the formation of III from 11β-hydroxyandrostenedione could not have resulted primarily from an intermediate 9,11-olefin.

The structure of the 9α-fluorosteroids was based on these considerations: (1) the biological activity of 9α-fluorodeoxycorticosterone acetate (see below) suggested the presence of an intact steroid nucleus. (2) The molecular rotatory contribution of the fluorine (ΔM_D^{XF-H} -45 to -93) corresponded to that found in other 9α-fluoro-11- or 12-hydroxylated steroids (ΔM_D -24 to -86).[4] (3) The n.m.r. spectrum of III in deuteriochloroform with added tetramethylsilane as an internal standard showed no hydrogen on the carbon atom holding the fluorine (region from 150 to 352 cps. was free of resonance bands) and showed angular methyl resonances (56 and 80 cps.) within 2 cps. of those expected from 9α-fluorosteroids.[5]

When corticosterone 21-acetate (VI) was treated with the hydrogen fluoride–pyridine reagent 9α-fluorodeoxycorticosterone 21-acetate (VII), m.p. 188–190°, $\lambda_{max}^{methanol}$ 238 (ϵ 17,900); $[\alpha]_D$ +169.5° ($CHCl_3$); (found: C, 70.49; H, 7.83); was obtained. Compound VII is twelve times as potent in the sodium retaining assay as deoxycorticosterone acetate.[6] This clearly indicates that the enhancement of hormonal activity by the introduction of a 9α-fluoro group is not necessarily mediated through the inductive effect of the fluoro group on an adjacent oxygen function.[1] It seems more probable that the 9α-fluoro group interferes with one of the normal metabolic mechanisms for the degradation of steroids, possibly with one involving 9-hydroxylation.[3,7]

(4) J. Fried, J. E. Herz, E. F. Sabo and M. H. Morrison, *Chem. and Ind.*, 1232 (1956); and references given in reference 1.

(5) We are indebted to Dr. LeRoy F. Johnson of Varian Associates, Palo Alto, California, for the determination and interpretation of this spectrum.

(6) C. M. Kagawa and R. S. Jacobs, Jr., *Proc. Soc. Exptl. Biol. Med.*, **104**, 60 (1960).

(7) R. M. Dodson and R. D. Muir, THIS JOURNAL, **80**, 5004(1958).

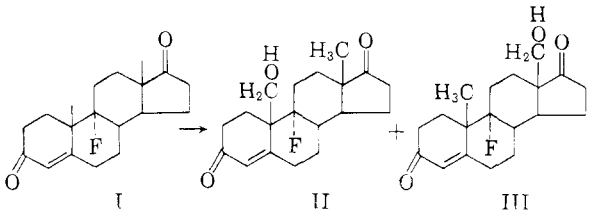
G. D. SEARLE AND COMPANY
P. O. BOX 5110 CLARENCE G. BERGSTROM
CHICAGO 80, ILLINOIS R. M. DODSON

RECEIVED MARCH 3, 1960

THE BIOLOGICAL HYDROXYLATION OF 9α-FLUOROANDROSTENEDIONE

Sir.

In order to provide further evidence for the position and configuration of the fluorine atom in 9α-fluoro-4-androstene-3,17-dione (I),[1] an attempt was made to convert I to the known 9α-fluoro-11β-hydroxyandrostenedione[2] by perfusion of a solution in blood through surviving adrenal glands.[3] However, none of the desired 11β-hydroxylated material was isolated. Instead, two new monohydroxy-9α-fluoro-4-androstene-3,17-diones were obtained: II, m.p. 238.5° dec., $\lambda_{max}^{ethanol}$ 240 mμ (ϵ 15,300); λ_{max}^{KBr} 3.01, 5.74, 6.02, 6.18, 9.16, 9.42–9.48, 9.81 μ; (found: C, 71.19; H, 7.74). III, m.p. 236–239°, slight dec.; $\lambda_{max}^{methanol}$ 237 mμ (ϵ 15,100); λ_{max}^{KBr} 2.95, 5.78, 6.00, 6.20, 9.46, 9.73 μ; $[\alpha]_D$ +134.8° ($CHCl_3$); (found: C, 71.09; H, 7.73). Both compounds gave qualitative tests[4] for the presence of fluorine.



