Further Studies on the Alkylation of Purines[1]

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The benzylation of a number of purines has been studied at different temperatures (60–150°) in dipolar aprotic solvents. The stability of a number of *N*-benzylhypoxanthinium bromides in these solvents at the same temperatures was also studied. These studies indicate that regardless of whether hypoxanthine or any of the monobenzylhypoxanthines (1-, 3-, 7-, or 9-) is benzylated, if the temperature is high enough and the reaction time long enough, the product isolated is always either 1,7-dibenzylhypoxanthine (IIIa), 1,9-dibenzylhypoxanthine (IIa), or a mixture of these two purines. Compounds IIa or IIIa can be reversibly benzylated to 1,7,9-tribenzylhypoxanthinium bromide, and thereby interconverted. These results are explained by the fact that at the minimum reaction temperature the initial point of attack on a purine is the nitrogen of greatest electron density, in either the neutral molecule or the anion, and the reaction is kinetically controlled. At this temperature an isolable, but thermodynamically unfavored, product may be formed and this product is converted at some higher temperature to the thermodynamically stable isomers (IIa and IIIa), if the reaction is allowed to come to equilibrium. The benzylation of 1-benzylpurine-6(1*H*)-thione (Ib), 6-methoxypurine (VII), and 9-benzyl-*N*,*N*-dimethyladenine (XX) were also studied. The benzylation of Ib both with and without base gave a mixture of 1,9- and 1,7-dibenzylpurine-6(1*H*)-thione (IIb and IIIb). The benzylation of 6-methoxylpurine is complex yielding both 1,3-dibenzylhypoxanthinium bromide (Va) and 7,9-dibenzylhypoxanthinium (XI) bromide. This result can be explained by postulating initial attack at N-3 of VII followed by demethylation of VIII and VII to give a mixture of 3-benzylhypoxanthine and hypoxanthine which in turn gives Va and XI. The benzylation of 9-benzyl-*N*,*N*-dimethyladenine (XX) was shown to take place at N-3 to give 3,9-dibenzyl-*N*,*N*-dimethyladeninium bromide.

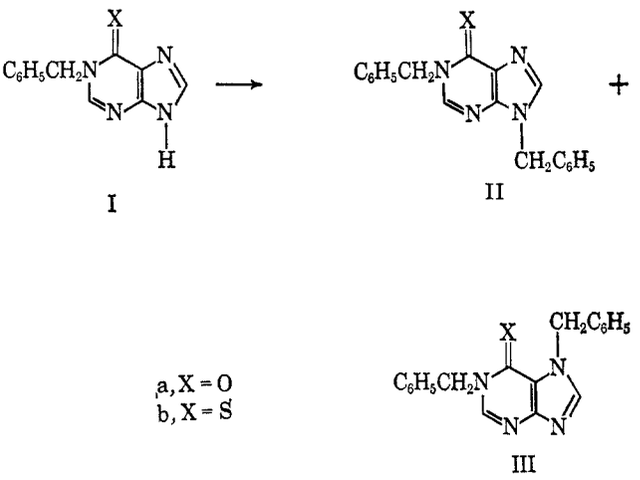
In the course of our studies leading to the synthesis of the nucleoside from pseudovitamin B_{12} (7-α-D-ribofuranosyladenine), we investigated the effect of substituents at N-1 and N-3 of purines on the position of attack on these purines by acylglycosyl halides and by alkyl halides.[2,3] Other factors can also determine the products of alkylation: the solvent,[4] the presence or absence of a proton acceptor,[2,5] and the nature of the alkyl halide.[5] More recently it has become evident that the reaction temperature can also govern the products formed.[6] The effect of temperature on the alkylation of hypoxanthines is a part of the present report, which also describes a reinvestigation of certain alkylation reactions previously described.[2]

1-Benzylhypoxanthine and 1-Benzylpurine-6(1*H*)-thione (Chart I).—The benzylation of 1-benzylhypoxanthine (Ia) in *N*,*N*-dimethylformamide in the presence of a proton acceptor has been reported to take place at N-9, but only a 20% yield of 1,9-dibenzylhypoxanthine (IIa) was obtained.[2,7] Furthermore the presence of 2,7-dibenzylhypoxanthine[2] (IIIa) in the reaction mixture could not be eliminated on the basis of ultraviolet spectral data or chromatographic data, since the ultraviolet spectra of the two isomers are similar and they could not, at that time, be separated chromatographically.

