[CONTRIBUTION FROM THE INSTITUTE OF APPLIED MICROBIOLOGY, UNIVERSITY OF TOKYO]

Steroid Studies. XVII.[1] On the Absolute Configuration of C-24-Ethyl of Stigmasterol

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The ozonolysis product of *i*-stigmasteryl methyl ether, 2-ethyl-3-methylbutanal (+) was converted through the corresponding alcohol (−) and its tosylate (−) to the reference compound, methylethylisopropylmethane (−) and the absolute configuration of the C-24-ethyl group of the stigmasterol series was thus shown to have the C-24 α configuration in Plattner's convention. Further, some evidence on the configuration of the C-24-methyl of the ergosterol series is presented.

Bergmann and Low[3,4] have deduced that the C-24 substituents of both stigmasterol and ergosterol have the same configuration, C-24b. The basis for their conclusion is: (i) that the oxidative fission products of the stigmasterol and ergosterol series, (+)-5-ethyl-6-methylheptanone-2[5,6] and (−)-5,6-dimethylheptanon-2,[7] respectively, must have the same configuration according to the empirical rule of Marker[8] and (ii) that the M_D differences between the 2,4-dinitrophenylhydrazones and semicarbazones of the corresponding ozonolysis products, 2-ethyl-3-methylbutanal[5,9,10] and 2,3-dimethylbutanal,[5,11−13] are in the same direction and of comparable magnitude (Freudenberg's rule of shift[14]).

Stokes and Bergmann[4,15−17] applied the van't Hoff principle of additivity[18] for the C-24-alkyl sterols. The molecular rotation of a 24-alkyl derivative of cholestanol can be considered to be made up of the molecular rotation of cholestanol and the molecular rotation due to the additional asymmetric center (C-24). The calculated shift due to the C-24-methyl group is numerically close to the molecular rotation of the closely analogous reference compound of optically active methylethylisopropylmethane. Thus they have deduced that the absolute configuration of the methyl group in the ergosterol series should be C-24β in Plattner's convention.[19]

Their deduction thus has a reasonable basis, but it is desirable to have direct chemical proof. This paper deals with such a proof of the configuration of the ethyl group of stigmasterol.

When *i*-stigmasteryl methyl ether (I)[20] is ozonized in methylene chloride containing one mole equivalent of pyridine at low temperature, according to Slomp and Johnson,[9] an aldehyde, (+)-2-ethyl-3-methylbutanal (II),[5,9,10] is formed in reasonably good yield. The dextrorotatory aldehyde was reduced with lithium aluminum hydride to the corresponding alcohol III[5] (levorotatory). Treatment of III with *p*-toluenesulfonyl chloride in pyridine gave a levorotatory tosylate which was distillable in high vacuum and which decomposed above 175° liberating *p*-toluenesulfonic acid. The tosylate was reduced with lithium aluminum hydride[21] to a hydrocarbon, (−)-methylethylisopropylmethane (V),[22−24] in contrast to that expected on the basis of Marker's rule.[25] Freudenberg, *et al.*,[23] correlated levorotatory V to dextrorotatory isopropylsuccinic acid whose absolute configuration[24,25] was definitely determined.[26−29] Therefore the absolute configuration of V is as shown in the formula and the ethyl group of stigmasterol must have the C-24α configuration in Plattner's convention. A repetition of the last step gave a sample of much more levorotatory V, confirming the result of the earlier experiment. Analyses of both samples by infrared spectra (liquid film) and gas chromatography (see Experimental) indicated that the hydrocarbon samples had no impurities which would affect the sign of rotation.

Analogous experiments were carried out in the ergosterol series. Ozonolysis of 5,6-dihydroergosterol (VIa)[30] or its acetate (VIb) in methylene chloride without pyridine afforded an ozonide which was directly reduced with lithium aluminum hydride to an alcohol, (−)-2,3-dimethylbutanol (VIII),[22] in poor yield. The yield was slightly

(1) Paper XVI, THIS JOURNAL, **82**, 1442 (1960).

