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Transformations in glycyrrhetinic acid: rearrangement of ring A

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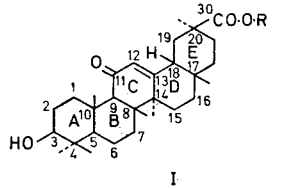
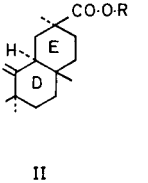
Methyl 11-oxo-18β-A-neo-oleana-3,12-dien-30-oate and the corresponding 18α-isomer have been prepared and tested for anti-inflammatory and antidiuretic activity. Relative to methyl glycyrrhetinate, both kinds of activity and toxicity are reduced by the rearrangement. Features of interest in the nuclear magnetic resonance spectra of 18α- and 18β-derivatives of glycyrrhetinic acid are recorded.

GLYCYRRHETINIC acid is known in two stereoisomeric forms: the 18 β-isomer (I; R = H) and the 18 α-isomer (II; R = H).



I



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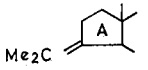

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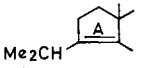

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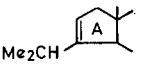

V

The 18β-isomer and a number of its derivatives are active anti-inflammatory agents (Finney & Somers, 1958; Finney & Tarnoky 1960; Kraus, 1960) and the hydrogen succinate of the 18 β-isomer has been shown to be of value in the treatment of gastric ulcer (Avery Jones, 1964). Little is known about the biological properties of the 18 α-isomer.

Methyl 18β-glycyrrhetinate was rearranged by phosphorus pentachloride in chloroform to give two compounds. The major component (methyl 11-oxo-18β-A-neo-oleana-3,12-dien-30-oate) was shown to have the structure III (R=Me) by the formation of acetone on ozonolysis and by the appearance of two methyl peaks at τ 8·21 and 8·37, absent in the nmr spectrum of the original ester, and assigned to the methyl groups of an isopropylidene group (Lehn & Ourisson, 1962). Structure IV (R=Me) has been assigned to the minor component (methyl 11-oxo-18β-A-neo-oleana-3(5),12-dien-30-oate). The methyls of the isopropyl group are not equivalent and in the nmr spectrum showed as two doublets (J = 7c/s); the position of the two methyl group resonances are 4c/s apart (see Lehn, 1962).

Methyl 18α-glycyrrhetinate was rearranged under similar conditions and the product crystallised to give a compound analogous to (III) (methyl 11-oxo-18α-A-neo-oleana-3,12-dien-30-oate). The mixture remaining in the mother liquors was isomerised by heating with acetic acid

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(Nowack, Jeger & Ruzicka, 1949) and gave a compound analogous to (IV) (methyl 11-oxo-18α-A-neo-oleana-3(5),12-dien-30-oate).

Rearrangement of the toluene-*p*-sulphonate of methyl 18β-glycyrrhetinate with sodium acetate and acetic acid gave a mixture which was isomerised as above to give a compound of proposed structure V (R=Me) (methyl 11-oxo-18β-A-neo-oleana-2,12-dien-30-oate). Unlike any of the above compounds this gave an nmr signal at τ 4·58 (proton at $C_{(2)}$) in addition to that at τ 4·26 (proton at $C_{(12)}$).

Table 1 lists the main nmr signals from a number of derivatives of 18α- and of 18β-glycyrrhetinic acids. For these derivatives the α-compounds can be differentiated from the β-compounds by the τ values of (*a*) the signal at highest field and (*b*) the signal from the proton at $C_{(12)}$.

During the course of this work a brief preliminary communication reporting a rearrangement of ring A of methyl 18β-glycyrrhetinate appeared (Tolstikov, Goriajev & Tolstikova, 1964). The results of these workers agree substantially with ours.

Pharmacology

The screening tests were by J. B. Dekanski, M. Khan and M. Cohen at the Pharmacology Department of Biorex Laboratories Research Division.

The cotton pellet granuloma test (Meier, Schuler & Desaulles, 1950) was used to evaluate the anti-inflammatory activity of the 18α- and 18β-isomers of methyl 11-oxo-A-neo-oleana-3,12-dien-30-oate and of the parent glycyrrhetinic acid esters from which these compounds were derived. A parallel evaluation of hydrocortisone acetate was also made.

A modification of the method described by Burn (1950) was used to determine antidiuretic activity over the 2 hr following the intraperitoneal administration of a single dose of 100 mg/kg to male rats.

