III from II presents strong evidence in support of assigned structure II.

The n.m.r. spectrum[7] (Fig. 2) of the $^{13}C_1$ satellite of compound II revealed remarkably high coupling constants both for $^{13}C_1-H$ and H_1-H_3 ($^{13}C_1-H = 190.1$ c.p.s., $H_1-H_3 = 14$ c.p.s.). Using an equation to correlate coupling constants with the extent of sp hybridization of the carbon atomic orbitals used in the bonds,[8] we obtain 38% s-character for the exocyclic orbital of the C_1 atom. This high degree of s-character is a clear demonstration of the great strain involved in this ring system.[9]

In view of the fact that long-range proton spin–spin coupling constants are in the order of 0.3–0.5 c.p.s. with methyl ketones,[10] the size of the coupling constant observed for H_1-H_3 of II is remarkable. Although the theoretical aspect of the long-range spin–spin interaction still remains unclear, this large coupling evidently arises from the unique geometrical arrangement of the two hydrogens and two carbon atoms, i.e., these four atoms lie in a straight line and the distance of the carbon atoms is considerably shorter than 2.7 Å. Under these circumstances, an appreciable interaction of the two C–H orbitals (in particular, because of their large s-character) seems reasonable.

The ultraviolet spectrum of II in methanol showed a maximum at 242 mμ (ϵ 1.46 $\times$ 10^4). Thus a distinctive conjugation effect of cyclopropanes is observable in the case of the bicyclobutane system.

We are observing unique reactions intrinsic to the bicyclobutane system as well as unusual solvolytic behaviors of the tosylate of 4,5-diphenyltricyclo-[1.1.1.0^{4,5}]pentan-2-ol. These results will be reported in a separate paper.

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[1.1.0]butane and tricyclo[2.1.1.0^{5,6}]hexane-5-t-butylcarboxamide. However, this difference is easily rationalized by the steric hindrance of the two phenyl groups of II.

(7) The author is grateful to Mr. Joey Holcombe for determining this n.m.r. spectrum with a Varian Associates HR-100 spectrometer.

(8) N. Muller and D. E. Pritchard, J. Chem. Phys., **31**, 1471 (1959).

(9) G. L. Closs and L. E. Closs, J. Am. Chem. Soc., **85**, 2022 (1963).

(10) N. van Meurs, Spectrochim. Acta, **19**, 1695 (1963), and references cited therein, B. L. Shapiro, unpublished.

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Microbiological 9α,12α- and 9α,18-Dihydroxylations of Androst-4-ene-3,17-dione

Sir:

Although a large number of papers dealing with the microbiological hydroxylation of steroids have appeared in recent years, the 18-hydroxylation has not been reported so far. We wish to report here the successful hydroxylation at the 9α-, 12α-, 14α-, 15β-, and 18-positions of androst-4-ene-3,17-dione (I). Among these, the 18-hydroxylation is believed to be the most important reaction because of its usefulness in preparing such 18-oxygenated steroids as aldosterone.

Transformation of I with the use of *Cercospora melonis* [*Corynespora melonis* (Cke) Lindau][1] produced four dihydroxylated compounds (II, III, IV, and V) by the method previously described.[4] These four products were obtained also when 9α-hydroxyandrost-4-

(1) By the use of this microorganism, Reichstein's substance S is converted into its 8β- and 15β-hydroxyl derivatives (see ref. 2 and 3, respectively).

(2) K. Tori and E. Kondo, Tetrahedron Letters, No. **10**, 645 (1963).

(3) E. Kondo, to be published.

(4) E. Kondo, K. Morihara, Y. Nozaki, and E. Masuo, J. Agr. Chem. Soc. Japan, **34**, 844 (1960).

ene-3,17-dione (VI)[5,6] was incubated with the same microorganism (see Scheme I, arabic numbers indicate the chemical shifts (τ) in deuteriochloroform solutions). Therefore, one of the two hydroxyl groups in each compound should be situated at the 9α-position.

