TABLE I

FORMATE-C^{14}, METHIONINE-METHYL-C^{14} AND SERINE-3-C^{14} AS C-24 METHYL DONORS[a]

Source of C^{14}	Non-radioactive additions	Non-saponifiable fraction Mg.	S. A. $\times 10^{-3}$	Total counts $\times 10^{-3}$	Yield, %
HCOOH	...	3.7	2.3	8.6	6
HCOOH	Homocysteine[b]	3.5	4.3	15.2	10
HCOOH	Homocysteine, folic acid	4.0	6.4	25.7	17
HCOOH	Homocysteine, aminopterin	4.2	0.8	3.5	2
HCOOH	Methionine	3.8	0.1	0.4	0.3
Methionine	...	3.9	9.5	37.2	25
Methionine	Formate	5.2	6.1	31.5	22
Methionine	Formate, homocysteine	3.3	2.5	8.5	6
Methionine	Formate, homocysteine, aminopterin	3.7	6.2	20.3	14
Methionine	Aminopterin	1.9	34.4	65.4	44
Methionine	Serine	3.6	28.4	102.1	68
Serine	...	3.5	4.7	16.6	11

[a] Each flask contained 4 ml. of homogenate made from 1 g. of dry yeast, 2×10^{-4} *M* ATP and 0.5 μc. of C^{14} (1 mc./mmole). [b] Additions per flask (where indicated): homocysteine, 5 mg.; folic acid, 1 mg.; aminopterin, 2 mg.; methionine, 15 mg.; HCOONa, 5 mg.; serine, 15 mg.

TABLE II

TRITIUM:CARBON^{14} RATIOS IN METHIONINE AND ERGOSTEROL

Each experiment consisted of 5 flasks, each containing 4 ml. of yeast extract, 5 mg. of serine, 2 mg. of aminopterin, 1 mg. of ATP, and 0.175 mg. of doubly labeled methionine. Incubation time was 48 hr.; radioactivities were determined on a Packard Tri-Carb Scintillation Counter. The values given are the averages of four samples.

Expt.	T:C^{14} Ratio Methionine	Ergosterol
1	1.12 ± 0.06	0.97 ± 0.06
2	1.12 ± 0.06	1.02 ± 0.02

methyl-T, was incubated with yeast homogenates and the resulting ergosterol rigorously purified, the T:C^{14} ratios of the substrate and product showed that all three hydrogen atoms of the methyl group of methionine are transferred to ergosterol. Were the methyl group oxidized to the level of formaldehyde, one third of the tritium would be lost, and the T:C^{14} ratio in ergosterol (Table II) would have been 67%. Since the ratio is 86–91%, at least some methyl groups of methionine must have been transferred intact to the carbon 24 of the sterol.

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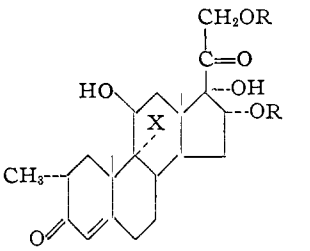
16-HYDROXYLATED STEROIDS. V.[1] THE SYNTHESIS OF THE 16α-HYDROXY DERIVATIVES OF 2α-METHYL-STEROIDS

Sir:

In view of our previous Communication[1] concerning the ability of the 16α-hydroxyl group to abolish the sodium retaining property of a steroid without destroying its glucocorticoid activity, we have investigated the effect of 16α-hydroxylation on the activities of 2α-methyl steroids.[2]

