71. *The Structure of 3-Methyladenine, and Methylation of 6-Dimethylaminopurine.**

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It has recently been suggested that 3-methyladenine exists in the amino- rather than the imino-form. The similarity of the ultraviolet spectra of 3-methyladenine and 6-dimethylamino-3-methylpurine confirms this view. The infrared spectrum of 3-methyladenine also indicates the absence of imidazole NH and the presence of an exocyclic NH_2 group. 3-Methyl-, 1-methyl-, and 9-methyl-6-dimethylaminopurine have been synthesized by methylation of 6-dimethylaminopurine. The infrared spectra of six alkyladenines have been obtained.

Although chemical evidence of reaction of alkylating agents with the adenine moiety of DNA has yet to be demonstrated, the possibility of such a reaction as a probable cause of mutagenesis by these agents cannot be eliminated. This is specially borne out by the results of the studies on the methylation of adenosine and adenylic acid. It was found that adenosine and adenylic acid are alkylated [1] *in vitro* at N-1 and N-3. Investigation of these reactions led us to re-examine the structure of 3-methyladenine which was synthesized and represented in the " imino-form " (I) by Elion.[2]

In adenine and presumably in alkyladenines the anionic pK arises from the dissociation of the imidazole NH.[3] Unlike 1-methyladenine, which exists in the " imino-form " and

Table 1.

Ultraviolet spectra (aqueous solutions) and pK values of adenines, methyladenines, and imidazoles.

	Spectra [a] $\lambda_{max.}$ (mμ)	pK_a Titrimetic	pK_a Spectral
Adenine	262·5(2) 260·5(7) 268(12)		
6-Methylaminopurine	267(1) 273(13) [b]		
6-Dimethylaminopurine	277(1) 281(13) [b]		4·4 10·1
1-Methyladenine	259(4) 270(13) [c]	6·95 11·9 [d]	7·2 11·0 [c]
3-Methyladenine	273(4) 272(12)	5·4 [d]	5·7
6-Dimethylamino-9-methylpurine	269·5(1) 276(12)		
6-Dimethylamino-3-methylpurine	289·5(1) 292(12)		5·8
5-Aminoimidazole-4-carboxamide	240, 267(2) 267(7) 276(12)		
5-Methylaminoimidazole-4-carboxamide	242·5 283(2) 282·5(7)		

[a] Figures in parentheses indicate the pH of determination. [b] Littlefield and Dunn, *Biochem. J.*, 1958, **70**, 642. [c] Ref. 1. [d] Ref. 5.

shows [1] pK at 7·2 and 11, 3-methyladenine shows a single pK of 5·7 (Table 1). Its ultraviolet absorption spectrum does not show any change between pH 7 and pH 13 (Figure) and it fails to migrate as an anion on paper electrophoresis even at pH 12. On the basis of these observations one of us suggested that 3-methyladenine may exist in the " amino-form " (II).[4] Recently Leonard and Deyrup [5] have arrived at the same conclusion independently, essentially on the same experimental evidence. Jones and Robins [6] have also suggested the " amino-form " of 3-methyladenine on the basis of its synthesis from

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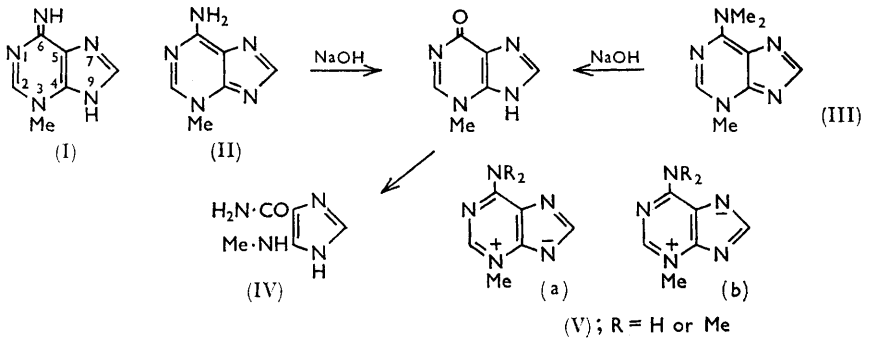
[1] Brookes and Lawley, *J.*, 1960, 539.
[2] Elion, Ciba Foundation Symposium on the Chemistry and Biology of Purines, Wolstenholme and O'Connor, editors, J. A. Churchill, London, 1957, p. 39.
[3] Bendich, " The Nucleic Acids," Chargaff and Davidson, editors, Vol. I, Academic Press, New York, 1955, p. 81.
[4] Pal, *Biochemistry*, 1962, **1**, 558.
[5] Leonard and Deyrup, *J. Amer. Chem. Soc.*, 1962, **84**, 2148.
[6] Jones and Robins, *J. Amer. Chem. Soc.*, 1962, **84**, 1914.