The distillates obtained after treatment of compounds II and III with aqueous sodium hydroxide gave positive reactions with chromotropic acid[5] indicating the presence of hydroxylated methyl groups in both steroids. A similar reaction was given by 19-hydroxyandrostenedione,[6] but reactions run with 9α-fluoro-11β-hydroxyandrostenedione[2] or without the addition of steroid were negative. A study of the change in ultraviolet spectrum with time of III and of 9α-fluoroandrostenedione (I) in 0.1 *N* ethanolic potassium hydroxide[7] showed no dramatic shifts, but only a slight decrease in extinction coefficient. The hydroxyl group in III is, therefore, isolated from the 3-keto-Δ^4-chromophore and should be at C_{18}. The molecular rotatory contribution of the new hydroxy group (ΔM_D^{18OH-H} $-49°$) is in good agreement with that calculated from 18-hydroxyestrone ($-28°$).[5]

The ultraviolet spectrum of II in 0.1 *N* ethanolic potassium hydroxide at zero time (approximately two minutes after preparing the solution) showed a maximum at 302 mμ (ϵ 17,400) and a point of inflection at 238 mμ (ϵ 5,850). After 24 hr. these changed to λ_{max} 292 mμ (ϵ 4,770) and λ_{max} 246 mμ (ϵ 9,250), respectively. Apparently, basic elimination of the 19-hydroxymethyl group occurred rapidly with concurrent elimination of the 9α-fluoro group to form 19-nor-4,10(9)-androstadiene-3,17-dione, which, in the basic solution slowly equilibrated with the δ,ϵ-unsaturated isomer(s). Thus, the sodium hydroxide chromotropic acid test and the study of change of ultraviolet spectra in ethanolic potassium hydroxide not only provided strong evidence for the positions of the hydroxyl groups in II and III, but also lent confirmatory evidence for the 9α-position of the fluoro group. The small bathochromic shift (2–3 mμ) associated with a 19-hydroxyl group is also noticed in a comparison of the ultraviolet spectra of 9α-fluoro-19-hydroxy-4-androstene-3,17-dione (II) ($\lambda_{max}^{ethanol}$ 240 mμ) and 9α-fluoro-18-hydroxy-4-androstene-3,17-dione (III) ($\lambda_{max}^{methanol}$ 237 mμ).[5]

(1) C. G. Bergstrom and R. M. Dodson, THIS JOURNAL, **82**, 3479 (1960).

(2) R. H. Lenhard and S. Bernstein, *ibid.*, **77**, 6665 (1955).

(3) R. W. Jeanloz, H. Levy, R. P. Jacobsen, O. Hechter, V. Schenker and G. Pincus, *J. Biol. Chem.*, **203**, 453 (1953). We are indebted to Dr. James J. Carlo and The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, for this perfusion.

(4) E. L. Bennett, C. W. Gould, E. H. Swift and C. Niemann, *Anal. Chem.*, **19**, 1035 (1947).

(5) K. H. Loke, G. F. Marrian and E. J. D. Watson, *Biochem. J.*, **71**, 43 (1959).

(6) A. S. Meyer, *Experientia*, **11**, 99 (1955); A. S. Meyer, M. Hayano, M. C. Lindberg, M. Gut and O. G. Rodgers, *Acta Endocrin.*, **18**, 148 (1955).

(7) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(8) F. W. Kahnt, R. Neher and A. Wettstein, *Helv. Chim. Acta*, **38**, 1237 (1955).

Most significant, however, is the difference in adrenal metabolism of androstenedione[3,6] and 9α-fluoroandrostenedione.

G. D. SEARLE AND COMPANY
P. O. BOX 5110 CLARENCE G. BERGSTROM
CHICAGO 80, ILLINOIS R. M. DODSON

RECEIVED MARCH 3, 1960

SEPARATION OF STEROIDS BY GAS CHROMATOGRAPHY

Sir:

The microanalytical separation of steroids by gas chromatographic techniques has not been achieved in any practical fashion up to the present time. This is because relatively high temperatures or long retention times have been necessary to achieve even limited success.[1]

Conditions suitable for effective separations have been found through use of a silicone gum (methyl-substituted type, SE-30) coated on Chromosorb W. The table lists retention times relative to cholestane for a series of model compounds separated under two different circumstances. The separations at 260° were carried out with a column containing 7/100 SE-30 silicone on Chromosorb W, 80–100 mesh. The disadvantages of high temperature operations were evident when hydroxy compounds and acetyl esters were involved; broad peaks and multiple components suggestive

TABLE I

RELATIVE RETENTION TIMES[a]

