[CONTRIBUTION FROM THE SYNTEX RESEARCH CENTER, PALO ALTO, CALIFORNIA]

Steroids. CCLXIX.[1] Spectra and Stereochemistry. XVII.[2] Nonadditivity of Angular Methyl Proton Frequency Shifts and Ring D Conformational Changes in 16-Methyl Steroids

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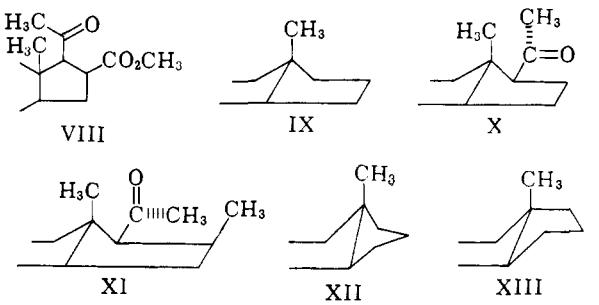
RECEIVED JULY 6, 1964

Nuclear magnetic resonance (n.m.r.) spectra have been recorded for 16-unsubstituted, 16α-methyl, and 16β-methyl steroids with varying substituents at C-17. The data suggest that introduction of a 16β-methyl group can lead to changes in the conformation of ring D and of the orientation of a 17β-acetyl side chain. Frequency shifts for the 18-proton resonance can be correlated with structural and conformational change but also indicate the need for caution in use of the frequency shift additivity principle.

Conspicuous among the profusion of applications of nuclear magnetic resonance spectroscopy to chemical problems is the use of this versatile technique in steroid chemistry. Following upon the classic investigations of Shoolery and Rogers[3] there have appeared several publications concerning the correlation of steroid structural modifications with resonance frequency shifts of angular methyl protons.[4] Several hundred steroids have been studied in establishing the principle of additivity of angular methyl proton frequency shifts and the latest results[4d] involve a computer analysis for the determination of the shift value to be associated with each substituent group. Although data accumulated in the Syntex Laboratories have permitted extension of this concept to other functional groups,[5,6] a greater effort has been directed toward defining the limitations of the frequency shift additivity principle. Thus, it was observed that for a 5β,6β-epoxy-$\Delta^{9(11)}$-steroid additivity does not hold owing to a conformational distortion.[6] Similarly, it was discovered that whereas 5α-bromo-3-ketones display additivity of the shifts for the 19-H resonance frequency[4a,7] the analogous 5α-cyano-3-ketones do not, again owing to a ring conformational change.[7] Zürcher has recorded numerous further examples where interaction of functional groups or ring conformational changes lead to a breakdown in the frequency shift additivity principle.[4c] More recently it was found that the shift of the 18-proton resonance attributed to a 16β-methoxycarbonyl group was substantially smaller for a 17α-pregnan-20-one than for the 17β-pregnan-20-one stereoisomer.[8] It was postulated that divergence from angular methyl proton frequency shift additivity for 16,17-disubstituted steroids would be observable in further compounds. An extension of such studies is now reported and amply supports the earlier suppositions. Moreover, the accumulated results indicate that great caution should be exercised before applying the frequency shift additivity principle to ring D polysubstituted steroids.[9]

For 16β,17β-disubstituted steroids the shift of the 18-proton resonance frequency associated with the 16β-substituent may be attributed to any one of, or a combination of, several effects. For example, for the earlier studied[8] pregnan-20-one-16-carboxylic acid methyl ester moiety VIII steric crowding across the β-face could conceivably lead to a conformational distortion of ring D, or to reorientation of the 20-carbonyl and/or ester carbonyl axes with repositioning of the 18-protons relative to the carbonyl shielding "cones." Dipolar interactions of the adjacent substituents might also be expected to exercise an influence over their orientation. To avoid these electrical effects, further studies were concentrated on 16-methyl steroids in which differences from the 16-unsubstituted parent compounds were expected to stem almost entirely from steric interactions.