3-methyl-6-methylthiopurine and ammonia, although this evidence cannot be regarded as rigorous.

To obtain further evidence in support of the "amino-form" (II) we prepared 6-dimethylamino-3-methylpurine, in which the amino-structure is fixed. It has been reported [4] that adenine, on methylation with dimethyl sulphate, forms 1-, 3- (major product), and 9-methyl-adenine. We therefore studied the methylation of 6-dimethylaminopurine with dimethyl sulphate and obtained three products (A, B, and C), identified as the 1-, 3-, and 9-methyl derivatives on the following grounds.

Comparison with the results of methylation of adenine suggests that dimethylaminopurine should give the 1-, 3-, and 9-methyl derivatives and that *C*-alkylation should not occur.[4] The ultraviolet spectra did not agree with that of the known 7-methyl-6-dimethylaminopurine,[7] but that of product (C) agreed with the published spectrum [7] of the 9-methyl derivative.

Product (A) almost immediately lost its ultraviolet absorption spectrum in alkali at room temperature and it could not be regenerated by acidification. This behaviour resembles that of 1-methyladenine, which is transformed into 6-methylaminopurine in alkali, presumably by a ring opening and closing mechanism.[1] Such a mechanism has been established by Brown for 1,2-dihydro-2-imino-1-methylpyrimidine.[8] With (A),



however, no such rearrangement is possible and the purine ring is apparently destroyed with loss of absorption in the ultraviolet spectrum. The sensitivity of product (A) towards alkali may be due to its inability to stabilize itself by forming an anion; it thus appears to be 6-dimethylamino-1-methylpurine.

Product (*B*) must then be 6-dimethylamino-3-methylpurine (III), and this is established by its hydrolysis to 3-methylhypoxanthine in alkali. Paper chromatography of the mixture obtained by heating 3-methyladenine and the purine (B) with alkali showed the presence of a second product apart from 3-methylhypoxanthine, and we have tentatively identified this as 5-methylaminoimidazole-4-carboxamide (IV). The compound gives a positive Pauly test for imidazole,[9] and the ultraviolet absorption spectra are similar to those of 5-aminoimidazole-4-carboxamide (Table 1).

The ultraviolet absorption spectra of 3-methyladenine and 6-dimethylamino-3-methylpurine are similar except that maxima of the latter are shifted by about 17 mμ towards longer wavelengths (Figure). A similar shift is observed in the spectra of 6-dimethylaminopurine compared to adenine (Table 1). The infrared spectra of 3-methyladenine also indicate the presence of an exocyclic amino-group and absence of imidazole NH. Thus, evidence from infrared and ultraviolet spectral data coupled with the absence of anionic p*K* conclusively prove that 3-methyladenine exists in the amino-form (II).

[7] Baker, Schaub, and Joseph, *J. Org. Chem.*, 1954, **19**, 638.
[8] Brown, *Nature*, 1961, **189**, 828.
[9] Smith, "Chromatographic and Electrophoretic Techniques," Vol. I, Interscience, New York, 1960, p. 296.

