[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A., MEXICO D.F., MEXICO]

Spectra and Stereochemistry. IX.[1,2] A Nuclear Magnetic Resonance Spectral Study of Some 16-Substituted Pregnenes and 17α-Pregnenes

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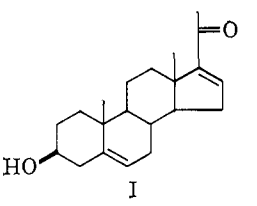
The stereochemistry at positions 16 and 17 of some 16α- and 16β-substituted pregnenes and 17α-pregnenes has been investigated in an n.m.r. examination of 16,17 proton–proton coupling constants and shieldings of the 18-methyl protons. Coupling constants for $J_{16,17}$ are calculated for the three possible conformations of ring D.

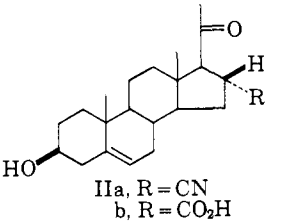
In recent years a number of detailed investigations have been reported dealing with the Michael addition of hydrogen cyanide to various pregna-5,16-dien-20-ones, and with the stereochemistry of the products arising from the alkaline hydrolysis of these adducts.[3,4] In one of the latest investigations[4] the discordant earlier results[3a,b] have been clarified. Concurrent with the chemical work studies were made of the stereochemistry at C-16 and C-17 utilizing the optical rotatory dispersion (O.R.D.) method.[5]

Nucleophilic attack by cyanide ion at position 16 of the pregn-16-en-20-one (I) was postulated to afford the 16α-cyano-20-ketone IIa, vigorous alkaline hydrolysis of which furnished an acid, initially formulated as IIb.[3a,b] Later work showed that the acid had in fact structure III.[3c] In view of the requirement of inversion at both C-16 and C-17 for the formation of the acid III from the nitrile IIa, it was considered pertinent to initiate n.m.r. studies with the 16-cyano compound. However, in a separate study of cyano steroids it became rapidly apparent that the phenomenon of long-range shielding of angular methyl protons by nitrile was a complicated one, especially so where dipolar repulsions affecting ring conformation were existent.[6] Therefore, a direct assignment of the configuration at C-16 and C-17 in the 16-cyano adduct, from inspection of the angular methyl frequencies, appeared untrustworthy. Our attention was diverted to the numerous derived 16,17-disubstituted steroids which had been prepared by chemical transformations.[3,4] A set of four pregnane 16-methoxycarbonyl-20-ketones,[4] IV, isomeric at C-16 and C-17, was available for the n.m.r. study. Elucidation of the stereochemistry of these four isomers was established by chemical[3,4] and O.R.D.[5] methods before the n.m.r. investigation ended. However, it was instructive to complete the latter to determine whether the same conclusions could be reached.

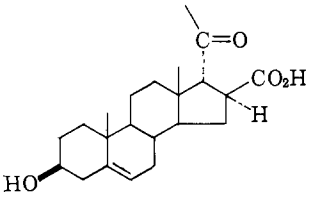
From the beginning it was anticipated that simple application of the Karplus relationship in its original[7] or modified[8,9] forms to the coupling constants for 16- and 17-protons would *not* solve the problem, for a number of reasons. First, it is known that coupling constants for protons on adjacent carbon atoms can vary considerably according to the nature of the other substituents borne by the carbon atoms, especially if the substituent is an electronegative group.[9-14] Furthermore, it is not known to what extent the strain inherent in rings of less than six carbon atoms affects coupling constant magnitude. Another factor which might be important is the dipolar interaction of the proximate carbonyl functions. Even if the constants for the Karplus relationship were known accurately for the case in hand there existed the complex question of ring D stereochemistry and, hence, of the magnitude of the angle subtended by adjacent C–H bonds.



