When cholesterol-[4-^{14}C + 7β-t] was used practically all the tritium remained in the cholic acid molecule. Mild oxidation of the isolated cholic acid to its 7-keto derivative resulted in complete loss of the tritium label. Consequently, 7α-hydroxylation involves displacement of the 7α-hydrogen with *at least* 93% and possibly complete specificity. The same stereochemical course has been observed for the hydroxylation of steroids at C_{11}, *i.e.*, displacement with retention of configuration.[8,9]

These data are reminiscent of the observation that hydroxylation of *cis*- and *trans*-decalin by ozone proceeds with retention of configuration to *cis*- and *trans*-9 hydroxydecalin, respectively,[10] and are in agreement with Bloom's evidence.[2] In addition, it seems relevant that *in chemical systems* electrophilic displacement at a saturated carbon atom has been found to occur preferentially with retention of configuration.[6a,11]

This work was supported by the National Institutes of Health (Grant H2842) and the Alfred P. Sloan Foundation.

(8) M. Hayano, M. Gut and D. H. Peterson, private communication.

(9) E. J. Corey, G. A. Gregoriou and D. H. Peterson, THIS JOURNAL, **80**, 2338 (1958).

(10) J. R. Durland and H. Adkins, *ibid.*, **61**, 429 (1939).

(11) S. Winstein, T. G. Traylor and C. S. Garner, *ibid.*, **77**, 3741 (1955); S. Winstein and T. G. Traylor, *ibid.*, **77**, 3747 (1955), **78**, 2597 (1956).

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THE STEREOCHEMISTRY OF 11α-HYDROXYLATION OF STEROIDS

Sir:

The enzymatic hydroxylation of steroids at C_{11}, a reaction which is presently of considerable commercial and medical importance, is subject to the same sort of stereochemical analysis which has been utilized generally for the study of displacement reactions involving tetrahedral carbon, if the 11α- and 11β-hydrogens are differentiated isotopically.[1] The required stereospecific labelling has now been accomplished in the pregnane-3,20-dione series and the enzymatic 11α-hydroxylation by *Rhizopus nigricans* has been shown to proceed by *stereospecific* displacement of the *11α-hydrogen (or deuterium) substituent, i.e.*, with over-all retention of configuration.

Microbiological oxidation of pregnane-3,20-dione-11β-*d* containing one deuterium/molecule[2] was carried out with *Rhizopus nigricans* using the techniques previously described[3] and yielded 11α-hydroxypregnane-3,20-dione-11β-*d* containing 0.98 ± 0.02 deuterium/molecule. Similar oxidation of pregnane-3,20-dione-11α-*d* having additional deuterium at C_9 and C_{12} and a total of 2.80 deuterium/molecule[4] resulted in complete loss of 11α-deuterium since the 11α-hydroxypregnane-3,20-dione which was produced possessed 1.77 deuterium/molecule.

Enzymatic hydroxylation of steroids at the 11β-[5] and 7α-positions[6] also has been found to proceed with retention of configuration, a course which, though under the control of specific enzymatic interactions as usual, may also be favored by the electrophilic nature of the displacing reagent.[6] All the data accumulated thus far[5,6] indicate a lack of hydrogen isotope effect on the rate of oxidation and permit an additional conclusion: either C–H bond rupture occurs after the rate determining step of the reaction or else chemical reaction is preceded by at least one slow physical step, *e.g.*, adsorption, which is insensitive to H isotope.

We take pleasure in thanking Mr. Josef Nemeth for the deuterium analyses, Dr. Robert Levin for gifts of steroids, and Mr. O. K. Sebek for experimental assistance and the Alfred P. Sloan Foundation for generous financial aid.

(1) See E. J. Corey, M. G. Howell, A. Boston, R. L. Young and R. A. Sneen, THIS JOURNAL, **78**, 5036 (1956).

(2) Synthesized by the sequence: pregnane-3,11,20-trione → pregnane-3,11,20-trione-3,20-bis-ethylene ketal → pregnane-3,20-dione-11β-ol-11α-*d*-3,20-bis-ethylene ketal (LiAlD$_4$) → $\Delta^{9,11}$-pregnene-3,20-dione-11-*d*-($POCl_3$-C_5H_5N, followed by HOAc) → pregnane-3,20-diol-11β-*d* (Pt, H_2, HOAc followed by deacetylation with LiAlH$_4$) → pregnane-3,20-dione-11β-*d* (CrO_3-HOAc).