Nuclear magnetic resonance spectra were recorded[11] for seven trios of related compounds, each trio consisting of a 16-unsubstituted steroid and the derived 16α-methyl and 16β-methyl analogs. Collected data appear in Table I. In the last column of the table are shown the shifts of the 18-proton resonance for the 16-methyl derivatives relative to the 16-unsubstituted parent steroid. Three sets of compounds, II–IV, are pregnan-20-ones and for these the values of $\Delta\nu_{18\text{-H}}$ show that the 18-proton resonance frequency *shifts*

(1) Steroids. CCLXVIII: J. A. Edwards, H. Carpio, and A. D. Cross, *Tetrahedron Letters*, in press.

(2) Spectra and Stereochemistry. XVI: A. D. Cross, *J. Am. Chem. Soc.*, **86**, 4011 (1964).

(3) J. N. Shoolery and M. T. Rogers, *ibid.*, **80**, 5121 (1958).

(4) *Inter al.* (a) J. Jacquesy, J. Lehn, and J. Levisalles, *Bull. soc. chim. France*, 2444 (1961); (b) R. F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961); (c) R. F. Zürcher, *ibid.*, **46**, 2054 (1963); (d) A. I. Cohen and S. Rock, Jr., *Steroids*, **3**, 243 (1964).

(5) A. D. Cross, H. Carpio, and H. J. Ringold, *J. Med. Chem.*, **6**, 198 (1963); B. Berkoz, A. D. Cross, M. E. Adame, H. Carpio, and A. Bowers, *J. Org. Chem.*, **28**, 1976 (1963); L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, P. Landis, and A. D. Cross, *J. Am. Chem. Soc.*, **85**, 1851 (1963); L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, and A. D. Cross, *J. Org. Chem.*, **29**, 2187 (1964).

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

(7) A. D. Cross and I. T. Harrison, *ibid.*, **85**, 3223 (1963).

(8) A. D. Cross and P. Crabbe, *ibid.*, **86**, 1221 (1964).

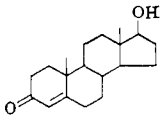
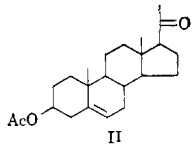
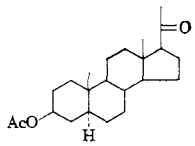
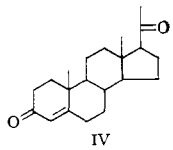
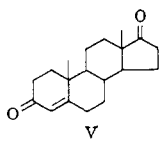
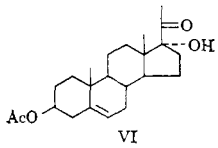
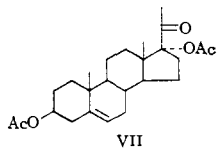
(9) In this connection it is of note that the additivity shift principle, as applied to the 18-proton resonance frequency in certain 16,17-disubstituted steroids, has been used recently for confirmation of assignment of stereochemistry at C-16 and C-17.[10] Although the structural conclusions arrived at appear to be correct, the precedent created is not devoid of hazard if attempts are made to extend these conclusions.

(10) J. E. Pike, M. A. Rebenstorf, G. Slomp, and F. A. MacKellar, *J. Org. Chem.*, **28**, 2499 (1963); J. E. Pike, G. Slomp, and F. A. MacKellar, *ibid.*, **28**, 2502 (1963).

(11) Spectra were determined using a Varian A-60 spectrometer and 5-8% deuteriochloroform solutions containing tetramethylsilane as an internal reference (0.0 c.p.s.). Chemical shifts, ν, are expressed in c.p.s. units downfield from the reference signal, and are accurate to better than ±1 c.p.s. Coupling constants, also expressed in c.p.s. units, have an accuracy of ±0.5 c.p.s. For any trio of compounds all spectra were recorded during one operating session to minimize errors in comparison. Thanks are due to Mr. E. Diaz and the Universidad Nacional Autónoma de México for the spectral measurements.

