Ritter for hormone analysis; and Mrs Possner and Mrs Schmidt for their help preparing the manuscript.

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ANTICONVULSANT SUGAR SULFAMATES. POTENT CYCLIC SULFATE AND CYCLIC SULFITE ANALOGUES OF TOPIRAMATE

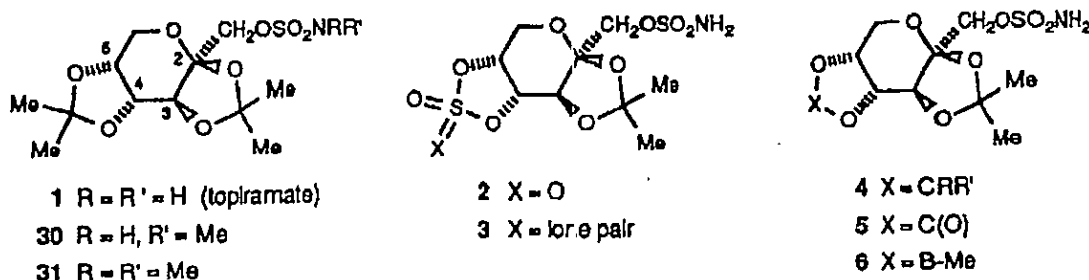
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 James I. Schupsky,[§] Marta E. Crtegon,[§] and Jeffrey L. Vaught[§]
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(Received in USA 5 August 1993; accepted 7 September 1993)

Summary: Exploration of structure-activity relationships surrounding the clinically effective antiepileptic drug topiramate (**1**) led to a series of potent anticonvulsants with a 4,5-cyclic sulfate or 4,5-cyclic sulfite functionality. Key derivative **2** (RWJ-37947) is ca. 8 times more potent than topiramate in mice; it also features a long duration of action and a very favorable neurotoxicity index.

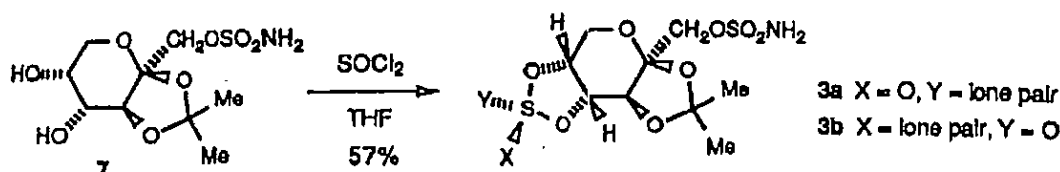
Epilepsy is a chronic neurological disorder characterized by seizures that result from the sudden, disorderly depolarization of neurons in the brain.¹ Anticonvulsant drugs, which function by inhibiting initiation and/or propagation of the causative neuronal discharges, are employed to control epileptic symptoms. However, the available drugs are limited in number, in kind, by various side-effects, and by ineffectiveness in selected patient populations. This has inspired a search for new, more effective, less toxic anticonvulsants.

Because of the multiple etiologies of epilepsy, and our lack of understanding about the responsible mechanisms, the discovery of novel antiepileptic drugs often involves empiricism. Indeed, we discovered topiramate (**1**) by using the standard maximal electroshock seizure (MES) test as a screening protocol.² Since the antiepileptic efficacy of topiramate has been confirmed through clinical trials in patients refractory to generally prescribed drugs,³ we have continued to pursue this novel "sugar sulfamate" structural class. In this communication, we report on a series of 4,5-cyclic sulfate and 4,5-cyclic sulfite analogues of topiramate, such as **2** and **3**, which exhibit potent anticonvulsant activity.

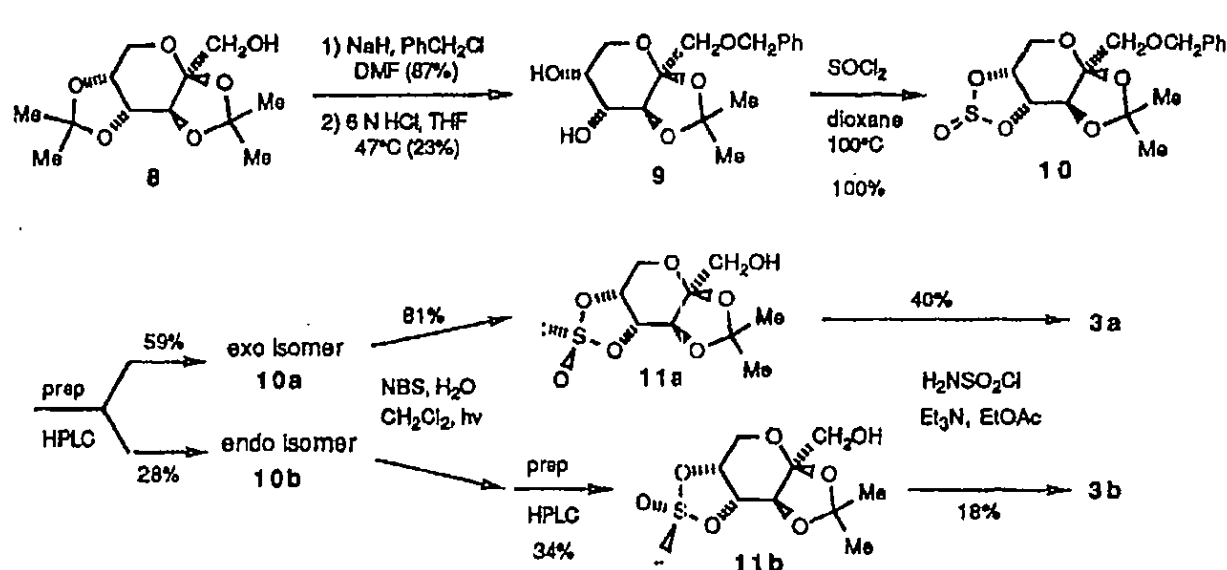


Our early work involved the investigation of various O-alkyl sulfamates as potential anticonvulsants, and was diverse from a structural standpoint.² Subsequently, we have conducted structure-activity studies with topiramate that entail more modest, systematic structural changes.⁴ We found that a high level of anticonvulsant activity requires the substituents on the 4,5-ring in **4** to be alkyl groups of optimal size in a narrow range (H <

R, R' < Et); furthermore, we found that a planar sp² atom in the 4,5-ring (as in 5 and 6) and a 6-membered 4,5-ring (not shown) are undesirable.⁴ These structural requirements seriously constrained our search for topiramate analogues with improved biological properties. Fortunately, our investigation of 1,3,2-dioxasulfane derivatives (*viz.* 2 and 3), commonly referred to as cyclic sulfates and cyclic sulfites, proved rewarding.