To resolve this problem the proton magnetic resonance (pmr) spectrum of a partially purified mixture of products was obtained. Singlets due to three different benzyl methylenes (eight protons) and four purine ring protons indicated that the reaction mixture did indeed contain both 1,9-dibenzylhypoxanthine (IIa) and 1,7-dibenzylhypoxanthine (IIIa), easily identified by the chemical shifts of their ring protons and methylene groups,[8] and furthermore, integration of the pmr spectrum of the mixture showed the relative amounts of the 1,9:1,7 isomer to be 1:2. When the benzylation was carried out on a larger scale, both isomers were obtained pure by fractional crystallization of the mixture; a 21% yield of the 1,9 isomer and a 42% yield of the 1,7 isomer were obtained, supporting the isomer ratio found by pmr spectrometry. An investigation of the benzylation of 1-benzylhypoxanthine (Ia) without base in *N*,*N*-dimethylacetamide gave similar results except that approximately equal quantities of the two isomers were formed.

The alkylation of 1-substituted purine-6(1*H*)-thiones in N,N-dimethylformamide in the presence of a proton acceptor has been reported to take place at

CHART I



(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

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

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

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

(5) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, **1**, 115 (1964).

(6) A preliminary account of part of this work has appeared: J. A. Montgomery and H. J. Thomas, *Chem. Ind.* (London), 1596 (1965).

(7) In contrast, a 72% yield of IIa was obtained from the benzylation of 9-benzylhypoxanthine.[2]

(8) The chemical shifts of the methylene protons of the benzyl group of *N*-benzylpurines appear to be dependent on the ring nitrogen to which it is attached, determined largely by the electron density at each ring nitrogen. These characteristic chemical shifts have diagnostic value.

TABLE I

PMR SPECTRA OF THE *N*-BENZYLHYPOXANTHINES AND *N*-BENZYLPURINE-6(1*H*)-THIONES

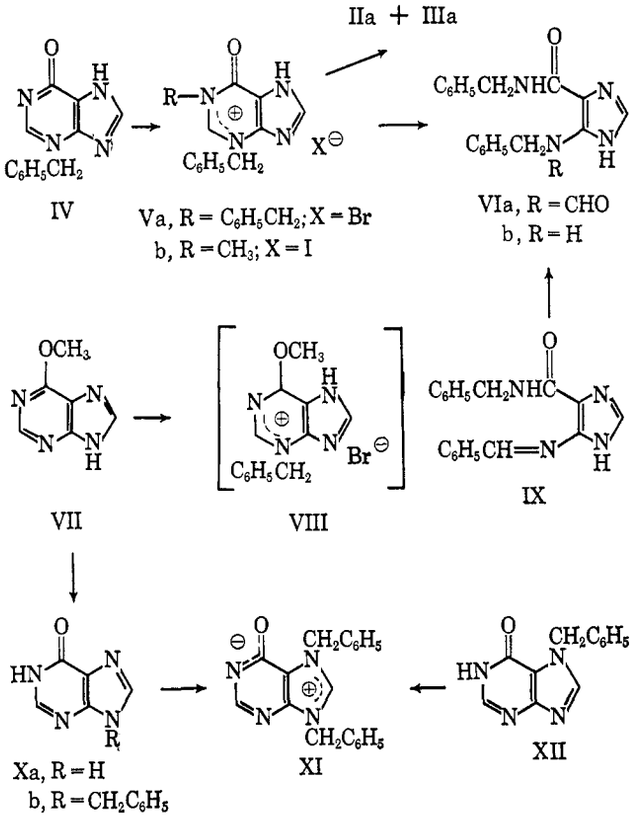
		τ, ppm						
No.	Compound	Ring	Protons	Phenyl protons	N_1-CH_2	N_3-CH_2	N_7-CH_2	N_9-CH_2
X	Hypoxanthine	1.88	2.03					
Ia	1-Benzylhypoxanthine	1.49	1.81	2.69	4.73			
IV	3-Benzylhypoxanthine	1.40	1.79	2.62		4.53		
XII	7-Benzylhypoxanthine	1.59	1.98	2.65			4.39	
Xb	9-Benzylhypoxanthine	1.79	1.95	2.68				4.61
Va	1,3-Dibenzylhypoxanthinium bromide	−0.80	1.50	2.59	4.25[a]	4.56[a]		
IIIa	1,7-Dibenzylhypoxanthine	1.49	1.57	2.70	4.77		4.40	
IIa	1,9-Dibenzylhypoxanthine	1.43	1.77	2.71	4.77			4.62
	3,7-Dibenzylhypoxanthine	1.45	1.63	2.58		4.59	4.38	
XI	7,9-Dibenzylhypoxanthine	0.58	1.87	2.63			4.50[a]	4.17[a]
XI	7,9-Dibenzylhypoxanthinium bromide	−0.23	1.56	2.57			4.37[a]	4.20[a]
Ib	1-Benzylpurine-6(1*H*)-thione	1.12	1.37	2.68	4.13			
IIIb	1,7-Dibenzylpurine-6(1*H*)-thione	1.08	1.37	2.69	4.14		3.85	
IIb	1,9-Dibenzylpurine-6(1*H*)-thione	1.10	1.57	2.67	4.10			4.57