(3) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *ibid.*, **74**, 5933 (1952).

(4) Prepared by the route: $\Delta^{9,11}$-pregnene-3,20-dione → $\Delta^{9,11}$-pregnene-3,20-diol (LiAlH$_4$) → pregnane-3,20-diol-9α,11α,12α-$d_{2.80}$ (D_2, DOAc, Pt) → pregnane-3,20-dione-9α,11α,12α-$d_{2.80}$ (CrO_3). The distribution of deuterium is probably: 9α:d_1, 11α:d_1 and 12α:$d_{0.8}$; see D. K. Fukushima and T. F. Gallagher, *ibid.*, **77**, 139 (1955).

(5) M. Hayano and M. Gut, private communication.

(6) S. Bergstrom, S. Linstedt, B. Samuelson, E. J. Corey and G. A. Gregoriou, *ibid.*, **80**, 2337 (1958).

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CYCLIC 16α,17α-KETALS AND ACETALS OF 9α-FLUORO-16α-HYDROXY-CORTISOL AND -PREDNISOLONE

Sir:

9α-Fluoro-16α-hydroxy-cortisol and -prednisolone (triamcinolone) are potent glucocorticoids and anti-inflammatory agents devoid of salt retaining properties.[1] We have now found that certain cyclic 16α,17α-ketals[2] and -acetals derived from these steroids possess considerably greater glucocorticoid and anti-inflammatory activity than the parent compounds.

The cyclic derivatives are formed in excellent yield when a suspension of the steroid in the ketone or aldehyde[3] is agitated at room temperature with

(1) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **76**, 5693 (1956).

(2) The preparation of the acetonide of triamcinolone was mentioned in a talk by Dr. Seymour Bernstein, Lederle Laboratories, at the Laurentian Hormone Conference, September, 1957.

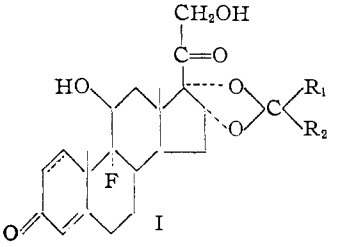
(3) The acetaldehyde derivatives were prepared with paraldehyde. They were obtained in crystalline form only after acetylation and hydrolysis of the crystalline acetates.

TABLE I

Derivative of	with	M.p., °C.	$[\alpha]_D^{CHCl_3}$	Analyses, found, % C	H	Liver glycogen[a] Cortisone acetate = 1	Anti-inflammatory[b] Cortisol acetate = 1
Δ^1-FF-16α-ol[c]	Acetaldehyde (I)	244–246	+102°	65.47	7.19	98 (62–155)[d]	50
FF-16α-ol	Acetaldehyde (II)	244–247	+145°	65.34	7.48	94 (57–157)	30
Δ^1-FF-16α-ol	Acetone (III)	292–294	+109°	66.49	7.31	92 (48–176)	40
FF-16α-ol	Acetone (IV)	270–273	+137°	66.03	7.92	121 (47–314)	18
Δ^1-FF-16α-ol	Methylethyl ketone (V)	255–260	+ 92°	67.01	7.41	77 (49–121)	35
Δ^1-FF-16α-ol	Diethyl ketone (VI)	265–268	+ 91°	67.62	7.24	26 (16–43)	11
Δ^1-FF-16α-ol	Methylisobutyl ketone[e] (VII)	256–258	+ 89°	68.10	7.72	12 (8–18)	8
Δ^1-FF-16α-ol	Methylisobutyl ketone (VIII)	185–188	+ 88°	67.87	7.74	4 (2–7)	3
Δ^1-FF-16α-ol	Cyclohexanone (IX)	278–281	+ 90°	67.97	7.53	16 (10–25)	5
Δ^1-FF-16α-ol	Acetophenone[f] (X)	281–283	+ 23°	69.91	7.04	12 (7–21)	15
Δ^1-6α-methyl-FF[g] (XI)						60 (34–106)	22

[a] Modifications of assay described by Pabst, *et al.*, *Endocrinology*, **41**, 55 (1947). [b] According to F. M. Singer and A. Borman, *Proc. Soc. Exptl. Biol. Med.*, **92**, 23 (1956). [c] FF = 9α-fluorocortisol. [d] The figures in parentheses represent the 95% confidence limits. [e] The two sets of values refer to the two stereoisomers about the new asymmetric center. [f] Shows infrared bands at 13.06 and 14.29 μ characteristic of mono-substituted phenyl. [g] We wish to express our sincere thanks to Dr. G. Schreiber of the Upjohn Company for supplying this sample.