TABLE I

N.M.R. SPECTRA DATA FOR 16-UNSUBSTITUTED AND 16-METHYL STEROIDS[a]

Basic steroid		16-Substitution	$\nu_{18\text{-H}}$	$\nu_{19\text{-H}}$	$\nu_{16\text{-Me}}$ (J_{apparent})	$\Delta\nu_{18\text{-H}}$[b]
 I	a		47.8	72.0		
	b	16α-Me[c]	49.5	71.5	63.5, 69.5 (*J* 6.0)	+ 1.7
	c	16β-Me[c]	48.5	72.0	57.5, 64.5 (*J* 7.0)	+ 0.7
 II	a		39.0	62.5		
	b	16α-Me[d,e]	40.0	61.4	54.0, *ca.* 61 (*J ca.* 7)	+ 1.0
	c	16β-Me[d,f]	58.2	62.0	*ca.* 60, 67.2 (*J ca.* 7)	+19.2
 III	a		36.5	49.8		
	b	16α-Me[d]	38.0	49.5	52.8, 59.7 (*J* 6.9)	+ 1.5
	c	16β-Me[d]	56.3	49.8	*ca.* 59, 65.8 (*J ca.* 7)	+19.8
 IV	a		40.6	72.0		
	b	16α-Me[d,e]	42.0	71.7	53.8, 60.6 (*J* 6.8)	1.4
	c	16β-Me[d]	60.5	72.0	*ca.* 60, 67.5 (*J ca.* 7)	+19.9
 V	a		55.6	74.0		
	b	16α-Me[c]	57.6	73.5	62.5, 70.0 (*J* 7.5)	+ 2.0
	c	16β-Me[c]	53.4	73.3	*ca.* 70, *ca.* 77 (*J ca.* 7)	− 2.2
 VI	a		43.9	62.1		
	b	16α-Me[g]	49.2	62.2	50.9, 58.5 (*J* 7.6)	+ 5.3
	c	16β-Me[h]	54.2	62.2	66.0, 73.0 (*J* 7.0)	+10.3
 VII	a	—	39.0	62.1		
	b	16α-Me[g]	42.0	61.5	50.4, 57.5 (*J* 7.1)	+ 3.0
	c	16β-Me[i]	42.6	62.4	78.5, 84.5 (*J ca.* 6)	+ 3.6

[a] Where resonances were partially obscurred or accurate frequency measurement otherwise impeded, the frequency is expressed as an integer, prefixed by the abbreviation *ca.* Coupling constant signs were not determined. Literature references are given for the less common steroids studied. [b] Positive values of $\Delta\nu_{18\text{-H}}$ are for shifts to lower fields while negative values indicate upfield shifts. [c] A. Bowers, P. G. Holton, E. Necoechea, and F. A. Kincl., *J. Chem. Soc.*, 4057 (1961). [d] J. Romo, J. Lepe, and M. Romero, *Bol. Inst. Quim. Univ. Nac. Autom. Mex.*, **4**, 125 (1952). [e] R. E. Marker and H. M. Crooks, *J. Am. Chem. Soc.*, **64**, 1280 (1942). [f] A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944). [g] E. Batres, T. Cárdenas, J. A. Edwards, G. Monroy, O. Mancera, and C. Djerassi, *J. Org. Chem.*, **26**, 871 (1961). [h] E. Shapiro, T. Legatt, L. Weber, M. Steinberg, A. Watnick, M. Eisler, M. G. Hennessey, C. T. Coniglio, W. Charney, and E. P. Oliveto, *J. Med. Pharm. Chem.*, **5**, 975 (1962). [i] R. Sciaky, *Gazz. chim. ital.*, 91, 562 (1951); sample kindly supplied by Dr. Sciaky.

