VII   VIII   IX

These results suggest that the structure of this yellow intermediate is 4(5),9(10)-diseco-3-hydroxyandrosta-1-(10),2-diene-5,9,17-trion-4-oic acid (XI). Treatment of XI with diazomethane afforded a methyl ester; its n.m.r. spectrum[12] showed a band at τ 6.16 characteristic of COOCH₃.

The participation of quinone in this aromatic ring fission reaction was eliminated by the following experiment. Incubation of I with mushroom tyrosinase afforded a quinone which readily condensed with [C¹⁴]-ethylenediamine to give a radioactive dihydropyrazine compound.[13] When cell-free extracts of *N. restrictus* were substituted for mushroom tyrosinase in this reaction no dihydropyrazine derivative was noted. Furthermore, 9,10-secoandrosta-1,5(10)-diene-3,4,9,17-tetraone, prepared by oxidation of I with Ag₂O,[14] was very slowly converted in low yields to II when compared to I.

On the basis of these results it appears that the metabolism of I by *N. restrictus* involves oxidative fission between C-4 and C-5 of the aromatic A ring. Addition of water to the ethylenic bond followed by hydrolytic cleavage, or *vice versa*, affords II and 2-oxo-4-hydroxycaproic acid (IV), the latter compound being lactonized during the isolation procedure.[15]
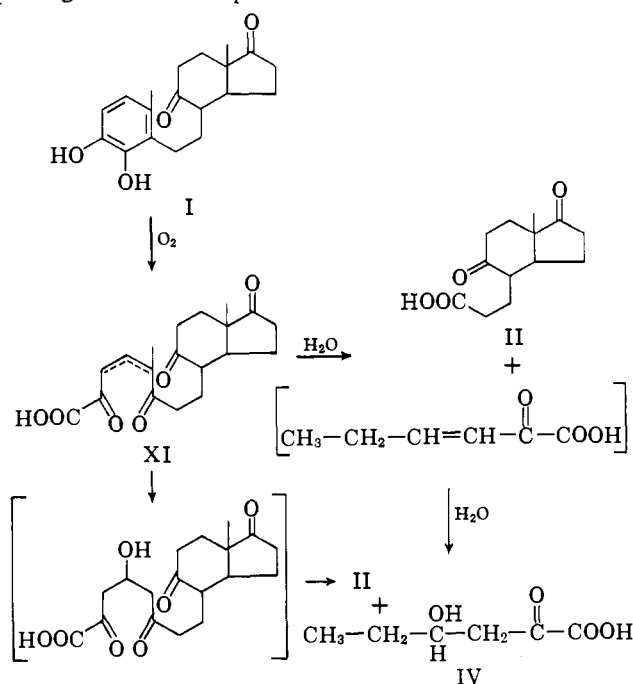
(12) The n.m.r. spectrum of this methyl ester is complex because it consists of a mixture of keto-enol forms. However, the spectrum is consistent for the structure proposed.

(13) P. H. Jellinck and L. Irwin, *Biochim. Biophys. Acta*, **78**, 778 (1963).

(14) R. Willstätter and F. Müller, *Ber.*, **41**, 2581 (1908).

(15) This investigation was supported in part by research grants from the National Institutes of Health (AM 4874 and AM 6110).

(16) Wellcome Travel Fellow.

Charles J. Sih, K. C. Wang
D. T. Gibson,[16] H. W. Whitlock, Jr.

School of Pharmacy and Department of Chemistry
University of Wisconsin, Madison 6, Wisconsin

A New Route to Estrone from Sterols

Sir:

The initial degradative reactions of steroid hormones by microorganisms is reasonably well understood.[1–3] In contrast, the mechanism by which sterols are degraded in nature is not clearly defined. Turfitt[4] found that cholesterol was first oxidized to cholestenone and then to 3-ketoetiochol-4-enic acid and isocaproic acid by *Proactinomyces erythropolis*. A-Nor-3,5-seco-cholestan-5-on-3-oic acid was also identified as an intermediate in this conversion. Intact cells of a *Mycobacterium*[5] incubated with 4-C¹⁴-cholesterol or 26-C¹⁴-cholesterol oxidized carbon 4 of the ring to carbon dioxide about four times as rapidly as carbon 26 of the side chain. It was suggested that some compound or compounds retaining the side chain but with ring A opened may be intermediates.[6] On the other hand, in some microorganisms it has been shown that radioactive C¹⁴O₂ appeared more rapidly from 26-C¹⁴-cholesterol than 4-C¹⁴-cholesterol.[7] Moreover, a *Nocardia* soil isolate in the presence of 8-hydroxyquinoline converted cholesterol in low yields to 3-ketobisnorchol-4-en-22-oic acid, 3-ketobisnorchola-1,4-dien-22-oic acid, androst-4-ene-3,17-dione, and androsta-1,4-diene-3,17-dione.[8] On the basis of these results it is not clear whether the side chain is cleaved prior to ring cleavage or *vice versa*. Thus, the attractive target of converting cholesterol into useful steroid intermediates by the microbial cleavage of the sterol side chain is still in its infancy.