Compound II [m.p. 224–226°, [α]^{24}D +144.2° (dioxane), λ_{max} 242 mμ (ϵ 15,400), ν_{max}^{Nujol} 3441, 1724, 1659, and 1617 cm.$^{-1}$][7] and its monoacetate (VII, sirup, $\nu_{max}^{CCl_4}$ 3466, 1745, 1674, 1622, and 1240 cm.$^{-1}$) show no signal corresponding to the 18-methyl group in their n.m.r. spectra.[8] Instead, oxygen-bearing methylene signals (about 6.28 τ in II and about 5.73 τ in VII) are observed. The 19-methyl signal of II appears at 8.66 τ shifted by -0.12 p.p.m. from that of I, owing to the 9α-hydroxyl group.[9,10] Chromium trioxide oxidation of II afforded an aldehyde [VIII, m.p. 208–211°, [α]^{25}D -7.3°, λ_{max} 240.5 mμ (ϵ 15,800), ν_{max}^{Nujol} 3400, 1747, 1692, 1646, and 1616 cm.$^{-1}$], which was verified by the appearance of the n.m.r. signal of an aldehydic proton at 0.47 τ. These results suggest that II is the 18-hydroxyl derivative of VI. Confirmatory evidence that II has an 18-hydroxyl group, not a 19-hydroxyl group, was obtained by the following degradation of II with *Bacillus sphaericus* (ATCC 7055) having the ability to 1-dehydrogenate various steroids.[11–13] Microbiological dehydrogenation of II afforded an expected 9,10-seco compound [IX, m.p. 148–150°, [α]$^{27.5}$D +91°, λ_{max} 279.5 mμ (ϵ 2350), ν_{max}^{Nujol} 3452, 1727, 1693, 1611, and 1512 cm.$^{-1}$]. The n.m.r. spectra of IX and its diacetate (X, sirup, $\nu_{max}^{CCl_4}$ 1751, 1713, 1612, 1591, 1502, and 1213 cm.$^{-1}$) show the signal of a methyl group attached to the benzene ring at 7.76 and 7.68 τ, respectively, and an AB-type quartet due to the oxygen-bearing methylene group. The angular methyl signal that appears in II is not observed in IX and X. For the purpose of comparison, 9,10-seco-3-hydroxyandrosta-1,3,5(10)-triene-9,17-dione[6,14] (XI, m.p. 122–123°, [α]^{27}D +96°) and its acetate (XII, m.p. 145–146°, [α]$^{27.5}$D +83°) were prepared by the same method. The main n.m.r. features of XI and XII resemble those of IX and X, respectively, except that the 18-methyl signals in XI and XII are replaced by a doublet of doublets in IX and X. Thus the structure of II was established to be 9α,18-dihydroxyandrost-4-ene-3,17-dione.

Compound III [m.p. 253–257°, [α]$^{23.5}$D +213.8°, λ_{max} 241 mμ, (ϵ 16,200), ν_{max}^{Nujol} 3434, 3359, 1720, 1678, and 1625 cm.$^{-1}$] was converted into its monoacetate [XIII, m.p. 254–256°, [α]^{24}D +201.3°, λ_{max} 241 mμ (ϵ 16,000), ν_{max}^{Nujol} 3565, 1740, 1661, 1617, and 1240 cm.$^{-1}$] by the usual acetylation. Chromium trioxide oxida-

(5) E. Kondo and T. Mitsugi, ibid., **35**, 521 (1961).

(6) R. M. Dodson and R. D. Muir, J. Am. Chem. Soc., **80**, 6148 (1958).

(7) Elementary analyses of the compounds described here gave satisfactory values. Unless otherwise noted, optical rotations were determined in chloroform containing 1% ethanol, and ultraviolet absorption spectra were observed on the solutions in 95% ethanol.

(8) All n.m.r. spectra were taken with a Varian A-60 spectrometer on 2–5% solutions in deuteriochloroform containing tetramethylsilane as an internal reference at room temperature. Chemical shifts are expressed in τ-values.

(9) The additivity rule for signal shifts of the angular methyl groups due to shielding by various functional groups has been well established [for example, see R. F. Zürcher, Helv. Chim. Acta, **44**, 1380 (1961)].