are independent of the substitution pattern in rings A and B. The values of $\nu_{18\text{-H}}$ do, however, reveal that relative to the three 3β-acetoxy-5α-pregnan-20-ones (III) $\nu_{18\text{-H}}$ is shifted consistently downfield by 2 c.p.s. in the three 3β-acetoxypregn-5-en-20-ones (II) and by 4 c.p.s. in each of the three pregn-4-ene-3,20-diones (IV). Zürcher's values for these structural changes are −0.5 and 4.0 c.p.s., respectively.[4c]

From the data for steroids I–V it is apparent that a 16α-methyl group leads, in these cases, to a constant downfield shift of 1–2 c.p.s. This adherence to the frequency shift additivity principle is in accord with expectations since in none of these 16α-methyl steroids is any serious extra nonbonded interaction introduced. However, comparison of $\Delta\nu_{18\text{-H}}$ values arising from introduction of a 16β-methyl substituent shows that the change from 17β-hydroxyl (I) to 17β-acetyl (II–IV) steroids is associated with a dramatic increase in the increment of downfield shift. Usually the introduction of a new 1:3 nonbonded diaxial (or pseudodiaxial) interaction with an angular methyl group leads to a noticeable downfield shift of the angular methyl proton resonance frequency.[4,8,12,13] Slomp and McGarvey[13] have recorded a downfield shift, $\Delta_{19\text{-H}}$ = 4.5 c.p.s.,

(12) *Inter al.* K. Tori and E. Kondo *Tetrahedron Letters*, 645 (1963); Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.*, **10**, 338 (1960); E. R. Malinowoski, M. S. Manhas, G. H. Müller, and A. K. Bose, *Tetrahedron Letters*, 1161 (1963).

(13) G. Slomp and B. R. McGarvey, *J. Am. Chem. Soc.*, **81**, 2200 (1959).

for the effect of a 6β-methyl substituent. It is concluded that the 18-proton downfield frequency shift by 0.7 c.p.s. attributed to 16β-methyl in the 17β-alcohol Ic is smaller than would be expected, while the large shift, $A\nu_{18\text{-H}}$ *ca.* 19.5 c.p.s., caused by 16β-methyl in the pregnan-20-ones (IIc–IVc) is much larger than expected, if no stereochemical changes are operating.

Brutcher and Bauer in a theoretical study of the ring D in steroids concluded that for 17β-alcohols and 17β-methyl steroids, bearing no other ring D substituents, the idealized form of ring D is as in IX.[14] This situation very probably is maintained in 17β-acetyl steroids since none of the extra interactions introduced on substitution of 17β-methyl by acetyl are removed by deformation of ring D, and the 17β-substituent retains the more favored equatorial configuration. For the 16β-methyl-20-ketones (IIc–IVc) the strong steric interactions which develop across the β-face of ring D are accompanied by a new severe interaction involving the 20-carbonyl function. An examination of Dreiding molecular models[15] suggests that this induced strain may be relieved by reorientation of the 17β-acetyl side chain so that the carbonyl oxygen is roughly at an equal distance from both 13β- and 16β-methyls. This change effectively moves the 18-protons from a position near the internodal surface of the shielding cone associated with the carbonyl group[16] to a less shielded position. Hence the large downfield shift of $\nu_{18\text{-H}}$ in 16β-methyl-20-ketones is comprehensible.[17]

At this point it is pertinent to consider other evidence bearing directly upon the question of a two-carbon 17β-side-chain conformation. For 16-unsubstituted steroids a preferred 20-ketone orientation as in X was suggested from hydrogenation experiments,[18] conformation analysis,[19] optical rotatory dispersion (O.R.D.) studies,[20] and dipole moment measurements.[21] Recently, 16β-substituted pregnan-20-ones have been studied by optical rotatory dispersion[22,23] and by circular dichroism (C.D.)[24,25] techniques. The more recent analysis indicates a conformational change of the side chain with a reorientation of the carbonyl axis relative to the steroid nucleus.[23] For the 16β-methyl-20-ketones the steric interactions are severe and are reflected in major changes in O.R.D. and C.D. curves. Interestingly, the 16β-substituent which causes the least distortion of such curves relative to the 16-unsubstituted steroids is the 16β-methoxycarbonyl group (CO_2Me) for which the changes are quite small.[22,26]

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

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

(16) *Cf.* L. M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, pp. 121–125.