[a] These pairs of benzyl methylene protons cannot be assigned with certainty.

N-7, although again high yields were not obtained.[2,4,9] Because of our results with 1-benzylhypoxanthine (Ia), we reinvestigated the benzylation of 1-benzylpurine-6(1*H*)-thione (Ib). Thin layer chromatography showed that here again in the presence or absence of a proton acceptor (potassium carbonate) two products were formed: 1,9-dibenzylpurine-6(1*H*)-thione (IIb), which has also been prepared by the thiation of 1,9-dibenzylhypoxanthine[10] and 1,7-dibenzylpurine-6(1*H*)-thione (IIIb).[2] Although not so easily as before, a quantitative analysis of the purified mixture could be carried out by means of pmr spectrometry, since the chemical shift of one of the ring protons of each purine is different, as in the chemical shift of the methylene protons of one of the benzyl groups of each purine (see Table I). Thus, the reaction mixture obtained with potassium carbonate was found by integration of its pmr spectrum to be a 1:2 mixture of 1,9-dibenzylpurine-6(1*H*)-thione (IIb) and 1,7-dibenzylpurine-6(1*H*)-thione (IIIb), and this ratio was later supported by isolation of the two compounds. A pmr spectral analysis of the reaction mixture resulting from the same reaction without base showed that in this case the ratio was reversed, *i.e.*, one part of 1,7-dibenzylpurine-6(1*H*)-thione (IIIb) to two-parts of 1,9-dibenzylpurine-6(1*H*)-thione (IIb).

3-Benzylhypoxanthine and 6-Methoxypurine (Chart II).—Although the benzylation of 3-benzylhypoxanthine (IV) in *N*,*N*-dimethylformamide in the presence of base was previously found to give only one product, 3,7-dibenzylhypoxanthine, in high yield;[2] the benzylation in *N*,*N*-dimethylacetamide without base has now been found to give none of this compound, but, when carried out in the usual manner at 110°, a mixture of approximately equal amounts of 1,9- and 1,7-dibenzylhypoxanthine (IIa and IIIa), the same result obtained with 1-benzylhypoxanthine (Ia). This result obviously requires an intra- or intermolecular migration of the benzyl group from N-3 to one of the other ring nitrogens. In an effort to further elucidate the path of this reaction, it was carried out in acetonitrile. From this reaction a high yield of a dibenzylhypoxanthinium bromide (A) was obtained.

CHART II



This same compound (A) along with still another dibenzylhypoxanthinium bromide (B) was obtained from the benzylation, without base, of 6-methoxypurine (VII). The latter compound (B) was also obtained from the benzylation, without base, of hypoxanthine (Xa) and of 7- and 9-benzylhypoxanthine (XII and Xb). From the latter bromide (B) could be obtained a pure base whose spectra resemble those of the betaine 7,9-dimethylhypoxanthine[11] and whose chromatographic behavior indicates a zwitterionic structure, and which was thus identified as 7,9-dibenzylhypoxanthine (XI). An attempt to prepare the purine base from compound A by careful neutralization of its aqueous solution gave a new compound that was

(9) J. A. Montgomery, R. W. Balsiger, A. L. Fikes, and T. P. Johnston, *J. Org. Chem.*, **27**, 195 (1962).

(10) J. A. Montgomery and H. J. Thomas, *ibid.*, **31**, 1411 (1966).

