The Noyes Chemical Laboratory, University of Illinois

Characterization of N_X,N_Y-Disubstituted Adenines by Ultraviolet Absorption Spectra (1a)

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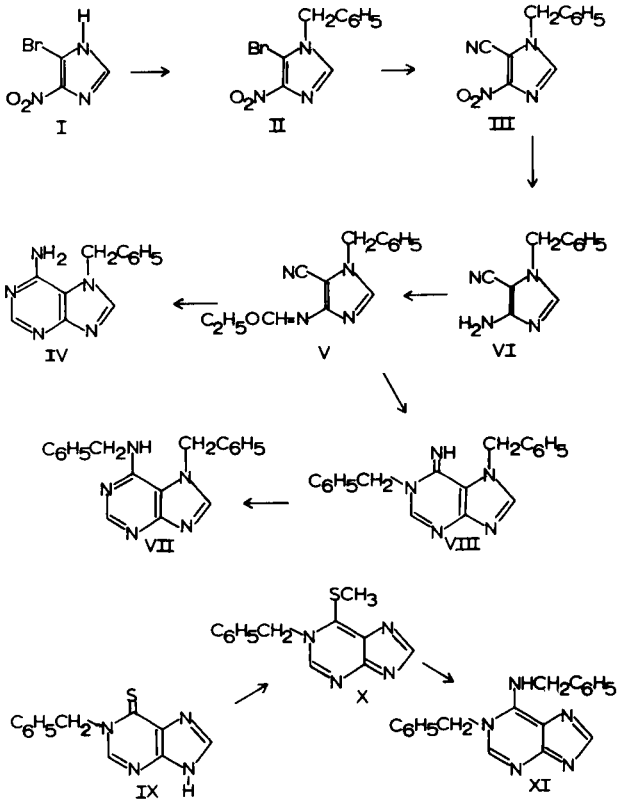
By the synthesis of representative N_X,N_Y-dibenzyladenines and determination of their ultraviolet absorption spectra in acidic, basic and neutral solution we have assembled a body of spectral characteristics which makes it possible to distinguish among possible N,N-disubstituted adenines.

The great interest in alkyl-substituted purines as chemotherapeutic agents (2) or cytokinins (3) and the attempts to clarify the action of biological alkylating agents (4) and to identify minor purine and pyrimidine components in the nucleic acids and other biological systems (5) have greatly multiplied the number of known alkylated purines. A major problem in all this work has been the determination of the nitrogen position on which the substituent group is located. This problem has been aggravated by the limited knowledge of the factors which are important in determining the position of alkylation in variously substituted purines under any given conditions, although among the adenine derivatives the substitution patterns have been substantially elucidated under a variety of conditions (4e, 4l, 6). The most useful factor in purine structural assignments has been ultraviolet analysis, which is particularly attractive because of the ease of operation, limited sample size, and simplicity of interpretation at a descriptive level. Structural assignments of new compounds have often been made in the past on the basis of comparison of certain features in the spectra obtained with those reported in the literature. The similarities in the spectra of many purines and the fact that insufficient spectral data are often reported have required occasional correction (7) of earlier structural assignments. These difficulties have led us to seek means by which the different purine derivatives can be described and differentiated on the basis of their ultraviolet spectra. In an earlier, joint contribution from Arizona State University and this Laboratory, some spectral features of importance in the monosubstituted adenines were delineated (7a). We would now like to expand these correlations to include the disubstituted adenines.

In connection with our studies of the effect of alkyl substitution on cytokinin activity (3, 6d) and on the physical properties of the adenine ring system, we have synthesized eight of the eleven possible N,N-dibenzyladenines (8). One other disubstituted adenine, a 3,9-disubstituted compound had previously been prepared as the cyclic derivative, pyrotriacanthine chloride (6a). Owing to the instability of the cyclic 3,9-disubstituted adenines in basic solution, the free bases have not yet been reported (6a, 10). Similar purine bases are known in which a zwitterionic structure is possible (11), and it has been suggested in those cases that the form isolated is in fact a zwitterion (11, 12). The remaining disubstituted adenines, the 1,3- and 7,9-dialkyl compounds, are as yet unknown and would of necessity be zwitterionic. On the basis of examination of the ultraviolet spectra of the eight different dibenzyladenines and many other mono- and dialkylated adenines, we have been able to establish a number of correlations of particular interest in structure determination. Our experience has shown that the general shape of the spectral curves is the most reliable characteristic, and that direct comparison should be employed whenever possible. However, the long-standing use of intensities and wave lengths of the maxima and minima under varying *p*H conditions is sufficient for many structural assignments. We have found 95% ethanol to give the most reliable and infromative spectra and to have sufficient solubilizing ability for a great variety of disubstituted derivatives. Since changes in positions of maxima and minima and even the disappearance of shoulders on some peaks have been observed on changing from ethanol to water as a solvent, it is strongly advised that critical comparisons be made only under identical conditions. The characterisitcs described below are those which are best representative and least variant for the particular compounds described. It should be recognized that underlying phenyl absorption is present in the ultraviolet absorption of the dibenzyl derivatives, but this does not detract from the generalities which can be made relating the dibenzyladenines to other dialkyladenines.

Pertinent ultraviolet spectral data for the dibenzyladenines are given in Table I. Some generalizations can be made with respect to the different classes of substitution, particularly in relation to their monosubstituted relatives. All 1-substituted adenines show a general gradual increase in absorption in basic solution from 350-330 mμ to about 290 mμ, where the specific absorption due to the characteristic maximum overcomes it. When monoalkylated adenines are substituted with an additional alkyl group on the N_6-position there is little change in the relative positions of the maxima in acidic,

CHART 1



neutral or basic solution. However, there is a general bathochromic shift with respect to the maxima of the monosubstituted compound. More specific differences are listed below. Shifts in wave length are recorded with respect to the corresponding value in neutral solution.

