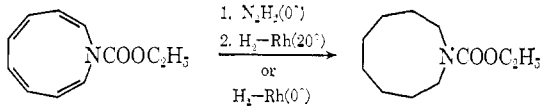

1 4

All the preliminary information presented herein unequivocally points to structure **1** for the photolysis product. Furthermore, the nmr data coupled with the pronounced thermal instability displayed by this compound attest to a nonaromatic, classical polyenic, character. The compound thus appears to lack aromaticity in spite of an all-*cis* geometry clearly implicated by the nmr data.[10] We are currently concentrating our efforts toward isolating **1** in the pure form in order to secure additional spectral and chemical information concerning its aromatic or classical character.[11]

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work. We are also grateful to Badische Anilin and Sodafabrik for a generous sample of cyclooctatetraene.

(10) To be sure, the nmr spectrum of this substance is consistent with any structure that possesses either a plane or a rotating axis of symmetry containing the nitrogen atom and bisecting the remote C–C bond. Among these, only the all-*cis* arrangement, shown in 1, ought to possess a reasonably stable σ frame. A "Dreiding" molecular model clearly points to a puckered all-*cis* arrangement possessing a twofold rotating axis of symmetry (C_2).

(11) NOTE ADDED IN PROOF. Subsequent to submittal of this paper, 1 was obtained pure by means of low-temperature column chromatography. We shall elaborate on the purification as well as the thermal and photochemical behavior of 1 in a subsequent report.

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Stereochemistry of Tritium at Carbon 15 in Cholesterol Derived from (3*R*,2*R*)-2T-Mevalonic Acid in Rat Livers

Sir:

Recent studies on the biosynthesis of sterols have centered on the changes occurring at C-7 and C-15 during the conversion of lanosterol to cholesterol. Canonica, *et al.*,[1,2] showed that the removal of the 14α-methyl group is accompanied by loss of the 15α-hydrogen which originates from the *pro*-2*S*-proton of mevalonic acid. Later work by Gibbons, *et al.*,[3] has confirmed this observation. Subsequently, it has been demonstrated that both 4,4-dimethylcholesta-8,14-dien-3β-ol[2] and cholesta-8,14-dien-3β-ol[4,5] are converted to cholesterol by rat liver preparations. More definitive evidence of the participation of 8,14-diene intermediates has been presented by Watkinson and Akhtar,[6] with the isolation of 4,4-dimethylcholesta-8,14-dien-3β-ol during cholesterol biosynthesis in rat livers. The same group[7] have shown that in the saturation of the Δ^{14}-double bond of the 8,14-diene, the 14α-hydrogen is derived from NADPH, and the C-15 hydrogen from a proton source. In this communication we concern ourselves with the stereochemical fate of the hydrogen at C-15, originating from the *pro*-2*R*-hydrogen of mevalonic acid.

Cholesterol (I) [7.2 $\times$ 10^5 dpm ^{14}C; T/^{14}C ratio 10.1; atomic ratio (ar) 5.00:5] biosynthesized from (3*R*,2*R*)-[2T-2-^{14}C]mevalonic acid in rat livers,[8] was incubated with a bovine adrenal mitochondrial preparation.[9] The crude residue from the reaction was fractionated by thin layer chromatography (tlc) in two systems and the zone corresponding to pregnenolone (II) was isolated. The extract (4.42 $\times$ 10^4 dpm ^{14}C) was diluted with inactive pregnenolone (100 mg) and crystallized to constant specific activity (85 mg; 2.89 $\times$ 10^4 dpm ^{14}C; T/^{14}C ratio 9.8; ar 2.91:3). Oppenauer oxidation of this material gave progesterone (III) (58 mg; 2.00 $\times$ 10^4 dpm ^{14}C; T/^{14}C ratio 9.1; ar 2.70:3). Progesterone derived by Jones oxidation of 20α-hydroxypregn-4-en-3-one, a by-product of the incubation, had a T/^{14}C ratio of 9.8 (ar 2.91:3). The small loss of tritium in the progesterone obtained by Oppenauer oxidation is not clear but probably involves some loss of isotopic hydrogen from the allylic C-7 position in pregnenolone.