It is well documented that one pathway of steroid degradation by microorganisms involves 1,2-dehydrogenation, followed by 9α-hydroxylation or *vice versa* with the rupture of the steroid ring B.[1] *Nocardia restrictus* (ATCC 14887) is an organism which degrades androst-4-ene-3,17-dione *via* this mechanism to yield 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione.[9] Since this organism is also capable of utilizing cholesterol as a sole carbon source, it appeared of interest to examine whether cholesterol is first degraded to C-19 steroids which are then metabolized by the conventional 9,10-seco pathway. When cholesterol was exposed to this organism, the only compound that one could isolate in reasonable yields was cholestenone; this product was then slowly metabolized in a series of reactions which eventually led to CO₂ and H₂O.

Previous studies have shown that 19-hydroxyandrost-4-ene-3,17-dione was rapidly converted into estrone by this organism[10] and the product, estrone, was not further metabolized. Figure 1 shows that 4-C¹⁴-

(1) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4627 (1961).

(2) C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *ibid.*, **87**, 1385 (1965).

(3) C. J. Sih, K. C. Wang, D. T. Gibson, and H. W. Whitlock, *ibid.*, **87**, 1386 (1965)

(4) G. E. Turfitt, *Biochem. J.*, **42**, 376 (1948).

(5) T. C. Stadtman, A. Cherkes, and C. B. Anfinsen, *J. Biol. Chem.*, **206**, 511 (1954).

(6) It should be emphasized that even though C¹⁴O₂ appeared more readily from 4-C¹⁴-cholesterol than 26-C¹⁴-cholesterol, this does not necessarily mean that the A ring is first ruptured prior to side-chain cleavage. It is quite conceivable that the side chain may be rapidly degraded, prior to ring fission, to a compound which in turn is slowly metabolized to C¹⁴O₂.

(7) G. E. Peterson and J. R. Davis, *Steroids*, **4**, 677 (1964).

(8) J. M. Whitmarsh, 435th Meeting of the Biochemical Society, University of Leicester, England, Dec. 1963.

(9) C. J. Sih, *Biochim. Biophys. Acta*, **62**, 541 (1962).

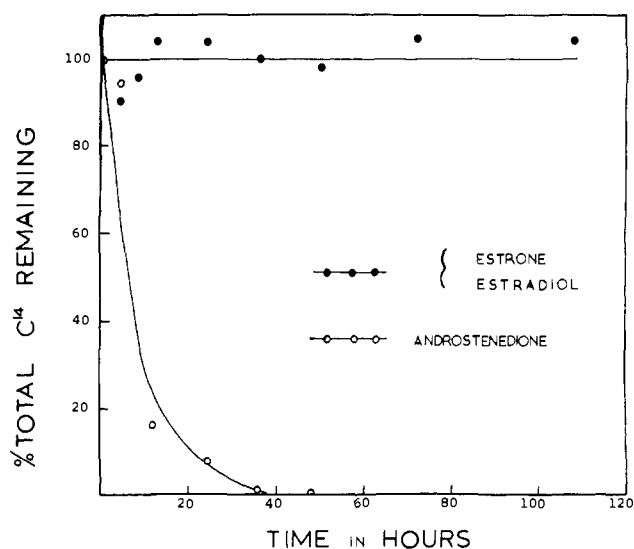(10) C. J. Sih and A. M. Rahim, *J. Pharm. Sci.*, **52**, 1075 (1963).

Figure 1. Comparative rates of metabolism of 4-C^{14}-androst-4-ene-3,17-dione and 4-C^{14}-estradiol by *Nocardia restrictus*.

androst-4-ene-3,17-dione was rapidly metabolized by *N. restrictus* whereas practically all of the radioactivity could be recovered either as 4-C^{14}-estrone or 4-C^{14}-estradiol after incubation of 4-C^{14}-estradiol with *N. restrictus*.