(11) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 1914 (1962).

initially identified on the basis of its elemental composition and spectra as *N*-benzyl-5- (or 4-) (*N*-benzylformamido)imidazole-4- (or 5-) carboxamide (VIa). Its structure was then established unequivocally by deformylation to *N*-benzyl-5- (or 4-) benzylaminoimidazole-4- (or 5-) carboxamide (VIb), which was also prepared by reaction of *N*-benzyl-5- (or 4-) aminoimidazole-4- (or 5-) carboxamide[12] with benzaldehyde and catalytic reduction of the resultant anil (IX). The infrared spectra of XI bromide and compound A show C=O absorption in the 1700- to 1750-cm^{-1} region, and in the pmr spectra the chemical shift of one ring proton of both compounds has an unusually low τ value (−0.23 and −0.80 ppm), as a result of deshielding by the quaternarized nitrogens (see Table I).

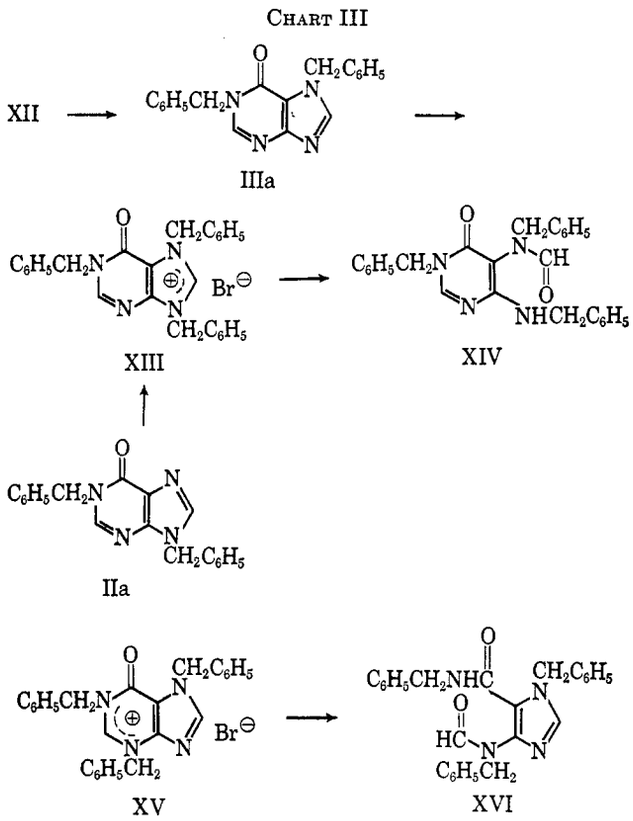
From these data it is obvious that compound A must be 1,3-dibenzylhypoxanthinium bromide (Va).[13] This same compound was then obtained in 8% yield, along with 17% yield of 1,7-dibenzylhypoxanthine, an 8% yield of 1,9-dibenzylhypoxanthine, and a 17% yield of 1,7,9-tribenzylhypoxanthinium bromide (see below) from the reaction of 1-benzylhypoxanthine with benzyl bromide in acetonitrile. When heated at 110° in *N*,*N*-dimethylacetamide, Va gave a reaction mixture essentially identical to that obtained by the benzylation of 3-benzylhypoxanthine (IV), from which 1,9- and 1,7-dibenzylhypoxanthine (II and IIIa) were isolated. Furthermore, the benzylation of 3-benzylhypoxanthine (IV) in N,N-dimethylacetamide at 60° rather than at 110° gave a good yield of Va. Apparently in the original benzylation reaction, quaternarization at N-1 destabilized the benzyl group at N-3 causing it to migrate at that temperature, perhaps as a result of attack of bromide ion on the benzylic carbon, to N-7 and N-9. Benzylation of 6-methoxypurine probably occurred first at N-3 to give 3-benzyl-6-methoxypurinium bromide (VIII). The bromide ions thus produced could then attack the methyl group of unreacted 6-methoxypurine to give hypoxanthine or the methyl group of 3-benzyl-6-methoxypurine to give 3-benzylhypoxanthine.[15] Further benzylation of these compounds would then give the two betaines Va and XI.

Since, in contrast to the above results, the methylation of 3-methylhypoxanthine is reported to take place at the carbonyl oxygen to give 6-methoxy-3-methylpurine,[17] we also methylated 3-benzylhypoxanthine with methyl iodide and obtained 3-benzyl-1-methylhypoxanthinium iodide (Vb). That *O*-methylation did not occur was evident from the carbonyl absorption in the infrared spectrum of Vb. The infrared spectrum of "6-methoxy-3-methylpurine" was not described, but its ultraviolet spectrum is similar to those of Va and b.