The radioactive progesterone (T/^{14}C ratio 9.1) was then incubated with *Calonectria decora*[10] to yield 12β,15α-dihydroxyprogesterone (IV)[11] (1.46 $\times$ 10^4 dpm ^{14}C), which had a T/^{14}C ratio of 6.2 (ar 1.84:3). In view of the earlier error[10] in the assignment of configuration at C-15, we confirmed the identity of the product as the 12β,15α-diol (IV) by its failure to undergo acid-catalyzed dehydration to a Δ^{14} compound[12] and from the chemical shift of the 18-methyl group (47 cps) in the nmr spectrum. Conclusive proof of the structure was derived from the fact that hydroxylation of stereospecifically labeled (15β-T)-(4-^{14}C)-progesterone (T/^{14}C ratio 10.8) with *Calonectria decora* gave (15β-T)-(4-^{14}C)-12β,15α-dihydroxyprogesterone (T/^{14}C ratio 10.5) which retained all the tritium. Controlled oxidation of IV with restricted amounts of Jones reagent gave 12β-hydroxypregn-4-en-3,15,20-trione (V)[11] (T/^{14}C ratio 6.6; ar 1.96:3). The assignment of structure V, rather than the alternative 15α-hydroxypregn-4-ene-3,12,20-trione structure VI, follows from the appearance of a peak at 1747 cm^{-1} (five-membered cyclic ketone), due to the C-15 ketone, in the ir spectrum, and the occurrence of the 12β-hydroxyl signal at low field (275 cps), due to hydrogen bonding between the hydroxyl and the C-20 ketone, and the shift of the 18-methyl group (49 cps), in the nmr spectrum. Complete oxidation of IV with Jones reagent gave pregn-4-ene-3,12,15,20-tetraone (VII)[11] (T/^{14}C ratio 6.4; ar 1.90:3).

The unchanged T/^{14}C ratio of the 12β-hydroxy-

(1) (a) L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala, G. Galli, E. Grossi Paoletti, and R. Paoletti, *J. Amer. Chem. Soc.*, 90, 3597 (1968); (b) L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala, G. Galli, E. Grossi Paoletti, and R. Paoletti, *Steroids*, 12, 445 (1968).

(2) L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala, G. Galli, E. Grossi Paoletti, and R. Paoletti, *J. Amer. Chem. Soc.*, 90, 6532 (1968).

(3) G. F. Gibbons, L. J. Goad, and T. W. Goodwin, *Chem. Commun.*, 1458 (1968).

(4) B. N. Lutsky and G. J. Schroepfer, *Biochem. Biophys. Res. Commun.*, 33, 492 (1968).

(5) M. Akhtar, I. A. Watkinson, A. D. Rahimtula, D. C. Wilton, and K. A. Munday, *Chem. Commun.*, 1406 (1968).

(6) I. A. Watkinson and M. Akhtar, *ibid.*, 206 (1969).

(7) M. Akhtar, A. D. Rahimtula, I. A. Watkinson, D. C. Wilton, and K. A. Munday, *ibid.*, 149 (1969).

(8) E. Caspi, J. B. Greig, P. J. Ramm, and K. R. Varma, *Tetrahedron Letters*, 3829 (1968).

(9) (a) I. D. K. Halkerston, J. Eichhorn, and O. Hechter, *J. Biol. Chem.*, 236, 374 (1961); (b) P. R. Raggatt and M. W. Whitehouse, *Biochem. J.*, 101, 819 (1966).

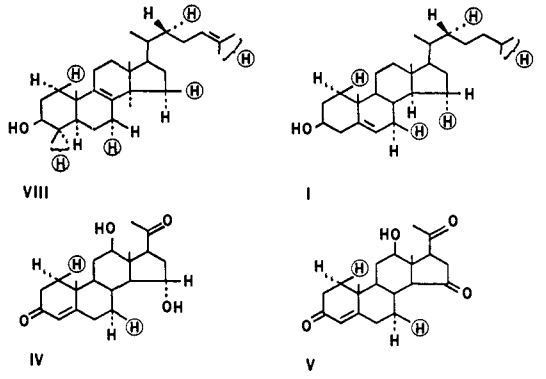
(10) A. Schubert, G. Langbein, and R. Siebert, *Ber.*, 90, 2576 (1957).