1-Benzyl-6-benzylaminopurine (Fig. 1)*.

Compounds with this disubstitution generally exhibit maxima from 260 to 280 mμ which occur at different wave lengths in acidic, basic and neutral solutions (λ acidic < λ basic < λ neutral). The basic maxima show a dual or broad peak. A second peak appears in neutral solution at about 230 mμ.

1,7-Dibenzyladenine (Fig. 2).

This representative compound has maxima of low intensity occurring from 265 to 280 mμ. The spectra in neutral and basic solution are essentially identical in shape although there is a slight hyperchromic shift in basic solution. Weak shoulders appear on the higher wave-length side of the maxima in neutral and basic solution. The acid spectrum shows a broad maximum, which is shifted toward longer wave-length and lower intensity, as well as a broad minimum which has higher extinction and longer wave length than the minimum shown in neutral solution.

1,9-Dibenzyladenine (Fig. 3).

This substitution results in an ultraviolet maximum at about 260 mμ which shows no appreciable shift in acidic or basic solution. Another characteristic of the spectral curves is the prominent shoulder on the high wave-length side of the maximum in neutral solution.

3-Benzyl-6-benzylaminopurine (Fig. 4).

A typical maximum is shown at about 290 mμ. The neutral and basic spectra are essentially identical and have very broad maxima. The acid spectrum shows pronounced hyperchromic and slight hypsochromic shifts. A second peak occurs in neutral solution near 220 mμ.

3,7-Dibenzyladenine (Fig. 5).

The maxima for acidic, basic and neutral solutions occur at about the same wave length but the acid maximum exhibits a slight hyperchromic shift while the basic maximum shows a slight hypsochromic shift. A second maximum in acid solution occurs at lower wave length (225-235 mμ).

3,9-Dialkyladenines.

These are difficult to characterize by their ultraviolet absorption. All presently known derivatives are cyclic compounds and exist as salts which show maxima near 275 mμ (6a,13). Their rapid decomposition in base is an important distinguishing feature.

6-Dibenzylaminopurine (Fig. 6).

Compounds of this type typically have spectra which exhibit bathochromic shifts in both acidic and basic media. A slight shoulder is present on the high wave-length side of the maximum in basic solution.

7-Benzyl-6-benzylaminopurine (Fig. 7).

This type has maxima at about 280 mμ. The spectra in neutral and basic solutions are essentially identical and slight shoulders occur on the lower wave-length side of the maxima. The spectrum in acid exhibits pronounced hyperchromic and bathochromic shifts of maximum and bathochromic shifts of minimum (6a,7a).

9-Benzyl-6-benzylaminopurine (Fig. 8).

The spectra at different *p*H show maxima at about 270 mμ with little variation in extinction coefficient. The acid maxima will show slight hypsochromic shifts for certain N^6,9-disubstituted compounds. In neutral and basic solutions the spectra exhibit similar maxima, but slight bathochromic shifts of minima occur in basic solution.

The generalities given above, while essentially descriptive in nature, are useful for identification

* In all the figures, the full line represents the spectrum in 95% ethanol; the dashed line, 0.1 *N* NaOH in 95% ethanol; the dotted line, 0.1 *N* HCl in 95% ethanol. In the examples of 1,N^6- and 3,N^6-disubstitution, one must assume equilibration of possible tautomeric forms in the hydroxylic solvent.

and for selection of positions of disubstitution on adenine. It is hoped that the curves and the numerical data may stimulate further theoretical treatment of the electronic excitations in substituted purine molecules.

Although most of the dibenzyl compounds reported in this study could be synthesized by modifications of general procedures (see Experimental), the route to the 1,7-disubstituted compounds bears special mention. A number of 1-alkyl-7-methyladenines have been prepared from 5-cyano-4-ethoxymethyleneamino-1-methylimidazole and the appropriate amine (14), but the use of this procedure has been limited to 7-methyl derivatives by the route to the precursor imidazole (15). As the starting point for the synthesis of the requisite 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (V) we used 4(5)-bromo-5(4)-nitroimidazole (I) (16). Benzylation of the sodium salt of this compound gave the two benzyl isomers in approximately equimolar amounts. A competitive experiment on this mixture showed (by n.m.r.) that only one of the isomeric bromides was readily displaced by cyanide. This result is in agreement with similar experiments by Taylor and Loeffler (14) and by Baddiley and his coworkers (17) although we observed no isomerization and reaction of the unreactive isomer. The reactive isomer, which was assigned the structure 1-benzyl-5-bromo-4-nitroimidazole (II), could be prepared in better yield by direct alkylation with benzyl bromide at high temperature. Displacement of bromide with cyanide in refluxing methanol gave the desired 1-benzyl-5-cyano-4-nitroimidazole (III), which could be converted to the ethoxymethyleneamino derivative (V) through VI by the previously reported methods (14, 15). Treatment with ammonia yielded 7-benzyladenine (IV), identical with an authentic sample (6c), establishing the position of the benzyl group in the precursor imidazoles as assigned. Reaction of V with benzylamine yielded 1,7-dibenzyladenine (VIII). This reaction can be extended to the preparation of other 1,7-dialkyladenines by the use of other alkylating agents in reaction I ⟶ II and other amines in reaction V ⟶ VIII.

The rearrangement of 1,7-dibenzyladenine (VIII) to the corresponding 7-benzyl-6-benzylaminopurine (VII) occurs readily in refluxing ethanol in the presence of hydroxide. We found that this rearrangement also occurs in the absence of added base by refluxing for longer periods of time in 50% aqueous ethanol.

We used a variation of the method employed by Townsend, Robins, Loeppky, and Leonard (7a) to prepare 1-benzyl-6-benzylaminopurine. The isomer of their 1-methyl-6-benzylthiopurine, namely 1-benzyl-6-methylthiopurine (X), was prepared from 1-benzyl-6-thiopurine (IX) (18) by treatment with methyl iodide in 0.4 *N* aqueous KOH solution. Treatment of X with benzylamine in ethanol yielded 1-benzyl-6-benzylaminopurine (XI).