The susceptibility of both 3-methyladenine and 6-dimethylamino-3-methylpurine (III) to attack by alkali may be explained by assuming the existence of such resonance forms as (Va) and (Vb). Such structures have been suggested by Robins and Jones to explain the lack of basicity of the amino-group in 3-methyladenine, as shown by failure to deaminate it with nitrous acid.[4,6]

Infrared Spectra.—The infrared spectra (Table 2) help to elucidate the tautomeric situation. A band at 1672 ± 8 cm.$^{-1}$ in the spectra of adenine, 7-methyl- and 9-methyladenine, isoguanine, 2-amino- and 2,6-diamino-purine, and 2-methyl-, 3-methyl-, 8-methyl-, 2,8-dimethyl-, 2-hydroxy-, 2-chloro-, and 2-methylamino-6-aminopurine is attributed to a primary amino-group [10,11] at the 2- or 6-position.[12] As expected, it is absent from the spectra of 6-dimethylaminopurine and its 2-methyl-, 3-methyl-, and 2-diethylamino-derivatives and from deuterated isoguanine and 6-amino-3-methylpurine. Bands 10, 12, 13—15 in the 3100 cm.$^{-1}$ region are attributed to NH stretching vibrations or to a C–H vibration of the pyrimidine ring.[11,12,16] If due to the former they should shift to lower

TABLE 2.

Principal infrared spectral bands (cm.$^{-1}$) of some purines.

(Based on the following sources: the present work; references 10, 12—14; Sadtler infrared spectra numbers 7006, 7007, 7510, 10,528, 16,805, 16,806, 16,826, 16,856—8, 16,861, 16,874, 17,159, 17,163; Brown and Mason, *J.*, 1957, 682; Katritzky, *Quart. Reviews*, 1959, **13**, 353; Randall, Fowler, Fuson, and Dange, " Infrared Determination of Organic Structures," Van Nostrand, New York, 1949, p. 209.)

Adenine: 3440s 3300ms 3060m 2960ms 2935ms 2880m 1742mw 1622vs 1578s 1550ms 1538m 1451ms 1395s 1330m 1272ms 1242m 1169s 1148ms 1112w sh 1066mw 1037mw 948w 868mw 776mw 752 w 656mw 630w

3-*Methyladenine*: 3420s 3365s sh 3200ms 3030ms 2960m 2930m 2870m 1693s 1673s 1652s 1630vs 1565ms 1527m 1462m w 1452mw 1430ms 1405s 1320m 1275m 1265mw 1235m 1212mw 1201mw sh 1173s 1125mw 1103m 997mw 947w 890mw 807ms 777m 698mw 660m 650m 630mw sh 615mw

6-*Dimethylamino-3-methylpurine*: 3440s 3295ms sh 3225m sh 3060m w 2960m 2940m 2870mw 1625vs 1577ms 1550m 1537m 1533mw 1450m 1425ms 1395s 1387ms sh 1330m 1272ms 1241m 1170s 1149m 1066mw 1036mw 947mw 867mw 776m 655m

6-*Methylaminopurine*: 3450ms 3280ms 3220ms bd 3060m bd 2990m 2940m bd 2780m 1662m sh 1630s 1617ms 1605s 1575sh? 1535mw 1487mw 1445m 1390ms 1358m 1334m 1302ms 1250ms 1158m 1132mw 1092mw 962w 934ms 896ms 888m sh 854m 797m 737mw 723mw 682mw 666ms 645s

6-*Dimethylaminopurine*: 3445s 3300m sh 3200m 3100m 3060m 2945ms 2870ms 2750mw bd sh 1750mw bd 1640mw sh 1597vs 1592s 1530m sh 1495mw sh 1465m sh 1422ms 1406ms 1342ms 1307ms 1261ms 1142mw 1082ms 967mw 945ms 872m 793ms 688ms 647ms

9-*Methyladenine*: 3430s bd 3360s 3295s bd 3105s 3030ms sh 2930ms 2880m sh 2695w 1890w 1737mw bd 1674vs 1650s 1603vs 1576s 1542mw sh 1525w 1477ms 1462mw sh 1440mw 1422ms 1411ms 1375m 1352mw 1328ms 1311ms 1256m 1231ms 1200mw 1150mw bd 1101w bd 1082mw 1048m 1022m 945m 904mw 843mw 797ms 743m 720ms 660m bd

