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]

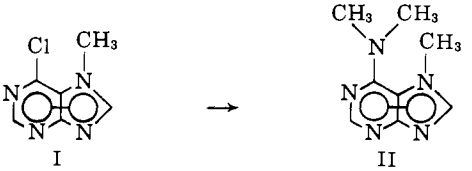
BY LEROY B. TOWNSEND, ROLAND K. ROBINS, RICHARD N. LOEPPKY,[2] AND NELSON J. LEONARD

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

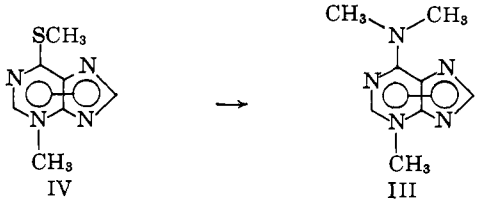


(1) Supported in part by American Cancer Society Grant No. T-181-B and in part by a research grant (USPHS-GM-05829-06) from the National Institutes of Health, U. S. Public Health Service.

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

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

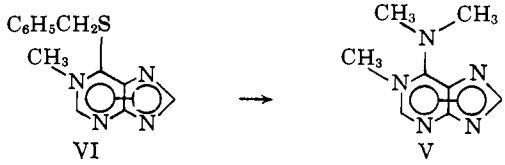
action of phosphorus oxychloride on 7-methylhypoxanthine, wherein the position of the methyl group had been secured by ring closure of 4-amino-1-methyl-5-imidazolecarboxamide.[3] Since the compound assigned structure II by Baker, Schaub, and Joseph[4] was reported to melt at 168–169°, a reinvestigation of the basis for their assignment seemed in order.[5] The synthetic precursor was 6-dimethylamino-2,8-bis(methylthio)purine,[6,7] which was methylated as previously described[4] using dimethyl sulfate and methanolic sodium hydroxide, followed by removal of the methylthio groups with Raney nickel to give two isomers described by Baker, Schaub, and Joseph[4] as 6-dimethylamino-9-methylpurine and 6-dimethylamino-7-methylpurine. The first of these structures was assigned correctly and the compound was identical with a sample of 6-dimethylamino-9-methylpurine, m.p. 119–120°, prepared by an unambiguous route.[8] The structure of the second isomer, m.p. 168-169°, seemed likely[9] to be that of 6-dimethylamino-3-methylpurine (III) in the light of the recently recognized preferential 3-substitution on adenine,[9–12] the occurrence of some 3-alkylation of adenine even in the presence of alcoholic alkali,[13] and the report, contemporary to this work, that compound III results from the direct methylation of 6-dimethylaminopurine.[14] It was necessary to provide an unequivocal route to III and complete the identification. Reaction of 3-methyl-6-methylthiopurine (IV)[9] with dimethylamine furnished authentic 6-dimethylamino-3-methylpurine (III), m.p. 167–168°, which proved to be the



IV III

compound described by Baker, Schaub, and Joseph[4] as 6-dimethylamino-"7"-methylpurine. Identity was established by the criteria of mixture melting points, ultraviolet and infrared spectra, n.m.r. spectra, and paper chromatography in four solvent systems. Molecular models of 6-dimethylamino-2,8-bis(methylthio)purine suggest steric interference with reaction at position 7, and by analogy it is probable that the compounds 6-dimethylamino-"7"-ethylpurine (m.p. 135–136°) and "7"-benzyl-6-dimethylaminopurine (picrate, m.p. 187–188° dec.) which were prepared similarly by alkylation of 6-dimethylamino-2,8-bis(methylthio)-purine[4] are also the 3-substituted isomers. The structural assignment of one of the products of benzylation of 6-chloropurine as 7-benzyl-6-chloropurine by Montgomery and Temple,[15] "by conversion to the known "7"-benzyl-6-dimethylaminopurine," is therefore invalid, but the assignment is actually correct as judged by comparison of the Montgomery and Temple product in one of our laboratories with 6-dimethylamino-7-methylpurine (I) by means of ultraviolet absorption and n.m.r. spectra.

The final N-methyl-substituted 6-dimethylaminopurine, namely, 6-dimethylamino-1-methylpurine (V), m.p. 199–200°, was synthesized unambiguously by treatment of 6-benzylthio-1-methylpurine[16] with aqueous dimethylamine.