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(17) W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1987 (1954).

(18) J. H. van't Hoff, "Die Lagerung der Atome in Raume," 2nd ed., Vieweg, Braunschweig, 1894, p. 119; P. A. Guge and A. Gautier, *Compt. rend.*, **119**, 740, 953 (1894).

(19) Pl. A. Plattner, *Helv. Chim. Acta*, **34**, 1693 (1951); Plattner convention originally defined the asymmetry at C-20, but it was later extended to other positions in the side chain as is cited in the Fiesers' book (ref. 4, p. 338). In this paper the authors use this extended Plattner convention.

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raised when the reaction was conducted in two steps, *i.e.*, ozonolysis of VIb by the method described earlier in the stigmasterol series gave 50% yield of (−)-2,3-dimethylbutanal (VII)[7,11–13] together with the corresponding acid. This aldehyde was then reduced with lithium aluminum hydride to VIII of the same sign. Since the levorotatory VIII has been correlated with V of the same sign by Levene and Marker,[22] the C-24-methyl in the ergosterol series should have the C-24β configuration in Plattner's convention. This deduction is of great importance because it contradicts Bergmann's conclusion of the same configuration for both sterols. Therefore, a repetition of their interconversion[31] was attempted, but unfortunately only certain parts of the work have so far been carried out. The results obtained are in agreement with those of Levene and Marker.

In Table I the values of α_D of various compounds described in this paper are summarized. Comparison of M_D of two compounds can lead to errors in assigning their configurations, especially when the compounds are not optically pure, such as VII, and their molecular weights differ widely. Comparison of α_D when molecular weights are similar gives a reliable answer and thus the differences between the aldehydes and alcohols seem to support our tentative assignment of C-24β for the ergosterol series.[32]

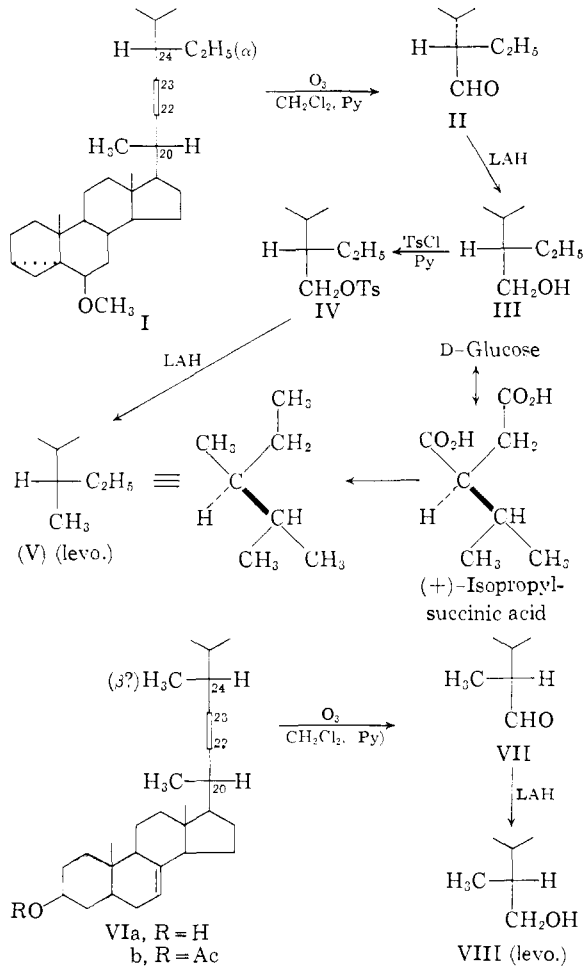

TABLE I

Stigmasterol series: Compounds	[α]D	Ergosterol series: Compounds	[α]D
II	+37°	VII	−65.2°
2,4-DNP of II	− 3.42	2,4-DNP of VII	−35.0
III	− 9.47	VIII	− 5.49
α-Naph.-urethan of III	− 3.76	α-Naph.-urethan of VIII	−12.65[a]
IV	− 8.05	1-Bromo-2,3-dimethylbutane	−11.7[a]
V	−10.22	3,4-Dimethylpentanol	−21.5[a]

[a] Calculated.

