RESEARCH PAPERS

16α,17α-ISOPROPYLIDENEDIOXY-6α-METHYLPREGNA-1,4-DIENE-3,20-DIONE (B.D.H. 3144). A NEW ANTI-INFLAMMATORY AGENT

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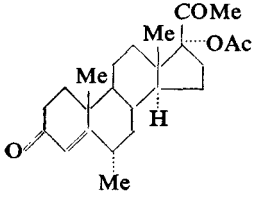
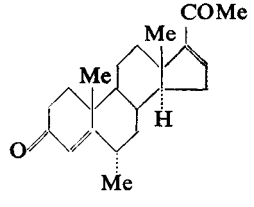
The preparation and anti-inflammatory properties of the title compound and of some related structures are reported. B.D.H. 3144 (16α,17α-Isopropylidenedioxy-6α-methylpregna-1,4-diene-3,20-dione) is found to be a potent anti-inflammatory agent which approaches prednisolone in activity.

AFTER the preparation by Barton, Ellis and Petrow (1959) of 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione (I), a highly potent progestational agent, we turned to the partial synthesis of the related 16α,17α- diacetoxy-6α-methylpregn-4-ene-3,20-dione (III; R = Ac). To this end 16α,17α-dihydroxy-6α-methylpregn-4-ene-3,20-dione (III; R = H) was prepared as described below. Its conversion into the diacetate (III; R = Ac), however, offered initial difficulty. In the meantime the parent diol (III; R = H) had been converted into the acetonide (IV) in excellent yield.



(I)



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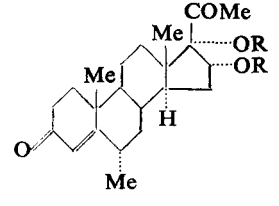

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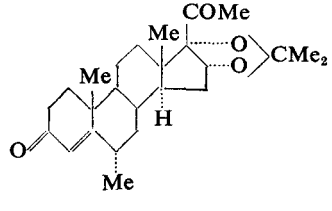

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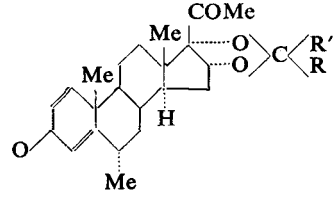

(V)

Biological study of the last compound confirmed its anticipated progestational potency, but also revealed a quite unexpected and substantial anti-inflammatory activity. In view of this significant result, (IV) was converted into its Δ^1 derivative (V; R = R' = Me; B.D.H. 3144), which forms the main subject of the present communication.

Cooley, Ellis, Hartley and Petrow (1955) had shown previously that oxidation of 3β-acetoxypregna-5,16-dien-20-one with potassium permanganate under controlled experimental conditions leads to the formation of the corresponding 16α,17α-dihydroxy derivative in moderate yield. Attempts to apply this reaction to the readily available 6-methyl derivative of 3β-hydroxypregna-5,16-dien-20-one, (Burn, Ellis, Petrow, Stuart-Webb and Williamson, 1957), however, proved uniformly unsuccessful owing to concomitant attack upon the C(5)-C(6) ditertiary unsaturated linkage. This difficulty was circumvented by using 6α-methylpregna-4,16-diene-3,20-dione (Burn and others, 1957) (II) as starting material when the required 16α,17α-dihydroxy-6α-methylpregn-4-ene-3,20-dione (III; R = H)

TABLE I

THE ANTI-INFLAMMATORY ACTIVITY OF B.D.H. 3144, CORTISONE, HYDROCORTISONE, PREDNISONE, AND PREDNISOLONE

Compound	Total dose mg./rat	No. of implants	Route	Granuloma	
				Mean wt. mg.	Per cent reduction
B.D.H. 3144	0·625	17	Subcut.	125	17
	1·25	20	,,	118	22
	2·5	20	,,	105	31
	5·0	20	,,	103	32
Hydrocortisone	5·0	17	,,	139	8
Controls	—	20	—	154	—
Cortisone acetate	10	20	Subcut.	98	38
Hydrocortisone	7·5	20	,,	106	32
Prednisolone acetate	5·0	19	,,	52	67
Controls	—	20	—	157	—
B.D.H. 3144	2·5	20	Oral	105	36
	10·0	20	,,	84	49
Hydrocortisone	2·5	20	,,	116	29
Prednisone	2·5	20	,,	75	54
Prednisolone acetate	2·5	19	,,	66	60
Controls	—	20	—	163	—

was obtained, albeit in only 25 per cent yield. The last compound condensed readily and nearly quantitatively with acetone in the presence of perchloric acid to give 16α,17α-isopropylidenedioxy-6α-methylpregn-4-ene-3,20-dione (IV). Conversion into its Δ^1 derivative (V; R = R′ = Me) was achieved using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (Burn, Kirk and Petrow, 1960), such oxidants as selenium dioxide having proved unsatisfactory.