For the preparation of 3-alkyl-6-alkylaminopurines, we found that relatively mild alkylating conditions could be employed. For example, we obtained 3-benzyl- and 3-methyl-6-benzylaminopurine in good yields by treatment of 6-benzylaminopurine at 30-35° with benzyl bromide and methyl iodide, respectively, in dimethylformamide.

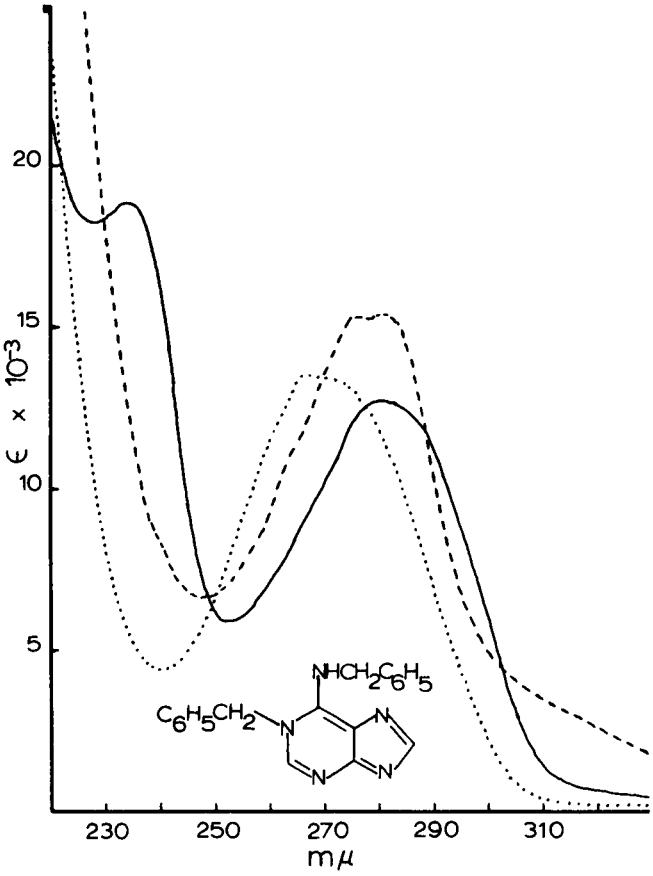


Fig. 1. 1-Benzyl-6-benzylaminopurine

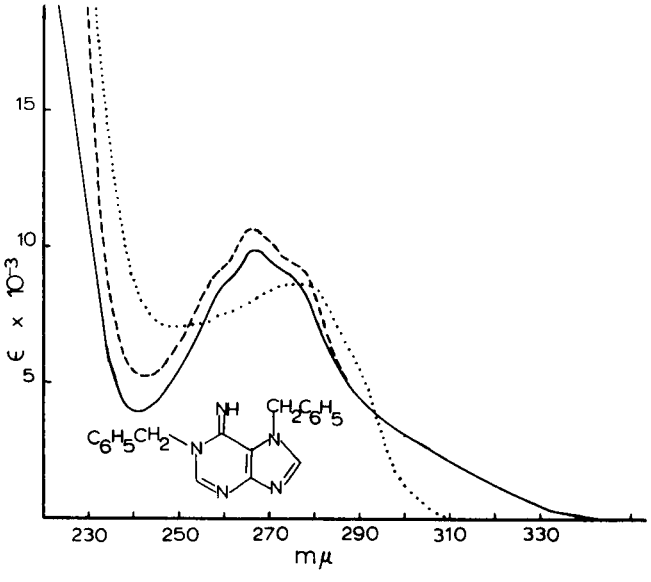


Fig. 2. 1,7-Dibenzyladenine

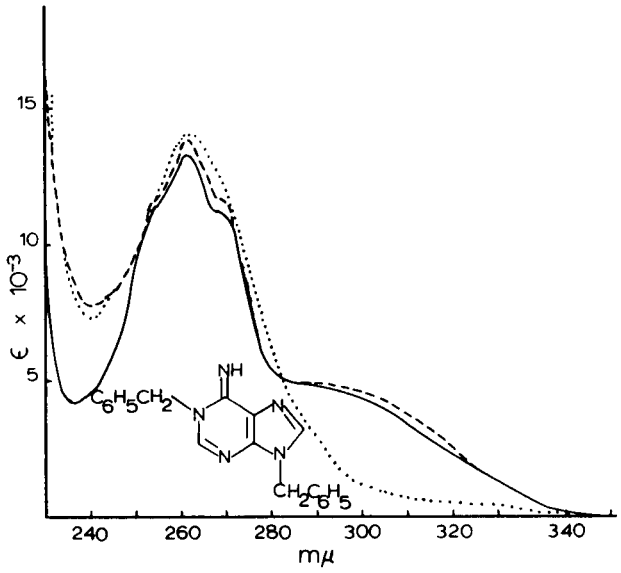


Fig. 3. 1, 9-Dibenzyladenine

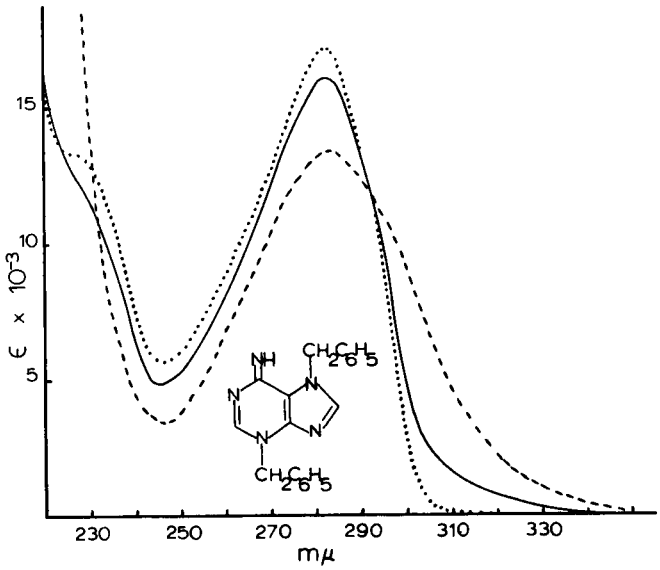


Fig. 5. 3, 7-Dibenzyladenine

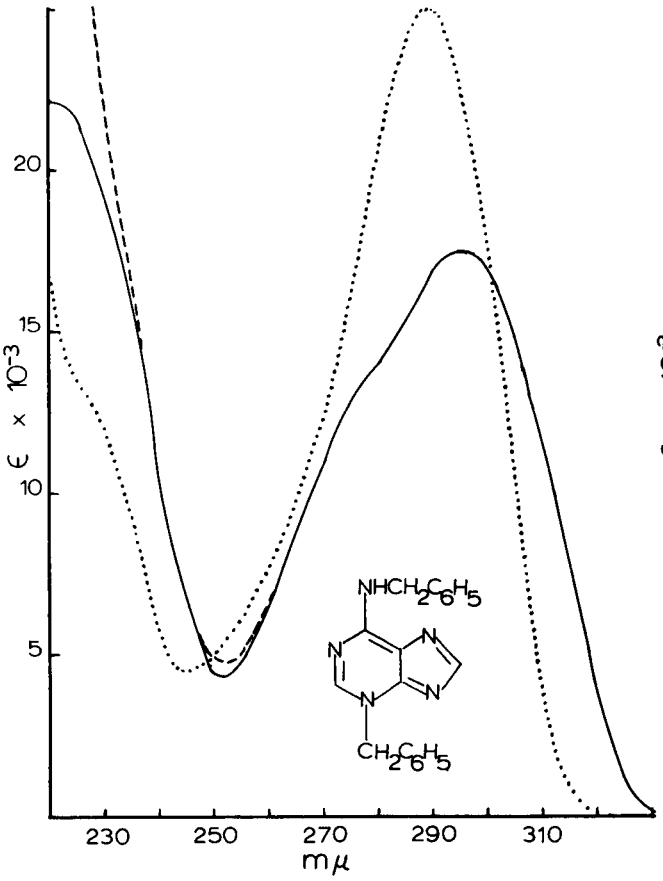


Fig. 4. 3-Benzyl-6-benzylaminopurine

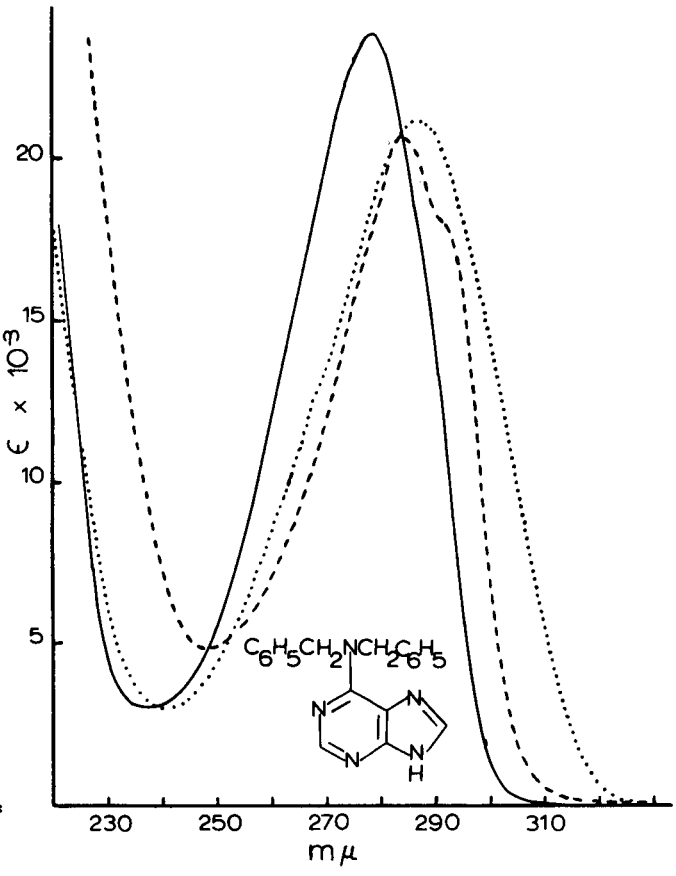


Fig. 6. 6-Dibenzylaminopurine

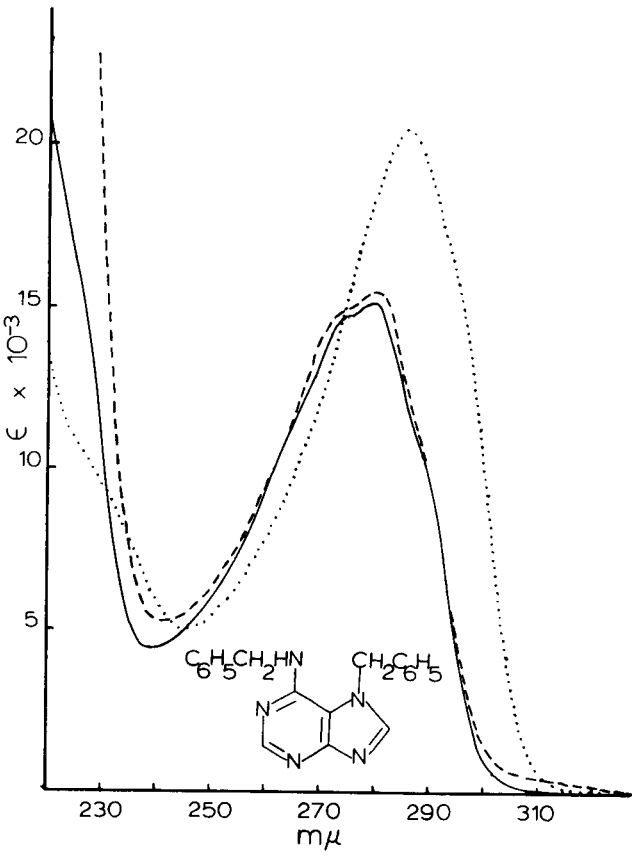


Fig. 7. 7-Benzyl-6-benzylaminopurine

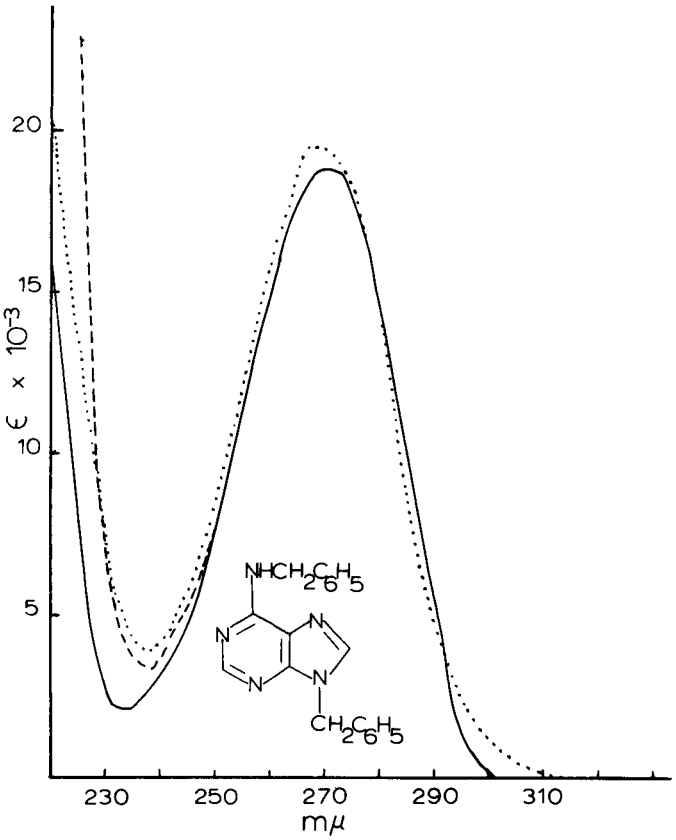


Fig. 8. 9-Benzyl-6-benzylaminopurine

TABLE I

Ultraviolet Spectra of Dibenzyladenines (a)

		0.1*N* HCl in 95% Ethanol				95% Ethanol				0.1*N* NaOH in 95% Ethanol			