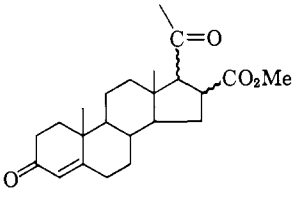
I



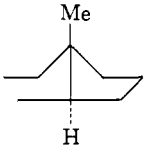
IIa, R = CN
b, R = CO_2H



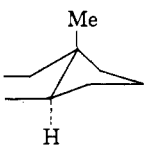
III



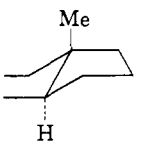
IVa, 16α-CO_2Me, 17β-Ac
b, 16β-CO_2Me, 17α-Ac
c, 16α-CO_2Me, 17α-Ac
d, 16β-CO_2Me, 17β-Ac



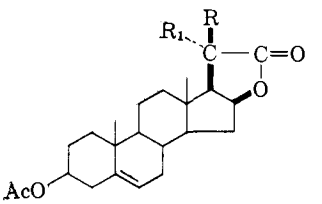
V



VI



VII



VIIIa, R = Me, R_1 = H
b, R = H, R_1 = Me

Brutcher and Bauer recently discussed in some detail the three possible conformations V–VII for the

(1) Part VIII: L. H. Knox, E. Velarde, and A. D. Cross, *J. Am. Chem. Soc.*, **85**, 2533 (1963).

(2) This paper constitutes Steroids. CCXLII; for part CCXLI, see P. G. Holton, A. D. Cross, and A. Bowers, *Steroids*, **2**, 71 (1963).

(3) (a) J. Romo, *Tetrahedron*, **3**, 37 (1958); (b) B. Ellis, V. Petrow, and D. Wedlake, *J. Chem. Soc.*, 3748 (1958); (c) R. H. Mazur and J. A. Cella, *Tetrahedron*, **7**, 130 (1959).

(4) P. Crabbé, L. M. Guerrero, J. Romo, and F. Sánchez-Viesca, *ibid.*, **19**, 25 (1963).

(5) (a) W. A. Struck and R. L. Houtman, *J. Org. Chem.*, **26**, 3883 (1961); (b) P. Crabbé, *Tetrahedron*, **19**, 51 (1963).

(6) A. D. Cross and I. T. Harrison, *J. Am. Chem. Soc.*, **85**, 3223 (1963).

(7) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(8) H. Conroy in "Advances in Organic Chemistry," Vol. 2., R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 311.

(9) K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).

(10) H. M. Hutton and T. Schaefer, *Can. J. Chem.*, **41**, 684 (1963).

(11) K. L. Williamson, *J. Am. Chem. Soc.*, **85**, 516 (1963).

(12) E. O. Bishop, *Ann. Rept. Progr. Chem.*, **58**, 55 (1961).

(13) R. U. Lemieux, J. D. Stevens, and R. R. Fraser, *Can. J. Chem.*, **40**, 1955 (1962).

(14) A. Nickon, M. A. Castle, R. Harada, C. E. Berkoff, and R. O. Williams, *J. Am. Chem. Soc.*, **85**, 2185 (1963).

TABLE I

CALCULATED COUPLING CONSTANTS FOR THE FOUR STEREOISOMERS IV IN EACH OF THREE RING D CONFORMATIONS (V–VII)[18]

Structure	Substituent orientation in IV 16	17	Proton orientation 16	17	Conformation V ϕ	Conformation V $J_{16,17}$, c.p.s.	Conformation VI ϕ	Conformation VI $J_{16,17}$, c.p.s.	Conformation VII ϕ	Conformation VII $J_{16,17}$, c.p.s.
IVa	α	β	β	α	148 ± 4°	5.9–7.1	134 ± 4°	3.7–5.0	111 ± 4°	0.5–1.4
IVb	β	α	α	β	96 ± 4	−0.3–0.0	108 ± 2	0.3–0.8	126 ± 4	2.4–3.7
IVc	α	α	β	β	29 ± 4	5.7–6.7	14 ± 3	7.5–7.9	8 ± 5	7.8–8.2
IVd	β	β	α	α	26 ± 3	6.2–6.9	11 ± 2	7.8–8.0	9 ± 3	7.9–8.1

cyclopentane ring D of steroids.[15] They concluded that a 17β-methyl or hydroxyandrostane exists with ring D having conformation V, and the 17α-methyl epimer with the conformation VII. For androstane itself, energy considerations suggest either conformation V or VI with the latter favored on steric grounds.[16] A steroid skeleton constructed from Dreiding models[17] has ring D in the half-chair conformation VI. Addition of acetyl and methoxycarbonyl groups at C-17 and C-16 introduces such a wealth of possible steric and dipolar interactions that the conformation of ring D for each isomer is not predictable.