VI V

The ultraviolet spectral data for all four isomers are included in Table I, as determined in aqueous solution under the conditions stipulated in the Experimental section. Certain generalities stated earlier concerning ultraviolet spectra are corroborated by the maxima and minima exhibited by this set of isomers. Thus the 3- and 7-isomers (III, II) show a great similarity, especially in the hyperchromic shift observed for the long wave length maximum, in going from neutral or alkaline to acid solution.[13,17–19] The 3-isomer can be differentiated from the 7-isomer by the observation of the appreciably negative value for λ_{min}(pH 1) − λ_{min}(pH 7) for the former.[18] The 3- and 1-isomers cannot be differentiated from each other on this basis since it will be seen that the empirical value relating the minima is also negative for the 1-isomer; however, the maximum for the 1-isomer does not undergo a hyperchromic shift on acidification as does the 3-isomer. A combination of values relating the intensities and wave lengths of maxima and minima under different pH conditions is accordingly sufficient to assign structures. Nevertheless, we do not wish to suggest too great reliance on the exact positions of maxima and minima. From visual inspection and comparison of the ultraviolet curves of about fifty substituted adenines we find that the shape and general appearance of the tracing is generally indicative of the position of substitution. We place some confidence in the representative characteristics of the ultraviolet absorption curves described below, based on the assumption that no other ultraviolet-absorbing groups are present in the molecules.

1-Alkyladenines show a hyperchromic shift in base, an isosbestic point on the short wave length side of the maxima, and a hypsochromic shift in acid.

3-Alkyladenines show a hyperchromic shift in acid and an isosbestic point on the long wave length side of the maxima. The basic and neutral spectra show very little difference.

(4) B. R. Baker, R. E. Schaub, and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).

(5) The statement on p. 6403 of ref. 3 must be regarded as premature, wherein a comparison was made which was not germane to the structure proof.

(6) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Org. Chem.*, **19**, 631 (1954).

(7) C. W. Noell and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 5997 (1959).

(8) R. K. Robins and H. H. Ling, *ibid.*, **79**, 490 (1957).

(9) J. W. Jones and R. K. Robins, *ibid.*, **84**, 1914 (1962).

(10) B. C. Pal, *Biochemistry*, 1, 558 (1962).

(11) N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, **85**, 2026 (1963).

(12) N. J. Leonard and T. Fujii, *ibid.*, **85**, 3719 (1963).

(13) N. J. Leonard and J. A. Deyrup, *ibid.*, **84**, 2148 (1962).

(14) B. C. Pal and C. A. Horton, *J. Chem. Soc.*, 400 (1964); Abstracts of Papers, 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962, p. 39c.

(15) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

(16) L. B. Townsend and R. K. Robins, *J. Org. Chem.*, **27**, 990 (1962).

(17) G. B. Elion, "Ciba Foundation Symposium on the Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957, p. 39.

(18) G. B. Elion, *J. Org. Chem.*, **27**, 2478 (1962).

(19) R. Denayer, A. Cavé, and R. Goutarel, *Compt. rend.*, **253**, 2994 (1961).

TABLE I

ULTRAVIOLET SPECTRA OF SUBSTITUTED ADENINES[a]

Compound, purine	M.p., °C.		λ_{max} mμ	λ_{max} ε × 10⁻³	λ_{max} mμ	λ_{max} ε × 10⁻³	λ_{min} mμ	λ_{min} ε × 10⁻³	λ_{min}(pH 1) − λ_{min}(pH 7), mμ[18]
6-Dimethylamino-1-methyl- (V)	199–200	pH 1	293	12.2	221	13.5	246	2.05	
		pH 7	298	12.6	215	14.0	257	3.6	−11
		pH 12	301	13.6	232	13.4	262	3.45	
6-Dimethylamino-3-methyl- (III)	167–168	pH 1	290[b]	20.4			243	2.5	
		pH 7	292	16.6	222	11.3	250	3.0	−7
		pH 12	293[c]	16.4			250	3.3	
6-Dimethylamino-7-methyl- (II)	111–112	pH 1	293	18.1	223	9.0	250	3.3	
		pH 7	291	14.4	222	13.0	246	3.3	+4
		pH 12	291	14.4			246	3.6	
6-Dimethylamino-9-methyl-	119–120	pH 1	269[d]	15.6			234	2.2	
		pH 7	276	16.0	214	15.5	235	2.0	−1
		pH 12	276[e]	16.3			237	1.75	
6-Dimethylamino-9-β-D-ribofuranosyl- (IX)	183–184	pH 1	268	18.4			234	2.8	
		pH 7	275	18.8	215	15.6	236	2.0	−2
		pH 12	275	19.2			237	2.3	
6-Dimethylamino-3-β-D-glucopyranosyl- (VII)	273–274	pH 1	292	19.6			245	2.8	
		pH 7	298	15.1	222	11.7	251	2.85	−6
		pH 12	299	15.0			252	2.8	
6-Dimethylamino-3-β-D-ribofuranosyl- (VIII)	200–201	pH 1	291	22.7			246	3.4	
		MeOH	298	18.0	226	15.0	251	2.8	−5[f]
		pH 12	298	17.5			253	3.1	