Experimental[33]

All boiling points and melting points are uncorrected. Optical rotations of liquid substances were measured in a 5-cm. tube at 25° without solvents for the comparison of Marker's rule. Evaporation of solvents and distillation of liquid products were performed under nitrogen.

2-Ethyl-3-methylbutanal (II).[5,9,10]—Ozonized air (3.14 g. of ozone/hr.) was passed into a solution of 60 g. of *i*-stigmasteryl methyl ether (I, prepared from stigmasteryl *p*-toluenesulfonate according to the method reported by Fernholz and Ruigh[20], m.p. 54°, $[\alpha]^{25}D$ +34.5° ($CHCl_3$, *c* 2.212)) in 750 ml. of methylene chloride containing 14.1 ml. of pyridine at −75° for about 4.5 hours according to Slomp and Johnson.[9] After 77.58 g. of glacial acetic acid and 37.5 g. of zinc powder was added to the ozonized solution, the temperature was quickly raised up to 20° and stirring was continued for 2.5 hours at the temperature. The cooled reaction mixture, after removal of zinc powder by filtration, was treated with 44.3 g. of sodium carbonate and steam distilled. The pyridine in the organic distillate was carefully eliminated by washing with 3% hydrochloric acid. The organic layer was washed successively with water, dilute sodium bicarbonate and water, dried over sodium sulfate and evaporated. The residue was distilled *in vacuo* to give 11.74 g. (73.5%) of II which on redistillation gave almost colorless liquid, b.p. 67–69° (74 mm.), $[\alpha]D$ +37°, d^{25}_4 0.8295, $n^{24}D$ 1.4100.

2,4-Dinitrophenylhydrazone.[5,9]—To a warm solution of 400 mg. of 2,4-dinitrophenylhydrazine in 60 ml. of 99% ethanol was added 230 mg. of the aldehyde and a few micro drops of concd. hydrochloric acid. The solution was digested for 10 minutes and then concentrated to 15 ml. under reduced pressure. Water was added and the deposited yellow mass was dissolved in hot ethanol and crystallized quickly in an ice-bath. Two recrystallizations gave a sample of m.p. 119°, $[\alpha]^{28.5}D$ −3.42° ($CHCl_3$, *c* 1.650).

Anal. Calcd. for $C_{13}H_{18}O_4N_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.04; H, 6.16; N, 19.07.

2-Ethyl-3-methylbutanol (III).[5]—To a stirred suspension of 7.35 g. of lithium aluminum hydride in 400 ml. of dry ether, was added dropwise at 0–5° a solution of 17.66 g. of II in 200 ml. of dry ether. Agitation was continued for 4 hours and the mixture was kept overnight at room temperature. Under cooling, the excess hydride was decomposed with ethyl acetate and water and 3% hydrochloric acid was added to clear the mixture. The aqueous layer which separated was extracted twice with ether. The combined ethereal solution was washed with water, dilute sodium bicarbonate, water and dried over sodium sulfate. Fractional distillation of the product gave 9.45 g. (79%) of 2-ethyl-3-methylbutanol (III) boiling at 91.5° (50 mm.), $[\alpha]D$ −9.47°, d^{25}_4 0.8326, $n^{29.5}D$ 1.4268; α-naphthylurethan (from petroleum ether, b.p. 40–60°), m.p. 71–72°, $[\alpha]^{25}D$ −3.76° ($CHCl_3$, *c* 2.126).