In spite of these limitations it was felt that some information on the stereochemistry of the isomers IV was obtainable from coupling constants. For each of the three conformations of ring D (V–VII) J-value ranges were calculated for the four possible stereoisomers IV, by measuring the angle, ϕ, subtended by the adjacent C-16 and C-17 protons and using the Karplus equation with its original constants.[7,18] The calculated coupling constant ranges (J for the lowest and highest measured value of ϕ was calculated for each case) are collected in Table I. Measured J-values and chemical shifts of various protons for the four stereoisomers, A,B,C, and D of IV, are assembled in Table II,[19] together with methyl proton resonance frequencies of progesterone and 17-isoprogesterone for comparative purposes.[20]

TABLE II

CHEMICAL SHIFTS, ν, AND $J_{H_{16}H_{17}}$ VALUES FOR THE FOUR STEREOISOMERS OF IV[18]

Compound	19-H	18-H	$J_{H_{16}H_{17}}$	$\nu_{17\text{-H}}$	Structure deduced
IV, stereoisomer A	71.5	41.4	8.9	175	IVa
IV, stereoisomer B	70.2	60.1	*Ca.* 1[a]	192	IVb
IV, stereoisomer C	70.1	59.9	*Ca.* 3[b]	194	IVc
IV, stereoisomer D	72.1	53.3	7.0	177.5	IVd
Progesterone		40.0	...	...	..
17-Isoprogesterone[c]		57	...	...	..

[a] The 17-H proton resonance was a broadened singlet. [b] In this particular stereoisomer the 17-proton resonance was not cleanly resolved, for reasons unknown. However, a related compound of the same stereochemistry at C-16 and C-17 showed a well-resolved doublet, J = 4.0 c.p.s. (J. Romo, L. Rodríguez, M. Martínez, and P. Crabbé, unpublished results). [c] G. Slomp, personal communication.

The fact that for one stereoisomer $J_{H_{16}H_{17}}$ has a magnitude as high as 8.9 c.p.s. shows that, although the effect of several factors (*vide supra*) upon the Karplus equation constants cannot be separately assessed, these constants cannot be substantially smaller in magnitude than those originally proposed.[7] From Table I it is readily apparent that, irrespective of the ring D conformation, only two of the four stereoisomers can have a very low value of $J_{H_{16}H_{17}}$.[22] Consequently, stereoisomer B (see Table II) must be either IVa or IVb. A decision in favor of structure IVb is possible on the basis of chemical shifts by the following argument.

The steroid literature is now replete with legion examples showing that shifts of the angular methyl proton resonances induced by groups substituted more than two carbon atoms distant from the methyl and attached to the steroid nucleus by a σ-bond are greatest when the substituent is spatially close to the methyl group.[24] Thus, large shifts of the 19-H resonance are caused by 2β-, 4β-, 6β-, and 11β-substituents which hold a 1,3-diaxial relation to the angular methyl group. Such downfield shifts may be as great as 15 c.p.s. (*e.g.*, 6β-Cl, 6β-Br). Conversely, similar substituent groups which are spatially distant from the angular methyl protons cause a negligible or small shift of the methyl proton resonance. Therefore, introduction of a 16α-methoxycarbonyl group should shift the 18-H resonance of progesterone or 17-isoprogesterone by not

(15) F. V. Brutcher and W. Bauer, *J. Am. Chem. Soc.*, **84**, 2236 (1962).

(16) For other considerations of the steroid ring D conformation and of cyclopentanes fused to cyclohexanes see D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953); E. L. Eliel and C. Pillar, *J. Am. Chem. Soc.*, **77**, 3600 (1955); L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959.

(17) A. S. Dreiding, *Helv. Chim. Acta*, **42**, 1339 (1959).

(18) Measurements were made on models[17] of the Δ^4-3-ketones. Two measurements of the subtended angle, ϕ, were made for each of three separate models of each 16,17-stereoisomer. By holding the D ring twisted into one or other of the envelope conformations, V and VII, measurements for these alternative stereochemical forms were obtainable. The measured values of ϕ generally fall within a range of ±4° of the arithmetical mean (quoted to the nearest degree). Although a change to a ring A/B saturated system, or to a Δ^5-steroid does lead to small changes in the angles subtended by the C-16 H and C-17 H bonds, these changes result in only minor alterations in calculated J values and in no way diminish the arguments presented later in this paper.