[a] In water unless otherwise stated. Reported[14]: [b] 289.5, [c] 292, [d] 269.5, [e] 276 mμ. [f] λ_{min}(pH 1) − λ_{min}(CH_3OH).

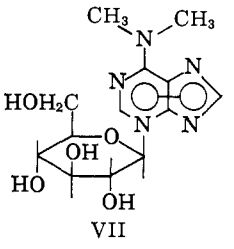
7-Alkyladenines exhibit a hyperchromic shift and possibly a bathochromic shift in acid, with an isosbestic point on the short wave length side of the maxima.

9-Alkyladenines possess spectra which are relatively insensitive to pH. A slight hypsochromic shift in acid and an isosbestic point near and on the short wave length side of the maxima are generally observed.

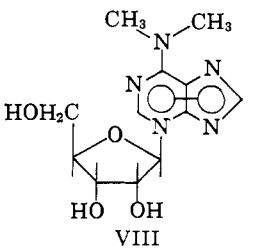
Among the sets of isomers examined which have spectra fitting these generalizations are the methyladenines,[3,9,13,17,18,20] the methyl-6-methylaminopurines[3,9] and allyladenines,[12,21] the γ,γ-dimethylallyladenines,[12,13,19,21] the benzyladenines[12,15,21] with subtraction of absorption for the phenyl moiety, the 6-dimethylaminomethylpurines (Table I), and the presently known ribosyladenine isomers.[11,22]

Since the structural assignment of a number of synthetic purine nucleosides related to puromycin have been based on comparison of the ultraviolet absorption spectra with that of 6-dimethylamino-"7"-methylpurine as a model compound, it appeared that certain of these "7"-glycosylpurines might indeed be the corresponding 3-glycosyl derivatives. In order to explore this possibility,[23] several of the 6-dimethylamino-"7"-substituted purine nucleosides described in the puromycin studies were resynthesized and their ultraviolet and n.m.r. spectra were examined.

Condensation of the chloromercury derivative of 6-dimethylamino-2,8-bis(methylthio)purine with α-bromoacetoglucose was reported[24] to yield 6-dimethylamino-"7"-β-D-glucopyranosyl-2,8-bis(methylthio)-purine tetraacetate, and, upon subsequent treatment with Raney nickel, to yield 6-dimethylamino-"7"-β-D-glucopyranosylpurine tetraacetate, m.p. 147–149°. The same compound was reported to result from the treatment of the chloromercury derivative of 6-dimethylaminopurine with α-bromoacetoglucose.[24] The latter route of synthesis has now been repeated exactly as described and the product deacetylated according to the directions of Baker and his co-workers to yield 6-dimethylamino-"7"-β-D-glucopyranosylpurine, m.p. 273–274° dec. (reported[24] m.p. 239–241° dec.). When the ultraviolet spectral values for our product are



VII



VIII

compared (Table I) with the corresponding data for 6-dimethylamino-7-methylpurine (II) (Fig. 1) and 6-dimethylamino-3-methylpurine (III) (Fig. 2) it can be seen, especially from the last column in the table, that this compound should be classified as a 3-substituted adenine, therefore 6-dimethylamino-3-β-D-glucopyranosylpurine (VII) (Fig. 3). A visual inspection of the ultraviolet absorption curve according to the general characteristics described above confirms the assignment. The possibility that the product might be the 1-substituted isomer of 6-dimethylaminopurine is readily eliminated by spectral comparison, especially of the hyperchromic shift for VII, like III, in going to acidic solution.