		λ max		λ min		λ max		λ min		λ max		λ min	
Compound	Fig.	mμ	ε x 10^{-3}	mμ	ε x 10^{-3}	mμ	ε x 10^{-3}	mμ	ε x 10^{-3}	mμ	ε x 10^{-3}	mμ	ε x 10^{-3}
1,N^6	1	267	12.8	240	4.1	280	12.8	252	5.7	275	15.3	247	6.1
						233	18.6			280			
1,7	2	277	8.4	249	6.8	266	9.7	239	2.8	266	11.0	242	5.2
						275 (sh)	8.7			275 (sh)	9.6		
1,9	3	261.5	14.5	240.5	6.3	261.5	13.6	236.5	4.0	261.5	14.4	240.5	7.4
						269 (sh)	11.2						
						290 (sh)	4.6			269 (sh)	12.4		
3,N^6	4	288	24.8	244	5.4	293	17.3	251	5.2	294	17.4	252	5.6
						218	22.6						
3,7 (b)	5	281	16.9	246	5.9	281	15.9	246	4.7	281	13.6	246	4.2
		224 (sh)	14.9										
N^6,N^6	6	287	22.0	242	3.4	278	23.9	238	3.2	284	21.5	248	5.5
										292 (sh)	18.6		
N^6,7	7	285	19.8	246	5.2	279	15.1	239	4.0	279	15.9	241	6.1
N^6,9	8	266	20.3	238	5.1	271	19.0	234	2.6	271	19.1	239.5	5.0

(a) 1,3-; 3,9- and 7,9-Dibenzyladenines are presently unknown. (b) *Cf.* ref. (4k) for 3,7-dimethyladenine hydriodide.

EXPERIMENTAL

Ultraviolet Spectra.

The following procedure was used to provide the analytical ultraviolet data given in Table I, employing a Cary Model 15 spectrophotometer: (1) Known quantities of the compound were weighed out and dissolved in 100 ml. of absolute ethanol. Quantity taken varied from 4 to 8 mg. depending on molecular weight and ϵ max of the compound. (2) Aliquots of 25 ml. each were transferred to three 100 ml. volumeric flasks and diluted to about 80 ml. with absolute ethanol. (3) Volumes of 5 ml. of 2 *N* HCl, H_2O or 2 *N* NaOH were added to the appropriate flasks and then the flask was filled to mark with absolute ethanol. (4) The ultraviolet spectra were then determined against a 95% ethanol blank: —— 95% EtOH; ----0.1 *N* NaOH in 95% EtOH; ····0.1 *N* HCl in 95% EtOH. This method has the advantage of giving relative values for maxima and minima directly on the chart paper. Therefore the quantitative spectra can be used to compare with qualitative spectra obtained by adding a drop of acid and a drop of base to ethanolic solutions of unknowns.

9-Benzyladenine.