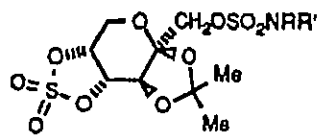


At the outset, cyclic sulfite 3 was prepared from diol 7² and thionyl chloride, as a 5.3:1 mixture of "exo" and "endo" diastereomers, 3a and 3b (from EtOH, mp 143-151°C; $[\alpha]^{25}_D$ -19.8°, c 0.883, MeOH).⁵ The potency of the 3a:3b mixture in the MES test was about eight times that of topiramate (Table). Since separation of the diastereomers was problematic, we used an alternative synthesis to obtain isomerically enriched samples.

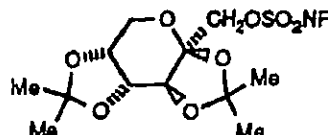


Diacetone fructose 8 was converted to diol 9,⁶ which was condensed with thionyl chloride to give 10. Reaction in THF at 5°C afforded a 12:1 mixture of 10a:10b, whereas reaction in dioxane at 100°C afforded a 1.6:1 mixture. Equilibration is precluded since 10a and 10b did not interconvert under the reaction conditions. The isomers from the dioxane reaction were separated by preparative HPLC (silica gel; EtOAc-hexane, 1:9) and each was debenzylated by free-radical bromination and solvolysis.⁷ The exo isomer 11a, obtained with 17:1 enrichment, was converted to target 3a, which was diastereomerically pure following recrystallization (from EtOH, mp 151-153°C; $[\alpha]^{25}_D$ -14.9°, c 1.00, MeOH). For the endo case, debenzylation unfortunately resulted in epimerization, such that a 2.4:1 mixture of 11b:11a was obtained. This was subjected to preparative HPLC (silica gel; Et₂O-hexane, 2:3), and pure 11b was converted to target 3b (from EtOH, mp 197-199°C, dec; $[\alpha]^{25}_D$ -43.5°, c 1.00, MeOH). Examination of 3a and 3b in the MES test revealed no difference in anticonvulsant potency relative to stereochemistry; the isomers were eight times more potent than topiramate (Table).

Table. Compounds and Their Anticonvulsant Activity



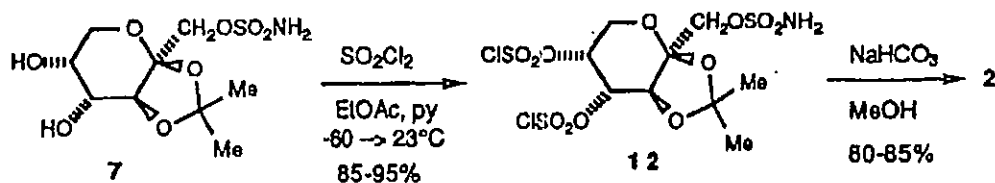
17-29



30, 31

compd ⁸	R	R'	mp, °C	dosage mg/kg, p.o.	MES test (mice, 4 h) % block (ED ₅₀)
2	H	H	139-141	10	80% (ED ₅₀ = 6.3 mg/kg)
3a/3b	H	H	148-151	10	70% (ED ₅₀ = 5.7 mg/kg)
3a	H	H	151-153	10	70% (ED ₅₀ = 6.3 mg/kg)
3b	H	H	197-199	10	70% (ED ₅₀ = 6.5 mg/kg)
17	H	Me	151-153	10	100% (ED ₅₀ = 7.4 mg/kg)
18	H	Et	glass	3; 35	0%; 100% (ED ₅₀ = 14.4 mg/kg)
19	H	Bu	111-113	10; 35	30%; 100%
20	H	allyl	75-77	1; 10	0%; 70%
21	H	octyl	syrup	10; 75	0%; 100%
22	H	Ph	foam	75; 300	0%; 0%
23	H	benzyl	foam	10; 75	0%; 100%
24	H	c-C ₃ H ₅	foam	1; 10	0%; 90% (ED ₅₀ = 7.3 mg/kg)
25	H	c-C ₄ H ₇	foam	1; 10	0%; 90%
26	H	cyclooctyl	foam	10; 75	0%; 30%
27	H	CH ₂ CF ₃	125-127	10; 75	40%; 90%
28	Me	Me	109-111	10; 35	70%; 100% (ED ₅₀ = 6.9 mg/kg)
29	Et	Et	foam	10; 75	10%; 100%
30 ²	H	Me		75; 200 ²	60%; 100%
31 ²	Me	Me		75; 200 ²	0%; 80%
topiramate (1) ²	H	H		60	60% (ED ₅₀ = 53.5 mg/kg)
phenytoin					ED ₅₀ = 6.4 mg/kg