The analogous synthesis reported[25] for 6-dimethylamino-"7"-β-D-ribofuranosylpurine, m.p. 200–201°, and 6-dimethylamino-9-β-D-ribofuranosylpurine (IX), m.p. 183–184°, using 1-chloro-2,3,5-tribenzoyl-D-ribofuranose and the chloromercury derivative of 6-dimethylamino-2,8-bis(methylthio)purine, was also repeated. The structure of IX (for ultraviolet spectrum, see Table I) was verified by direct comparison with the

(20) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
(21) N. J. Leonard and T. Fujii, *Proc. Natl. Acad. Sci., U. S.*, **51**, 73 (1964).
(22) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **85**, 2672 (1963).
(23) J. A. Deyrup, Ph.D. Thesis, University of Illinois, 1961, p. 55.
(24) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954).
(25) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

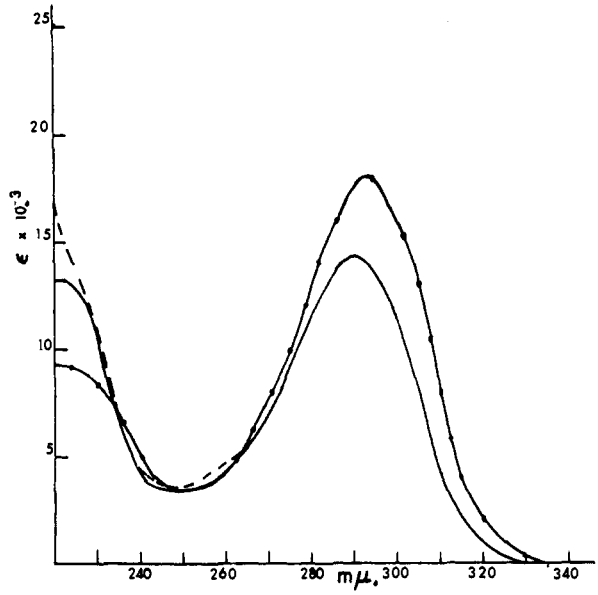


Fig. 1.—6-Dimethylamino-7-methylpurine (II): —·—·—, pH 1; ———, pH 7; – – – –, pH 12.

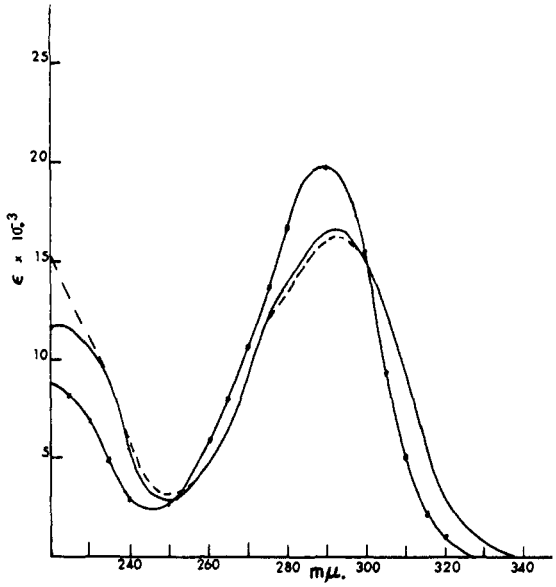
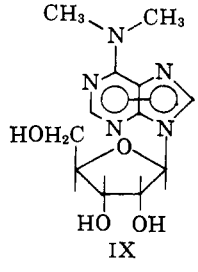


Fig. 2.—6-Dimethylamino-3-methylpurine (III).

authentic 9-substituted 6-dimethylaminopurine prepared from 6-chloro-9-β-D-ribofuranosylpurine.[26,27] The ultraviolet spectra of the isomeric product of m.p.



IX

200–201° determined in methanol and in water at pH 1 and 12 (Table I) were closely analogous to those for VII under the same conditions, both with respect to wave length of the maxima and visual inspection of the

(26) R. K. Robins, *J. Am. Chem. Soc.*, **82**, 2654 (1960); see also *Biochem. Prepn.*, **10**, 145 (1963).

(27) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963).