(19) N.m.r. spectra were recorded at 60 Mc.p.s. using 5–8% w./v. solutions of the steroid in deuteriochloroform or chloroform containing tetramethylsilane (TMS) as an internal reference. Resonance frequencies, ν, are quoted as c.p.s. downfield from the TMS reference (0.0 c.p.s.) and are accurate to ±1 c.p.s. Coupling constants, J, also expressed in c.p.s. units, are accurate to ±0.5 c.p.s. Cleanly resolved, unobscured doublets for the C-17 proton were obtained for three of the four stereoisomers IV (see Table II and footnotes). First-order analysis was applied for all measurements recorded in this paper. Thanks are due to the Universidad Nacional Autónoma de México for time on the A-60 spectrometer.

(20) Advances in instrumentation and the variety of reference standards employed probably account for the wide range of resonance frequencies which has been reported for the angular methyl protons of progesterone.[21] For our further argument we determined the value 40.0 c.p.s. for the 18-proton resonance of progesterone. This value may be compared with 36 c.p.s. by J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958); 43.2 c.p.s. by B. G. Christensen, R. G. Strachan, N. R. Trenner, B. H. Arison, R. Hirschmann, and J. M. Chermerda, *ibid.*, **82**, 3995 (1960); 41.4 c.p.s. by Y. Kawazoe, Y. Sato, M. Natsume, H. Hagegawa, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.*, (Tokyo), **10**, 328 (1962); and 40.8 c.p.s. by R. C. Tweit, R. M. Dodson, and R. D. Muir, *J. Org. Chem.*, **27**, 3654 (1962).

(21) For comparative purposes all literature data have been expressed as c.p.s. relative to TMS by employing the following chemical shifts in the calculations: $\nu_{(benzene)} - \nu_{(TMS)}$ = 384 c.p.s.; $\nu_{(water)} - \nu_{(TMS)}$ = 282 c.p.s.; $\nu_{(cyclohexane)} - \nu_{(TMS)}$ = 86.4 c.p.s. Frequencies measured originally at 40 Mc.p.s. have been multiplied by 3/2 to express all data for a 60 Mc.p.s. oscillator.

(22) Implicit in the argument used here is an acceptance of the general shape of the Karplus equations curve but with the need for great caution in their application when other influential factors operate. In such cases it is safe to assume that for ϕ = 75–105° J is small (*i.e.*, <2 c.p.s.), and for ϕ = 0–20° or 160–180° J is usually large (*i.e.*, >6 c.p.s.). Karplus himself has very recently drawn attention to the necessity of such caution and to questionable applications of the equations.[23]

(23) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

(24) *Inter alia* (a) R. F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961); (b) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. Pharm Bull.*, (Tokyo), **10**, 338 (1962); (c) A. D. Cross, H. Carpio, and H. J. Ringold, *J. Med. Chem.*, **6**, 198 (1963); (d) J. Jacquesy, J. Lehn, and J. Levisalles, *Bull. soc. chim. France*, 2444 (1961); (e) G. Slomp and B. McGarvey, *J. Am. Chem. Soc.*, **81**, 2200 (1959); (f) R. C. Tweit, *J. Org. Chem.*, **27**, 2693 (1962).

more than a few c.p.s. On the other hand, a 16β-methoxycarbonyl could have a more marked effect, depending upon the conformation of ring D. This conclusion presupposes that in these esters the dipolar repulsion between ester and ketone carbonyl groups is insufficient to cause major reorientation of the 20-carbonyl axis, since this would change the long-range shielding of the 18-protons by the carbonyl (*vide infra*).[25]

Now, stereoisomer B must be either IVa or IVb (*vide supra*). If it is IVa the 18-H resonance frequency shift caused by the distant 16α-ester group must be 20.1 c.p.s. [60.1 − 40.0 (progesterone)] which is inconceivably large. On the other hand, allocation of structure IVb to stereoisomer B indicates that the 16β-ester group leads to deshielding of the 18-H resonance by 3.1 c.p.s. [60.1 − 57 (17α-isoprogesterone)] which is an eminently reasonable value (*cf.* 18-H shift caused by 16β-methyl = 5 c.p.s.[28]). This structural assignment is in complete agreement with the chemical evidence[3c] by which the acid corresponding to ester IVb had earlier been identified as III.