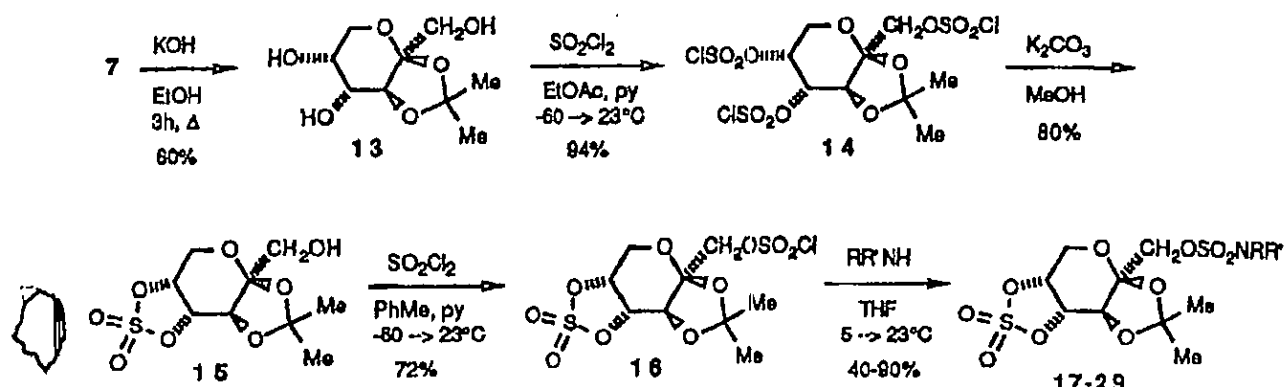
Conversion of 7 to bis-chlorosulfate 12,^{9,10} followed by dechlorosulfation furnished cyclic sulfate 2 (from 50% EtOH:H₂O, mp 139-141°C, dec; [α]_D²⁵ -27.7°, c 1.00, MeOH). MES testing of 2 showed it to be



about eight times more potent than topiramate (Table). Consequently, a series of N-substituted analogues was synthesized by preparing key chlorosulfate cyclic sulfate 16 from 7, as shown, and reacting it with various

amines. These compounds, 17-29, are listed in the Table along with their MES data.¹¹

Use of a 4,5-cyclic sulfate group in place of a 4,5-acetonide group substantially enhances anticonvulsant potency for the fructopyranose structural series (cf. 2 with 1, 17 with 30, and 28 with 31). Hence, robust activity is realized for cyclic sulfates that have alkyl substituents on nitrogen (17, 18, 20, 24, 25, and 28). Inspection of the monoalkylated analogues reveals that MES activity is optimal when the size of R' is relatively small. However, a cycloalkyl group confers better potency to moderately sized alkyl groups (cf. 25 and 19). Potency was lower for R' = CH₂CF₃ (cf. 27 and 18), and fell off dramatically for R' = Ph (22).



At 16 h in mice p.o., 2 (RWJ-37947) had an ED₅₀ of 41.5 mg/kg, compared to an ED₅₀ of 720 mg/kg for 1, showing 2 to be a potent anticonvulsant with a long duration of action. Compound 2 also shows an excellent therapeutic index relative to neurotoxicity in the rotarod test: ED₅₀/TD₅₀ >150.

¹H NMR spectral data for 2 are consistent with a skew conformation (³S₀) for the pyranose ring, as was reported for topiramate.² This conformation is also observed for 2 in the solid state by X-ray crystallography.⁴

Acknowledgment. We thank Samuel Nortey and Delene Mitchell for technical assistance.

References and Notes

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- Maryanoff, B. E.; Costanzo, M. J.; Nortey, S. O.; Shank, R. P.; Schupsky, J. J.; Ortegón, M. E.; Vaught, J. L., unpublished results, manuscript in preparation.
- The S=O stereochemistry was assigned by H-1 NMR chemical shifts. The 4,5-protons syn to the sulfinyl group are deshielded relative to the anti 4,5-protons. See: Wang, Y.; Hogenkamp, H. P. C. *Carbohydr. Res.* 1979, 76, 131-140; Pritchard, J. G.; Lauterbur, P. C. *J. Am. Chem. Soc.* 1961, 83, 2105-2110.
- The hydrolysis also produced the tetraol product (43%), resulting from removal of both acetonide groups.
- Binkley, R. W.; Hehmann, D. G. *J. Org. Chem.* 1990, 55, 378-380.
- All new compounds, except 10, were purified by chromatography on silica gel; all target sulfamates were characterized by H-1 NMR, C-13 NMR, MS, and elemental analysis (C, H, N, S).
- Martin, O. R.; Korppi-Tommola, S.-L.; Szarek, W. A. *Can. J. Chem.* 1982, 60, 1857-1862.
- We could also prepare 2 from 3 by use of NaIO₄/RuCl₃ (Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* 1988, 110, 7538-7539); however, we tend to favor the direct condensation method.
- ED₅₀ values are reported for reference drugs and several noteworthy test compounds. Data for other test compounds are reported as % block, with high and low doses to afford a sense of relative potency.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020/
Bogotá, D.C.,

Doctor
Ian Raisbeck
Apoderado

Oficio N° 6926

REFERENCIA

Radicación	02 108566
Trámite	002
Evento	001
Actuación	430
Folios	014

Como resultado del examen de patentabilidad de la solicitud de patente de invención N° 02 108566, realizado según el artículo 45 de la decisión 486 de la comisión de la Comunidad Andina, se notifica:

Documentos estudiados:

- . Descripción: folios 70-91
- . Ejemplos: folios 91-93
- . Capítulo reivindicatorio estudiado: folios 94-99
- . Se reivindica prioridad, presentada en Alemania el 31.05.00, bajo el número 10027887.6

Objeto de la invención

Según las reivindicaciones 1 a 28 de la presente solicitud, se refiere a sulfamatos que actúan como profármacos o sustancias portadoras de diferentes principios activos que presentan un alto efecto de primer paso, mejorando su administración por vía oral.

Clasificación internacional de patentes A 61 K 47/00; 47/20

Claridad de la solicitud

El art. 30 de la decisión 486 contempla que *“Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción”*

La reivindicación 1 es tan general que puede abarcar un sinnúmero de compuestos que comprendan el grupo –SO₂NR₁R₂, incluyendo aquellos que no se encuentran revelados en la descripción.

20%
207

Los términos “acilo”, “alquilo”, “cicloalquilo”, “arilo” de la reivindicación 1 son muy generales y puede abarcar compuestos que no se encuentran revelados en la descripción.