(10) The shift value of the 19-methyl due to a 9α-hydroxyl group was reported to be about -0.14 p.p.m.[2] It should be noted that the signal shift of the 4-proton by about -0.15 p.p.m. is characteristic for the introduction of a 9α-hydroxyl group into a Δ^4-3-keto steroid.

(11) T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlatt, and R. Miller, Arch. Biochem. Biophys., **59**, 304 (1955).

(12) E. Kondo and E. Masuo, Ann. Rept. Shionogi Res. Lab., **10**, 103 (1960).

(13) It has been well known that 9α-hydroxy-Δ^4-3-keto steroids are transformed to their 9,10-secophenol derivatives by microorganisms capable of introducing a Δ^1-double bond.[6,14]

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

SCHEME I
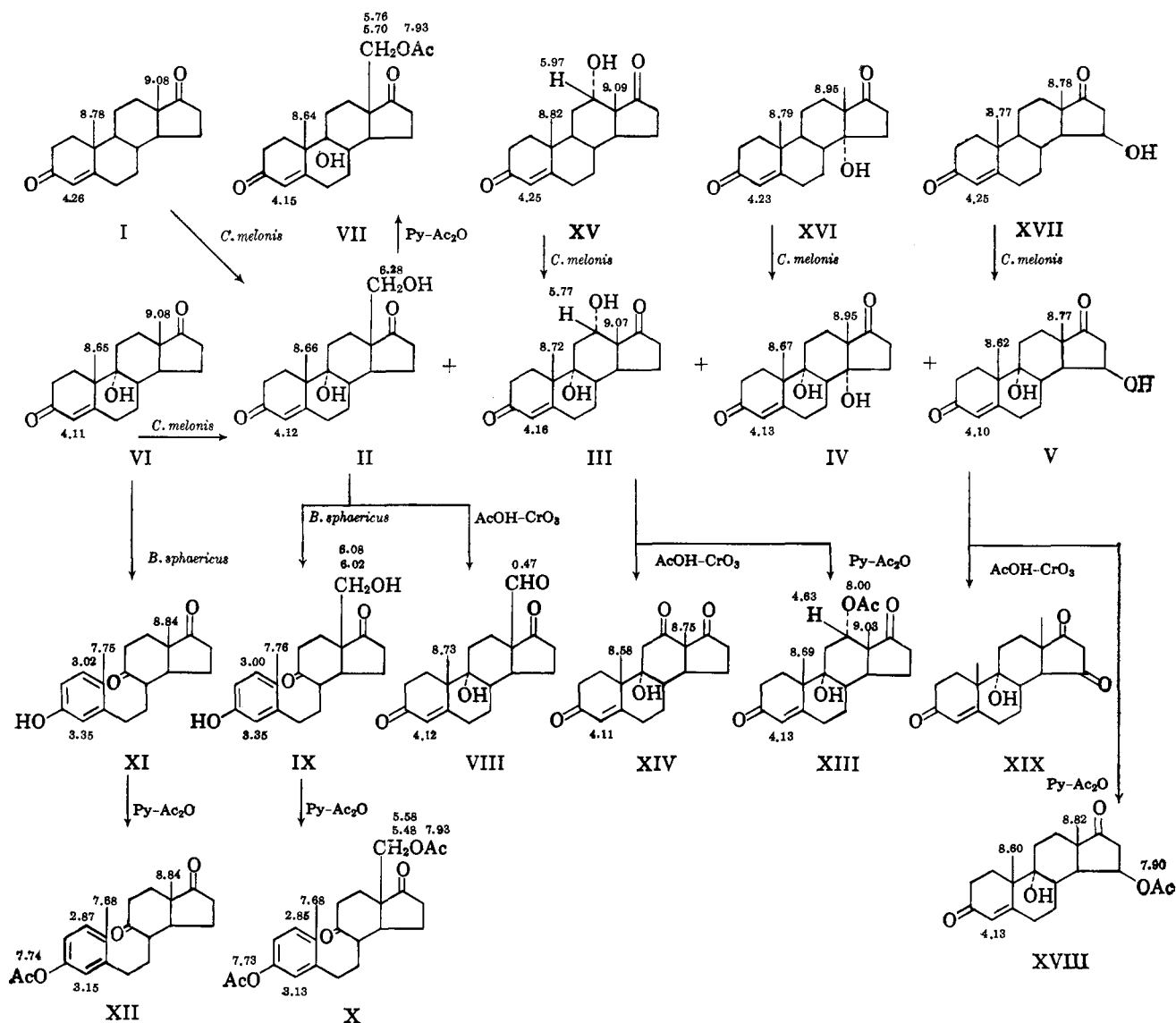
tion of III gave a triketone [XIV, m.p. 210–213°, $[\alpha]^{24}$D +265.1°, λ_{max}^{MeOH} 240.5 mμ (ϵ 16,100), ν_{max}^{Nujol} 3483, 1749, 1703, 1674, and 1622 cm.$^{-1}$], whose angular methyl signals are shifted characteristically from those of VI (−0.07 p.p.m. for the 19-methyl and −0.33 p.p.m. for the 18-methyl) to suggest the presence of the 12-oxo group.[15] The n.m.r. signal of the proton on the oxygen-bearing carbon in III and XIII shows a distinct triplet, implying α-configuration of the assumed 12-hydroxyl group. Further, III was obtained from 12α-hydroxyandrost-4-ene-3,17-dione (XV) with *C. melonis*. Accordingly, III was proved to be 9α,12α-dihydroxyandrost-4-ene-3,17-dione.