The sodium salt of adenine was formed by stirring a suspension of 25 g. (0.19 mole) of dry adenine and 5 g. (0.21 mole) of sodium hydride (as an oil emulsion) in 300 ml. of dimethylformamide. To this suspension was added dropwise 25 ml. of benzyl bromide, and stirring was continued overnight. The product precipitated on cooling and was recrystallized from absolute ethanol as colorless needles, m.p. 233.5-235.5° (reported (20, 22) 235°), yield 11.7 g. (27%), homogeneous by thin layer chromatography on silica gel.

1,9-Dibenzyladenine.

To a suspension of 3.0 g. (13.3 mmoles) of 9-benzyladenine in 50 ml. of dry dimethylacetamide was added 2.0 ml. of benzyl bromide. After about 45 hours of stirring at room temperature the suspension cleared to a colorless solution. This solution was poured into 500 ml. of water after 70 hours and stirred further until all precipitated material dissolved. Addition of concentrated ammonium hydroxide to *p*H 10 gave a fluffy colorless precipitate. Filtration and recrystallization from benzene gave 1.8 g. of fine colorless needles, m.p. 162-165.5°, homogeneous by thin layer chromatography on silica gel. A second crop of 0.5 g., m.p. 161.5-163.5°, was recovered from the benzene mother liquor (over-all yield 55%). A second recrystallization from benzene gave an analytical sample, m.p. 163-164°.

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.43; H, 5.37; N, 22.40.

9-Benzyl-6-benzylaminopurine.

A solution of 700 mg. (2.2 mmoles) of 1,9-dibenzyladenine in 45 ml. of ethanol containing 5 ml. of 2 *N* sodium hydroxide was heated under reflux for 1 hour, chilled and filtered to give 562 mg. of colorless needles, m.p. 173-175°. The mother liquor after dilution with water and refrigeration overnight yielded a further 122 mg., m.p. 174-175.5° (total yield 98%). An analytical sample was obtained as colorless fluffy needles from benzene, m.p. 174.5-175.5° (reported (9b) 174-175°).

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.39; H, 5.47; N, 22.21.

3,7-Dibenzyladenine.

A sample of 3,7-dibenzyladenine hydrobromide (6c,g) was dissolved in warm water and brought to *p*H 12 with 2 *N* sodium hydroxide. The resulting gum was cooled and scratched until solidification was complete. Recrystallization of this white solid from benzene gave the analytical sample as clusters of fine colorless needles, m.p. 125.5-127.5°.

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.55; H, 5.44; N, 22.33.

1-Benzyl-5-bromo-4-nitroimidazole (II).

A solution of 60.0 g. (0.31 mole) of 4(5)-bromo-5(4)-nitroimidazole (I) (16) and 75 ml. of benzyl bromide in 600 ml. of dimethylformamide was heated under reflux 2-3 hours, then at 130° overnight. The solvent was removed *in vacuo*, and the resulting dark residue was shaken thoroughly with water. The water was decanted, and the remaining solid mass was recrystallized from ethanol with charcoal treatment to yield 68.7 g. (78%) of colorless needles, m.p. 144-145.5°. A single recrystallization from ethanol gave the analytical sample as fine colorless needles, m.p. 145-146°.

Anal. Calcd. for $C_{10}H_8BrN_3O_2$: C, 42.58; H, 2.87; N, 14.90. Found: C, 42.44; H, 2.86; N, 14.82.

1-Benzyl-5-cyano-4-nitroimidazole (III).

To a stirred suspension of 19.6 g. (0.07 mole) of 1-benzyl-5-bromo-4-nitroimidazole (II) in 500 ml. of methanol was added 10.0 g. (0.15 mole) of potassium cyanide and 1.0 g. of potassium iodide, and the mixture refluxed for 5 hours. After cooling to room temperature, the methanol was removed *in vacuo*, and the residue was shaken thoroughly with about 700 ml. of chloroform and filtered. The dark filtrate was evaporated to a brown solid, which was recrystallized from ethanol with charcoal treatment to yield 4.85 g. of a pale yellow solid, m.p. 127-129°. Two further crops, obtained by partial evaporation, provided a combined 4.00 g. (over-all yield 56%) of pale yellow needles after one recrystallization from ethanol, m.p. 128-130°. A small amount of the second crop yielded an analytical sample as pale yellow needles, m.p. 130.5-131.5°, after one recrystallization, ν max (Nujol) 2250 cm^{-1} (C≡N).

The ultraviolet spectra indicated the following: 0.1 *N* HCl in 95% ethanol - λ max 290 mμ (ϵ, 6,600), λ min 260 (3,600); 95% ethanol - λ max 290 (5,700), λ min 260 (3,500); 0.1 *N* NaOH in 95% ethanol - λ max 292.5 (6,700), λ min 260 (4,000). The n.m.r. spectrum ($CDCl_3$) showed signals at τ-values 4.64 (singlet, CH_2), 2.55 (singlet, C_6H_5), 2.30 (singlet, CH).

Anal. Calcd. for $C_{11}H_9N_4O_2$: C, 57.89; H, 3.54; N, 24.56. Found: C, 57.99; H, 3.46; N, 24.52.

4-Amino-1-benzyl-5-cyanoimidazole (VI).

A mixture of 1.00 g. (4.4 mmoles) of 1-benzyl-5-cyano-4-nitroimidazole (III) and about 0.8 g. of wet Raney nickel in 100 ml. of ethanol was shaken under a hydrogen pressure of 2 atm. for 3 hours. The catalyst was removed by filtration and was washed thoroughly with ethanol. The orange filtrate was evaporated to a dark brown solid, which was washed with ether and filtered to give 0.85 g. (99%) m.p. 197-206° (d), recrystallization from ethanol with melting point unchanged as yellow-green needles, ν max (Nujol) 2200 cm^{-1} (C≡N).