One stereoisomer having been identified, allocation of the structures IVa, IVc, and IVd to stereoisomers A, C, and D, respectively, followed from further considerations of chemical shifts. As noted above, a 16α-methoxycarbonyl substituent is expected to cause, at most, only a small shift of the 18-H resonance. Only one stereoisomer has an 18-H resonance close to that of progesterone (see Table II). Accordingly, stereoisomer A is 16α-methoxycarbonylprogesterone (IVa, $\nu_{18\text{-H}}$ 41.4 c.p.s.). Similarly, stereoisomer C for which the 18-H resonance (59.9 c.p.s.) is slightly downfield from 17α-isoprogesterone (57 c.p.s.) must be 16α-methoxycarbonyl-17α-isoprogesterone. As expected, the shift of the 18-H resonance caused by a 16α-methoxycarbonyl substituent is small and of similar magnitude in both the progesterone and 17α-isoprogesterone series. By elimination, stereoisomer D has structure IVd.

The last assignment merits further attention since the inferred value of $\Delta\nu$ for the 18-H resonance is no less than 13.3 c.p.s. (53.3 − 40, see Table II), in striking contrast to the $\Delta\nu$ value 3.1 c.p.s. for the 17α,16β-stereoisomer. In 16β-methoxycarbonylprogesterone the β-face of ring D is subject to serious crowding by *three* large groups — 13β-methyl, 17β-acetyl, and 16β-methoxycarbonyl. Such crowding is known to cause substantial downfield shifts of angular methyl protons. Thus, the 18-protons of 20β-methylsapogenins resonate 7 c.p.s. to lower fields than the 20α-epimer.[29] We have examined another pair of stereoisomers where a similar phenomenon is manifest. An appropriate pair of γ-lactones of known stereochemistry at C-16, C-17, and C-20 was kindly made available to us by Prof. F. Sondheimer.[30] The lactone VIIIa bearing a 20β-oriented methyl had an 18-H resonance showing an 6 c.p.s. downfield shift relative to the 20α-methyl isomer VIIIb. The C-21 protons also showed a 2–3 c.p.s. downfield shift in VIIIa. Structure IVd may well have the 16β-ester group constrained by dipolar or steric repulsions into such an orientation that the 18-protons are also subject to deshielding by the ester carbonyl.

Having solved the stereochemical problem presented by the four esters IV, it was germane to return to the stereochemistry of the original 16-cyano-20-ketone IIa, the spectrum of which showed $J_{H_{16}H_{17}} = 8.7$ c.p.s. This value is strikingly similar to that recorded for 16α-methoxycarbonylprogesterone and is much higher than the corresponding J-values for isomers IVc and IVb (Table II).[31]

Of the remaining two possible structures for the cyano ketone, IIa or the 16β-cyano epimer, a 16α-cyano group (as in IIa) is most suitably oriented to cause the observed small over-all shielding of 2.3 c.p.s. (40-37.7 c.p.s.).[33]

Inspection of Tables I and II reveals how unjustifiable it would have been to rely solely upon coupling constant data and the Karplus equations to assign stereochemistry. The observed fluctuations in J-values in no way cast suspicion on the validity of the Karplus equations. However, the results do point to the need to consider all possible conformers before deciding whether or not the equations are directly applicable.[22] It is to be expected that further examples of this sort will be encountered and reported.