(17) Besides the reorientation of the side-chain in the 16β-methyl derivatives IIc–IVc there may also be a conformational change in ring D, but no n.m.r. evidence was obtainable for or against this possibility. In any case ring D conformational changes alone would certainly not be expected to cause such drastic shifts of the 18-proton resonance.

(18) W. Klyne, *Ciba Foundation Colloquia on Endocrinology*, **7**, 127 (1953).

(19) S. Rakhit and Ch. R. Engel, *Can. J. Chem.*, **40**, 2163 (1962).

(20) C. Djerassi, I. Forneguera, and O. Mancera, *J. Am. Chem. Soc.*, **81**, 2383 (1959).

(21) N. L. Allinger and M. A. DaRooge, *ibid.*, **83**, 4526 (1961).

(22) P. Crabbé, *Tetrahedron*, **19**, 51 (1963).

(23) J. C. Danilewicz and W. Klyne, *J. Chem. Soc.*, in press.

(24) G. Snatzke, H. Pieper, and R. Tschesche *Tetrahedron*, **20**, 107 (1964).

(25) P. Crabbé, F. McCapra, F. Comer, and A. I. Scott, forthcoming publication.

However, the conclusion to be drawn from O.R.D., C.D. and the present n.m.r. studies is that for 16β-substituted 20-ketopregnanes the side-chain conformation is usually as in XI.

For the 17β-alcohols I the smaller than expected shift of the 18-proton resonance on introduction of a 16β-methyl substituent (*vide supra*) can only be accounted for by a ring D conformational distortion. There is some evidence from coupling constant magnitudes to support this conclusion. Earlier,[8] we published calculated coupling constants for the four possible 16,17-disubstituted steroid stereoisomers in each of the three idealized ring D conformations (IX, XII, and XIII) of Brutcher and Bauer.[14] For 16α-methyl-17β-hydroxyandrost-4-en-3-one (Ib) ring D is expected to hold the conformation IX of the 16-unsubstituted analog (*vide supra*). This supposition finds strong support in the proton–proton coupling constant $J_{16\beta,17\alpha}$ measured from the 17α-proton resonance doublet. The observed value, 6.8 c.p.s., falls neatly into the calculated range of 5.9–7.1 c.p.s. for conformation IX, while being comfortably outside the calculated ranges for the other two ring D conformers XII, $J_{16\beta,17\alpha}$ 3.7–5.0 c.p.s., and XIII, $J_{16\beta,17\alpha}$ 0.5–1.4 c.p.s. For the 16β-methyl isomer Ic the measured value of $J_{16\alpha H,17\alpha H}$ was 9.5 c.p.s., a value indicative of conformer XII (calcd. $J_{16\alpha,17\alpha}$ 7.8–8.0 c.p.s.) or XIII (calcd. $J_{16\alpha,17\alpha}$ 7.9–8.1 c.p.s.) rather than of conformer IX (calcd. $J_{16\alpha,17\alpha}$ 6.2–6.9 c.p.s.). The coupling constant change tends therefore to support the concept of ring D distortion in Ic.[27]

With the androst-4-en-3,17-diones Va–c the pattern of $\nu_{18\text{-H}}$ shifts changes once more. Although the 16α-methyl derivative Vb shows additivity of frequency shift (*vide supra*), with introduction of the 16β-methyl group (Vc) there is a net *shielding* effect and the 18-proton resonance frequency suffers a small upfield shift. This can only be attributed to a ring D conformational distortion as a result of which the 18-protons in Vc are now in a more shielded position relative to the 17-carbonyl shielding cone than are the 18-protons in the 16-unsubstituted (Va) and 16α-methyl (Vb) analogs. Moreover, this increase in shielding is powerful enough to exceed the normally observed net downfield shift of $\nu_{18\text{-H}}$ on introduction of a 1,3-diaxial methyl–methyl interaction.