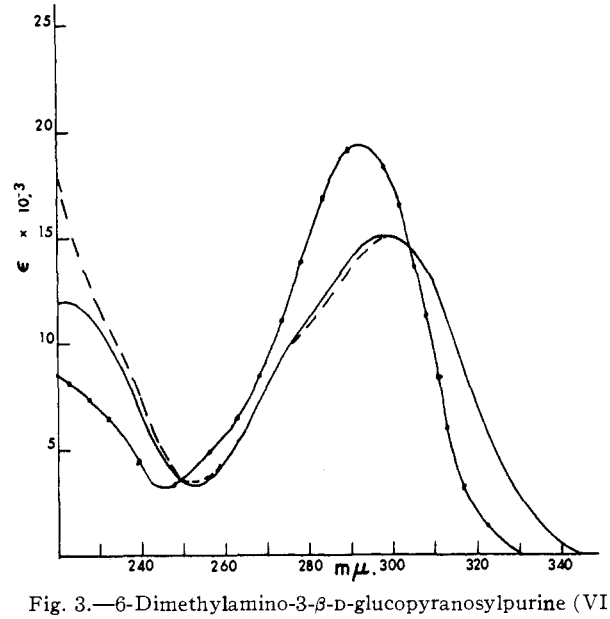


Fig. 3.—6-Dimethylamino-3-β-D-glucopyranosylpurine (VII).

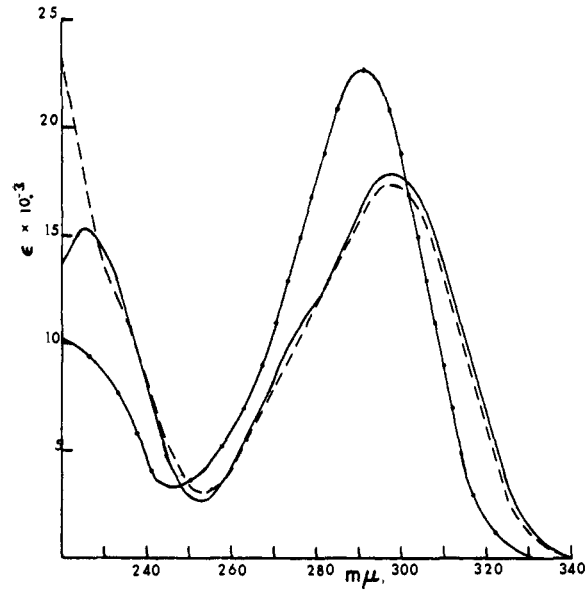


Fig. 4.—6-Dimethylamino-3-β-D-ribofuranosylpurine (VIII).

curves (Fig. 4). Accordingly, the structure should be revised to 6-dimethylamino-3-β-ribofuranosylpurine (VIII). Even the melting points of the isomerically substituted 6-dimethylaminopurines may serve as a guide in the preliminary evaluation of structure assignments, since it will be seen from the physical properties of the compounds included in Table I and others here represented that the melting points of the 1- or 3-substituted dimethylaminopurines are higher than those of the correspondingly substituted 7- or 9-substituted dimethylaminopurines.[28] Reasoning by analogy to the formation of VII and VIII and on the basis of the limited physical and spectral data available, we suggest that the 6-dimethylamino-"7"-β-D-xylofuranosylpurine, m.p. 214–215° dec., obtained[29] as one of the products from the chloromercury derivative of 6-dimethylaminopurine and 1-bromo-2,3,5-tribenzoyl-D-

(28) This guideline must obviously be applied within caution since in some cases, *e.g.*, 6-dimethylamino-3-β-D-glucopyranosylpurine tetraacetate, m.p. 147–149°, and 6-dimethylamino-9-β-D-glucopyranosylpurine tetraacetate, m.p. 141–143°,[24] the melting points are very close, and in the substituted 6-NH_2 series the correlation does not exist.

(29) B. R. Baker and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 5900 (1955).

xylofuranose, actually has the xylofuranosyl moiety attached at the 3-position. The contaminant, with the 9-isomer, described as 7-(di-O-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine,[30] but which was not isolated or characterized, may have been the 3-glycoside.

From the present studies, it is clear for the first time that a halosugar and the mercury salt of a purine do not necessarily result in glycosidation in the imidazole ring *only*, but that the possible N-sites of attack on the purine nucleus depend upon the nature and size of the group(s) already attached.[24,31]

Examination of the work of Blackburn and Johnson[32] in view of our findings makes it clear why these authors failed to obtain the desired true 7-glycosylpurine derivatives. The substitution of large groups such as benzyl on the 6-amino function merely added to the steric hinderance at the 7-position preventing the desired 7-glycosidation. The 6-(N-benzyl-N-methylamino)-"7"-(tetra-O-acetyl-β-D-glucopyranosyl)-purine reported[32] by these workers from 6-(N-benzyl-N-methylamino)purine is very probably another example of 3-substitution instead of 7. By contrast, Montgomery and Thomas[33,34] were able to synthesize 7-glycosylpurines of proven structure by blocking nucleoside formation at position 3 by prior benzylation. It has recently been shown[12] that the 3-blocking procedure is a general method of accomplishing 7-alkylation.