Las reivindicaciones 2-5 caracterizan a un compuesto por el tipo de absorción que éste tiene, lo cual no es claro. Se le recuerda al solicitante que un compuesto debe caracterizarse por su fórmula química específica para que se diferencie de aquellos que se encuentran en el estado de la técnica.

Parece que las reivindicaciones 6-7 ampliarán la materia de la reivindicación 1, puesto que incluye la presencia de un “separador”.

Las reivindicaciones 9, 11 caracterizan a un compuesto por un efecto fisiológico, lo cual no es claro ni diferencia de manera clara la materia que se desea proteger.

Los términos “alquilo”, “acilo”, “alquileno”, “arileno”, “alquilarileno”, “alquilenarilenoalquileno” de la reivindicación 14 son muy amplios y pueden incluir compuestos que no se encuentran revelados en la descripción.

Los términos usados en la reivindicación 20 caracterizan a un principio activo por su actividad farmacológica, lo cual es muy amplio y puede llegar a incluir compuestos que no se revelan en la descripción.

Favor hacer las aclaraciones y correcciones del caso

Excepciones a la patentabilidad

El artículo 14 de la decisión 486 de la comisión de la comunidad andina establece que: *"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"*

El contenido de las reivindicaciones 27-28, que trata de “Uso de un compuesto...”, no es patentable, debido a que a legislación vigente no contempla los usos como objeto de patente.

Determinación del estado de la técnica:

Consultadas las fuentes de información con que cuenta este despacho, se encontraron los siguientes documentos, anteriores a la fecha de prioridad de la presente solicitud - 31.05.00-, relacionados con la materia de la presente solicitud:

Nº	Documento	Título	Fecha de publicación
1		“Influencia de de la administración subcrónica de estradiol, etinilestradiol y sulfamato de estradiol en el flujo de bilis, excreción ácida de bilisy el estado del glutatión biliar y hepático en ratas”	1997
2		“Sulfamatos de varios estrógenos son prodrogas con estrogenicidad sistémica aumentada y estrogenicidad hepática disminuida en aplicación oral”	1995
3	WO 9714712	“Derivados de sulfamato de 1,3,5(10) derivados de estratieno, métodos de producirlos y compuestos que los contienen”	27.04.97
4	WO 9305064	“Esteroides inhibidores de sulfatasa”	18.03.93

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209

5	WO 9605216	"Derivados de estra-1,3,5,(10) trieno. Métodos para preparar tales compuestos t composiciones farmacéuticas que los contienen"	22.02.96
6	US 4792569	"Sulfamatos de anticonvulsivantes de fenetilo"	20.12.88
7	US 5025031	"Sulfamatos de aril y ariloxialquil esterres útiles como anticonvulsivantes"	18.06.91
8	WO 9414827	"Sulfamatos de alticonvulsivantes de sorbopiranososa"	07.07.94
9	WO 9426702	"Ésteres (o tioesteres) de n-acil ácido sulfámico, n-acil sulfonamidas y ésteres (o tioesteres) del ácido n-sulfonil carbámico como agentes hipocolesterolémicos"	24.11.94
10		"Sulfamatos de azúcar de anticonvulsivantes. Sulfatos cíclicos potentes y análogos de sulfito cíclico de topiramato"	1993
11	DE 1203042	"Medios fungicidas"	14.10.65
12	US 4512006	"Derivados de sulfamatos de anticonvulsivantes"	23.04.85

Evaluación de los requisitos de patentabilidad:

El artículo 14 de la decisión 486 de la comisión de la comunidad andina establece que: *"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 del artículo 14 de la decisión 486 de la comisión de la comunidad andina, 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 artículo 16 de la decisión 486 establece que *"Una invención se considerará nueva cuando no está comprendida en el estado de la técnica"*

Los documentos WO 9714712, WO 9305064, WO 9605216, US 4792569, US 5025031, WO 9414827, WO9426702, DE 1203042, US 4513006, así como los artículos "Influencia de de la administración subcrónica de estradiol, etinilestradiol y sulfamato de estradiol en el flujo de bilis, excreción ácida de bilis y el estado del glutatión biliar y hepático en ratas", "Sulfamatos de varios estrógenos son prodrogas con estrogenicidad sistémica aumentada y estrogenicidad hepática disminuida en aplicación oral" y "Sulfamatos de azúcar de anticonvulsivantes. Sulfatos cíclicos potentes y análogos de sulfito cíclico de topiramato", revelan compuestos que cumplen con la definición dada por la presente solicitud, es decir compuestos que contienen el grupo -SO₂NR₁R₂ acompañando a un principio activo, lo cual puede afectar la novedad de la presente solicitud.

Evaluación del NIVEL INVENTIVO

El artículo 18 de la decisión 486 establece que *"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"*

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El artículo *“Sulfamatos de varios estrógenos son prodrogas con estrogenicidad sistémica aumentada y estrogenicidad hepática disminuida en aplicación oral”* enseña que los sulfamatos de estrógenos presentan una absorción sistémica aumentada gracias a la acción del grupo sulfamato dentro de la molécula.

Teniendo en cuenta el objeto de la solicitud, el cual es proveer sulfamatos de compuestos activos que presentan una absorción sistémica aumentada, es una derivación evidente del estado de la técnica ya que una persona versada en la materia utilizaría las enseñanzas del artículo citado y, junto con sus conocimientos usuales, fabricaría los compuestos que se pretenden proteger, sabiendo de antemano que la absorción oral se vería aumentada.