(31) The assistant editor and two referees also pointed out in the preliminary paper (ref. 25) that the deduction must be drawn from the result of the trace of Marker's interconversion.

(32) The referee also pointed out in the preliminary paper (ref. 25) that the $\Delta\alpha D$ seems to support our deductions.

(33) The authors are indebted to Mr. T. Onoe, Miss C. Furukawa and Miss H. Otsuka for microanalysis. Thanks are also due to Dr. M. Maruyama and Mr. S. Senoh for the analyses of gas chromatography. The authors wish to express their appreciations to Messrs. H. Shindo and H. Higuchi for the analyses of infrared spectra.

Anal. Calcd. for $C_{16}H_{22}O_2N$: C, 75.75; H, 8.12; N, 4.91. Found: C, 75.73; H, 8.13; N, 5.00.

2-Ethyl-3-methylbutanol *p*-Toluenesulfonate (IV).—A solution of 4.9 g. of 2-ethyl-3-methylbutanol (III) in 10 ml. of pyridine was added to a solution of 8.1 g. of *p*-toluenesulfonyl chloride in 18.4 g. of pyridine at 0–5° and the mixture was kept for 20 hours at room temperature. After treating with water for 3 hours in a refrigerator, the reaction mixture was washed with water and dilute hydrochloric acid (50.6 g. of 35% hydrochloric acid was used) and then extracted three times with ether. The extract was washed as usual, dried over sodium sulfate and the solvent was evaporated through a short column. The residue (9.33 g.) was distilled to give 8.11 g. (70.4%) of colorless tosylate, b.p. 116° (0.007 mm.) (bath temperature, 135°), $[\alpha]_D$ −8.05°, d^{25}_4 1.0727, $n^{29}D$ 1.4968 ($n^{29.5}D$ 1.4971).

Anal. Calcd. for $C_{14}H_{22}O_3S$: C, 61.49; H, 8.10; S, 12.25. Found: C, 61.47; H, 8.11; S, 12.27.

Another sample of IV ($[\alpha]_D$ −5.03°) boiled at 125–127° (0.01–0.005 mm.) and the tosylate was decomposed at 175° and 5 mm., liberating *p*-toluenesulfonic acid even under nitrogen.

Methylethylisopropylmethane (V).[23-24]—A solution of 8.11 g. of the tosylate ($[\alpha]_D$ −8.05°) in 100 ml. of dry ether was added to a stirred suspension of 14.5 g. of lithium aluminum hydride in 400 ml. of dry ether under cooling and the stirring was continued for 20 hours at room temperature (25°). The excess hydride was decomposed with hydrous ether under cooling; during this time about 200 ml. of ether was collected in a trap which was connected to the top of the reflux condenser and which was cooled with Dry Ice–acetone. To the combined organic layer was added dilute hydrochloric acid (57 g. of 35% hydrochloric acid was used) to clear the layer and the aqueous layer separated was re-extracted three times with ether. The whole ethereal solution was washed successively with dilute hydrochloric acid and saturated aqueous solution of sodium chloride, 2% sodium bicarbonate, again with saturated aqueous solution of sodium chloride and dried over sodium sulfate. The ether was evaporated through a short column and the residue was carefully distilled over sodium through an efficient packed column (*ca.* 30 × 1 cm.) with electric heating to give 0.7 g. (23.6%) of methylethylisopropylmethane, b.p. 90°, $[\alpha]_D$ −10.22°, d^{25}_4 0.6950, $n^{25}D$ 1.3949. The reported optical constants[22,23] are b.p. 90°, $M^{21}D$ +2.9: calcd. maximum M_D +28.3, d^{21}_4 0.695 (for the antipode of V); b.p. 89.2°, $[\alpha]^{20}D$ −11.4, d^{20}_4 0.6950, $n^{20}D$ 1.3921 [for V from (−)-(2S)-2-isopropyl-1,4-dibromobutane[34]].