The synthesis of purine nucleosides with a sugar moiety at position 3 is of particular interest in view of the recent work on the isolation and structure of 3-ribosyluric acid[35] which occurs naturally in beef blood.

The n.m.r. spectra have also proved of value for differentiation between isomerically substituted adenines and purines, the most useful region of the spectra being that in which the 2- and 8-proton signals occur (δ = 7.5–10.0 downfield from tetramethylsilane = 0.0). Matsuura and Goto[36] and Schweizer, Chan, Helmkamp, and Ts'o[37] have recently shown that, of the aromatic protons in purine itself, the 6-proton is the least shielded, followed by the 2-proton, while the 8-proton is at highest field. This is in contrast to the earlier assignment by Jardetzky and Jardetzky[38] of the 6-proton as the most shielded. At this time, we merely wish to call attention to the chemical shift (difference) between the signals observed for the 2- and 8-aromatic protons rather than to indicate firm assignments to the separate protons in each example.[39] The differences listed in Table II are therefore not accorded positive or negative values but fulfill a very useful role without regard to sign. Where sets of isomerically substituted adenine derivatives are considered, the 3- or 1-substituted derivatives show, in their n.m.r. spectra taken in dimethyl sulfoxide, a relatively greater difference between the signals for the 2- and 8-protons than do the corresponding 7- or 9-substituted derivatives.[40] The accumulated data lead to an empirical generalization which is especially useful in the adenine series where only one ring nitrogen is substituted and which will hold in the absence of extraneous shielding or deshielding of the aromatic protons by neighboring groups. It will be seen that the structural assignments made earlier in this paper to certain isomeric glycosides encountered in the synthesis of nucleosides related to puromycin, especially 6-dimethylamino-3-β-D-glucopyranosylpurine (VII) and 6-dimethylamino-3-β-D-ribofuranosylpurine (VIII), are corroborated by the large chemical shift between the two aromatic protons. Such specific n.m.r. spectral data are accordingly useful, along with the ultraviolet spectra, for differentiating between substitution on the pyrimidine or imidazole ring of adenine and other substituted 6-aminopurines.

TABLE II

DIFFERENCE IN CHEMICAL SHIFT FOR THE 2- AND 8-PROTONS[a]

Compound	Δδ, c.p.s.
6-Dimethylamino-	
purine	8
1-methylpurine (V)	22
3-methylpurine (III)	32
7-methylpurine (II)	2
9-methylpurine	11
3-β-D-glucopyranosylpurine (VII)	47
3-β-D-ribofuranosylpurine (VIII)	48
9-β-D-ribofuranosylpurine (IX)	8
Adenosine	12
Adenine	2
3-β-D-Ribofuranosyl-	48
1-Methyl-	26[b]
3-Methyl-	28
7-Methyl-	6
9-Methyl-	3

[a] Spectra run in dimethyl sulfoxide-d_6. [b] In trifluoroacetic acid, using a Varian Associates A-60 high-resolution spectrometer, with tetramethylsilane as an internal standard.

Finally, other data which we have found useful for comparative characterization of substituted adenines are readily obtainable from paper chromatography.

Inspection of Table III reveals that the chromatographic behavior of the 1- and 3-methyladenines is characteristically different from that of the 7- and 9-methyl derivatives, depending of course upon the choice of solvents. Bergmann and his co-workers[41] first noticed that a number of 3-methylpurines exhibited a smaller R_f value than similarly substituted 7- or 9-substituted derivatives. In the solvents employed the difference was sufficient to be diagnostic. Jones and Robins[20] have shown that various polar or zwitterionic purine structures could be correlated with a smaller

(30) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 5905 (1955).