7-*Methyladenine*: 3445ms 3345s 3210ms sh 3160s 3075m sh 2960m 2930m 2870mw bd sh 2665w 2605w 2425mw 2400w 1770mw 1670s 1648m 1617ms 1562ms 1552ms 1487s 1465mw 1450w 1426m 1387s 1345m 1312mw 1260m 1235mw 1166m 1092mw 1067m 1015mw 911mw 893m 840mw 826m 797ms 761w 727w bd 700mw 691ms 657w bd

Isoguanine: 3460m 3400m 3250ms 3080s 2970ms 2940ms 2910ms 2880m 1717vs 1665s 1650s 1620ms 1535ms 1412s 1390ms sh 1243m 1179m 1125m 1115m sh 1030mw 943m 915mw bd 853m 802mw 776m 759mw 680mw 637m

s = strong, m = medium, w = weak, sh = shoulder, bd = broad.

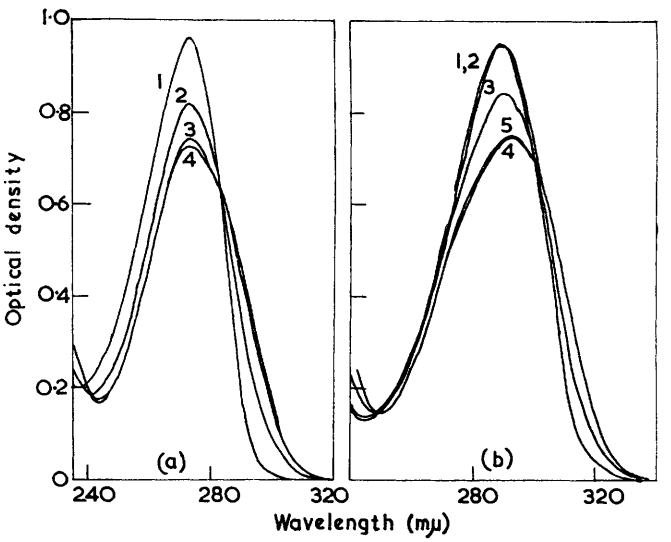
frequencies on deuteration or replacement by alkyl groups. The spectra of all the compounds, including those without hydroxy- or amino-substituents, but excluding 2-chloro- and 3-methyl-6-dimethylaminopurine, have a band at 3100 cm.$^{-1}$. The spectra of deuterated adenine and 7-methyladenine have a weak band in this region whilst the strong band of 9-methyladenine remained strong after such treatment. The 3300 cm.$^{-1}$ band, attributed to an NH group, is absent from the spectra of dialkylamino-derivatives except 6-dimethylamino-3-methylpurine (III), and less intense for alkylamino- than for amino-derivatives. The band was absent from the spectra of deuterated adenine and deuterated

[10] Angell, *J.*, 1961, 504.
[11] Bellamy, " The Infra-red Spectra of Complex Molecules," Wiley, New York, 1958.
[12] Montgomery and Holum, *J. Amer. Chem. Soc.*, 1958, **80**, 404.

7-methyladenine but remained at about the same intensity for 6-amino- and 6-dimethylamino-3-methylpurine (III). This anomaly may arise from interference by hydroxy-bands from undeuterated compounds in the pellets and residual water, since all spectra showed a band in the 3400—3500 cm.$^{-1}$ region. Thus, presence of 3100 and 3300 cm.$^{-1}$ bands can only be used for confirmation of amino-groups if supported by other bands.

A band between 1695—1715 cm.$^{-1}$ has been attributed to the influence of an N^1-proton of a 6-amino-vibration in adenine salts.[10] A shoulder is found here in the spectra of the neutral compounds examined except for 6-amino-2-methyl-, 2-amino-, 2,6-diamino- and perhaps 3-amino-8-methyl-purines. These shoulders may be due to uncancelled water-vapour bands.