Table II offers further evidence for the above stereochemical assignments. The large shift of the 18-H resonance in 17α-isoprogesterone as compared with progesterone ($\Delta\nu$ 17 c.p.s.) must be due mainly to more powerful deshielding of 18-H by the 20-carbonyl in the 17α-isomer.[34] Molecular models[17] show that the orientations of the C-20 carbonyl axis in 17α- and 17β-isomers which are necessary to give greater deshielding of 18-H in the 17α-isomer should also give rise to stronger deshielding of the 17β-proton in 17α-isoprogesterone relative to the 17α-proton of progesterone. In accordance with this conclusion it is seen (Table II) that both the stereoisomers (IVb,c) allocated a 17α-acetyl side chain show the 17β-proton resonance *ca.* 17 c.p.s. downfield from the 17α-H resonance for the progesterones (IVa,d). Furthermore, the similarity of the 17-proton resonances for both pairs of 17α- and 17β-isomers constitutes extra evidence for the largely undisturbed orientations of the 20-carbonyl axis (*vide supra*).[25]

A corollary of the variations discussed above concerning the interrelationships of coupling constant magnitudes, deshielding, carbonyl axis orientations, and ring D conformations is that additivity of the 18-H resonance frequency shifts by the C-16 and C-17 substituents is not expected to hold in these cases. Brief study of the tabulated 18-H frequencies illustrates

(25) The validity of this assumption is amply verified by numerous O.R.D. curves[6] where some irregularity is observed for only one of the four esters. In this case, IVc, one extremum is more negative than expected and is compatible with a *small* displacement of the C-20 carbonyl axis. Any large displacement would reposition the bulky steroid nucleus relative to the 20-carbonyl and, from considerations of the octant rule,[26] *substantial* changes in the amplitude and shape of the O.R.D. curve would be expected. That no major effects are detected is sound evidence for the largely undisturbed orientation of the 20-carbonyl group.[27]

(26) W. Moffitt, A. Moscowitz, R. B. Woodward, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).

(27) For general considerations of the O.R.D. curves of pregnan-20-ones, see C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 51; C. Djerassi, O. Halpern, V. Halpern, O. Schindler, and Ch. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958). For specific consideration of the orientation of the 20-carbonyl group, see C. Djerassi, I. Fornaguera, and O. Mancera, *J. Am. Chem. Soc.*, **81**, 2383 (1959); C. Djerassi, "Optical Rotatory Dispersion," pp. 128–129; N. L. Allinger and M. A. DaRooge, *J. Am. Chem. Soc.*, **83**, 4256 (1961); S. Rakhit and Ch. R. Engel, *Can. J. Chem.*, **40**, 2163 (1962).

(28) G. Slomp, personal communication.

(29) W. E. Rosen, J. B. Ziegler, A. C. Shabica, and J. N. Shoolery, *J. Am. Chem. Soc.*, **81**, 1687 (1959).

(30) Y. Mazur, N. Danieli, and F. Sondheimer, *ibid.*, **82**, 5889 (1960).

(31) Since this work was completed a 16β-cyano-17α-pregn-20-one derivative has been synthesized, and, as expected, showed $J_{16,17}$ = *ca.* 1 c.p.s.[32]

(32) J. Romo, L. Rodríguez, M. Martínez, and P. Crabbé, unpublished results.

(33) Chemical[4] and O.R.D.[5] evidence conclusively demonstrated the correctness of this structural consignment.

(34) For a general consideration of long-range shielding by carbonyl see L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, pp. 121–125.

this point. Similar observations have been made elsewhere for nonadditivity of 19-H frequency shifts due to substituents on six-membered rings where steric strain or interactions leads to ring conformational changes.[6,24a,e,35]

(35) A. D. Cross, *J. Am. Chem. Soc.*, **84**, 3206 (1962).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS 14, MINN.]

Biosynthesis of the Nicotiana Alkaloids. X. The Incorporation of Glycerol-2-C^{14} into the Pyridine Ring of Anabasine[1]

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The administration of glycerol-2-C^{14} to *Nicotiana glauca* plants leads to the formation of radioactive anabasine which has 38% of its activity located in the pyridine ring. A systematic degradation is described whereby the distribution of activity around the pyridine ring can be determined. The anabasine derived from glycerol-2-C^{14} was found to have substantial activity at C-2, C-3, and C-5, with only low activity at C-4 and C-6. These results are consistent with the hypothesis that nicotinic acid, the precursor of the pyridine ring of anabasine, is formed in *Nicotiana* species from glycerol and succinic acid or closely related metabolites.