The 2-methyl derivative of (V; R = R′ = Me) was prepared by treating 16α,17α-isopropylidenedioxy-6α-methylpregn-4-ene-3,20-dione (IV) with diethyl oxalate in the presence of sodium hydride to give the sodio-salt of the 2-ethoxalyl derivative. Methylation with methyl iodide, followed by alkaline hydrolysis furnished 16α,17α-isopropylidenedioxy-2α,6α-dimethyl-pregn-4-ene-3,20-dione, which was converted into the required Δ^1 derivative by reaction with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. The remaining congeners of (IV) and (V) included in the present biological study were prepared by an alternative route, which will be reported elsewhere.

Anti-inflammatory Assay

The anti-inflammatory activity was estimated by a modification of the methods described by Meier, Schuler and Desaulles (1950), Cresseri and Meli (1953) and Christian and Williamson (1958). An agar pellet containing 5 per cent turpentine and measuring approximately 0·5 cm. × 1·0 cm. and weighing 220·2 ± 2·5 mg. was implanted subcutaneously under the dorsal skin on either side of the vertebral spine in young albino rats weighing between 70 and 90 g. The implantation was made under ether anaesthesia through a small medial incision. Immediately after the implantation the animals were given the steroid in an aqueous suspending medium either subcutaneously or orally twice daily for 5 days. Twenty-four hr. after the last injection the animals were killed, the granuloma capsule freed from the implant and weighed wet on a torsion balance.

TABLE II

ANTI-INFLAMMATORY ACTIVITY OF STEROIDS ADMINISTERED SUBCUTANEOUSLY

	Steroid	Activity
1.	6α-Methylpregn-4-ene-3,20-dione	none
2.	17α-Hydroxy-6α-methylpregna-1,4-diene-3,20-dione	none
3.	16α,17α-Dihydroxy-6α-methylpregn-4-ene-3,20-dione	none
4.	16α,17α-Furfurylidenedioxy-6α-methylpregn-4-ene-3,20-dione	none?
5.	6α-Methylpregna-1,4-diene-3,20-dione	+
6.	16α,17α-Isopropylidenedioxy-2,6α-dimethylpregna-1,4-diene-3,20-dione	+ (+)
7.	16α,17α-*p*-Fluorobenzylidenedioxy-6α-methylpregn-4-ene-3,20-dione	++
8.	16α,17α-Isopropylidenedioxy-6-methylpregna-1,4,6-triene-3,20-dione	++ (+)
9.	17α,21-Dihydroxypregn-4-ene-3,11,20-trione	+++
10.	16α,17α-Ethylidenedioxy-6α-methylpregna-1,4-diene-3,20-dione	+++
11.	6α-Methyl-16α,17α-(1-methylpropylidenedioxy)pregna-1,4-diene-3,20-dione	+++
12.	16α,17α-Isopropylidenedioxy-6α-methylpregn-4-ene-3,20-dione	+++ (+)
13.	16α,17α-Isopropylidenedioxy-6α-methylpregna-1,4-diene-3,20-dione (B.D.H. 3144)	++++
14.	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	++++ (+)

The control animals were given the vehicle only. For comparative purposes cortisone, hydrocortisone, prednisone and prednisolone were included in the studies. The per cent reduction in the weight of the granuloma capsule represents a measure of the anti-inflammatory activity. The results are recorded in Tables I and II.