A band in the 2700 cm.$^{-1}$ region has been assigned to an N^7–H [13] vibration, but bands at 2700—2940 cm.$^{-1}$ have also been assigned to C–H in purine,[15] at 2500 cm.$^{-1}$ to the methyl



(a). Ultraviolet absorption spectra of authentic specimen of 3-methyladenine at different pH values: curve 1, pH 4; curve 2, pH 5·9; curve 3, pH 6·9; curve 4, pH 7·8 (Cary recordings). Spectra at pH 1·0 and 13 were the same as those at pH 4·0 and 7·8, respectively.

(b). Ultraviolet absorption spectra of 6-dimethylamino-3-methylpurine at different pH values: curve 1, pH 1; curve 2, pH 4; curve 3, pH 5·9: curve 4, pH 8; curve 5, pH 13 (Cary recordings).

of methyl-substituted pyrimidines,[16] and at 2540—2800 cm.$^{-1}$ to N–H group vibrations.[12] Of compounds whose spectra are available only 6-amino- and 6-dimethylamino-3-methylpurine and 7-methyladenine cannot have a N^7–H group tautomer providing that the 6-substituent is an NH_2-group. A weak band was then noted at 2660, 2750, and 2690 cm.$^{-1}$, respectively; other compounds also had weak bands in that region. These bands did not change appreciably on deuteration, indicating either that the N^7-hydrogen atom was non-labile or that this group does not cause vibration in this region.

It has been reported [12] that the N^9–H group exhibits broad, often intense, absorption bands at 870 cm.$^{-1}$. 6-Amino- and 6-dimethylamino-3-methylpurine and 9-methyladenine should, therefore, not show this band; other purines could. The spectrum of 9-methyladenine has no band at 870 $\pm$ 8 cm.$^{-1}$, but that of the deuterated material had

[13] Willits, Decius, Dille, and Christenson, *J. Amer. Chem. Soc.*, 1955, **77**, 2569.
[14] Katritzky and Jones, *J.*, 1959, 3674.
[15] Blout and Fields, *J. Amer. Chem. Soc.*, 1950, **72**, 479.
[16] Brownlie, *J.*, 1950, 3062.

a sharp band of medium intensity at 878 cm.$^{-1}$; 6-amino-3-methylpurine shows no band there nor does the deuterated sample; 6-dimethylamino-3-methylpurine shows a medium-weak band at 868 cm.$^{-1}$, the deuterated sample does not. The spectra of all other compounds for which information is available have a band between 865—878 cm.$^{-1}$ of weak to medium intensity not influenced by deuteration. The weak band in 6-dimethylamino-3-methylpurine could have been due to an impurity, since it disappears on deuteration.

Experimental

All m. p.s are uncorrected. The ultraviolet spectra were taken in a Cary recording spectrophotometer model 14 PM. Spectra were recorded for the same solution in the same cuvette after addition of very small amounts of concentrated acid, alkali, or buffer solutions, to adjust the pH. Infrared spectra were recorded for 0·1% or lower dispersions of the sample in potassium bromide pellets by using a Beckman IR-7 spectrometer or Perkin-Elmer model 21 spectrometer. Spectra were recorded also after replacement of labile hydrogen atoms by deuterium by refluxing the compounds with heavy water containing potassium bromide, and freeze drying.

Descending paper chromatography was carried out on Whatman No. 3 MM paper, with following solvents: (1) propan-2-ol–concentrated hydrochloric acid–water (68 : 16·4 : 15·6); (2) propan-2-ol–water–aqueous ammonia (*d* 0·88) (85 : 15 : 1·3); (3) n-butanol–water (86 : 14). 6-Dimethylaminopurine, 6-methylaminopurine, isoguanine, and 4-aminoimidazole-5-carboxamide were obtained from Mann Research Laboratories, New York. Specimens of 3-, 7-, and 9-methyladenine, and 3-methylhypoxanthine were obtained through the courtesy of Dr. G. B. Elion of the Wellcome Research Laboratories, Tuckahoe, New York.

Methylation of 6-*Dimethylaminopurine.*—6-Dimethylaminopurine (81 mg., 0·5 mmole) was dissolved in 0·01M-phosphate buffer (4 ml.; pH 7·0) and ethanol (1 ml.). Dimethyl sulphate 200 λ (*ca.* 2·0 mmole) was added and the mixture was stirred at room temperature in a pH-stat, Radiometer, Type TTTlc, Copenhagen, N-sodium hydroxide being added from the syringe burette to maintain the pH at 7·0. The reaction was complete in 3—4 hr.