To learn more about the mechanism of migration, and equimolar mixture of 1,3-dibenzylhypoxanthinium bromide (Va) and adenine in *N*,*N*-dimethylacetamide was heated at 110° for 4 hr. Isolation of 1-benzylhypoxanthine (Ia) and 3-benzyladenine from this mixture provides proof that, at least in part, the reaction is intermolecular in nature, perhaps *via* the re-formation of benzyl bromide. This finding does not eliminate the possibility that transfer from N-3 to N-9 is intramolecular.

7-Benzylhypoxanthine (Chart III).—Reaction of 7-benzylhypoxanthine (XII) with 3 equiv of benzyl chloride in the presence of potassium carbonate has been reported to give a low yield of 1,7-dibenzylhypoxanthine (IIIa).[2] We have now found that the yield of IIIa can be increased to 51% by using only 1 equiv of benzyl bromide; a small amount of 3,7-dibenzylhypoxanthine is also formed. From the reaction using 3 equiv of benzyl bromide we have isolated, in 28% yield, another product (C) whose elemental analyses indicate the empirical formula, $C_{26}H_{25}N_4O_2$, that in turn suggests the introduction of two benzyl groups into XII followed by cleavage of either the imidazole or pyrimidine ring of the resultant tribenzylhypoxanthine. This same compound (C) was then isolated from the reaction of 1,7-dibenzylhypoxanthine (IIIa) with benzyl bromide in the presence of base. Without potassium carbonate, IIIa gave a tribenzylhypoxanthinium bromide that could be converted with base to compound C. Since 1,7-dibenzylhypoxanthine does not undergo benzyl group migration even under *acid* conditions (see below), the tribenzylhypoxanthine must be either 1,7,9- or 1,3,7-tribenzylhypoxanthinium bromide (XIII or XV), and compound C, which would result from cleavage of the ring containing the quaternarized nitrogen, could be either 1-benzyl-4-benzylbenzylamino-5-(*N*-benzylformamido)pyrimidin-6(1*H*)-

CHART III



(12) E. Shaw, *J. Am. Chem. Soc.*, **80**, 3899 (1958).

(13) The ease of opening of the pyrimidine ring of V in aqueous base is compatible with its structure, since quaternarization of N-1 creates an unusual electron deficiency at C-2 allowing facile attack by hydroxyl ions. Other relatively easy pyrimidine ring openings have been reported.[14]

(14) W. C. Curran and R. B. Angier, *J. Org. Chem.*, **26**, 2364 (1961).

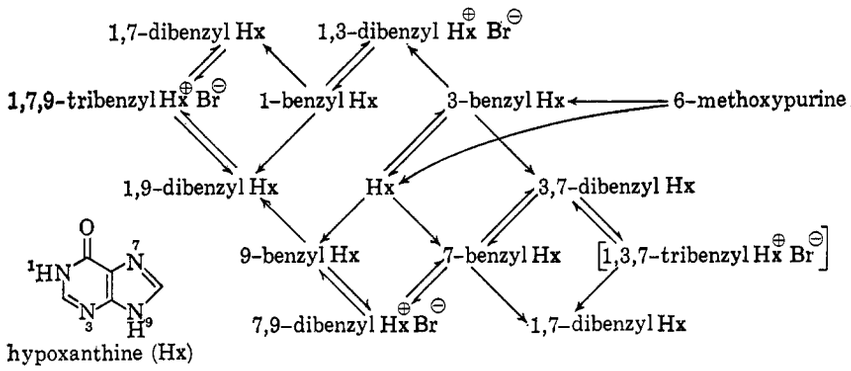
(15) This type of *O*-demethylation is well known in the pyrimidine series; *e.g.*, see the Hilbert–Johnston nucleoside synthesis.[16]

(16) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959). See also T. L. V. Ulbricht, *Proc. Chem. Soc.*, 298 (1962).

(17) F. Bergmann, M. Kleiner, Z. Neiman, and M. Rashi, *Israel J. Chem.*, **2**, 185 (1964).