(11) R. M. Dodson, G. Langbein, R. D. Muir, A. Schubert, R. Siebert, Ch. Tamm, and E. Weiss-Berg, *Helv. Chim. Acta*, 48, 1933 (1965).

(12) We have shown that under analogous conditions, 15β-hydroxy compounds in the pregnane series (prepared by sodium borohydride reduction of the corresponding ketones) undergo a very facile dehydration to the Δ^{14} compounds (to be published).

trione (V) and the tetraone (VII), with respect to the 12β,15α-diol (IV), indicates that the loss of tritium is associated entirely with the introduction of the 15α-hydroxyl group. Since, in microbial hydroxylations the hydroxyl group assumes the stereochemistry of the displaced hydrogen,[13] the loss in this step indicates the presence of a 15α-tritium in the progesterone and hence in the parent cholesterol. It follows that the saturation of the Δ^{14}-double bond occurs with the addition of hydrogens in the 14α and 15β configurations, *i.e.*, a *trans* addition, thereby paralleling the similar process in the saturation of the Δ^7 double bond.[14] The over-all result of events occurring at C-15 during the conversion of lanosterol to cholesterol is, therefore, the inversion of the proton originating from the *pro*-2*R*-hydrogen of mevalonic acid, from the 15β configuration in lanosterol[8] to the 15α orientation in cholesterol.

In summary, we have shown in this and an earlier communication,[8] that during the conversion of lanosterol (VIII) to cholesterol (I) in rat livers, of the three hydrogens derived from the *pro*-2*R*-hydrogen of mevalonic acid which occur in the steroid nucleus, only one (1β) retains its stereochemistry, while those at C-7 and C-15 undergo inversion. The biological significance of these results, together with our findings of stereochemical differences in the introduction of the Δ^7 double bond into C_{27} sterols in rats and in yeast[15] is at present under active investigation in our laboratories.



Encircled hydrogens represent protons derived from the pro-2R-hydrogen of mevalonic acid and in the case of radioactive materials, are indicative of tritium atoms.

	T/^{14}C ratio	Atomic ratio
Cholesterol	10.1	5.00:5
Pregnenolone	9.8	2.91:3
Progesterone (from pregnenolone)	9.1	2.70:3
Progesterone (from 20α-hydroxy-pregn-4-en-3-one)	9.8	2.91:3
12β,15α-Dihydroxyprogesterone	6.2	1.84:3
12β-Hydroxypregn-4-ene-3,15,20-trione	6.6	1.96:3
Pregn-4-ene-3,12,15,20-tetraone	6.4	1.90:3

Acknowledgment. This work was supported by Grants P(500H) from the American Cancer Society and K3-16614 from the National Institute of Health.

(13) (a) W. Charney and H. L. Herzog, "Microbial Transformations of Steroids," Academic Press, New York, N. Y., 1967, p 18; (b) M. Hayano in "Oxygenases," O. Hayaishi, Ed., Academic Press, New York, N. Y., 1962, p 182; (c) R. I. Dorfman and F. Ungar, "Metabolism of Steroid Hormones," Academic Press, New York, N. Y., 1965, pp 224, 382; (d) A. A. Akhrem and U. A. Titov, "Microbiological Transformations of Steroids," Nauka Publishing House, Moscow, 1965.

(14) D. C. Wilton, K. A. Munday, S. J. M. Skinner, and M. Akhtar, *Biochem. J.*, 106, 803 (1968).

(15) E. Caspi and P. J. Ramm, *Tetrahedron Letters*, 181 (1969).