Discussion

6α-Methylpregn-4-ene-3,20-dione (No. 1*) is one of the simplest structures to show anti-inflammatory activity, which may be demonstrated by direct injection of the steroid into the granuloma pouch (Glenn, Richardson and Bowman, 1959). It is inactive in our assay. The introduction of an unsaturated bond in the 1,2 position into 6α-methylpregn-4-ene-3,20-dione, producing 6α-methylpregna-1,4-diene-3,20-dione (No. 5), however is accompanied by the appearance of slight anti-inflammatory potency, which is destroyed by the introduction of an 17α-hydroxyl group (No. 2). 16α,17α-Dihydroxy-6α-methylpregn-4-ene-3,20-dione (No. 3), the parent compound of the present series, is likewise without activity in the present assay. Conversion of the 16α and 17α hydroxyl groups into the acetonide group results in the production of a highly potent compound (No. 12),

* The numbers refer to Table II.

which is approximately twice as active as cortisone. A similar potentiation of anti-inflammatory activity through acetonide formation has been observed in the triamcinolone series (Bernstein, Heller, Littell, Stolar, Lenhard, Allen and Ringler, 1959). A further increase in anti-inflammatory activity is produced by introduction of an unsaturated bond in the 1,2 position into No. 12 to give B.D.H. 3144, which now approaches prednisolone in potency. Attempts to increase activity still further by methylation at the 2 position (No. 6), by increase of conjugation (No. 8), or by modification of the isopropylidene moiety (Nos. 4, 7, 10 and 11) resulted in all cases in loss of potency.

B.D.H. 3144, in common with other anti-inflammatory steroids, also possesses thymolytic activity and causes a loss in body and adrenal weights. This latter action, however, can be prevented by the simultaneous administration of ACTH. It has glucocorticoid activity as shown in the liver glycogen deposition test and it prolongs the survival time in adrenalectomised rats. It has some anti-anaphylactic, progestational and claudogenic activity (cf. Petrow, 1960), but no apparent androgenic, oestrogenic or mineralocorticoid properties. It is hoped to report these results in detail at a later date.

B.D.H. 3144 has been tested for topical activity in human volunteers using the patch test of Schlagel and Northam (1959). In this assay the irritant property of tetrahydrofurfuryl alcohol, when applied to the skin of the forearm, is balanced against the anti-inflammatory activity of the steroid which is itself dissolved in the irritant material. The lowest concentration of steroid required to prevent erythema of the skin completely, then furnishes a measure of its potency. B.D.H. 3144 proved equal to hydrocortisone in this assay, both compounds being tested at 1 per cent concentration.

The marked potency of B.D.H. 3144 on topical application shows that its anti-inflammatory activity is an inherent property of its unique structure and not the property of a metabolic (and more highly oxygenated) product formed from it *in vivo*. It differs markedly from current anti-inflammatory steroids, in the absence of an 11β-hydroxyl group [c.f. prednisolone (No. 14)] which has hitherto been regarded as mandatory for significant activity of this type. B.D.H. 3144 consequently may be regarded as a new type of anti-inflammatory agent.

Experimental

Optical rotations were measured in a 1 dm. tube for chloroform solutions. Ultra-violet absorption spectra were kindly determined (for ethanol solutions) by Mr. M. T. Davies, B.Sc. B.D.H. chromatographic alumina was used.

16α,17α-*Dihydroxy*-6α-*methylpregn*-4-*ene*-3,20-*dione* (III; R = H).—A solution of potassium permanganate (9 g.) in aqueous actone (450 ml. of 85 per cent) was added during 30 min. to a stirred solution of 6α-methylpregna-4,16-diene-3,20-dione (Burn and others, 1957) (14·7 g.) in a mixture of acetone (450 ml.) and acetic acid (4·2 ml,). After treatment with sulphur dioxide, the pale yellow solution was poured into water (3 l.), from which

a crystalline product (7 g., m.p. 190–197°) separated during 45 min. Repeated crystallisation of this material first from aqueous ethanol and then from aqueous acetic acid gave the *diol*, needles, m.p. 232–235°, $[\alpha]_D^{21°}$ + 82° (c=0·94), λ_{max} 240 mμ (log ε 4·19) (Found: C, 73·5; H, 8·8. $C_{22}H_{32}O_4$ requires C, 73·3; H, 8·95 per cent).

16α,17α-*Isopropylidenedioxy*-6α-*methylpregn*-4-*ene*-3,20-*dione* (IV). A suspension of the foregoing compound (100 mg.) in acetone (10 ml.) was treated with 2 drops of perchloric acid (72 per cent w/w), and the mixture stirred for 15 min. The product obtained by the addition of water was purified from aqueous methanol to give the *acetonide*, leaflets, m.p. 166–167°, $[\alpha]_D^{21°}$ + 118° (c=0·82), λ_{max} 240 mμ (log ε 4·18) (Found: C, 74·8; H, 8·85. $C_{25}H_{36}O_4$ requires C, 75·0; H, 9·1 per cent).