CHART IV

THE BENZYLATION OF HYPOXANTHINES

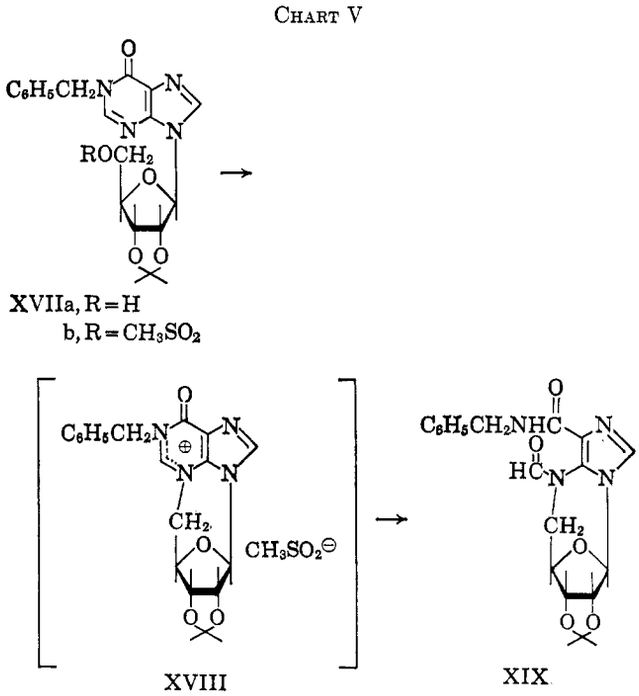


one (XIV) or 1,*N*-dibenzyl-4-(*N*-benzylformamido)-imidazole-5-carboxamide (XVI). To resolve this structural problem the benzylation of 3,7-dibenzylhypoxanthine and 1,9-dibenzylhypoxanthine (IIa) without base was carried out. Heating 3,7-dibenzylhypoxanthine for 4 hr with benzyl bromide in acetonitrile followed by recrystallization of the crude product from ethanol gave a small amount of the same tribenzylhypoxanthinium bromide obtained from IIIa, but principally a new compound identified by its elemental analyses and spectra as 1,*N*-dibenzyl-4-(*N*-benzylformamido)imidazole-5-carboxamide (XVI), which must result from rupture of the pyrimidine ring of the presumed intermediate 1,3,7-tribenzylhypoxanthinium bromide (XV). On the other hand, the only tribenzylhypoxanthinium bromide obtained from 1,9-dibenzylhypoxanthine was found to be identical with that isolated from IIIa. Thus the tribenzylhypoxanthinium bromide must be the 1,7,9-isomer (XIII) and compound C must be the pyrimidine (XIV). Refluxing 98% formic acid slowly converted XIV to 1,7,9-tribenzylhypoxanthinium formate.

Thermal Stability (Chart IV).—The results described above caused us to investigate the thermal stability of 7,9-dibenzylhypoxanthinium (XI) bromide. Heating XI bromide for 14 hr at 110° in *N*,*N*-dimethylacetamide gave a mixture of XI bromide and 9-benzylhypoxanthine (Xb) [and a very small amount of a mixture of 1,9- and 1,7-dibenzylhypoxanthine (IIa and IIIa)]. At 150° a mixture was obtained from which 1,9-dibenzylhypoxanthine (IIa), 1,7-dibenzylhypoxanthine (IIIa), and 9-benzylhypoxanthine (Xb) were isolated (1:1:2). 3,7-Dibenzylhypoxanthine and 1 equiv of hydrogen bromide in *N*,*N*-dimethylacetamide at 110° gave a mixture of 1,7-dibenzylhypoxanthine (30%), 1,9-dibenzylhypoxanthine (30%), 1,7,9-tribenzylhypoxanthinium bromide (15%), 7,9-dibenzylhypoxanthinium bromide (10%), and 7-benzylhypoxanthine (10%). Under the same conditions 3-benzylhypoxanthine gave primarily hypoxanthine and some 1-benzylhypoxanthine; 1,7-dibenzylhypoxanthine (XII), however, remained unchanged. These experiments establish the general nature of these intermolecular migrations.[18] It would appear that at the minimum reaction temperature the *N*-benzylation of a purine is an equilibrium reaction governed by the relative electron densities of the nitrogen atoms (including exocyclic nitrogens if present[5]), but at higher temperatures the products isolated depend on their relative thermodynamic stabilities. Thus under certain conditions 1,3-dibenzylhypoxanthinium bromide (Va), 3,7-dibenzylhypoxanthine, and 7,9-dibenzylhypoxanthinium (XI) bromide are formed. At higher temperatures these thermodynamically less stable isomers are converted to a mixture of the more stable 1,9-dibenzylhypoxanthine (IIa) and 1,7-dibenzylhypoxanthine (IIIa). With excess benzyl bromide IIa and IIIa can be converted to 1,7,9-tribenzylhypoxanthinium bromide (XIII), but this reaction is reversible, so that IIa and IIIa are interconvertible. Furthermore, it would appear that alkylation of many purines in the absence of base (*i.e.*, the neutral molecule) takes place preferentially at a tertiary nitrogen by an SE2′ mechanism, rather than at the imino groups by an SE2 mechanism.[19] These alkylations and interconversions are summarized in Chart IV.