Extension of the studies to 3β,17α-dihydroxypregn-5-en-20-one 3β-acetates (VI) and to the derived 3β,17α-diacetates VII disclosed that for these trios not only is there no consistent shift of $\nu_{18\text{-H}}$ attributed to a 16β-methyl substituent but $\Delta\nu_{18\text{-H}}$ for 16α-methyl substituents also deviates from the value of 1–2 c.p.s. found

(26) Four 16ξ-methoxycarbonyl-17ξ-acetyl stereoisomers were chosen for an earlier n.m.r. study in which the arguments rested, in part, on the 20-carbonyl retaining a largely undisturbed orientation in all four stereoisomers.[8] The subsequent studies for other 16β-substituents, summarized above, confirm that this choice was quite correct, though fortuitous.

(27) The coupling constant evidence must be regarded with considerable caution, however. Factors such as ring strain and substituent electronegativity are known to lead to deviations from the Karplus equations[28] upon which the coupling constant range calculations were based.[8,29] Coupling constant dependence upon the configuration of electronegative groups has also been proposed.[30] Nevertheless, the large observed value for $J_{16\alpha,17\alpha}$, 9.5 c.p.s. for Ic, suggests that any deviations from Karplus' equations are not excessive and, by ring D distortion, the 16β-methyl will tend to occupy a pseudo-equatorial configuration, as does a 16α-methyl group in conformation IX.

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

(29) See footnote 8 and ref. 9–14 therein for a summary of deviations from the Karplus equations.

(30) D. H. Williams and N. S. Bhacca, *J. Am. Chem. Soc.*, **86**, 2742 (1964).

for structures Ib–Vb. The 16α-methyl group generates new steric interactions in the 17α-substituted compounds VIb and VIIb, which can clearly influence the conformation of ring D and the side-chain orientation. These multiple effects cannot be analyzed separately by the n.m.r. technique though it is clear that collectively, or individually, they could lead to the observed breakdown in the additivity principle as applied to $\Delta_{18\text{-H}}$ and the 16α-methyl group.

Discussion so far has centered on the effects of structural change upon the 18-proton resonance frequency. Not unexpectedly, the substitution of hydrogen at C-16 by 16α- or 16β-methyl groups leads to no important frequency shifts of the 19-proton resonances (Table I) or of the resonances for any other protons of the distant rings A and B or their substituent groups. Thus, the the nine Δ^4-3-ketones (I, IV, V) show $\nu_{4\text{-H}}$ 345 ± 2 c.p.s., and the 3β-acetate protons (II, VI, VII) resonate at 121.5 ± 1 c.p.s. The 21-proton resonance shows no significant variation which can be assigned to the influence of 16α- or 16β-methyls. The nine 17α-unsubstituted pregnen-20-ones (II–IV) show $\nu_{21\text{-H}}$ 126.5 ± 1.5 c.p.s., the variation within any trio being less than 2 c.p.s. A 17α-hydroxy substituent causes a downfield shift of $\nu_{21\text{-H}}$ to 136 ± 0.5 c.p.s. There are no significant shifts in the 21-proton resonances of the three 17α-acetoxy compounds VII where $\nu_{21\text{-H}}$ moves from 127.2 c.p.s. for the unsubstituted derivative VIIa to 129.7 and 128.3 c.p.s., respectively, for the 16α-methyl (VIIb) and 16β-methyl (VIIc) homologs; n.m.r. spectral data for the pregn-4-en-3-one analogous to compound VIIc have been reported by Shapiro and co-workers.[31] Their values are in excellent agreement with those recorded here, with the expected shifts arising from the structural changes in rings A and B, and confirm that no ring D expansion has taken place during acetylation of the 17α-hydroxyl.