Compound	Temperature 260°[b]	222°[c]
Androstane	0.17	0.11
Androstan-17-one	.30	.22
Androstan-3,17-dione	.56	.47
4-Androsten-3,17-dione	.68	.57
Pregnan-3,20-dione	.74	.67
Allopregnan-3,20-dione	.82	.74
Allopregnan-3β,20β-diol		.70
Allopregnan-3,11,20-trione	1.05	.99
Coprostane		.90
Cholestane	1.00[d]	1.00[e]
Cholestanyl methyl ether	1.58	1.78
Cholesteryl methyl ether	1.47	1.72
Cholestan-3-one	2.00	2.17
4-Cholesten-3-one	2.37	2.72
Cholestanol	1.70	1.99
Cholesterol	1.21 (broad)	1.98
Cholestanyl acetate	1.15 (v. broad)	2.84
Cholesteryl acetate	1.18 (broad)	2.81
β-Sitosterol	1.82 (v. broad)	3.26
β-Sitosteryl acetate		4.62
Stigmastane		1.65
Stigmasterol	1.62 2.29	2.84

[a] Argon ionization detector, 6 ft. × 4 mm. i.d. columns. [b] Pressure, 20 p.s.i.; 7/100 SE-30 on Chromosorb W, 80–100 mesh. [c] Pressure, 10 p.s.i.; 2–3/100 SE-30 on Chromosorb W, 80–100 mesh. [d] Time, 19.3 min. [e] Time, 17.6 min.

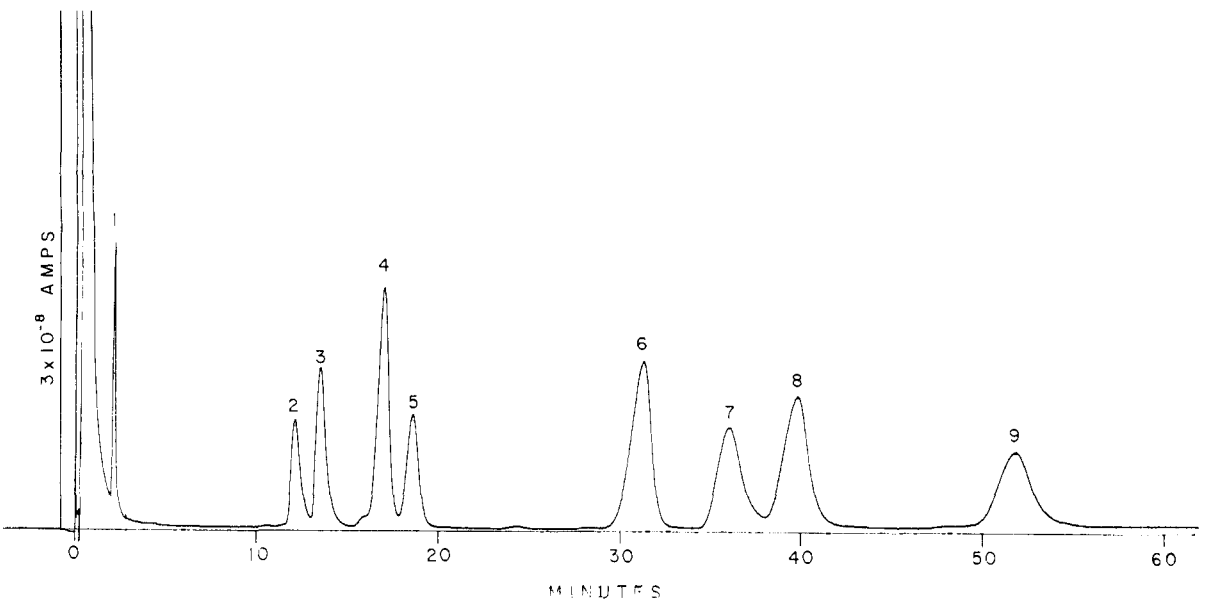


Fig. 1.—Separation of 1, androstane; 2, pregnane-3,20-dione; 3, allopregnane-3,20-dione; 4, coprostane; 5, cholestane; 6, stigmastane; 7, cholesterol; 8, cholestan-3-one; 9, stigmasterol. Conditions:—3% SE-30 silicone gum on Chromosorb W (80-100 mesh); 6 ft. × 4 mm. column; 222°; argon inlet pressure, 10 psi.

of decomposition were obtained for substances of this kind. Hydrocarbons, ethers and ketones were not affected. When a column containing 2–3/100 SE-30 silicone on the same support was used, there was a major change in the observed effects. At 222° all of the compounds in the table were eluted as single components with no sign of decomposition; these included hydrocarbons, ketones, alcohols, ethers and acetyl esters. The short retention times observed at this relatively low temperature for comparatively high molecular

(1) For example, G. Eglinton, R. J. Hamilton, R. Hodges and R. A. Raphael, *Chem. & Ind.*, 955 (1959), reported a retention time of about 4 hours for cholestanone at 220° with an Apiezon L phase (46 in. col.). An ethylene glycol–isophthalate polyester has been used at 270° for steroid separations (C. C. Sweeley and E. C. Horning, *Nature*, in press).