Nucleosides (Chart V).—Prior to these benzylation studies, an attempt was made to carry out the nucleo-

CHART V



(18) In addition, see B. Shimizu and M. Miyaki, *Tetrahecron Letters*, 2059 (1965).

(19) The *N* alkylation of imidazoles has been shown to occur by an SE2′ mechanism.[20]

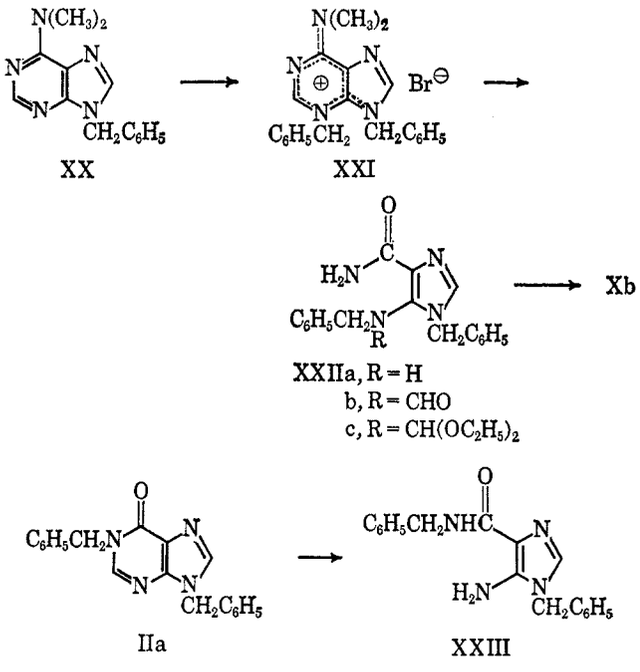
(20) A. Grimison, J. H. Ridd, and B. V. Smith, *J. Chem. Soc.*, 1352, 1357 (1960).

philic displacement of the 5′-mesyloxy group of 1-benzyl-2′,3′-*O*-isopropylidene-5′-*O*-mesylinosine (XVIIb), since it seemed reasonable that substitution at N-1 might prevent anhydronucleoside formation. In view of the present results it is not surprising that, even though the ease of anhydronucleoside formation (to give XVIII) relative to 2′,3′-*O*-isopropylidene-5′-*O*-tosylinosine[21] was decreased, it could not be avoided under conditions necessary for the nucleophilic displacement reaction. From the reaction was isolated the anhydronucleoside from *N*-benzyl-5-formamido-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (XIX), which resulted from the opening of the pyrimidine ring of XVIII.

The current work also explains the fact that an attempt to prepare 3-benzyl-7-β-D-ribofuranosylhypoxanthine by the fusion of 3-benzylhypoxanthine and tetra-*O*-acetylribofuranose in the presence of acid gave a low yield of 1-benzylinosine and no other nucleoside.

9-Benzyl-*N*,*N*-dimethyladenine (Chart VI).—Studies on the benzylation of 9-benzyl-*N*,*N*-dimethyladenine (XX)[22] are included here because the results obtained

CHART VI



are related to those discussed above. Benzylation of this purine with benzyl bromide in *N*,*N*-dimethylformamide gave a single product that analyzed for a dibenzylpurinium bromide, presumably either the 1,9 or the 3,9 isomer (XXI). Refluxing aqueous base converted this product to a dibenzylimidazole, which failed to give a positive Bratton–Marshall test[24] indicating that it is 5-benzylamino-1-benzylimidazole-4-carboxamide (XXIIa) and, therefore, that benzylation of XX took place at N-3 not N-1 to give 3,9-dibenzyl-*N*,*N*-dimethyladenium bromide. To confirm this assignment the other possible imidazole isomer, 5-amino-1,*N*-dibenzylimidazole-4-carboxamide (XXIII) was prepared by opening the pyrimidine ring of 1,9-dibenzylhypoxanthine (IIa) with aqueous base, and indeed, the two imidazoles were not identical; furthermore, XXIII readily gave a positive Bratton–Marshall test. Attempts to convert XXIIa to 3,9-dibenzylhypoxanthine, the only remaining dibenzylhypoxanthine, have so far been unsuccessful.