A portion (0·2 ml.) of the solution was diluted to ~10 ml., the pH adjusted to ~11 with concentrated ammonia solution, and the solution then applied to a column (10 cm. × 1·0 cm.^{2}) of Dowex-1. The column was washed with ammonia solution (pH 10) until the eluate had no significant absorption at 290 mμ. Paper chromatography of the eluate in solvents (1) and (2) indicated the absence of 6-dimethylaminopurine.

The eluate from the anion-exchange column was adjusted to pH 4 with glacial acetic acid and applied to a column (same size) of Dowex-50. The column was developed by using a gradient of 0·5M-ammonium formate, fractions (20 ml.) were collected, and the optical densities of the fractions were measured at 270 and 290 mμ. The first 67 fractions contained all three products. The yield of each product was calculated on the assumption that the values of $\varepsilon_{max.}$ at pH 2 were the same as that of 6-dimethylaminopurine. The yields were 9- 8·3%, 3- 66%, and 1-methyl-6-dimethylaminopurine 22·9%.

Fractions 16—19 contained 9-methyl-6-dimethylaminopurine. The fractions were combined, the pH was adjusted to 4·0 by adding concentrated hydrochloric acid, and the solution was applied to a column (same size) of Dowex-50. The column was washed with water, and the product was eluted with 0·5M-ammonium hydrogen carbonate solution. The combined eluates were evaporated to dryness *in vacuo* and extracted with ethanol. The ethanolic extract on evaporation left a crude product, m. p. 115° (from n-heptane) (lit.,[7] 114—115°). The picrate had m. p. 244—245° (decomp.) (from ethanol) (Found: C, 41·4; H, 3·3; N, 27·6. $C_{14}H_{14}N_8O_7$ requires C, 41·4; H, 3·45; N, 27·6%).

Fractions 31—44 contained 6-dimethylamino-3-methylpurine, which was treated as for the 9-methyl compound. The free base had m. p. 169—170°. The picrate formed needles (from ethanol), m. p. 190° (Found: C, 41·6; H, 3·6; N, 28·0).

Fractions 59—67 contained 6-dimethylamino-1-methylpurine, which was treated as for the 9-methyl compound. The picrate had m. p. 219° (from ethanol) (Found: C, 41·4; H, 3·4; N, 27·4).

Action of Alkali on 3-*Methyladenine,* 6-*Dimethylamino*-3-*methylpurine, and* 3-*Methylhypoxanthine.*—Each of these substances (2—3 mg.), dissolved in N-sodium hydroxide (1 ml.), was heated at 100° for 2—4 hr. Each reaction mixture was chromatographed on paper in

solvent (2). 3-Methylhypoxanthine and 5-methylaminoimidazole-4-carboxamide were present in all three mixtures. Their identities were established spectrophotometrically and chromatographically in three solvent systems.

Pauly test on the carboxamide (VI) obtained in each of the three cases was carried out by Smith's method.[9] A deep blue colour was obtained which rapidly faded to brown. Pauly tests on 3-methyladenine, 6-dimethylamino-3-methylpurine, and 3-methylhypoxanthine were negative.

Electrophoresis of 3-Methyladenine.—Paper electrophoresis was carried out according to Markham and Smith's method.[17] Samples of 3-methyladenine, 1-methyladenine (prepared by Pal's method [4]), adenine, and adenosine were applied at the centre of the paper to minimize the effect of endosmosis. About 30 mA was passed for 1·5 hr. at 8—12 v/cm., 0·05M-sodium hydroxide (pH 12·2) being used. Adenine and 1-methyladenine spots moved about 6·7 cm. towards the anode while adenosine and 3-methyladenine spots moved 0·7 cm. and 2·3 cm., respectively, towards the cathode.

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[17] Markham and Smith, *Biochem.*, 1952, **52**, 552.