En conclusión los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

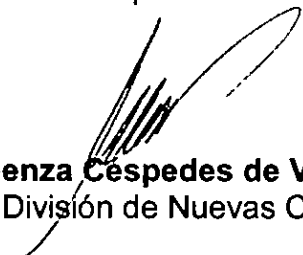
Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
1	"Influencia de de la administración subcrónica de estradiol, etinilestradiol y sulfamato de estradiol en el flujo de bilis, excreción ácida de bilisy el estado del glutatión biliar y hepático en ratas"	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
2	"Sulfamatos de varios estrógenos son prodrogas con estrogenicidad sistémica aumentada y estrogenicidad hepática disminuida en aplicación oral"	1-8, 11-13, 20. 23-25	Novedad
		1-26	Novedad
3	WO 9714712	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
4	WO 9305064	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
5	WO9605216	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
6	US 4792569	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
7	US 5025031	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
8	WO 9414827	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
9	WO 9426702	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
10	"Sulfamatos de azúcar de anticonvulsivantes. Sulfatos cíclicos potentes y análogos de sulfito cíclico de topiramato"	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo

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11	DE 1203042	1-8, 11-13, 20. 23-25	Novedad
		1-26	Nivel Inventivo
12	US 4513006	1-8, 11-13, 20. 23-25	Novedad
		1-26	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 el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo 5 del título primero de la Circular Única N° 10 de 2001


Álix Carmenza Céspedes de Vergel
Jefe de la División de Nuevas Creaciones

08 JUN 2007

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

CONCEPTO TÉCNICO Número 0803	EXAMINADOR TÉCNICO Código 202024
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WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro
INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE
INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation ⁶ : C07J 41/00, A61K 31/565		A2	(11) Internationale Veröffentlichungsnummer: WO 97/14712
		(43) Internationales Veröffentlichungsdatum:	24. April 1997 (24.04.97)
(21) Internationales Aktenzeichen: PCT/DE96/01823		(81) Bestimmungsstaaten: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO Patent (KE, LS, MW, SD, SZ, UG), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) Internationales Anmeldedatum: 19. September 1996 (19.09.96)		Veröffentlicht <i>Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.</i>	
(30) Prioritätsdaten: 195 40 233.2 19. Oktober 1995 (19.10.95) DE			
(71) Anmelder: JENAPHARM GMBH [DE/DE]; Otto-Schott-Strasse 15, D-07745 Jena (DE).			
(72) Erfinder: SCHWARZ, Sigfrid; Ottogerd-Mühlmann-Strasse 17, D-07743 Jena (DE). ELGER, Walter; Schorlemmerallee 12b, D-14195 Berlin (DE). REDDERSEN, Gudrun; Hügelstrasse 25, D-07749 Jena (DE). SCHNEIDER, Birgitt; Damaschkeweg 19, D-07745 Jena (DE). THIEME, Ina; B. Siedlung 12, D-07616 Graitzschen (DE). RICHTER, Margit; Boegeholdstrasse 19, D-07745 Jena (DE).			
(74) Anwalt: WABLAT, Wolfgang; Potsdamer Chaussee 48, D-14129 Berlin (DE).			
(54) Title: SULPHAMATE DERIVATIVES OF 1,3,5(10)-ESTRADIENE DERIVATIVES, METHODS OF PRODUCING THEM AND PHARMACEUTICAL COMPOUNDS CONTAINING THEM			
(54) Bezeichnung: SULFAMAT-DERIVATE VON 1,3,5(10)-ESTRADIEN-DERIVATEN, VERFAHREN ZU IHRER HERSTELLUNG UND DIESE VERBINDUNGEN ENTHALTENDE PHARMAZEUTISCHE ZUSAMMENSETZUNGEN			
(57) Abstract			
The invention relates to novel sulphamate derivatives of 1,3,5(10)-estradiene derivatives of general formula (I) in which the 3-sulphamate group is acylated, sulphonated or amido-sulphonated. Also disclosed are methods of producing these compounds and pharmaceutical compounds containing them. The claimed compounds have estrogen-like properties.		(I)	
(57) Zusammenfassung Die Erfindung betrifft neue Sulfamat-Derivate von 1,3,5(10)-Estradien-Derivaten der allgemeinen Formel (I), bei denen der 3-Sulfamatrest acyliert, sulfoniert oder amidosulfoniert ist. Ferner werden Verfahren zur Herstellung der Verbindungen und diese Verbindungen enthaltende pharmazeutische Zusammensetzungen beschrieben. Die erfindungsgemäßen Verbindungen sind estrogen wirksam.			

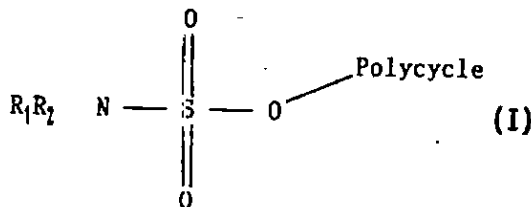


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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07J 41/00, A61K 31/565	A1	(11) International Publication Number: WO 93/05064 (43) International Publication Date: 18 March 1993 (18.03.93)
(21) International Application Number: PCT/GB92/01587 (22) International Filing Date: 28 August 1992 (28.08.92) (30) Priority data: 9118478.8 29 August 1991 (29.08.91) GB (71) Applicant (for all designated States except US): IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE [GB/GB]; Sherfield Building, London SW7 2AZ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : REED, Michael, John [GB/GB]; 42 Wimborne Gardens, London W13 8BZ (GB). POTTER, Barry, Victor, Lloyd [GB/GB]; 95 Dovers Park, Bathford, Bath, Avon BA1 7UE (GB).	(74) Agents: LAMBERT, Hugh, Richmond et al.; D. Young & Co., 10 Staple Inn, London WC1V 7RD (GB). (81) Designated States: AU, BB, BG, BR, CA, CS, FL, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published <i>With international search report.</i>	

(54) Title: STEROID SULPHATASE INHIBITORS



(57) Abstract

Novel steroid sulphatase inhibitors are disclosed as well as pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. The novel steroid sulphatase inhibitors are: sulphamate esters of formula (I), where R₁ and R₂ are each H, alkyl, alkenyl, cycloalkyl or aryl, or together represent an alkylene group optionally containing a heteroatom e.g. -O- or -NH-; and -O-polycycle represents the residue of a polycyclic alcohol, preferably a sterol, most preferably a 3-sterol. Preferred compounds are oestrone-3-sulphamate and N,N-dimethyl oestrone-3-sulphamate.