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Resonance Interactions in Substituted Ethylenes

Sir:

We wish to report that the integrated intensity (Table I) of the CC stretching mode near 1630 cm^{-1} of monosubstituted ethylenes[1] is closely proportional to the square of the σ_R^0 value of the substituent. Intensities for 18 compounds are plotted against σ_R^0 values[2] in Figure 1; a least-squares treatment of this data gives eq 1, with a correlation coefficient of 0.998.

$$A_{eth} = 27{,}300(\sigma_R^0)^2 + 80 \quad (1)$$

$$A_{mono} = 17{,}600(\sigma_R^0)^2 + 100 \quad (2)$$

This result is significant for a number of reasons. (a) Equation 1 is of the same form as eq 2 which correlates[3] the intensity of the ν_{16} ring-stretching bands of benzene in the 1600-cm^{-1} region, demonstrating the fundamental similarity of the interactions between the substituent and the carbon π bond(s) in the two systems. (b) Equation 2 has been used to calculate σ_R^0 values but is not accurate for $|\sigma_R^0| < 0.1$ because of the uncertainty due to the second term in the equation which is a correction factor needed because a combination band of C–H out-of-plane bending modes occurs in the same spectral region. A similar complication arises for eq 1 as the first overtone of the CH_2 in-plane rocking vibration interferes; however, the relative value of the correction term is only half the magnitude of that in eq 2. Therefore, relation 1 should be particularly suited to the measurement of small σ_R^0 values.[4] (c) Relation 2 has been shown[5] to hold in a modified form for di- and trisubstituted benzenes, and to afford considerable information on steric and electronic interactions between substituents; it may be expected that the intensities of poly-substituted ethylenes can be treated similarly.[4] (d) Relation 2 indicated that the intensity of ν_{16} in monosubstituted benzenes was largely due to the motion of the ring carbon atoms and suggested the possibility of molecular orbital calculations of absolute infrared intensities, which have succeeded;[6] similar calculations should be possible in the ethylene series.[4]

We wish to report preliminary extensions of this work along the lines just indicated. *trans*-1-Chloro-1-propene has $A = 268$; if eq 3 holds for *trans*-disubstituted ethylenes (based on analogy with *para*-disubstituted benzenes;[5a] as these compounds possess no CH_2 group, the overtone correction does not apply), then we deduce

$$A_{t-1,2} = 27{,}300[\sigma_R^0(1) - \sigma_R^0(2)]^2 \quad (3)$$

(1) A. X. Wexler, *Spectrochim. Acta*, 21, 1725 (1965), has previously reported precise integrated intensities for the $\nu_{C=C}$ of some 1-alkenes; our values for 1-hexene (500) and styrene (339) are in good agreement with this (500; 400). We have used the values quoted for 1-pentene (470) and 4-methyl-1-pentene (460).

(2) The values for σ_R^0 used in the plot are those deduced from the ir of monosubstituted benzenes[3] except that for substituents CH_2Cl_2 and CH_2OH the ^{19}F values (R. W. Taft, E. Price, I. R. Fox, I. C. Lewis, K. K. Andersen, and G. T. Davis, *J. Amer. Chem. Soc.*, **85**, 3146 (1963)) are used because the ir values are uncertain as a result of the overtone correction. The substituent CH_2Br is not included in the plot as no ^{19}F value is available.

(3) R. T. C. Brownlee, R. E. J. Hutchinson, A. R. Katritzky, T. T. Tidwell, and R. D. Topsom, *ibid.*, **90**, 1757 (1968).

(4) Work along these lines is in hand.

(5) (a) P. J. Q. English, A. R. Katritzky, T. T. Tidwell, and R. D. Topsom, *ibid.*, **90**, 1767 (1968); (b) A. R. Katritzky, M. V. Sinnott, T. T. Tidwell, and R. D. Topsom, *ibid.*, **91**, 628 (1969); (c) M. V. Sinnott, unpublished work.

(6) R. T. C. Brownlee, A. R. Katritzky, M. V. Sinnott, M. Szafran, L. Yakhontov, and R. D. Topsom, *Tetrahedron Letters*, 5773 (1968).