binding of the two salts: the zinc sulfate is fixed to a greater extent than is the zinc pantothenate (17 *versus* 8%).

The efficient distribution and diffusion of zinc pantothenate in the skin and the fur, as well as its less important retention in the liver, would suggest a therapeutic use in the treatment of disorders of the skin and scalp.

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Epiandrosterone- and Dehydroepiandrosterone-3β-alkanesulfonates as Inhibitors of Mouse Glucose-6-phosphate Dehydrogenase Activity

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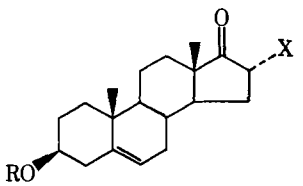
Abstract □ 3β-Alkanesulfonates of dehydroepiandrosterone, 16α-bromodehydroepiandrosterone, epiandrosterone, and 16α-bromoepiandrosterone were prepared in 54–95% yield *via* the reaction of long chain alkanesulfonyl chlorides with the corresponding dehydroepiandrosterone, and epiandrosterone analogues. These compounds inhibit mouse glucose-6-phosphate dehydrogenase activity.

Keyphrases □ 3β-Alkanesulfonates—synthesis, screened as glucose-6-phosphate dehydrogenase inhibitors □ Long-chain alkanesulfonyl chlorides—synthesis, antitumor agents

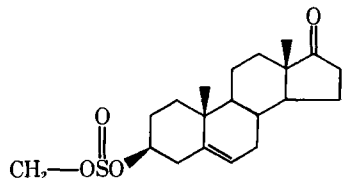
In a series of clinical and epidemiological studies several investigators have found that women with subnormal plasma concentrations of the adrenal steroid dehydroepiandrosterone (Ia) have a high risk of developing breast cancer (1–3). We have found that long-term treatment of various mouse strains with Ia inhibits the development of spontaneous breast cancer (4) and chemically induced lung (5) and colon tumors (6). It also blocks the 12-*O*-tetradecanoylphorbol-13-acetate (tumor promoter) stimulation of DNA synthesis in mouse epidermis (7) and inhibits the rate of [^{3}H]thymidine incorporation in various cell lines in culture (4).

Compound Ia is a potent noncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH) (8, 9). Inhibition of this enzyme and the pentose-phosphate shunt, a major source of extramitochondrial NADPH, may account for the reduction in DNA synthesis rate by the steroid (10). Dehydroepiandrosterone in human plasma is largely sulfated. According to Oertel and Hoppe-Seyler, the predominant sulfated form of Ia in human plasma is not dehydroepiandrosterone sulfate (Ib), but an ester of Ib and a diglyceride, known as dehydroepiandrosterone sulfatide (II) (11). The sulfatide was found to be a more potent inhibitor of G6PDH than Ia (9).

We have observed that II is more effective than Ia in inhibiting mouse epidermal G6PDH as well as in blocking 12-*O*-tetradecanoylphorbol-13-acetate stimulation of DNA synthesis in mouse epidermis (7). On the other hand II is a labile material and decomposes readily within a few days, even



Ia, R = H, X = H
Ib, R = SO_3H
Ic, R = H, X = Br
Id, R = $SO_2(CH_2)_7CH_3$, X = H
Ie, R = $SO_2(CH_2)_7CH_3$, X = Br
If, R = $SO_2(CH_2)_{15}CH_3$, X = H
Ig, R = $SO_2(CH_2)_{15}CH_3$, X = Br



II

when stored at −5°C, to the sulfate Ib, resulting in loss of biological activity. Replacement of the sulfate functionality with sulfonate should lead to more stable analogues. In the search for stable and potent derivatives of II, we decided to incorporate the long chain alkanesulfonates into compounds which have already demonstrated a greater activity as inhibitors of glucose-6-phosphate dehydrogenase such as 16α-bromoepiandrosterone (7). Accordingly, 3β-alkanesulfonates of Ia, 16α-bromodehydroepiandrosterone (Ic), epiandrosterone (IIIa), and 16α-bromoepiandrosterone (IIIb) were prepared.

RESULTS AND DISCUSSION

Chemistry—3β-Alkanesulfonates of dehydroepiandrosterone and epiandrosterone (I and V) were synthesized by the reaction of a long chain alkanesulfonyl chloride IVa–e with the corresponding dehydroepiandrosterone or epiandrosterone analogue in the presence of dry triethylamine (Scheme I).

Long-chain alkanesulfonyl chlorides IVa–e were prepared in 60–85% yields from the corresponding fatty alcohol *via* a modification of the King and Aslam procedure (12) in which the hydroxyl group was replaced by the isothiuronium functionality and then the sulfur moiety was oxidized with chlorine in aqueous medium (Scheme II).