In the above tests all compounds were injected as 1–3% suspensions in saline, with polysorbate 80 as suspending agent.

The LD50 was determined intraperitoneally in mice, over 7 days, the dose only being administered on the first day of the test. In these acute toxicity studies isopropyl myristate was used as the vehicle.

The results of the tests are summarised in Table 2. Thus, when ring A of methyl glycyrrhetinate is rearranged to give structure (III), anti-inflammatory activity and toxicity are reduced in both the 18α- and 18β-isomers. A similar picture seemed to emerge from the antidiuretic results.

Experimental

Melting-points are uncorrected and are taken on samples dried *in vacuo* at 100°. Optical rotations are measured on 2% solutions in chloroform, and ultraviolet spectra on solutions in chloroform. Analyses are by Mr. G. S. Crouch, School of Pharmacy, London.

Methyl 11-*oxo*-18β-*A-neo-oleana*-3,12-*dien*-30-*oate*. Methyl 18β-glycyrrhetinate (m.p. 245-248·5°, $[\alpha]_D^{20}$ + 157·4°) (8·1 g) was dissolved in alcohol-free chloroform (Vogel, 1956) (600 ml) which had been dried by

TABLE 1. NUCLEAR MAGNETIC RESONANCE SPECTRA* OF 18α- AND 18β-METHYL GLYCYRRHETINATE AND THEIR DERIVATIVES (τ VALUES)

Compound	$C_{(12)}-H$	$C_{(2)}-H$	$C_{(20)}-CO\cdot O\cdot CH_3$	C–H								
18β-Methyl glycyrrhetinate (I; R = Me)	4·23	—	6·27	8·41	—	8·58	—	—	8·82	8·95	9·14	
Methyl 11-oxo-A-homo-3*b*-aza-18β-olean-12-en-30-oate	4·25	—	6·26	8·42	—	8·59	8·66	—	8·82	—	9·16	
Methyl 11-oxo-18β-A-neo-oleana-3,12-dien-30-oate(III; R = Me)	4·23	—	6·27	8·37	—	8·61	—	—	8·83	—	9·13	
Methyl 11-oxo-18β-A-neo-oleana-2,12-dien-30-oate(V; R = Me)	4·26	4·58	6·26	8·45	—	8·61	—	—	8·82	9·03	9·14	
Methyl 11-oxo-18β-A-neo-oleana-3(5),12-dien-30-oate (IV; R = Me)	4·18	—	6·26	—	—	—	8·68	8·76	8·84 8·88	8·93 ‡, 9·05 ‡ 9·00 ‡, 9·12 ‡	9·15	
Methyl 18α-glycyrrhetinate (II; R = Me)	4·41	—	6·30	8·43	—	8·63	—	8·77 8·79	8·85	8·99	9·19	9·28
Methyl 11-oxo-18α-A-neo-oleana-3,12-dien-30-oate (III; R = Me)	4·31	—	6·28	8·37	—	8·64	—	8·75	8·83	—	9·09	9·25
Methyl 11-oxo-A-homo-3*b*-aza-18α-olean-12-en-30-oate	4·36	—	6·27	8·39	8·53	8·62	8·67	8·75	8·81	—	—	9·26
Methyl 11-oxo-18α-A-neo-oleana-3(5),12-dien-30-oate (IV; R = Me)	4·28	—	6·28	8·41	8·56	—	8·71	8·78	8·83	8·93 ‡, 9·05 ‡ 9·00 ‡, 9·12 ‡	—	9·25

* In $CDCl_3$ with tetramethylsilane as internal standard on Varian A-60 spectrophotometer. Main peaks occurring between τ 4 to 6·5 and τ 8·3 to 10.
‡ = J = 7c/sec.

distilling from phosphorus pentoxide. The solution was cooled to 4° in an ice-bath, with stirring, and anhydrous sodium acetate (5·4 g), dried at 100°, added. Stirring was continued and after 10 min phosphorus pentachloride (5·4 g) was added. The mixture was stirred for 35 min, sodium bicarbonate solution (3·3%, 480 ml) was added and stirring was continued for 3 hr. The chloroform layer was separated, washed with water until the washings were neutral and dried (Na_2SO_4). After removing the chloroform under reduced pressure, a white solid remained. This was dissolved in chloroform (40 ml), and methanol (309 ml) was rapidly added to the solution with rotation of the container so that a crystalline product (4·85 g) separated. This was removed by filtration and crystallised from ethyl acetate to give *methyl* 11-*oxo*-18β-*A-neo-oleana*-3,12-*dien*-30-*oate* (3·0 g) as colourless plates, m.p. 227–229° (softening at 225°), $[\alpha]_D^{20}+205°$ (unchanged on further crystallisation from chloroform-methanol), λ_{max} 251 mμ (log ϵ 4·1). Found: C, 79·6; H, 9·8. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%.