PCT
WELTORGANISATION FÜR GE
Internationales I
INTERNATIONALE ANMELDUNG VERÖFFENTL.
INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)



WO 9605216A1

(51) Internationale Patentklassifikation 6 : C07J 41/00, A61K 31/56, C07J 53/00, 43/00	A1	(11) Internationale Veröffentlichungsnummer: WO 96/05216 (43) Internationales Veröffentlichungsdatum: 22. Februar 1996 (22.02.96)
(21) Internationales Aktenzeichen: PCT/DE95/00877 (22) Internationales Anmeldedatum: 3. Juli 1995 (03.07.95) (30) Prioritätsdaten: P 44 29 397.6 9. August 1994 (09.08.94) DE (71) Anmelder (für alle Bestimmungsstaaten ausser US): JE- NAPHARM GMBH [DE/DE]; Otto-Schott-Strasse 15, D-07745 Jena (DE). (71) Anmelder (nur für US): SIEMANN, Christel (Erbin des verstorbenen Erfinders) [DE/DE]; Fritz-Ritter-Strasse 6, D- 07747 Jena (DE). (72) Erfinder: SIEMANN, Hans-Joachim (verstorben). (72) Erfinder; und (75) Erfinder/Anmelder (nur für US): SCHWARZ, Sigfrid [DE/DE]; Ottogerd-Mühlmann-Strasse 17, D-07743 Jena (DE). ELGER, Walter [DE/DE]; Schorlemmerallee 12b, D-14195 Berlin (DE). REDDERSEN, Gudrun [DE/DE]; Hügelstrasse 25, D-07749 Jena (DE). SCHNEIDER, Birgitt [DE/DE]; Damaschkeweg 19, D-07745 Jena (DE).	(74) Anwalt: WABLAT, Wolfgang; Potsdamer Chaussee 48, D- 14129 Berlin (DE). (81) Bestimmungsstaaten: AU, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MN, NO, NZ, PL, RO, RU, SK, UA, US, UZ, VN, europäisches Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Veröffentlicht Mit internationalem Recherchenbericht.	
(54) Title: ESTRA-1,3,5(10)-TRIENE DERIVATIVES, METHODS OF PREPARING SUCH COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM		
(54) Bezeichnung: ESTRA-1,3,5(10)-TRIEN-DERIVATE, VERFAHREN ZU IHRER HERSTELLUNG UND DIESE VERBINDUNGEN ENTHALTENDE PHARMAZEUTISCHE ZUSAMMENSETZUNGEN		
(57) Abstract		
The invention concerns novel estra-1,3,5(10)-triene amidosulphamates with an R-SO₂-O- group in the 3-position, R being an R¹R²N-group in which R¹ and R², independently of each other, are a hydrogen atom, an alkyl group with 1 to 5 C-atoms or, together with the N-atom, a polymethyleneimino group with 4 to 6 C-atoms or a morpholino group. The compounds described are suitable for use in hormonal contraception and in climacteric hormone-replacement therapy (HRT), as well as in the treatment of gynaecological and andrological conditions. The compounds described thus only have a low hepatic oestrogenicity. Also described are methods of preparing the compounds described, plus the preparation of pharmaceutical compositions containing them.		
(57) Zusammenfassung		
Die Erfindung betrifft neue Estra-1,3,5(10)-trien-Amidosulfamate, welche an der 3-Position eine R-SO₂-O-Gruppe tragen, wobei R eine R¹R²N-Gruppe ist, worin R¹ und R² unabhängig voneinander ein Wasserstoffatom, einen Alkylrest mit 1-5 C-Atomen oder zusammen mit dem N-Atom einen Polymethyleniminorest mit 4 bis 6 C-Atomen oder einen Morpholinorest bedeuten. Die erfindungsgemäßen Verbindungen sind zur hormonalen Kontrazeption und in der klimakterischen Hormon-Replacement-Therapie (HRT) sowie zur Behandlung gynäkologischer und andrologischer Krankheitsbilder geeignet. Daher weisen die erfindungsgemäßen Verbindungen nur eine geringe hepatische Estrogenität auf. Ferner werden Verfahren zur Herstellung der erfindungsgemäßen Verbindungen beschrieben, sowie zur Herstellung pharmazeutischer Zusammensetzungen.		

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United States Patent [19]
Maryanoff et al.

[11] Patent Number: 4,792,569
[45] Date of Patent: Dec. 20, 1988

[54] ANTICONVULSANT PHENETHYL
SULFAMATES

[75] Inventors: Bruce E. Maryanoff, New Hope;
Samuel O. Nortey, Lamott, both of
Pa.

[73] Assignee: McNellab, Inc., Spring House, Pa.

[21] Appl. No.: 89,880

[22] Filed: Aug. 27, 1987

[51] Int. Cl.⁴ A61K 31/255; C07C 143/68

[52] U.S. Cl. 514/517; 558/48;
558/53

[58] Field of Search 558/48; 514/517

[56] References Cited

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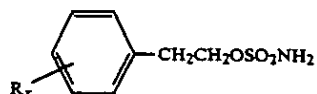
J. Med. Chem. 1987, 30, 880-887, B. E. Maryanoff et al.

Primary Examiner—Nicky Chan

Attorney, Agent, or Firm—David J. Levy

[57] ABSTRACT

Sulfamates of the following formula (I) are anticonvul-
sants and are useful in the treatment of epilepsy:



(I)

wherein

R is one or more substituents such as alkyl, halo, alkoxy,
CH₃ or nitro; and
x is 0-3.

18 Claims, No Drawings

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244

United States Patent [19]

Lo et al.

[11] Patent Number: 5,025,031

[45] Date of Patent: Jun. 18, 1991

[54] ARYL AND ARYLOXYALKYL SULFAMATE ESTERS USEFUL AS ANTICONVULSANTS

[75] Inventors: Young S. Lo; David A. Walsh, both of Richmond; Ibrahim M. Uwaydah, Chesterfield, all of Va.

[73] Assignee: A. H. Robins Co., Inc., Richmond, Va.