(31) *Cf.* (a) M. L. Wolfrom, P. McWain, F. Shafizadeh, and A. Thompson, *ibid.*, **81**, 6080 (1959); (b) M. L. Wolfrom, P. McWain, and A. Thompson, *ibid.*, **82**, 4353 (1960); (c) R. H. Iwamoto, E. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963); (d) H. J. Schaeffer and H. J. Thomas, *J. Am. Chem. Soc.*, **80**, 4896 (1958); (e) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957); (f) R. H. Iwamoto, E. M. Acton, and L. Goodman, *ibid.*, **27**, 3949 (1962); (g) H. Zinner and E. Wittenburg, *Chem. Ber.*, **95**, 1866 (1962); (h) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, **17**, 301 (1962); (i) T. Yamane and N. Davidson, *J. Am. Chem. Soc.*, **83**, 2599 (1961).

(32) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4347 (1960).

(33) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963).

(34) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **85**, 2672 (1963).

(35) R. Lohrmann, J. M. Lagowski, and H. S. Forrest, *J. Chem. Soc.*, 451 (1964). See also D. Hatfield, R. R. Rinehartdt, and H. S. Forrest, *ibid.*, 899 (1963), and H. S. Forrest, D. Hatfield, and J. M. Lagowski, *ibid.*, 963 (1961).

(36) S. Matsuura and T. Goto, *Tetrahedron Letters*, **22**, 1499 (1963).

(37) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964).

(38) C. D. Jaredtzky and O. Jardetzky, *ibid.*, **82**, 222 (1960); however, *cf.* F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1964).

(39) Full discussion of the n.m.r. spectra of variously substituted adenines and purines will be found in sequential papers in press from our laboratories.

(40) A similar observation has been made by Dr. T. Fujii, University of Illinois, for sets of allyl-, benzyl-, and-γ,γ-dimethylallyladenines

(41) F. Bergmann, G. Levin, A. Kalmas, and H. Kwietny-Govrin, *J. Org. Chem.*, **26**, 1504 (1961).

TABLE III

R_{Ad} FOR CERTAIN METHYLATED ADENINE DERIVATIVES

Compound	R_{Ad}[a] Solvent A	R_{Ad}[a] Solvent B
Adenine		
1-Methyl-	0.94	1.69
3-Methyl-	1.20	1.61
7-Methyl-	1.38	1.28
9-Methyl-	1.40	1.04
Aminopurine		
6-Methyl-	1.83	1.19
1-Methyl-6-methyl-	1.47	1.78
3-Methyl-6-methyl-	1.69	1.65
7-Methyl-6-methyl-	2.24	1.40
9-Methyl-6-methyl-	2.24	1.24
6-Dimethyl-	2.38	1.29
Methylpurine		
6-Dimethylamino-1-	1.60	1.83
6-Dimethylamino-3-	2.29	1.68
6-Dimethylamino-7-	2.50	1.68
6-Dimethylamino-9-	2.95	1.31

[a] Solvent A: dimethylformamide, 25 parts–aqueous ammonia 28%, 10 parts–isopropyl alcohol, 65 parts by volume. Solvent B: isopropyl alcohol, 1 part–5% aqueous ammonium sulfate, 19 parts by volume.

R_f value owing to the fact that the more polar compounds are likely to be retarded in the aqueous stationary phase. This holds true for the 1- and 3-methyladenines in solvent A (Table III). However, when a solvent system is employed in which the mobile phase is highly ionic (solvent B) the more polar 1- and 3-substituted derivatives, in general, have a greater R_f value than the corresponding 7- and 9-substituted derivatives. For a more valid comparison the R_{Ad} values (R_f of compound/R_f of adenine)[18] have been recorded.

Experimental

Ultraviolet Absorption Spectra.—All spectra (except VIII) were taken on a Cary Model 15 spectrophotometer. The wave length of this instrument was calibrated and the absorbance checked with a sodium chromate solution prepared according to National Bureau of Standards directions and was found to be satisfactory. The pH of the aqueous solutions was varied in the following manner. The cell was filled with 3 ml. of solution, the pH of which was close to 7.0 (between 6.5 and 7.5) as determined by a Leeds and Northrup pH meter, and the spectrum was taken. (For comparison, in the case of 6-dimethylamino-1-methylpurine (V) a solution buffered at pH 7 with 0.05 *M* phosphate exhibited very similar maximum (297 mμ, ε 11,000) and minimum values (255 mμ, ε 3500) to those reported in Table I for V in water alone.) One drop of 12 *N* HCl was then added to the solution and the resulting pH was found to vary between 1 and 1.2. The spectrum was then taken on the same chart and it was determined that the addition of 1 drop to 3 ml. results in a decrease in absorbance of 0.006. This correction was applied in all calculations. This sample was then discarded and a new 3-ml. sample was introduced into the cell with 1 drop of 4 *N* NaOH. This resulted in a pH of 12. A base line was run for acid, neutral, and basic solutions, and the appropriate corrections were applied.