Anal. Calcd. for C_7H_{16}: C, 83.90; H, 16.10. Found: C, 83.64; H, 16.01.

Another run of the experiment, starting with a tosylate of $[\alpha]_D$ −5.03°, by the same method described above, except for stirring for 6 hours and digestion at room temperature overnight, yielded a sample (26.3%) of b.p. 89–90.5°, $[\alpha]_D$ −2.74°, d^{25}_4 0.6952, $n^{25}D$ 1.3950. The infrared spectrum of the former was simple (hydrocarbon) and identical with that of the latter. The R_t-values of the peaks of both hydrocarbons, using C.E.C. Type 26-201 Gas Chromatograph (stationary phase, 6 ft. long dinonyl phthalate; carrier, He, 60 ml./min.; temperature, 80°; detector bridge current, 200 ma.; chart speed, 0.5''/min.), were 16.42 and 16.26. No other significant peaks were observed.

2,3-Dimethylbutanal (VII).[7,11-13]—Ozonized air was passed into a solution of 44.07 g. (0.1 mole) of 5,6-dihydroergosteryl acetate[30] in 750 ml. of methylene chloride containing 16 g. (0.2 mole) of pyridine at −78° for 4 hours. To the cold reaction mixture was added 50 g. of zinc powder and 100 g. of glacial acetic acid and the temperature was quickly raised to 22° and stirred for 2 hours. After removal of the zinc powder, the solution was treated with 67.1 g. of sodium carbonate under cooling and steam distilled. The organic distillate was washed successively with dilute hydrochloric acid (25.2 g. of 35% hydrochloric acid was used), saturated aqueous solution of sodium chloride, dilute sodium bicarbonate, saturated aqueous solution of sodium chloride and dried over sodium sulfate. The solvent was evaporated through a short column and the residue was distilled to give 4.57 g. (45.7%) of colorless liquid of 2,3-dimethylbutanal (VII), b.p. 69–72° (160 mm.), $[\alpha]_D$ −65.2°, $[\alpha]^{26}D$ −62.6° ($CHCl_3$, *c* 5.648), d^{25}_4 p.8300, $n^{25}D$ 1.4029. The yield of VII varied from 25.6 to 45.7%.

2,4-Dinitrophenylhydrazone[12,13]—was prepared by the same method as described in II; m.p. 124.5–125.5°, $[\alpha]^{28.5}D$ −35.0° ($CHCl_3$, *c* 1.440).

Anal. Calcd. for $C_{12}H_{16}O_4N_4$: C, 53.42; H, 5.52; N, 19.17. Found: C, 53.48; H, 5.52; N, 19.17. Found: C, 53.48; H, 5.50; N, 19.20.

The higher boiling fraction of 2,3-dimethylbutyric acid, b.p. 75° (7.5 mm.), was obtained together with VII on the distillation; S-benzylthiuronium salt, m.p. 154°.

Anal. Calcd. for $C_{14}H_{21}N_2O_2S$: C, 59.98; H, 7.52; N, 9.97. Found: C, 60.09; H, 7.16; N, 10.05.

2,3-Dimethylbutanol (VIII).[22] **A.** ***Via*** **the Ozonide.**—Ozonized air (3.15 g. of ozone/hr.) was passed into a solution of 10 g. of 5,6-dihydroergosterol, m.p. 176–177°, $[\alpha]^{25}D$ −19.0° ($CHCl_3$, *c* 2.050), in 550 ml. of methylene chloride at −75°, until the content of ozone in the exhausted air had returned to the initial value. (One mole equivalent of ozone was insufficient to yield VII in better yield and the selectivity[30b] of ozone to attach first to the side-chain double bond of this sterol was not observed in the conditions employed.) The solvent was evaporated *in vacuo* under cooling, to the residue was successively added 400 ml. of ether and 12 g. of lithium aluminum hydride and the mixture was left to stand overnight and heated gently under reflux for 6 hours. The ether solution separated, after decomposition of the excess hydride with hydrous ether, was washed with water, dried over sodium sulfate and evaporated. The aldehyde-free pale yellow liquid was steam distilled and the distillate, saturated with sodium chloride, was extracted with ether. The solvent was evaporated and the residue was distilled to give 540 mg. (21%) of colorless liquid, b.p. 64° (25.5 mm.), $[\alpha]^{28}D$ −2.95° ($CHCl_3$, *c* 60.521); α-naphthylurethan, m.p. 92–93° (from petroleum ether, b.p. 40–100°), $[\alpha]^{28}D$ −6.80° ($CHCl_3$, *c* 2.054).