The ultraviolet spectra showed the following: 0.1 *N* HCl in 95% ethanol - λ max 264 mμ (ϵ, 6,500), 236 (9,900); λ min 252 (5,700); 95% ethanol - λ max 267 (8,000), 231 (4,500); λ min 240 (4,200); 0.1 *N* NaOH in 95% ethanol - λ max 267 (8,500); λ min 242 (5,000). The n.m.r. spectrum (CF_3COOH) showed signals at τ-values 4.53 (singlet, CH_2), 2.39 (singlet, C_6H_5), 1.63 (singlet, CH).

Anal. Calcd. for $C_{11}H_{10}N_4$: C, 66.64; H, 5.09; N, 28.27. Found: C, 66.85; H, 5.19; N, 28.04.

1-Benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (V).

A mixture of 3.0 g. (15 mmoles) of VI, 20 ml. of ethyl orthoformate and 40 ml. of dry dimethylformamide was heated under reflux for 4 hours with exclusion of moisture. The dark solution was evaporated to a gum, which was washed thoroughly with ether and filtered. The filtrate was evaporated to a brown gum, which was recrystallized from cyclohexane with charcoal treatment to yield 2.20 g. of yellow needles, m.p. 84.5-85.5°. A second crop of identical material (0.15 g., total yield 61%) was obtained from the cyclohexane mother liquor. Further recrystallization from cyclohexane yielded an analytical sample of tiny colorless needles, m.p. 84.5-85.5°, ν max ($CHCl_3$) 2220 (C≡N), 1630 cm^{-1} (C=N).

The ultraviolet spectra indicated the following: 0.1 *N* HCl in 95% ethanol - λ max 264 mμ (ϵ, 7,200), 236 (10,300); λ min 251 (6,300), 223.5 (7,700); 95% ethanol - λ max 236.5 (13,900), 209 (20,300); λ min 232.5 (6,500); 0.1 *N* NaOH in 95% ethanol - λ max 280.5 (14,300); λ min 241 (5,700).

Anal. Calcd. for $C_{14}H_{14}N_4O$: C, 66.12; H, 5.55; N, 22.03. Found: C, 66.35; H, 5.68; N, 22.27.

7-Benzyladenine (IV).

A solution of 63 mg. (0.25 mmole) of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (V) in 15 ml. of ethanol was saturated with ammonia and allowed to stand for 10 hours at room temperature. Evaporation of the ethanol gave a residue which was washed and filtered with ether to yield 50 mg. (90%) of slightly yellow solid, m.p. 237-240° dec. Two recrystallizations from ethanol gave a pure sample of the 7-benzyladenine, m.p. 240-242° dec., identified by melting point, mixture melting point and ultraviolet spectral comparisons with an authentic sample (6c).

Anal. Calcd. for $C_{12}H_{11}N_5$: C, 63.98; H, 4.93. Found: C, 63.70; H, 4.76.

1,7-Dibenzyladenine (VIII).

To a solution of 0.87 g. (3.4 mmoles) of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (V) in 25 ml. of ethyl acetate was added 0.5 ml. of benzylamine and the solution was heated under reflux for 6 hours. Evaporation of the ethyl acetate and residual benzylamine *in vacuo* yielded a yellow gum, which solidified when triturated with ether. This solid was filtered to give 815 mg. of 1,7-dibenzyladenine, m.p. 121-123.5°. A second crop of 53 mg., m.p. 120-123° (over-all yield 80%) was obtained on partial evaporation of the ether. Recrystallization from ethyl acetate gave an analytical sample as colorless feathery needles, m.p. 125.5-127°.

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.08; H, 5.53; N, 22.32.

7-Benzyl-6-benzylaminopurine (VII).

A solution of 313 mg. (1 mmole) of 1,7-dibenzyladenine (VIII) in 22.5 ml. of ethanol containing 2.5 ml. of 2 *N* aqueous sodium hydroxide was refluxed 1 hour. The solution was neutralized with Dowex-50 (H^+ form), and the resin was filtered and washed well with ethanol. The filtrate was evaporated to about 30 ml. and diluted with an equal volume of water. Upon partial evaporation colorless needles crystallized from the solution, yielding 146 mg. on filtration, m.p. 113-126°. A further yield of 40 mg. of identical material was obtained by chilling the mother liquor. Analytical data suggested this compound was a monohydrate. The analytical sample, recrystallized from ethanol as colorless needles, melted slowly 118-132°, then effervesced violently. Total yield, as monohydrate, was 56%.

Anal. Calcd. for $C_{19}H_{17}N_5 \cdot H_2O$: C, 68.45; H, 5.74; N, 21.01. Found: C, 68.73; H, 5.77; N, 21.19.

This compound was also prepared (75% yield) by refluxing 1,7-dibenzyladenine in 50% aqueous ethanol for 30 days.

1-Benzyl-6-methylthiopurine (X).

To 50 ml. of 0.4 *N* aqueous potassium hydroxide cooled in an ice bath was added 2.2 g. of 1-benzyl-6-thiopurine (IX) (18). After all the starting material was in solution 1.85 ml. of methyl iodide was added and stirring was continued in the ice bath for 3.3 hours. The white precipitate which formed was filtered to yield 1.4 g. of product containing a trace of 1-benzyl-6-thiopurine (by ultraviolet analysis). Two crystallizations from small quantities of benzene yielded an analytical sample, m.p. 169-171°.

Anal. Calcd. for $C_{13}H_{12}N_4S$: C, 60.91; H, 4.72; N, 21.86. Found: C, 60.70; H, 4.71; N, 20.80, 23.17.

1-Benzyl-6-benzylaminopurine (XI).

A solution of *ca.* 250 mg. of 1-benzyl-6-methylthiopurine (X) in 50 ml. of ethanol and 10 ml. of benzylamine was stirred for 24 hours at room temperature. The solvent was removed under reduced pressure and the resulting syrup was poured into 250 ml. of anhydrous ether. After standing overnight in the refrigerator the ether was decanted and evaporated under reduced pressure. The resulting syrup was suspended in hot water and enough ethanol was added to effect complete solution. After standing and cooling, 230 mg. of tan needles, m.p. 195-198°, was collected from the solution. Two crystallizations from benzene yielded 94 mg. of white solid, m.p. 200-202° dec.