Ozonolysis. The above compound (1·85 g) in methylene dichloride (120 ml) was cooled to −30° and ozonised oxygen passed into the solution for 2·5 hr. The solvent was removed at room temperature under reduced pressure and the residue heated under reflux with water (60 ml) for 50 min. The mixture was cooled and then distilled until 42 ml of distillate had been collected. A portion (3·3 ml) of the distillate was shown to contain acetone by comparison with an authentic specimen by vapour-phase chromatography on a "Pye" panchromatograph. To the remainder of the distillate, iodine solution (10%, 6 ml) was added and then sodium hydroxide solution (20%). Iodoform (0·22 g) was precipitated and was recrystallised from ethanol (m.p. and mixed m.p. 115°).

The mother liquors from the fractionation of the crude rearrangement product by chloroform and methanol were taken to dryness under reduced pressure. The residue (3·2 g) was crystallised from methanol (285 ml). The first crop of crystals was rejected and the crops formed by removing (*a*) 135 ml and (*b*) a further 100 ml of methanol were combined and twice recrystallised from methanol to give *methyl* 11-*oxo*-18β-*A-neo-oleana*-3(5),12-*dien*-30-*oate* (0·59 g) as colourless needles, m.p. 184–187°, $[\alpha]_D^{20}$ + 202°, λ_{max} 251 mμ (log ϵ 4·1). A sample further crystallised from methanol for analysis, had m.p. 186–7° Found: C, 80·0; H, 10·2. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%.

Methyl 11-*oxo*-18α-*A-neo-oleana*-3,12-*dien*-30-*oate*. Methyl 18α-glycyrrhetinate (m.p. 260–262°,$[\alpha]_D^{20}$ + 97°) (10 g) was dissolved in dry alcohol-free chloroform (1 litre) and the rearrangement carried out as described above using anhydrous sodium acetate (6·7 g) and phosphorus pentachloride (6·7 g). The crude product was crystallised directly from ethyl acetate (500 ml) to give *methyl* 11-*oxo*-18α-*A-neo-oleana*-3,12-*dien*-30-*oate* (6·6 g) as colourless plates, m.p. 267–270°,$[\alpha]_D^{20}$ + 139° (unchanged on further crystallisation from chloroform-methanol),λ_{max} 250 mμ (log ϵ 4·1). Found: C, 80·2; H, 9·8. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%.

Ozonolysis. The above compound (1·04 g) in methylene dichloride (50 ml) was ozonised as previously described and, when treated with

TABLE 2. PRELIMINARY ASSESSMENT OF ANTI-INFLAMMATORY AND ANTIDIURETIC ACTIVITY OF THE DERIVATIVES IN RATS AND THEIR ACUTE TOXICITY IN MICE

Compound	Anti-inflammatory activity			Antidiuretic activity* (Dose 100 mg/kg)		Acute toxicity (i.p.) Groups of 4 mice	
	Route	Dose (mg/rat/day for 4 days) 5 male wistar rats/dose	Mean % reduction in granulation tissue weight compared with controls	% Effect†		Conc. %	LD50 (approx.) mg/kg
				Urine volume	Na excretion		
Hydrocortisone acetate	s.c.	12	51	—	—	—	—
Methyl 18β-glycyrrhetinate	s.c.	12 3	28 49 24	59	33	4	600
Methyl 18α-glycyrrhetinate	s.c.	12 3	37 27	39	46	4	400
Hydrocortisone acetate	i.p.	10	44	—	—	—	—
Methyl 11-oxo-18β-A-neo-oleana-3,12-dien-30-oate	i.p.	30 10	23 12	77	59	5	1000
Methyl 11-oxo-18α-A-neo-oleana-3,12-dien-30-oate	i.p.	30 20 10	32 22 8	68	56	6	900
Control	—	—	—	124	83	—	—