Anal. Calcd. for $C_{17}H_{21}O_2N$: C, 75.24; H, 7.80; N, 5.16. Found: C, 75.24; H, 7.96; N, 5.31.

B. ***Via*** **2,3-Dimethylbutanal (VII).**—A solution of 3.534 g. of VII in 500 ml. of dry ether was added to a suspension of 1.75 g. of lithium aluminum hydride in 450 ml. of dry ether under stirring and cooling and kept overnight at room temperature. The excess hydride was decomposed with water and the ether layer was washed successively with dilute hydrochloric acid (19.2 g. of 35% hydrochloric acid was used), a saturated aqueous solution of sodium chloride, 2% sodium bicarbonate, again with a saturated aqueous solution of sodium chloride three times and dried over sodium sulfate. The ether was evaporated through a short column and the residue was distilled to give 2.98 g. (82.5%) of colorless liquid of VIII, b.p. 101° (148.5 mm.), $[\alpha]_D$ −5.49°, d^{25}_4 0.8237, $n^{27}D$ 1.4190. Its naphthylurethan was identical with the one described in the method of A.

1-Bromo-2,3-dimethylbutane.[22]—A solution of 9.61 g. of 2,3-dimethylbutanol (VIII, $[\alpha]D$ −5.11°) in 12 ml. of chloroform was added dropwise under cooling and vigorous stirring to 55 g. of phosphorus pentabromide covered with chloroform. The stirring was continued for 13 hours at room temperature and then heated for 2.5 hours at 50°. The cooled mixture was poured onto ice and the chloroform layer separated was washed with water, dilute sodium bicarbonate and water, and dried over sodium sulfate. The solvent was evaporated and the residue was distilled to give crude bromide which was shaken with concd. sulfuric acid and redistilled to give 7.13 g. (46%) of 1-bromo-2,3-dimethylbutane, b.p. 140°, $[\alpha]^{25}D$ −10.88°, d^{25}_4 1.187, $n^{25}D$ 1.4508. The reported constants[22] are b.p. 140°, $M^{25}D$ +3.1, calcd. max. $M^{25}D$ +30.6, d^{25}_4 1.190 (for the antipode). Analytical sample was further purified by distillation.

Anal. Calcd. for $C_6H_{13}Br$: C, 43.60; H, 7.95; Br, 48.40. Found: C, 43.26; H, 8.19; Br, 48.87.

Several runs of the experiment using chloroform and phosphorus tribromide gave the bromide ($[\alpha]_D$ −8.98 to −10.86°) in 31.1–35.2% yield.

3,4-Dimethylpentanol.[22]—Under cooling and the current of nitrogen, gaseous formaldehyde generated from paraformaldehyde was passed into the Grignard reagent which was prepared from 7 g. of the bromide ($[\alpha]_D$ −10.88°), 1.05 g. of magnesium turnings and 50 ml. of dry ether. After a test with Michler ketone reagent was negative, the mixture was further stirred for 30 minutes, then decomposed with water

(34) R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia*, **12**, 81 (1956).