Biological Activity—The compounds listed in Table I were evaluated for their abilities to inhibit mouse epidermal glucose-6-phosphate dehydrogenase (Table II). Among the 3β-alkanesulfonates, compound Vf and its 16α-bromo analogue (Vg), both with the hexadecanesulfonyl side chain attached to the 3β-position of the epiandrosterone molecule, are the most potent inhibitors.

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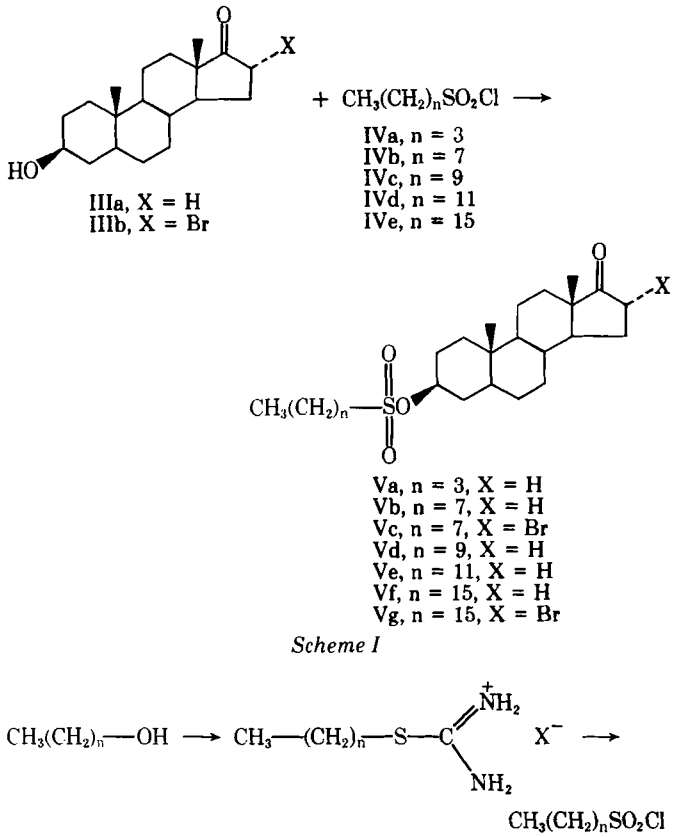

Scheme I

IVa–e (X = halogen)

Scheme II

Compound Vg is twice as active as the parent steroid Ia, showing 88% inhibition compared with the 46% inhibition produced by Ia.

Inhibition of this enzyme will consequently inhibit the pentose-phosphate shunt which generates ribose-phosphate and the bulk of extramitochondrial reduced nicotinamide adenine diphosphate (NADPH) (13), both of which are necessary for deoxyribonucleotide and fatty acid syntheses.

EXPERIMENTAL SECTION

Chemical Syntheses—All melting points were determined on apparatus[1] and are uncorrected. IR spectra were recorded on a spectrophotometer[2]. Elemental analyses were performed by Galbraith Laboratories.

Dehydroepiandrosterone Sulfatide (II)—This compound was prepared in 68% yield from the reaction of Ia-silver sulfate with dipalmitoyl-L-2-iodopropylene glycol following a reported procedure (14).

Alkanesulfonyl Chlorides IVa–d—These compounds were prepared from long-chain fatty alcohols following reported procedures (7). Dodecansulfonyl chloride IVe was similarly prepared in 75% yield, bp 126–130°C at 4 mm Hg.

Anal.—Calc. for $C_{12}H_{25}ClO_2S$: C, 53.60; H, 9.27; Cl 13.18; S, 11.92. Found: C, 53.75; H, 9.42; Cl, 12.90; S, 11.83.

General Procedure for the Preparation of 3β-Alkanesulfonates I, V, and VI—A modified procedure of Tesser and Balvert-Geev (15) was used in which 0.05 mol of the steroid was dissolved in 25 mL of dichloromethane and stirred. To this solution was added 0.65 g of dry triethylamine. The solution was cooled in an ice bath for 15 min and 0.006 mol of the appropriately substituted alkanesulfonyl chloride was added over a period of 10 min. The mixture was stirred for an additional 5 min, then was washed four times with cold water, dried (sodium sulfate), and evaporated under reduced pressure. The white solid formed was recrystallized from a suitable solvent (Table I).