Hydrolysis of 21-acetoxy-3,20-bis-ethylenedioxy-5-pregnene-11β,16α,17α-triol (I)[3] in dilute acetic acid afforded 21-acetoxy-20-ethylenedioxy-11β,16α,-17α-triol-4-pregnen-3-one (II), m.p. 262–263°, $[\alpha]^{25}D$ + 85° ($CHCl_3$); (*Anal.* Found: C, 64.77; H, 8.03). Treatment of compound II with ethyl oxalate and sodium methoxide in *t*-butyl alcohol formed the sodium enolate of 20-ethylenedioxy-2-ethoxyoxalyl- 11β,16α,17α,21 -tetrahydroxy-4-pregnen-3-one (III) as a pale yellow amorphous solid. Methylation of III with methyl iodide and potassium carbonate in acetone followed by removal of the ethoxyoxalyl group by sodium methoxide in methanol gave a glass. After removal of the 20-ketal group with dilute ethanolic sulfuric acid, partition chromatography[4] yielded 11β,16α,17α,21-tetrahydroxy- 2α -methyl- 4 -pregnene- 3,20 -dione (IVa) apparently with one molecule of acetone of crystallization, m.p. 201–203°, λ_{max}. 240–241 mμ (ϵ 16,600),[5] $[\alpha]^{25}D$ + 145° ($CHCl_3$); (*Anal.* Found: C, 65.59; H, 8.39). Acetylation gave the 16α,21-diacetate IVb, m.p. 253–254°, λ_{max}. 240–241 mμ (ϵ 17,500), ν_{max}^{KBr}. 3450, 1743, 1726 (shoulder), 1654, 1620 and 1238 cm.$^{-1}$, $[\alpha]^{25}D$ + 92° ($CHCl_3$); (*Anal.* Found: C, 65.43; H, 7.75). Oxidation of IVb with chromium trioxide–pyridine reagent[6] gave 16α,21-diacetoxy-17α-hydroxy-2α-methyl-4-pregnene-3,11,20-trione (V), m.p. 240.5–241.5°, $[\alpha]^{25}D$ + 129° ($CHCl_3$); (*Anal.* Found: C, 65.49; H, 7.30).

IVa, X = H, R = H
IVb, X = H, R = Ac
XIIIa, X = F, R = H
XIIIb, X = F, R = Ac



Acetylation of 3,20-bis-ethylenedioxy-5-pregnene-11β,16α,17α,21-tetrol (VIa)[7] yielded the 16α,-21-diacetate (VIb), m.p. 129–135°,[8] $[\alpha]^{25}D$ − 61.5° ($CHCl_3$); (*Anal.* Found: C, 62.49; H, 7.81). Treatment with phosphorus oxychloride in pyridine afforded 3,20-bis-ethylene-dioxy-16α,21-diacetoxy-5.9(11)-pregnadien-17α-ol (VII), m.p. 221–224°, $[\alpha]^{25}D$ − 48° ($CHCl_3$); (*Anal.* Found: C, 65.52; H, 7.67). Hydrolysis of VII in dilute acetic acid gave 16α,21-diacetoxy-20-ethylenedioxy-17α-hydroxy-4,9(11)-pregnadien-3-one (VIII), m.p. 184.5–186°, $[\alpha]^{25}D$ ± 0° ($CHCl_3$); (*Anal.* Found: C, 66.63; H, 7.65).

The sodium enolate (IX) of the 2-ethoxyoxalyl

(1) Paper IV, S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956).

(2) J. A. Hogg, F. H. Lincoln, R. W. Jackson and W. P. Schneider, *ibid.*, **77**, 6401 (1955).

(3) W. S. Allen and S. Bernstein, *ibid.*, **78**, 3223 (1956).

(4) R. Littell and S. Bernstein, *ibid.*, **78**, 984 (1956).

(5) The ultraviolet spectra were determined in absolute alcohol solutions.

(6) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(7) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956).