and made clear by the addition of dilute sulfuric acid. The ethereal solution was washed with water, dilute sodium bicarbonate and water. After drying over sodium sulfate, the ether was evaporated and the residue was distilled to give 800 mg. of colorless liquid of b.p. 103–117° (130–137 mm.) and 1.45 g. of higher boiling liquid. Both fractions were combined and refluxed with methanol containing a few drops of concd. sulfuric acid for 5 hours. Water was added and the mixture was extracted with ether. After the usual processing, the extract afforded 1.62 g. (32.85%) of colorless liquid, c.p. 118–119° (150 mm.) or 165° (760 mm.), $[\alpha]D$ −20.02°, d^{52}_4 0.8035, $n^{28}D$ 1.4258. The reported constants[22] are b.p. 164°, $M^{25}D$ +3.2, calcd. max. $M^{25}D$ +31.2, d^{25}_4 0.828 (for the antipode).

Anal. Calcd. for $C_7H_{16}O$: C, 72.35; H, 13.88. Found: C, 72.39; H, 14.00.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. CXXXVII.[1] Synthesis of a New Class of Potent Cortical Hormones. 6α,9α-Difluoro-16α-hydroxyprednisolone and its Acetonide

BY J. S. MILLS, A. BOWERS, CARL DJERASSI AND H. J. RINGOLD

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The syntheses of 6α,9α-difluoro-16α-hydroxyprednisolone (XVb) and related corticoids are described. In one sequence, 16α,17α-oxido-Δ⁵-pregnene-3β,21-diol-20-one 21-acetate (I) was converted to 6α-fluoro-16α-hydroxy substance "S" (IXb) which on adrenal incubation gave 6α-fluoro-16α-hydroxyhydrocortisone (Xa) transformed to XV by the Fried sequence followed by selenium dioxide oxidation. Alternately 6α-fluorohydrocortisone and 6α,9α-difluorohydrocortisone were hydroxylated at C-16α by *Streptomyces roseochromogenus*. 6α,9α-Difluoro-16α-hydroxyprednisolone and its corresponding acetonide (XVc) exhibited high anti-inflammatory activity without retaining sodium.

Following the basic finding of Fried and his coworkers[2] that the glycogenic, anti-inflammatory and mineralocorticoid activity of cortical hormones may be potentiated by the substitution of halogen, in particular fluorine, at C-9α, considerable effort has been devoted to the synthesis of cortical hormone analogs bearing substituents at other sites of the molecule in the hopes of obtaining high anti-inflammatory activity without undesirable sodium retention. This search has led to the preparation, among others, of 2α-methyl-,[3] 4-methyl-,[4] 4-halo-,[5] 6α-methyl-,[6] 6α-chloro-,[7] 6α-fluoro-,[8] 6α-nitro-,[9] 7β-methyl-,[10] 11α-methyl-,[11] 12α-chloro-,[12] 14α-hydroxy-,[13] 16α-hydroxy-,[8d,14] 16α-methyl-[8c,e,f,15] and 16β-methyl-[15b,16] cortical hormone analogs with the 6- and 16-substituted compounds appearing to be of particular interest. Thus, the 6-methyl, -chloro and -fluoro groups potentiate anti-inflammatory activity and promote sodium excretion but as single modificants are incapable of completely overcoming the profound sodium retention induced by a 9α-fluoro atom. Introduction of a 16α-hydroxy group into 9α-fluoroprednisolone leads to a compound devoid of sodium retention[14d] but with anti-inflammatory activity considerably lower[14d,e] than the parent 9α-fluoro compound, while 16α-[15c,d] and 16β-methyl-9α-fluoro-prednisolone,[16] also free of sodium retention, are more potent than the parent compound. Peculiarly conversion of 16α-hydroxy-

(1) Paper CXXXVI, J. A. Edwards, H. J. Ringold and C. Djerassi, THIS JOURNAL, **82**, 2318 (1960).

(2) See J. Fried and E. F. Sabo, *ibid.*, **79**, 1130 (1957), and earlier references therein.

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