[21] Appl. No.: 443,146

[22] Filed: Nov. 30, 1989

[51] Int. Cl.³ A61K 31/415; A61K 31/255

[52] U.S. Cl. 514/399; 514/517; 514/397; 514/383; 548/336; 558/47; 558/48

[58] Field of Search 548/265.8, 346, 336; 514/383, 397, 399, 517; 558/47, 48

[56] References Cited

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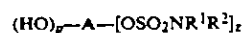
1554976 12/1968 France 558/47

Primary Examiner—Mary C. Lee

Assistant Examiner—Lenora Miltenberger

[57] ABSTRACT

Herein disclosed is a method of treating convulsions with a pharmaceutical composition containing a compound of the formula:



where A is an aryl, arylalkyl, or aryloxyalkyl group and is substituted on 1 or more carbon atoms with a sulfamate group ($-OSO_2NR^1R^2$) wherein R^1 and R^2 , same or different, are hydrogen or loweralkyl wherein p is 0 or 1 and is the number of untreated hydroxyl groups and z is 1 or 2 and is the number of $-OS(O_2)NR^1R^2$ groups. Aryl is selected from phenyl, substituted phenyl, pyridinyl, naphthyl, quinolinyl, and the like. Phenyl substituents are selected from hydrogen, halo, hydroxy, phenyl, phenoxy, benzoyl, loweralkyl, loweralkoxy, carboxy, amino, loweralkylamino, diloweralkylamino, acetamido, cyano, nitro, loweralkoxy-carbonyl, aminosulfonyl, imidazolyl, triazolyl, and the like. Novel compounds not previously disclosed are also described.

14 Claims, No Drawings

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau216
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07H 9/04, 11/00, A61K 31/70	A1	(11) International Publication Number: WO 94/14827 (43) International Publication Date: 7 July 1994 (07.07.94)
(21) International Application Number: PCT/US93/11983 (22) International Filing Date: 7 December 1993 (07.12.93) (30) Priority Data: 07/994,735 22 December 1992 (22.12.92) US (71) Applicant: McNEILAB, INC. (US/US); McKean and Welsh Roads, Spring House, PA 19477-0776 (US). (72) Inventors: COSTANZO, Michael, J.; 14 Breckenridge Drive, Ivyland, PA 18974 (US). MARYANOFF, Bruce, E.; 3204 Aquetong Road, New Hope, PA 18938 (US). (74) Agents: CLAMPORCERO, Audley, A., Jr. et al.; Johnson and Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).	(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.	
(54) Title: ANTICONVULSANT SORBOPYRANOSE SULFAMATES (I) (57) Abstract Sulfamate derivatives having formula (I), wherein R₁, R₂, R₃, R₄, R₅ and R₆ are as herein defined, have been found to exhibit anticonvulsant activity and are thus useful in the treatment of conditions such as epilepsy. Further, the present invention encompasses pharmaceutical compositions containing a compound of formula (I) as well as methods for their use and novel intermediates are disclosed.		

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau217
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 307/02, 311/51, A61K 31/18, 31/325		A1	(11) International Publication Number: WO 94/26702 (43) International Publication Date: 24 November 1994 (24.11.94)
(21) International Application Number: PCT/US94/05233 (22) International Filing Date: 11 May 1994 (11.05.94) (30) Priority Data: 08/062,515 14 May 1993 (14.05.93) US 08/223,932 13 April 1994 (13.04.94) US (71) Applicant: WARNER-LAMBERT COMPANY [US/US]; 2800 Plymouth Road, Ann Arbor, MI 48105 (US). (72) Inventors: LEE, Helen, Tsenwhci; 3625 Fox Hunt, Ann Arbor, MI 48105 (US). PICARD, Joseph, Armand; 5764 Princeton Place, Ypsilanti, MI 48197 (US). SLISKOVIC, Drago, Robert; 4860 Cole Boulevard, Ypsilanti, MI 48197 (US). WIERENGA, Wendell; 1968 Woodlily Court, Ann Arbor, MI 48103 (US). (74) Agents: ANDERSON, Elizabeth, M.; Warner-Lambert Com- pany, 2800 Plymouth Road, Ann Arbor, MI 48105 (US) et al.			(81) Designated States: AU, CA, CZ, FI, HU, JP, KR, NO, NZ, RU, SK, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.
(54) Title: N-ACYL SULFAMIC ACID ESTERS (OR THIOESTERS), N-ACYL SULFONAMIDES, AND N-SULFONYL CARBAMIC ACID ESTERS (OR THIOESTERS) AS HYPERCHOLESTEROLEMIC AGENTS			
(57) Abstract The present invention is directed to compounds useful for the regulation of cholesterol of formula (I), methods for using them and pharmaceutical compositions thereof. In Formula (I) X and Y are oxygen, sulfur, or (CR'R'')_n wherein n is 1 to 4; R is hydrogen, alkyl, or benzyl; R₁ and R₂ are phenyl, substituted phenyl, naphthyl, substituted naphthyl, an aralkyl group, an alkyl chain, adamantyl, or a cycloalkyl group.			
$\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{R}_1 - \text{X} - \text{S} - \text{N} - \text{C} - \text{Y} - \text{R}_2 \\ \parallel \quad \\ \text{O} \quad \text{R} \end{array} \quad (\text{I})$			

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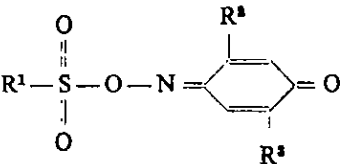
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Deutsche Kl.: 451 - 9/16

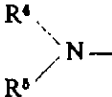
Nummer: 1 203 042
Aktenzeichen: Sch 34196 IV a/451
Anmeldetag: 21. November 1963
Auslegungstag: 14. Oktober 1965

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Zu den bekanntesten Mitteln mit fungizider Wirkung, die in der Landwirtschaft Verwendung finden, gehört das Kupferoxychlorid.
Es wurde nun gefunden, daß Verbindungen der allgemeinen Formel



in der R¹ Methyl, gegebenenfalls durch Methyl, Chlor oder Carboxy substituiertes Phenyl, Naphthyl oder die Gruppe



in der R⁴ und R⁵ gleich oder verschieden sein können und niedere Alkyle darstellen oder gemeinsam mit dem Stickstoffatom einen gegebenenfalls weitere Heteroatome enthaltenden Ring bilden, und R² und R³ gleich oder verschieden sein können und niedere Alkyle bedeuten, sehr wirksame Fungizide darstellen und eine bessere Wirkung als Kupferoxychlorid aufweisen.