* Groups of five male Wistar albino rats, approximately 200 g, were starved for about 17 hr before water-loading and during urine collection, but allowed water *ad lib.* The water-load, administered by stomach tube, was 5 ml of distilled water per 100 g body weight. Immediately after loading the animals were placed in metabolism cages and faeces-free urine was collected 2 and 6 hr later. The urines were stored below 4° until analysis. The urine volumes at 2 and 6 hr were noted and the samples analysed for sodium, potassium and chloride content. The water-loading and urine collections were repeated twice weekly. Doses of the compound under test were administered intraperitoneally, simultaneous with the third water-load. The dose employed was 100 mg/kg. The drugs were administered as 1% suspensions in water, with polysorbate 80 as suspending agent. Control groups received the same volume of vehicle alone. Sodium and potassium were estimated using the E.E.L. flame photometer. Chloride was estimated by the method of Schales & Schales (1941).

† As % of mean 2 hr excretion levels determined on previous days.

iodine and sodium hydroxide, gave iodoform (0·112 g), which was crystallised from ethanol (m.p. and mixed m.p. 117–118°). A second portion (0·5 g) was ozonised as above and acetone was shown to be present in the distillate by vapour-phase chromatography.

The ethyl acetate mother liquors (500 ml above) were taken to dryness under reduced pressure and the residual solid (2·5 g) refluxed for 18 hr with glacial acetic acid (500 ml). The solution was cooled and the acetic acid removed under reduced pressure to low volume. The remaining solution was poured into water and the mixture filtered, washed with water and dried in a vacuum oven at 40°. The product (2·45 g), was three times crystallised from methanol-ethyl acetate 1 : 1 (about sixty times the weight of solid) to give *methyl* 11-*oxo*-18α-*A-neo-oleana*-3(5),12-*dien*-30-*oate* (0·49 g) as colourless plates, m.p. 237–238°, $[\alpha]_D^{20}$ + 151·6°, λ_{max} 247 mμ (log ε 4·06). Found: C, 79·4; H, 9·9. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%.

Toluene-p-*sulphonate of methyl* 18β-*glyrrhetinate.* Methyl 18β-glycyrrhetinate (7·1 g) was dissolved in pyridine (100 ml, distilled from KOH) and the solution cooled in an ice-bath. Toluene-*p*-sulphonyl chloride (19·6 g) was added and the solution, protected by a calcium chloride tube, set aside for three days. The solution was poured into ice and water and the mixture extracted with ether. The ethereal solution was washed with 2N hydrochloric acid (100 ml), then with saturated sodium bicarbonate solution and finally with water. The solution was dried (Na_2SO_4), the ether removed by distillation and the residue refluxed with light petroleum (b.p. 100–120°, 500 ml). The hot solution was decanted from the oily residue and allowed to cool, whereupon colourless needles of the toluene-*p*-sulphonate were deposited. The mother liquors were used to extract the residue and obtain further crops. Yield 7·73 g, m.p. 139–140° (decomp.), $[\alpha]_D^{20}$ + 110°. Found: C, 71·4; H, 8·5; S, 4·9. $C_{38}H_{54}O_6S$ requires C, 71·45; H, 8·5; S, 5·0%. The toluene-*p*-sulphonate can also be crystallised from methanol (150 ml/g).

Rearrangement of the toluene-p-*sulphonate of methyl* 18β-*glycyrrhetinate.* The toluene-*p*-sulphonate (4·1 g) and anhydrous sodium acetate (2·3g) were dissolved in acetic acid (540 ml). The solution, in a flask with condenser and calcium chloride tube, was heated in a water-bath for 6 hr at 90–95°.

Water was added to the solution to dilute the acetic acid to about 10% and the solid removed by filtration and dried. This product (1 g) was heated under reflux with acetic acid (130 ml) for 12 hr. The solution was poured into water, extracted with ether and the ethereal solution washed and dried (Na_2SO_4). Removal of the ether by distillation left a resinous product (1·08 g), which was extracted with boiling methanol (5 ml) to leave a solid (0·76 g), m.p. 161–172°. Recrystallisation from ethyl acetate and then from methanol gave crystals (0·17 g), m.p. 186–188·5°, which depressed the m.p. of methyl 11-oxo-18β-A-neo-oleana-3(5),12-dien-30-oate. $[\alpha]_D^{20}$ + 214°, λ_{max} 249 mμ (log ε 4·1). Found: C, 80·3; H, 10·0. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%. This compound has been tentatively assigned the constitution *methyl* 11-*oxo*-18β-*A-neo-oleana*-2,12-*dien*-30-*oate*.

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