In our previous publication on the biosynthesis of anabasine[4] we reported that the administration of sodium acetate-2-C^{14} to *Nicotiana glauca* led to the formation of radioactive anabasine which had 37% of its activity located in the pyridine ring. It was shown that all this activity was located at C-2 and C-3 and was divided approximately equally between these positions. It was suggested that the acetate-2-C^{14} enters the Krebs cycle leading to the formation of succinic acid-2,3-C^{14} which is then incorporated into nicotinic acid, the established precursor of the pyridine ring of anabasine.[5] Ricinine, and the pyridine ring of nicotine, are also derived from nicotinic acid, and recent investigations on their biosynthesis[6-8] are consistent with this hypothesis. We considered that carbons 4,5, and 6 of the pyridine ring of nicotinic acid were derived from glycerol or a closely related three carbon metabolite. When glycerol-2-C^{14} was fed to *N. glauca* plants, radioactive anabasine was indeed obtained. Oxidation of the anabasine yielded nicotinic acid which was decarboxylated affording pyridine having 38% of the total activity of the alkaloid. We have now developed a systematic degradation of nicotinic acid (I) which enables us to determine the distribution of activity around the pyridine ring. Initial steps in this degradation have been previously described[4] and result in the formation of 1,3-dimethyl-2-phenylpiperidine (II). Oxidation of this piperidine derivative with chromic acid afforded a mixture of benzoic acid (representing the activity at C-2) and acetic acid which was further degraded to methylamine (C-7) and carbon dioxide (C-3) by the Schmidt reaction. The piperidine II was converted to its methiodide III which on treatment with sodium in liquid ammonia afforded 1-dimethylamino-4-methyl-5-phenylpentane (IV). Vapor phase chromatography of the crude reaction product from the Emde reduction indicated that only one of the two possible isomers was obtained. The n.m.r. spectrum of the product was consistent with structure IV. A doublet at 7.43 τ was assigned to the benzylic hydrogens at C-5 (the benzylic hydrogens of ethylbenzene absorb at 7.38 τ[9]). The hydrogen at C-1 in the isomeric structure VI would be expected to produce a doublet at about 6.68 τ (the position at which the benzylic hydrogens of N,N-dimethylbenzylamine absorb[9]). No absorption was present in this region of the spectrum. A doublet was present at 7.78 τ which was assigned to the hydrogens at C-1 (the hydrogens on the α-carbons of triethylamine absorb at 7.58 τ[9]). Furthermore, subsequent steps in the degradation confirmed the structure IV. A Hofmann elimination reaction on the methiodide of IV yielded 4-methyl-5-phenyl-1-pentene (V) which had an absorption in the ultraviolet similar to toluene. A similar series of reactions on the amine VI would have yielded a styrene derivative having quite a different ultraviolet spectrum. The alkene V was cleaved with sodium metaperiodate in the presence of a catalytic amount of osmium tetroxide yielding formaldehyde, collected as its dimedone derivative (C-6), and 3-methyl-4-phenylbutanal (VIII). A solution of this aldehyde in acetone was oxidized with a calculated amount of chromic acid in sulfuric acid to 3-methyl-4-phenylbutanoic acid (VII).[10] On treatment of this acid with sodium azide in sulfuric acid, carbon dioxide (C-5) was obtained in 33% yield. A neutral by-product which was assigned the structure IX was also obtained in this reaction. It presumably arises by cyclization of the butanoic acid to 3-methyltetralone (X) followed by ring enlargement with hydrazoic acid. The lactam IX was also obtained by a Beckmann rearrangement on the oxime of 3-methyltetralone. Lack of material prevented us from proceeding further to determine the activity at C-4 directly. In the present work we have calculated the activity at this position by difference.

The activities of the degradation products obtained from the radioactive anabasine isolated from the plant which had been fed glycerol-2-C^{14} are recorded in Table I. The distribution of activity in the pyridine ring was: C-2, 11; C-3, 11; C-4, 2.4; C-5, 12; C-6, 1.6%. Low activity at C-4 and C-6 with high activity at C-5 is consistent with the direct participation of the three carbons of glycerol-2-C^{14} in the biosynthesis of this part of the pyridine ring. A similar pattern of labeling was found at C-4, C-5, and C-6 of ricinine obtained from *Ricinus communis* which had been fed glycerol-2-C^{14}.[11]

The appreciable activity which was found at C-2 and C-3 may be rationalized by postulating that the glyc-

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