With this information available, it appeared desirable to synthesize 19-hydroxycholest-4-en-3-one (I). If the organism cleaves the side chain first to yield 19-hydroxy-androst-4-ene-3,17-dione, the latter compound should be converted into estrone which then should accumulate. When I (1.2 g.) was incubated with *N. restrictus*, an 8% yield[11] of estrone (65 mg.) was indeed obtained, m.p. 257–259°, identical (mixture melting point and infrared spectrum) with an authentic sample. When 1.2 g. of I was exposed to CSD-10,[12] 230 mg. of estrone (30%) was obtained.[13] Similarly, 19-hydroxy-β-sitost-4-en-3-one (II) was also converted into estrone by CSD-10 in 10% yield.[13]
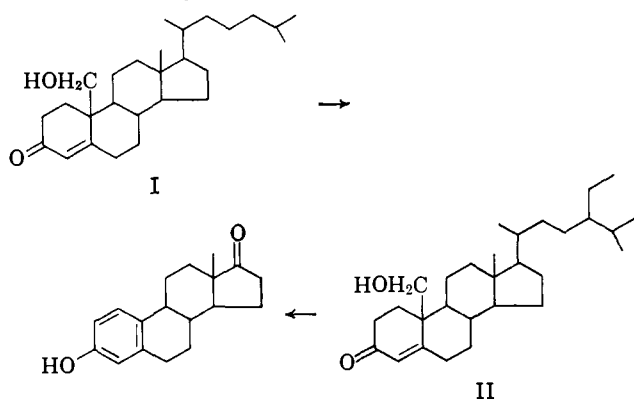
Cholestenone is not only a very poor inducer of the steroid 1-dehydrogenase and the 9α-hydroxylase but also it is a very poor substrate for these enzymes when compared to androst-4-ene-3,17-dione.[14,15] Thus it is

(11) The major portion of the remaining 92% can be accounted for as unmetabolized substrate and phenolic acids.
(12) CSD-10 is an organism isolated from soil utilizing cholesterol as a sole carbon source.
(13) Practically all the steroidal material was recovered in these fermentations, the main portion being unmetabolized substrate.
(14) C. J. Sih and R. E. Bennett, *Biochim. Biophys. Acta*, **56**, 584 (1962).
(15) F. N. Chang and C. J. Sih, *Biochemistry*, **3**, 1551 (1964).

unlikely that ring fission preceded side-chain cleavage. All these results support the view that the major pathway of sterol breakdown among microorganisms involves first the removal of the sterol side chain to yield C-19 steroids. The reason for the nonaccumulation of C-19 steroidal intermediates from cholesterol is due to their rapid metabolism *via* the conventional 9,10-seco pathway, for when the A ring is first aromatized estrone accumulates, and practically all of the steroidal material is recovered.[16]

In view of the accessibility of I and II from cholesterol and β-sitosterol, respectively,[17] this appears to be an attractive route for the synthesis of estrone from these readily available sterols.[18]

Acknowledgment. We wish to thank Mr. Y. Y. Tsong and Mr. F. N. Chang for their technical assistance.

Charles J. Sih, K. C. Wang
School of Pharmacy, University of Wisconsin
Madison 6, Wisconsin
Received January 30, 1965

Valence Tautomerism in Cyclooctatetraene–Iron Carbonyl Complexes

Sir:

The concept of facile valence tautomerism has been invoked to rationalize apparently conflicting physical data recently obtained for several organometallic π-complexes. For example, although X-ray data suggest[1] a structure of cyclooctatetraene–iron tricarbonyl (I) in which the $Fe(CO)_3$ residue is bonded to only four carbon atoms of the C_8 ring, nonetheless the n.m.r. spectrum of the complex indicates all eight protons to be equivalent.[2] A diene–iron tricarbonyl formulation with rapid rotation of the $Fe(CO)_3$ group about the ring, to produce equivalent valence-tautomeric structures (Ia, etc.), would reconcile these data.

Analogous degenerate valence tautomerism has been proposed to occur in the $C_7H_7Fe(CO)_3$[3] and $C_7H_7Fe_2(CO)_6$ cationic systems,[4] and similar behavior is indicated for a cyclopentadienyl–cycloheptatrienyl–Mo(CO)_2 complex.[5]

In each of these systems the valence tautomers possess the same type of metal–ligand electronic

(1) B. Dickens and W. N. Lipscomb, *J. Chem. Phys.*, **37**, 2084 (1962).
(2) (a) T. A. Manuel and F. G. A. Stone, *J. Am. Chem. Soc.*, **82**, 366 (1960). (b) M. D. Rausch and G. N. Schrauzer, *Chem. Ind.* (London), 957 (1959). (c) We have not been able to find evidence for the broadening of this peak at temperatures as low as −60°.
(3) J. E. Mahler, D. A. K. Jones, and R. Pettit, *J. Am. Chem. Soc.*, **86**, 3589 (1964).
(4) G. F. Emerson, J. E. Mahler, R. Pettit, and R. Collins, *ibid.*, **86**, 3590 (1964).
(5) R. B. King, *Tetrahedron Letters*, 1137 (1963).