The help of Mr. Kermit Carraway is gratefully acknowledged in assembling and analyzing the ultraviolet data for many more compounds than are here recorded.

6-Chloro-7-methylpurine (I).—6-Chloro-7-methylpurine obtained by chlorinating 7-methylhypoxanthine[4] was subjected to paper chromatography, and a bright blue fluorescent contaminant was observed. This contaminant occurs in such a small amount that an analytical sample can be obtained without separation of the two compounds. Also, other compounds derived from this compound can be analyzed but they also contain a small amount of a bright blue fluorescent contaminant. For purification of the 6-chloro-7-methylpurine, the product obtained from the chlorination was extracted 48 hr. with petroleum ether (60–110°) in a Soxhlet apparatus. The petroleum ether was removed *in vacuo* on a steam bath, and the residue was recrystallized twice from toluene to yield chromatographically pure 6-chloro-7-methylpurine, m.p. 198–199°.[3] Compounds derived from this sample were also chromatographically pure, exhibiting no indication of a bright blue fluorescent contaminant.

6-Dimethylamino-7-methylpurine (II).—A solution of 500 mg. of 6-chloro-7-methylpurine (I) in 50 ml. of dimethylamine (25% aqueous) was evaporated to dryness on the steam bath. The residue was extracted with 200 ml. of boiling benzene, and heptane was added dropwise to the boiling solution to a cloud point. The solution was then allowed to stand at 10° for 24 hr. and the solid which separated was filtered and recrystallized from benzene–heptane; yield 340 mg., m.p. 111–112°.

Anal. Calcd. for $C_8H_{11}N_5$: C, 54.2; H, 6.3; N, 39.5. Found: C, 54.0; H, 6.0; N, 39.5.

6-Dimethylamino-3-methylpurine (III).—One gram of 3-methyl-6-methylthiopurine was dissolved in 50 ml. of methanol containing 20 ml. of dimethylamine (25% aqueous). The solution was then evaporated to dryness on a steam bath. The residue was extracted with 250 ml. of boiling benzene, and the volume was reduced to 100 ml. on a steam bath. Heptane was added slowly to the boiling solution to the cloud point, and the solution was allowed to stand at 10° for 24 hr. The solid which separated was filtered and recrystallized from benzene–heptane solution; yield 0.6 g., m.p. 167–168°.

Anal. Calcd. for $C_8H_{11}N_5$: C, 54.2; H, 6.3; N, 39.5. Found: C, 54.3; H, 6.3; N, 40.0.

6-Dimethylamino-9-β-D-ribofuranosylpurine (IX).—6-Chloro-9-β-D-ribofuranosylpurine (2.5 g.) was added to 50 ml. of methanol and 50 ml. of acetone containing 2.5 g. of anhydrous dimethylamine at 25°. After stirring for 8 hr. at room temperature, the reaction mixture was allowed to stand at 10–15° for 12 hr. The solid which separated was filtered and washed with 200 ml. of anhydrous diethyl ether. The product was then dissolved in 400 ml. of boiling acetone, treated with charcoal, filtered, and cooled at 10–15° for 24 hr. The precipitate which had separated was filtered, washed with anhydrous diethyl ether, and dried at 60° to yield 1.5 g. of product, m.p. 183–184°. This compound prepared from 6-methylthio-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine is reported[42] to melt at 174–175°.

Anal. Calcd. for $C_{12}H_{17}N_5O_4$: N, 23.7. Found: N, 24.0.

6-Dimethylamino-1-methylpurine (V).—6-Benzylthio-1-methylpurine (VI,[16] 2.0 g.) was added with stirring to 100 ml. of absolute ethanol containing 10 g. of anhydrous dimethylamine at −5°. The solution was stirred at room temperature for 5 hr. and then evaporated to dryness *in vacuo* at room temperature. The resulting residue was recrystallized twice from ethyl acetate–heptane; yield 1.1 g. of colorless product, m.p. 199–200°.

Anal. Calcd. for $C_8H_{11}N_5$: C, 54.2; H, 6.3; N, 39.5. Found: C, 54.1; H, 6.5; N, 40.0.

(42) M. Ikehara, E. Ohtsuka, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 173 (1961).