16α,17α-*Isopropylidenedioxy*-6α-*methylpregna*-1,4-*diene*-3,20-*dione* (V; R = R′ = Me). A solution of the foregoing compound (3·5 g.) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (3·5 g.) in benzene (35 ml.) was heated under reflux for 22 hr. The mixture was poured into 10 per cent aqueous sodium hydroxide (100 ml.) and the product isolated with ether. Crystallised from aqueous methanol, the *dienedione* formed plates, m.p. 219–220°, $[\alpha]_D^{20°}$ + 60° (c=0·82), λ_{max} 243·5 mμ (log ε 4·17) (Found: C, 75·0; H, 8·5. $C_{25}H_{34}O_4$ requires C, 75·3: H, 8·6 per cent).

16α,17α-*Isopropylidenedioxy*-2α,6α-*dimethyl-pregn*-4-*ene*-3,20-*dione*. Sodium hydride (12 g. of a 50 per cent dispersion in oil) was added to 16α,17α-isopropylidenedioxy-6α-methylpregn-4-ene-3,20-dione (12 g.) in dry benzene (240 ml.) and redistilled diethyl oxalate (12 ml.), and the mixture set aside, under nitrogen, for 3 days. After careful addition of methanol (until effervescence ceased), treatment of the mixture with light petroleum (700 ml.) gave a yellow crude sodio-derivative which was collected, washed well with light petroleum and air-dried. This material (30 g.) was suspended, together with potassium carbonate (12 g.), in acetone (270 ml.) and methyl iodide (48 ml.), and the mixture heated under reflux for 48 hr. The product was isolated with ether and treated for 18 hr. at room temperature with sodium ethoxide (from 1·2 g. sodium) in ethanol (120 ml.). The product, isolated with ether, was chromatographed on alumina (160 g.), employing light petroleum and light petroleum-benzene mixture (9 : 1) as eluants, to give material which crystallised from aqueous ethanol. 16α,17α-*Isopropylidenedioxy*-2α,6α-*dimethyl-pregn*-4-*ene*-3,20-*dione* separated in blades, m.p. 209–210°, $[\alpha]_D^{20°}$ + 120° (c=0·72), λ_{max} 238·5 mμ (log ε 4·15) (Found: C, 75·6; H, 9·4. $C_{26}H_{38}O_4$ requires C, 75·3; H, 9·2 per cent).

Isopropylidenedioxy-2,6α-*dimethyl-pregna*-1,4-*diene*-3,20-*dione* prepared from the foregoing compound, crystallised from aqueous methanol in rods, m.p. 205–206°, $[\alpha]_D^{21°}$ + 89° (c=1·05), λ_{max} 243·5 mμ (log ε 4·18) (Found: C, 75·8; H, 9·2. $C_{26}H_{36}O_4$ requires C, 75·7; H, 8·8 per cent).

References

Barton, S. P., Ellis, B., and Petrow, V. (1959). *J chem Soc.*, 478–479.
Bernstein, S., Heller, M., Littell, R., Stolar, S. M., Lenhard, R. H., Allen, W. S., and Ringler, I. (1959). *J. Amer. chem. Soc.*, **81**, 1696–1701.

Burn, D., Ellis, B., Petrow, V., Stuart-Webb, I. A., and Williamson, D. M. (1957). *J. chem. Soc.*, 4092–4098.
Burn, D., Kirk, D. N., and Petrow, V. (1960). *Proc. chem. Soc.*, 14.
Christian, J. J., and Williamson, H. O. (1958). *Proc. Soc. exp. Biol. N.Y.*, **99**, 385–387.
Cooley, G., Ellis, B., Hartley, F., and Petrow, V. (1955). *J. chem. Soc.*, 4373–4377.
Cresseri, A., and Meli, A. (1953). *Arch. Sci. Biol.*, **37**, 551–562.
Glenn, E. M., Richardson, S., and Bowman, B. J. (1959). *Metab.*, **8**, 265–285.
Meier, R., Schuler, W., and Desaulles, P. (1950). *Experientia*, **6**, 469–471.
Petrow, V. (1960). *J. Pharm. Pharmacol.*, **12**, 704.
Schlagel, C. A., and Northam, J. I. (1959). *Proc. Soc. exp. Biol. N.Y.*, **101**, 629–932.