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.03; H, 5.41; N, 22.19.

3-Benzyl-6-benzylaminopurine.

A solution of 1 g. of 6-benzylaminopurine in 25 ml. of dry dimethylformamide was stirred with 0.76 g. of benzyl bromide for 25 hours at 30°. The solvent was removed *in vacuo* and the resulting yellow syrup was taken up in 25 ml. of hot ethanol. Upon cooling, the ethanol solution deposited 1.2 g. of yellow solid. The solid was taken up in 300 ml. of hot water containing 25 ml. of ethanol, and the solution was adjusted to *p*H 9 (4 *N* NaOH). The fine white precipitate which formed upon cooling was collected and crystallized from ethanol, colorless needles, m.p. 179-180°.

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.55; H, 5.46; N, 22.37.

6-Dibenzylaminopurine.

This compound was prepared by the method of Bullock, Hand and Stokestad (19), m.p. 190-191° (reported (9a) 184°).

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.20. Found: C, 72.14; H, 5.51; N, 21.93.

REFERENCES

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(2) R. K. Robins, *J. Med. Chem.*, 7, 186 (1964) and references therein.

(3a) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza, and F. M. Strong, *J. Am. Chem. Soc.*, 78, 1375 (1956); (b) F. M. Strong, "Topics in Microbial Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 98-157; (c) C. O. Miller, *Ann. Rev. Plant Physiol.*, 12, 395 (1961); (d) G. Beauchesne and R. Goutarel, *Physiol. Plant.*, 16, 630 (1963); (e) J. Rogozinska, J. Helgeson, and F. Skoog, *ibid.*, 17, 165 (1964); (f) H. Q. Hamzi and F. Skoog, *Proc. Natl. Acad. Sci.*, 51, 76 (1964); (g) D. S. Letham, J. S. Shannon, and I. R. McDonald, *Proc. Chem. Soc.*, 230 (1964).

(4a) P. D. Lawley, *J. chim. phys.*, 58, 1011 (1961); (b) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960); (c) B. Reiner and S. Zamenhoff, *J. Biol. Chem.*, 228, 475 (1957); (d) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, 84, 1914 (1962); (e) J. W. Jones and R. K. Robins, *ibid.*, 85, 193 (1963); (f) A. Wacker and M. Ebert, *Z. Naturforsch.*, 14b, 709 (1959); (g) O. M. Friedman, G. N. Mahapatra, and R. Stevenson, *Biochem. Biophys. Acta*, 68, 144 (1963); (h) H. G. Windmueller and N. O. Kaplan, *J. Biol. Chem.*, 236, 2716 (1961); (i) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 3923 (1961); (j) J. A. Haines, C. B. Reese, and Lord Todd, *ibid.*, 1406 (1964); (k) A. D. Broom, L. B. Townsend, J. W. Jones, and R. K. Robins, *Biochemistry*, 3, 494 (1964); (l) B. C. Pal, *ibid.*, 1, 558 (1962).

(5a) R. H. Hall, *ibid.*, 4, 661 (1965); (b) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, pp. 39-51.

(6a) N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, 84, 2148 (1962); (b) N. J. Leonard and R. A. Laursen, *ibid.*, 85, 2026 (1963); (c) N. J. Leonard and T. Fujii, *ibid.*, 85, 3719 (1963); (d) N. J. Leonard and T. Fujii, *Proc. Natl. Acad. Sci.*, 51, 73 (1964); (e) R. Denayer, A. Cavé, and R. Goutarel, *Compt. rend.*, 253, 2994 (1961); (f) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, 85, 2672 (1963); (g) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, 1, 115 (1964); (h) C. J. Abshire and L. Berlinguet, *Can. J. Chem.*, 42, 1599 (1964).

(7a) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, 86, 5320 (1964) and references therein; (b) W. F. Hemmens, *Biochem. Biophys. Acta*, 68, 284 (1963); (c) R. Shapiro and C. N. Gordon, *Biochem. Biophys. Res. Commun.*, 17, 160 (1964).

(8) Previous syntheses have been recorded for the 3,7-, 3,N^6-, 9,N^6-, and N^6,N^6-dibenzyladenines (6c,6g,9). Identifying characteristics of the first two have been reported only for the salts.

(9a) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4347 (1960); (b) Von H. Goldner and E. Carstens, *J. prakt. Chem.*, 12, 242 (1961).

(10) B. R. Baker and J. P. Joseph, *J. Am. Chem. Soc.*, 77, 15 (1955).

(11) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, 28, 3483 (1963).

(12a) K. C. Murdock and R. B. Angier, *Tetrahedron Letters*, 415 (1962); (b) K. C. Murdock and R. B. Angier, *J. Am. Chem. Soc.*, 84, 3748 (1962).

(13) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(14) E. C. Taylor and P. K. Loeffler, *J. Am. Chem. Soc.*, 82, 3147 (1960).

(15a) R. N. Prasad and R. K. Robins, *ibid.*, 79, 6401 (1957); (b) J. Sarasin and E. Wegmann, *Helv. Chim. Acta*, 7, 713 (1924).

(16) I. E. Balaban and F. L. Pyman, *J. Chem. Soc.*, 121, 947 (1922).

(17) J. Baddiley, J. G. Buchanan, F. E. Hardy, and J. Stewart, *ibid.*, 2893 (1959).

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

(19) M. W. Bullock, J. J. Hand, and E. L. R. Stokestad, *J. Am. Chem. Soc.*, 78, 3693 (1956).

(20) J. A. Montgomery and C. Temple, Jr., *ibid.*, 83, 630 (1961).

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