(8) This compound seemed to be solvated and could not be brought to a better melt or analytical value.

derivative of VIII (free steroid) was obtained as an amorphous solid in the manner described above. Treatment with methyl iodide and potassium carbonate in acetone followed by reaction with sodium methoxide in methanol and finally hydrolysis in dilute methanolic sulfuric acid yielded after partition chromatography[4] 16α,17α,21-trihydroxy-2α-methyl-4,9(11)-pregnadiene-3-20-dione, (Xa), m.p. 203–207, $[\alpha]^{25}D$ + 103° ($CHCl_3$); (*Anal.* Found: C, 70.75; H, 8.29). Acetylation afforded the 16α,21-diacetate (Xb), m.p. 221.5–224°, $[\alpha]^{25}D$ + 104° ($CHCl_3$); (*Anal.* Found: C, 68.10; H, 7.53).

Addition of N-bromoacetamide and 10% perchloric acid to a solution of Xb in dioxane gave the bromohydrin XI as an amorphous solid, m.p. 131–134° which could not be purified. Treatment of XI with potassium acetate in acetone furnished the 9β,11β-epoxide XII, m.p. 222–223°, $[\alpha]^{25}D$ − 34° ($CHCl_3$); (*Anal.* Found: C, 65.57; H, 7.49). Hydrofluoric acid converted XII to 16α,21-diacetoxy- 9α -fluoro -11β,17α- dihydroxy- 2α -methyl-4-pregnene-3,20-dione (XIIIb), m.p. 140–200°[8], λ_{max} 237–238 mμ (ϵ 16,300), ν_{max}^{KBr} 3420, 1740, 1732, 1725, 1660, 1627 (shoulder) and 1235 cm^{-1}; (*Anal.* F, 3.87. Found: F, 4.29). The corresponding 16α,21-diol XIIIa formed from XIIIb by potassium hydroxide hydrolysis melted at 231–234° d., λ_{max} 237–238 mμ (ϵ 15,100), ν_{max}^{KBr} 3450, 1720, 1660, and 1635 $cm.^{-1}$, $[\alpha]^{25}D$ + 115° (pyridine); (*Anal.* Found: C, 64.30; H, 7.66; F, 4.57).

Bio-assays.[9]—Preliminary assay (rat liver glycogen procedure) of 11β,16α,17α,21-tetrahydroxy-2α-methyl-4-pregnene-3,20-dione (IVa) indicated definite activity (less than that of hydrocortisone); the 16α,21-diacetate IVb and the 16α,21-diacetoxy-11-one V were inactive. In the same assay, 9α-fluoro - 11β,16α,17α,21 - tetrahydroxy - 2α - methyl-4-pregnene-3,20-dione (XIIIa), and its diacetate XIIIb were found to be at least two times as active as hydrocortisone.

In the rate electrolyte (sodium retention) assay, IVa, IVb and V were inactive. The 9α-fluoro-compounds XIIIa and XIIIb exhibited minor activity (much less than that of desoxycorticosterone).

(9) The assays were done by L. Bortle, E. Heyder, J. Perrine, E. Ross, and I. Ringler (Experimental Therapeutics Research Section of these Laboratories).

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STEROIDS. LXXXIX.[1] 19-NORDIHYDROTESTOSTERONE DERIVATIVES. A POTENT CLASS OF ANTI-ESTROGENIC COMPOUNDS.

Sir:

Following Birch's[2] synthesis of 19-nortestosterone (Ia) in 1949 a number of 19-nor analogs of the steroid hormones and metabolites have been prepared[3a–h] and many of these substances exhibited unusual biological activity.

We now wish to describe the synthesis of a new series of biologically active 19-nor compounds, namely, the 4,5-dihydroallo derivatives of nortestosterone and 17α-alkyl substituted nortestosterones as well as the corresponding 3β,17β-diols.