There are two resonances where the structural changes do cause pronounced effects. In the 17β-alcohol Ia the 17α-proton resonance appears at 220 c.p.s. as an ill-defined triplet.[3] For the 16β-methyl homolog Ic this proton appears as a doublet, J 9.5 c.p.s., at 219 c.p.s., but the corresponding doublet in the 16α-methyl isomer is centered upfield at 188 c.p.s. For the 16-methyl proton resonances, although apparent coupling constants[32] show no significant variation, it is of interest to note that in all the 17- and 20-ketones the 16β-methyl protons resonate at lower fields than the 16α-methyl protons (see Table I). In the 17β-alcohols the situation is the reverse.

From the data analyzed above it is clear that assignment of stereochemistry in 16,17-disubstituted steroids on the basis of 18-proton resonance frequency shifts must be approached with considerable caution.

Acknowledgment.—The authors wish to express sincere thanks to Dr. W. Klyne, P. Crabbe, and D. H. Williams for disclosure of their results prior to publication.

(31) E. Shapiro, T. Legatt, M. Steinberg, A. Watnick, M. Eisler, M. G. Henessey, C. T. Coniglio, W. Charney, and E. P. Oliveto, *J. Med. Chem.*, **5**, 975 (1962).

(32) F. A. L. Anet (*Can. J. Chem.*, **39**, 2662 (1961)) has drawn attention to the fact that the observed coupling constants for methyl protons in environments similar to those existing in these steroids are not the true values.

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZONA, AND THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS]

Purine Nucleosides. VIII. Reinvestigation of the Position of Glycosidation in Certain Synthetic "7"-Substituted 6-Dimethylaminopurine Nucleosides Related to Puromycin[1]

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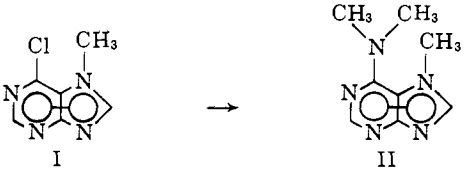
RECEIVED AUGUST 3, 1964

The compound previously described as 6-dimethylamino-"7"-methylpurine[4] obtained by a direct methylation procedure has now been shown to be dimethylamino-3-methylpurine (III) by unambiguous, independent syntheses of both compounds. This finding necessitated a reinvestigation of several purine nucleosides related to puromycin, the structure of which had been assigned previously by comparison with 6-dimethylamino-"7"-methylpurine on the basis of ultraviolet absorption spectra. By providing sets of comparably substituted adenines, it has been possible for us to develop further the spectral methods for differentiation between adenine derivatives substituted at various nitrogen atoms. With the utilization of the ultraviolet absorption data supplemented by proton magnetic resonance spectra, the structures of a number of previously described 6-dimethylamino-"7"-glycosylpurines have now been reassigned as the corresponding 3-glycosyl derivatives. Thus, conversion of a purine to its mercuric salt does not necessarily direct glycosidation exclusively to the imidazole ring.

As a result of the preparation and spectral examination of some model 3- and 7-substituted adenines, we have been able to develop further the spectral methods for differentiation between adenine derivatives substituted at various nitrogen atoms. Accordingly, we have reinvestigated a number of compounds related to puromycin and find that we can reassign certain structures reported to result from the glycosidation of 6-dimethylaminopurine.

6-Dimethylamino-7-methylpurine (II), m.p. 111–112°, was synthesized by treatment of 6-chloro-7-methylpurine (I) with dimethylamine. The structure of compound II may be regarded as unequivocal since 6-chloro-7-methylpurine[3] was prepared by the



I II

(1) Supported in part by American Cancer Society Grant No. T-181-B and in part by a research grant (USPHS-GM-05829-06) from the National Institutes of Health, U. S. Public Health Service.

(2) National Institutes of Health Postdoctoral Fellow, 1963–1964.

(3) R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6401 (1957).