a trace of a mineral acid, preferably perchloric acid, until solution has occurred. Characterizing data and biological properties for some representative derivatives are shown in Table I.[4] They reduce tetrazolium reagent and form monoacetates *e.g.*, 9α-fluoro-16α-hydroxyprednisolone acetonide 21-acetate (m.p. 266°; $[\alpha]^{23}D$ +92° (*c* 0.59 in $CHCl_3$); λ_{max}^{Nujol} 3.01, 5.71, 5.79, 6.01–6.04, 6.21–6.24 μ. *Anal.* Found: C, 65.49; H, 6.81), and are therefore formulated as 16α,17α-ketals or acetals of structure I. In contrast to the extreme



ease of hydrolysis of most cyclic ketals the acetonide of 9α-fluoro-16α-hydroxyprednisolone remains unchanged during 4 hours of refluxing with 0.1 *N* sulfuric acid in aqueous methanol. The biological data indicate a progressive increase in activity with decreasing molecular weight of R_1 and R_2, with the exception of R_1 = phenyl. The majority of the derivatives listed show considerably greater glucocorticoid and anti-inflammatory activity than the parent steroids,[5] the more active ones surpassing 6α-methyl-9α-fluoroprednisolone[6] the most potent glucocorticoid heretofore described. The anti-inflammatory glucocorticoid activity ratios are in each case greater than those found for the respective parent compounds. Compounds I, II, III, IV and V cause sodium excretion in the rat, IX and X cause retention, and VI and XI effect neither retention nor excretion. In view of the altered physiological properties and of the unusual acid stability this group of compounds, in our opinion, is biologically active *per se* rather than after hydrolysis to the parent compounds.

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(4) All infrared spectra (Nujol) show bands characteristic of 20-keto (5.80–5.85 μ), Δ^4-3-keto (6.01 and 6.15 μ) and $\Delta^{1,4}$-3-keto groups (5.99–6.02, 6.15–6.19 and 6.21–6.24 μ), respectively.

(5) The liver glycogen and anti-inflammatory values determined in our laboratories are: 9α-fluoro-16α-hydroxyprednisolone, 14 (9–22) and 4; 9α-fluoro-16α-hydroxycortisol, 11 (7–19) and 1.

(6) G. B. Spero, J. L. Thompson, F. H. Lincoln, W. P. Schneider and J. A. Hogg, THIS JOURNAL, **79**, 1515 (1957).

THE SYNTHESIS OF DIHYDROSPHINGOMYELIN

Sir:

The correctness of structure I for the sphingomyelins has been proved recently by Fujino,[1] and by Stotz and co-workers.[2,3] Depending upon their origin, these natural products differ by the substituent RCO– which may be a palmitic, stearic, nervonic, or lignoceric acid residue. In this paper we wish to announce the synthesis of two dihydro derivatives of I, namely, palmitoyldihydrosphingomyelin (VIIIa) and stearoyldihydrosphingomyelin (VIIIb).

For an unambiguous synthesis we chose as key intermediate the oxazoline III, a derivative of dihydrosphingosine in which both the secondary hydroxyl and the amino group are blocked.

Methyl *threo*-α-benzamido-β-hydroxystearate[4] was cyclized with thionyl chloride to *cis*-2-phenyl-4-carbomethoxy-4-pentadecyl-2-oxazoline (II), m.p. 43–45°. (Found: C, 75.38; H, 10.11; N, 3.36.) Reduction with lithium aluminum hydride yielded 85% of the hydroxymethyloxazoline III, m.p. 98–99°. (Found: C, 77.6; H. 10.3; N, 3.0).

Treatment of III with β-chloroethylphosphoryl dichloride in the presence of pyridine led to the phosphate ester IV which was isolated in pure form as its barium salt (m.p. 143–145°) in a 30%

(1) Y. Fujino, *J. Biochem.* (*Japan*), **39**, 45 (1952).

(2) G. Rouser, J. F. Berry, G. Marinetti and E. Stotz, THIS JOURNAL, **75**, 310 (1953).

(3) G. Marinetti, J. F. Berry, G. Rouser and E. Stotz, *ibid.*, **75**, 313 (1953).

(4) H. E. Carter, J. B. Harrison and David Shapiro, *ibid.*, **75**, 4705 (1953).