The remaining two products, IV [m.p. 241–244°, $[\alpha]^{23.5}$D +139°, λ_{max} 242 mμ (ϵ 15,900), ν_{max}^{Nujol} 3406, 1737, 1665, 1642, and 1621 cm.$^{-1}$] and V [m.p. 236–238°, $[\alpha]^{23.5}$D +138°, λ_{max} 242.5 mμ (ϵ 16,500), ν_{max}^{Nujol} 3470, 3405, 3310, 1724, 1659, 1644, and 1617 cm.$^{-1}$], were assumed to have a 14α- and a 15β-hydroxyl group, respectively, as derived from their chemical and n.m.r. features.[16] When acetylation or chro-

mium trioxide oxidation was carried out, IV was recovered unchanged, whereas V was converted into its monoacetate [XVIII, m.p. 171–175°/223–227°, $[\alpha]^{23.5}$D +90°, λ_{max} 242 mμ (ϵ 16,100), ν_{max}^{Nujol} 3502, 1732, 1657, 1617, 1257, and 1238 cm.$^{-1}$] and a five-membered β-diketone [XIX, m.p. 182–185°, $[\alpha]^{27.5}$D +117.2°, λ_{max}^{MeOH} 242.5 (ϵ 16,800) and 275 mμ (shoulder), ν_{max}^{Nujol} 3549, 1763, 1727, 1666, and 1625 cm.$^{-1}$]. In addition, IV and V also were obtained from 14α-hydroxyandrost-4-ene-3,17-dione (XVI)[5] and 15β-hydroxyandrost-4-ene-3,17-dione (XVII),[3,17] respectively, by using *C. melonis*. Therefore, IV was identified as 9α,14α-dihydroxyandrost-4-ene-3,17-dione and V as 9α,15β-dihydroxyandrost-4-ene-3,17-dione.

(15) B. C. Christensen, R. G. Strachan, N. R. Trenner, B. H. Arison, R. Hirschmann, and J. M. Chemerda, *J. Am. Chem. Soc.*, **82**, 3995 (1960).

(16) The shift value of the 18-methyl due to a 14α- and a 15β-hydroxyl group has been reported to be about −0.11 and −0.27 p.p.m., respectively [see ref. 2 and Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 338 (1962)].

(17) S. Bernstein, L. I. Feldman, W. S. Allen, R. H. Blank, and C. E. Linden, *Chem. Ind.* (London), 111 (1956).

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