tifications were established by comparisons against infra-red spectra of known standards. Compounds isolated from the above experiments are listed in Table I together with their specific activities.

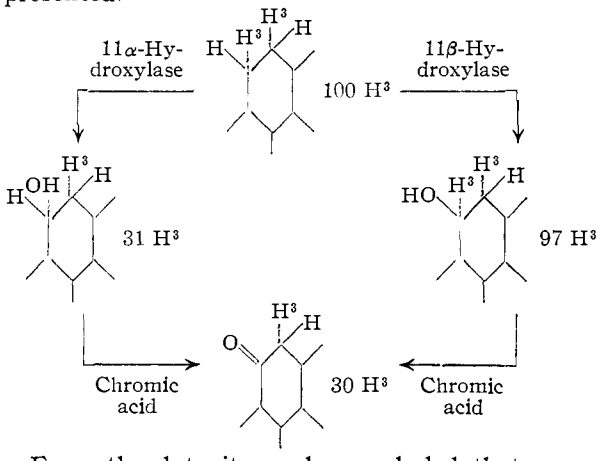
The results showed a loss of 69% of the total count of pregnanedione-H^3 on 11α-hydroxylation, and essentially no loss (2–3%) on 11β-hydroxylation. Mild chromic acid oxidation according to the method of Poos[5] of the hydroxylated products to their keto analogs (Table II) showed a loss of 70% of the total count in the case of the 11β-hydroxylated structure, while there was essentially no change (1%) in counts in the 11α series.

TABLE II

CHROMIC ACID OXIDATION OF 11-HYDROXYLATED STEROIDS

11α-Hydroxy-pregnanedione	11-Keto-pregnanedione
m.p. 123–125°	m.p. 159–161°
8.43×10^4 counts/min./μM	8.49×10^4 counts/min./μM
Corticosterone-21-acetate	11-Dehydro-corticosterone-21-acetate
m.p. 156–157°	m.p. 180–182°
3.21×10^4 counts/min./μM	0.98×10^4 counts/min./μM

A schematic representation of the results is presented.



From the data it can be concluded that enzymatic steroid hydroxylations proceed by a mechanism in which there is a simple replacement of the hydrogen in the position to be hydroxylated. This is the second instance noted in points of similarity in the mechanism of reaction of the steroid hydroxylases, irrespective of source, the first being their ability of the utilization of molecular oxygen directly in the formation of the hydroxyl function.[6,7] Further mechanism studies involving this group of enzymes are now in progress.

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

(6) M. Hayano, M. C. Lindberg, R. I. Dorfman, J. E. H. Hancock, and W. v. E. Doering, *Arch. Biochem. Biophys.*, **59**, 529 (1955).

(7) M. Hayano, A. Saito, D. Stone and R. I. Dorfman, *Biochim. Biophys. Acta*, **21**, 380 (1956).

THE STEREOCHEMISTRY OF 7α-HYDROXYLATION IN THE BIOSYNTHESIS OF CHOLIC ACID FROM CHOLESTEROL

Sir:

Recent evidence on the course of enzymatic hydroxylation of steroids at a saturated carbon atom indicates that such reactions occur by a direct replacement mechanism and not by hydration of olefinic intermediates.[1–3] However, the chemical nature of the enzymatic reagent and the type of mechanism involved are still undetermined. The stereochemistry of enzymatic hydroxylation at C_7 of the steroid nucleus has now been examined to provide geometrical evidence regarding mechanism.

During the biochemical conversion of cholesterol to cholic acid a 7α-hydroxyl group is introduced, quite possibly as the initial step since nuclear hydroxylation of cholesterol proceeds side-chain degradation[4] and since 7α-hydroxycholesterol is transformed into cholic acid in the rat.[5] The stereochemical course of this 7α-hydroxylation in rats has been investigated by double-labelling experiments using cholesterol stereospecifically labelled with tritium at position 7[6] and cholesterol-4-^{14}C.[7]

TABLE I

		Activity ratio T/C^{14}	Per cent. retention of tritium in cholic acid
1	Cholesterol-4-^{14}C-7α-*t*	1.75	
2	Cholic acid rat 4	0.31	7.4
3	Cholic acid rat 5	0.12	6.8
4	Cholesterol-4-^{14}C-7α-*t*	1.24	
5	Cholic acid rat 6	0.07	5.6
6	Cholic acid rat 7	0.09	7.2
7	Cholesterol-4-^{14}C-7β-*t*	1.04	
8	Cholic acid rat 9	0.95	91.5
9	Cholic acid rat 10	1.03	100

When cholesterol-[4-^{14}C + 7α-*t*] was administered to rats the isolated cholic acid retained only *ca.* 7% of tritium relative to radiocarbon (Table I).

(1) M. Hayano and R. I. Dorfman, *J. Biol. Chem.*, **211**, 227 (1954).

(2) B. M. Bloom and G. M. Shull, THIS JOURNAL, **77**, 5767 (1955).

(3) M. Hayano, A. Saito, D. Stone and R. I. Dorfman, *Biochem. and Biophys. Acta*, **21**, 380 (1956).

(4) See S. Bergstrom and B. Bergstrom, *Ann. Rev. Biochem.*, **25**, 177 (1956).

(5) S. Linstedt, *Acta Chem. Scand.*, **11**, 417 (1957).

(6) (a) Cholesterol-7α-*t* and cholesterol-7α-*d* were synthesized stereospecifically by the sequence: 7α-bromo-6-ketocholestanyl acetate → 7α-^{n}H-6-ketocholestanyl acetate (Zn-^{n}HOAc) → 7α-^{n}H-6β-hydroxycholestanyl acetate ($NaBH_4$) → 7α-^{n}H-cholesterol ($POCl_3$-C_5H_5N, followed by $LiAlH_4$). Cholesterol-7β-*t* and cholesterol-7β-*d* were prepared by a similar process starting with 5α,7β-$^n H_2$-7α-bromo-6-ketocholestanyl acetate using unlabelled acetic acid in the debromination step. The cholesterol-7α-*d* and -7β-*d* (infrared max. 2102, 2127 cm.$^{-1}$ and 2147, 2160 cm.$^{-1}$, respectively) were analyzed by infrared absorption [E. J. Corey, M. G. Howell, A. Boston, R. L. Young and R. A. Sneen, THIS JOURNAL, **78**, 5036 (1956)] and the isotope orientation was found to be stereospecific within the analytical sensitivity of ±4%. It follows that the tritium labelling is 96 ± 4% stereospecific; (b) see E. J. Corey and G. A. Gregoriou, *Abstracts*, 131st A.C.S. meeting, p. 15-O.

(7) *Ca.* 2 mg. of a mixture of cholesterol-4-^{14}C and cholesterol-7α-*t* or cholesterol-7β-*t* (one microcurie of each isotope) was injected intraperitoneally into rats with bile fistula. The bile was collected and cholic acid was isolated as described earlier [S. Bergstrom and A. Norman, *Proc. Soc. Exptl. Biol. Med.*, **83**, 71 (1953)], further purified by dilution with 100 mg. of unlabelled cholic acid and recrystallization, and analyzed for isotopes according to R. Glascock, *Isotopic Gas Analysis for Biochemists*, Academic Press, N. Y., 1954.

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.