Glucose-6-phosphate Dehydrogenase Assay—Epidermal G6PDH was prepared by a modification of the procedure of Ziboh *et al.* (16). Male ICR mice (6–8 weeks old) in hair resting phase were shaved 2–3 d before use. Only those mice showing no hair regrowth were used. Mice were sacrificed and a depilatory[3] was applied to the shaved area. After 5 min, the depilatory was washed off, the skin was cut out, placed in 55°C water for 30 s and the epidermis was scraped off with a scalpel. The scrapings were frozen and thawed three times in liquid nitrogen and then homogenized in 10 volumes of 0.25 M sucrose. The homogenate was centrifuged at 105,000×*g* for 1 h at 0°C. The supernatant was concentrated to one-fifth of its original volume[4] and the concentrated cytosol was used in the G6PDH assay. Steroid inhibition of G6PDH activity was determined according to the procedure of Oertel and Rebelein (9). Steroids were dissolved in dioxane immediately before testing. The reaction medium consisted of 3 mL of 0.05 M triethanolamine–0.005 M EDTA buffer (pH 7.6), 0.01 M NADP[5] solution, and 0.02 mL of dioxane or steroid in dioxane. The reaction was run at 25°C and was initiated by the addition of 0.005 mL of a 0.03 M glucose-6-phosphate[5] solution. Activity was measured in minutes from changes in absorbance at 366 nm on a recording spectrophotometer[6]. The inhibition produced by the added steroid was expressed as percent of control value. (The inhibition values in Table II were obtained by subtracting the percent control activity from 100.)

[1] Thomas-Hoover Unimelt.
[2] Unicam SP-1000.
[3] Nair.
[4] Sephadex G-25.
[5] Sigma.
[6] Gilford 2400-S.

Table I—3β-Alkanesulfonates of Dehydroepiandrosterone and Epiandrosterone

Compound	mp, °C	Yield, %	IR ν_{max}·cm^{-1} (SO_3, CO)	Formula[e]
Va	132–133[a]	91	1370, 1155, 1735	$C_{23}H_{38}SO_4$[f]
Vb	52–53[b]	80	1350, 1140, 1730	$C_{27}H_{46}SO_4$
Id	97–98[c]	70	1170, 1357, 1747	$C_{27}H_{44}SO_4$
Vc	75–76[c]	54	1172, 1368, 1751	$C_{27}H_{46}BrSO_4$
Ie	145–146[c]	54	1168, 1359, 1751	$C_{27}H_{43}BrSO_4$
Vd	60–61[b]	82	1210, 1400, 1740	$C_{29}H_{50}SO_4$
Ve	33–35[a]	68	1135, 1365, 1735	$C_{31}H_{53}SO_4$
If	88–89[c]	65	1168, 1355, 1748	$C_{35}H_{59}SO_4$
Ig	118–120[c]	61	1162, 1360, 1745	$C_{35}H_{57}BrSO_4$
VI[g]	74–76[c]	71	1167, 1367, 1740	$C_{35}H_{59}SO_5$
Vf	45–48[d]	95	1160, 1375, 1745	$C_{35}H_{61}SO_4$
Vg	68–71[d]	78	1170, 1380, 1735	$C_{35}H_{61}BrSO_4$

[a] Recrystallized from ethanol-hexane. [b] Recrystallized from hexane-petroleum ether. [c] Recrystallized from $CHCl_3$-petroleum ether. [d] Recrystallized from petroleum ether. [e] Compounds Id–Ig, Va–Vg, and VI were analyzed for C, H, and S; in addition, Ie, Ig, Vc, and Vg were analyzed for Br. Unless otherwise indicated, all values were within ±0.4% of the theoretical value. [f] Calc. for Br, 12.49; found, 11.94. [g] The five-membered ring D is expanded to a six-membered ring lactone. The structure of VI is:

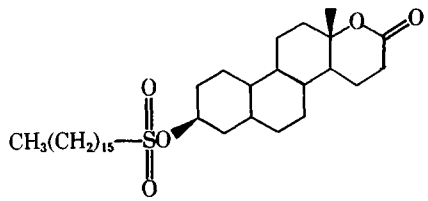

Table II—Inhibition of Mouse Epidermal G6PDH Activity by 3β-Alkanesulfonates of Dehydroepiandrosterone and Epiandrosterone[a]

Steroid	Concentration, M	Percent Inhibition[b]
Ia	10^{-5}	46 ± 2.4
Ia	10^{-7}	10 ± 1.7
II	10^{-5}	54 ± 3.7
II	10^{-7}	36 ± 2.6
Va	10^{-5}	18 ± 1.7
Vb	10^{-5}	23 ± 4.0
Id	10^{-5}	26 ± 4.4
Vc	10^{-5}	7 ± 1.5
Ie	10^{-5}	22 ± 3.0
Vd	10^{-5}	18 ± 1.4
Ve	10^{-5}	34 ± 2.8
If	10^{-5}	28 ± 2.1
Ig	10^{-5}	7 ± 0.7
VI	10^{-5}	12 ± 1.4
Vf	10^{-5}	58 ± 5.0
Vg	10^{-5}	88 ± 0.7

[a] Conditions are as described in the *Experimental Section*. [b] Values are averages of two or more replicates ± *SD*.