Aus der deutschen Patentschrift 456 848 ist zwar bereits die Anwendung von Nitrosophenolsalzen zur Bekämpfung schädlicher Pilze und aus der deutschen Patentschrift 1 025 204 die Anwendung von Nitrosophenolen oder deren Salzen zur Bekämpfung von Viren in lebenden Pflanzen und deren Organen

Fungizide Mittel

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bekanntgeworden. Vergleichsversuche, zu denen verschiedene Nitrosophenole — im folgenden als 1,4-Benzochinon-1-oxime bezeichnet — herangezogen wurden, zeigten jedoch, daß die beanspruchten Mittel diesen gegenüber ebenfalls eine weitaus stärkere fungizide Wirkung besitzen und die fungiziden Eigenschaften außerdem überraschenderweise zum Teil sogar systemischer Natur sind.

Die Herstellung der in den erfindungsgemäßen Mitteln enthaltenen Verbindungen erfolgt nach an sich bekannten Methoden, z. B. durch Umsetzung der entsprechenden Sulfon- bzw. Amidosulfonsäurehalogenide mit einem Alkali-, Ammonium- oder Aminsatz des entsprechenden 2,5-Dialkyl-1,4-benzochinon-1-oxims in Wasser oder einem indifferenten organischen Lösungsmittel.

Die folgende Tabelle enthält die physikalischen Konstanten einiger dieser Verbindungen, die in der Literatur bis auf zwei Ausnahmen bisher noch nicht beschrieben wurden.

Nr.	Verbindung	Physikalische Konstante
1	Benzolsulfonsäure-(2,5-dimethyl-1,4-benzochinon-1-oxim)-ester	F. = 109 bis 110°C
2	Benzolsulfonsäure-(1,4-thymochinon-1-oxim)-ester	F. = 79 bis 80°C
3	Benzolsulfonsäure-(1,4-thymochinon-4-oxim)-ester	F. = 61 bis 62°C
4	Benzolsulfonsäure-(2-methyl-5-tert.-butyl-1,4-benzochinon-1-oxim)-ester	F. = 67 bis 68°C
5	p-Toluolsulfonsäure-(2,5-dimethyl-1,4-benzochinon-1-oxim)-ester	F. = 106 bis 107°C
6	p-Toluolsulfonsäure-(1,4-thymochinon-1-oxim)-ester	F. = 85,5 bis 86,5°C
7	p-Toluolsulfonsäure-(1,4-thymochinon-4-oxim)-ester	F. = 89 bis 90°C
8	p-Toluolsulfonsäure-(2-methyl-5-tert.-butyl-1,4-benzochinon-1-oxim)-ester	F. = 95 bis 97°C
9	p-Chlorbenzolsulfonsäure-(2,5-dimethyl-1,4-benzochinon-1-oxim)-ester	F. = 131 bis 131,5°C
10	p-Chlorbenzolsulfonsäure-(1,4-thymochinon-1-oxim)-ester	F. = 105 bis 105,5°C
11	p-Chlorbenzolsulfonsäure-(2-methyl-5-tert.-butyl-1,4-benzochinon-1-oxim)-ester	F. = 135 bis 136°C

United States Patent [19]
Maryanoff et al.

[11] **Patent Number:** **4,513,006**
[45] **Date of Patent:** **Apr. 23, 1985**

[54] **ANTICONVULSANT SULFAMATE
DERIVATIVES**

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Pa.
- [21] **Appl. No.:** **535,475**
- [22] **Filed:** **Sep. 26, 1983**
- [51] **Int. Cl.³** **A61K 31/35; C07D 311/78;**
C07D 311/94; C07D 309/06
- [52] **U.S. Cl.** **514/23; 549/426;**
549/396; 549/387; 549/338; 549/337; 549/336;
549/427; 536/54; 260/456 A; 514/459; 514/517
- [58] **Field of Search** **549/387, 396, 426, 337,**
549/338; 260/456 A; 424/283, 303; 536/54

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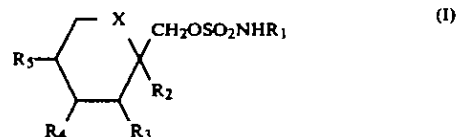
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Primary Examiner—Nicky Chan
Attorney, Agent, or Firm—David J. Levy

[57] **ABSTRACT**

Sulfamates of the following formula (I):



wherein X is O or CH₂ and R₁, R₂, R₃, R₄ and R₅ are as
herein defined have been found to exhibit anticonvul-
sant activity and are thus useful in the treatment of
conditions such as epilepsy. Further, pharmaceutical
compositions containing a compound of formula (I) as
well as methods for their use and intermediates form
part of the present invention.

12 Claims, No Drawings

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EXAMEN TÉCNICO DE FONDO			
Nº Radicación	07-108566	Fecha de presentación	29.11.02
Nº Prioridad	DE 1007887	Fecha de Prioridad	31.05.00
Palabras clave			
Sulfamato			
antagónico			

CLASIFICACIONES INTERNACIONALES DE BÚSQUEDA			
A61K 47/00			

DOCUMENTOS CORRESPONDIENTES EN OTROS PAÍSES			
50 01 9177			
Patente Original			

BASES DE DATOS CONSULTADAS			

INFORMES DE EXÁMENES EN OTRAS OFICINAS		
Oficina	Fecha	Reiv. afectadas

ANTERIORIDADES ENCONTRADAS		
Documento	Relevancia	Reiv. afectadas
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Observaciones	Act 46

Fecha del examen	07.02.02 ; 29.05.02
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