While catalytic hydrogenation of Ia, Ib and Ic led to mixtures of the rings A/B *cis* and *trans* compounds, it was found that reduction of the unsaturated ketones in ether–dioxane solution with lithium in liquid ammonia[4] followed by ammonium chloride decomposition, furnished in excellent yield the dihydroallo derivatives: 19-norandrostan-17β-ol-3-one (IIa) (m.p. 130–132°, $[\alpha]D$ + 60°.[5] Found for $C_{18}H_{28}O_2$: C, 78.34; H, 9.94); 17α-methyl-19-norandrostan-17β-ol-3-one (IIb) (m.p. 145–146°, $[\alpha]D$ +35°. Found for $C_{19}H_{30}O_2$: C, 78.49; H, 10.40); and 17α-ethyl-19-norandrostan-17β-ol-3-one (IIc) (m.p. 212–213°, $[\alpha]D$ +33°. Found for $C_{20}H_{32}O_2$: C, 78.47; H, 10.49). Reduction of the 17-vinyl (Id) and the 17-ethynyl (Ie) compounds by this technique resulted in saturation of the 4,5-double bonds only, furnishing, respectively, 17α-vinyl-19-norandrostan-17β-ol-3-one (IId) (m.p. 192–193°, $[\alpha]D$ +47°. Found for $C_{20}H_{30}O_2$: C, 79.18; H, 10.05) and 17α-ethynyl-19-norandrostan-17β-ol-3-one (IIe) (m.p. 222–223°, $[\alpha]D$ +6°. Found for $C_{20}H_{28}O_2$: C, 80.30; H, 9.52). That the unsaturated side-chains had withstood the reduction conditions was demonstrated conclusively by the conversion of IIe to IId by partial hydrogenation (palladium on calcium carbonate–pyridine) and the derivation of IIc from either IId or IIe by reduction over palladium–carbon in methanol solution. The A/B allo configuration for compounds II which could be predicted on thermodynamic grounds,[6] is firmly established by the rotatory dispersion curves[7] of these dihydro compounds, the curves being virtually identical with that of androstan-17β-ol-3-one.

Treatment of IIa through IIe with sodium borohydride in aqueous dioxane gave the corresponding 19-norandrostan-3β,17β-diols: IIIa (m.p. 168–170°, $[\alpha]D$ +37°. Found for $C_{18}H_{30}O_2 \cdot 2C_3H_6O$: C, 72.88; H, 10.94); IIIb (m.p. 174–176°, $[\alpha]D$ ±0°. Found for $C_{19}H_{32}O_2 \cdot 2C_3H_6O$: C, 73.76; H, 11.12); IIIc (m.p. 181–183°, $[\alpha]D$ +2°. Found for $C_{20}H_{34}O_2$: C, 78.20; H, 11.03[8]); IIId (m.p. 167–169°, $[\alpha]D$ +9°. Found for $C_{20}H_{32}O_2$: C,

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(2) A. J. Birch, *J. Chem. Soc.*, 367 (1950).

(3) (a) L. Miramontes, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3540 (1951); **75**, 4440 (1953); (b) A. Sandoval, L. Miramontes, G. Rosenkranz, C. Djerassi and F. Sondheimer, *ibid.*, **75**, 4117 (1953); (c) A. L. Wilds and N. A. Nelson, *ibid.*, **75**, 5366 (1953); (d) C. Djerassi, L. Miramontes, G. Rosenkranz and F. Sondheimer, *ibid.*, **76**, 4092 (1954); (e) A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, *ibid.*, **76**, 6210 (1954); (f) B. J. Magerlein and J. A. Hogg, *ibid.*, **79**, 1508 (1957); (g) F. B. Colton, L. N. Nysted, B. Riegel and A. L. Raymond, *ibid.*, **79**, 1123 (1957); (h) F. B. Colton, U. S Patent 2,725,389 (1955).

(4) *Cf.* F. Sondheimer, R. Yashin, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **74**, 2695 (1952).

(5) All melting points are uncorrected and rotations were determined at 20° in chloroform. Thanks are due Mr. E. Denot for his able technical assistance and to Mr. E. Avila for rotations and spectra.

(6) See D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 (1954), and references cited therein.

(7) We are grateful to Professor C. Djerassi, Wayne State University, for determination and comparison of rotatory dispersions.

(8) Analytical sample sublimed in high vacuum.