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Nalmefene: Radioimmunoassay for a New Opioid Antagonist

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Received December 5, 1983, from [¶]*Key Pharmaceuticals, Inc., Miami, FL 33169*, [*]*Innotech Laboratories, Inc., Ft. Lauderdale, FL 33314*, the [‡]*Institute for Clinical Pharmacology, St. James Hospital, Dublin, Ireland*, and [§]*Rockefeller University, New York, NY 10021.* Accepted for publication February 3, 1984.

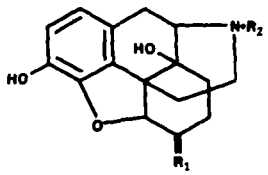
Abstract □ A specific radioimmunoassay (RIA) has been developed for the quantitation of a new opioid antagonist, nalmefene, in human plasma. The method employs a rabbit antiserum to an albumin conjugate of naltrexone-6-(*O*-carboxymethyl)oxime and [^{3}H]naltrexone as the radioligand. Assay specificity was achieved by extraction of nalmefene from plasma at pH 9 into ether prior to RIA. The procedure has a limit of sensitivity of 0.2 ng/mL of nalmefene using a 0.5-mL sample of plasma for analysis. The intra- and interassay coefficients of variation did not exceed 5.6 and 11%, respectively. The specificity of the RIA was established by demonstrating excellent agreement (r = 0.99) with a less sensitive and more time consuming HPLC procedure in the analysis of clinical plasma samples. The use of the RIA for the pharmacokinetic evaluation of nalmefene is illustrated with plasma concentration profiles of the drug in humans following intravenous and oral administration.

Keyphrases □ Nalmefene—human plasma, RIA, compared with HPLC □ RIA—compared with HPLC, nalmefene, human plasma

Nalmefene [17-(cyclopropylmethyl)-4,5α-epoxy-6-methylenemorphinan-3,14-diol] is a potent, orally active narcotic antagonist (1) which is undergoing clinical evaluation in humans. The drug is a structural analogue of naltrexone with an exocyclic methylene group in the 6-position. Hahn *et al.* (1) indicated in preliminary pharmacology studies that orally administered nalmefene was about 50 times more potent than naloxone in its ability to antagonize the antinociceptive activity of an ED_{95} dose of morphine in either the mouse hot-plate or tail-clip procedures. Furthermore, nalmefene was 16 times more potent than naloxone in precipitating narcotic withdrawal in morphine-dependent rhesus monkeys. Recently, nalmefene has been shown to decrease the food and water intake and weight gain in obese and lean Zucker rats, lending further support to a possible role for opioids in the control of food intake (2).

The present study was undertaken to develop a simple and specific radioimmunoassay (RIA) for nalmefene which would provide greater sensitivity and ease of operation than a recently reported high-performance liquid chromatographic (HPLC) procedure (3). It was hoped that the RIA might be utilized in a wide variety of human tolerance and pharmacokinetic studies that are under way with nalmefene.

The initial approach taken in the development of the RIA was to produce an antiserum which would cross-react almost equally well with nalmefene and naltrexone. In this way, high specific activity, commercially available [^{3}H]naltrexone could be used as the radioligand for the assay. Furthermore, the hapten which was likely to elicit the production of such an antiserum could be readily prepared from naltrexone in a fashion similar to the recently reported synthesis of naloxone-6-(*O*-carboxymethyl)oxime (4).



Nalmefene, R_1 = CH_2, R_2 = CH_2—◁
Naltrexone, R_1 = O, R_2 = CH_2—◁
Naloxone, R_1 = O, R_2 = $CH_2CH{=}CH_2$
Hapten, R_1 = N—O—CH_2COOH, R_2 = CH_2—◁

EXPERIMENTAL SECTION

Preparation of Immunogen and Antibody Production—The hapten, naltrexone-6-(*O*-carboxymethyl)oxime[1], was covalently coupled to bovine serum albumin using the mixed anhydride procedure of Erlanger *et al.* (5). The resulting conjugate was dialyzed against 0.05 M $NaHCO_3$, then water, and was then isolated by lyophilization. UV analysis of the immunogen indicated that ~17 moles of hapten were covalently coupled to 1 mol of albumin.

Two New Zealand White rabbits were immunized intradermally and boosted intravenously with the immunogen as previously described by Dixon and Crews (6). The antiserum with the highest titer of antibodies to nalmefene, obtained following the second booster immunization, was used for all subsequent studies. The antiserum was divided into 1-mL aliquots, lyophilized, and stored at −20°C.

Radioimmunoassay Procedure—Plasma samples (0.1–0.5 mL) containing

[1] Synthesized according to the procedure reported for naloxone-6-(*O*-methyl)oxime (4). The NMR, IR, and mass spectra were compatible with the proposed structure.

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