Treatment of XXIIa with formic acid for a short period of time gave 5-(*N*-benzylformamido)-1-benzylimidazole-4-carboxamide (XXIIb) and a trace of 9-benzylhypoxanthine (Xb), resulting from ring closure and acidic debenzylation. When the reflux period was increased to 3 days, the major product was 9-benzylhypoxanthine (Xb) contaminated with some XXIIb. Treatment of XXIIa with diethoxymethyl acetate quite surprisingly gave the formyl derivative XXIIb, presumably *via* the diethoxymethyl derivative XXIIc, and some 9-benzylhypoxanthine (Xb). Ethyl orthoformate and a catalytic amount of concentrated hydrochloric acid gave essentially the same results.

Experimental Section

The melting points reported were determined on a Kofler Heizbank, unless otherwise stated. The ultraviolet spectra were determined in aqueous solution with a Cary 14 spectrophotometer; the infrared spectra were determined in pressed potassium bromide discs with a Perkin-Elmer Model 221-G or 521 spectrophotometer; the proton magnetic resonance spectra were determined in 10% (w/v) solution in DMSO-d_6 with a Varian Associates Model A-60 spectrometer. SilicAR TLC-7 (Mallinckrodt) silica gel or silica gel H (Brinkmann) was used for the chromatographic separations. Usually the chromatograms were developed with a mixture of chloroform and methanol (in various proportions). Spots were detected with an ultraviolet light after spraying the plates with "Ultraphor WT highly concentrated" (BASF Colors and Chemicals, Inc., Charlotte, N. C.).

The Benzylation of 1-Benzylhypoxanthine (Ia). A. With Base.—A vigorously stirred suspension of 1-benzylhypoxanthine (16.4 g, 72.5 mmoles), anhydrous potassium carbonate (10.1 g, 72.5 mmoles), and benzyl chloride (16.4 ml, 18.0 g, 145 mmoles) in 1 l. of *N*,*N*-dimethylformamide (spectral grade) was heated at 95° for 22 hr. After removal of the potassium carbonate by filtration, the solution was evaporated to dryness *in vacuo* at 60°. The residue was suspended in water and concentrated to dryness three times, followed by a similar treatment with ethanol. This residue was crystallized from 750 ml of ethanol. A second recrystallization from ethanol gave, after drying *in vacuo* at 78° for 16 hr, 4.8 g (21%) of 1,9-dibenzylhypoxanthine (IIa):[2] mp 207°; λ_{max}, in mμ (ε × 10^{-3}), pH 1—252 (11.3), pH 7—253 (11.2), pH 13—252 (11.1). The compound was shown to be homogeneous by thin layer chromatography (chloroform–methanol, 99:1).

The mother liquor from the recrystallization of IIa was concentrated to a very small volume and cooled in a Dry Ice–acetone bath. Scratching with a glass rod induced crystallization. The solid was collected and recrystallized twice from ethanol: yield, 9.5 g (41.6%) of 1,7-dibenzylhypoxanthine (IIIa); mp 105° (a mixture melting point with 1,7-dibenzylhypoxanthine[2] was the same); λ_{max}, in mμ (ε 10^{-3}), pH 1—257 (8.25), pH 7—257 (7.57), pH 13—256 (7.83).

B. Without Base in Acetonitrile.—A suspension of 226 mg (1.00 mmole) of 1-benzylhypoxanthine (Ia) in 50 ml of acetonitrile containing 171 mg (1.00 mmole) of benzyl bromide was refluxed for 48 hr. The 1-benzylhypoxanthine that had reprecipitated (68 mg) was collected.

The filtrate was evaporated to dryness and the residue was dissolved in 2 ml of methanol. This solution, found to be a mixture of more unreacted 1-benzylhypoxanthine (Ia), 1,3-dibenzylhypoxanthinium bromide (Va), 1,7-dibenzylhypoxanthine (IIIa), 1,9-dibenzylhypoxanthine (IIa), and 1,7,9-tribenzylhypoxanthinium bromide (XIII), was resolved by means of chromatography on a silica gel plate. The yield of 1,3-dibenzylhypoxanthinium bromide was 8%; 1,7-dibenzylhypoxanthine, 17%; 1,9-dibenzylhypoxanthine, 8%; and 1,7,9-tribenzylhypoxanthinium bromide, 17%.

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

(22) Studies on the methylation of 6-dimethylamino-9-methylpurine, which are in agreement with those published here, have recently appeared.[23]

(23) J. W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965).

(24) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

Benzylation of 1-Benzylpurine-6(1*H*)-thione (Ib). A. With Base.—A solution of 121 mg (0.50 mmole) of 1-benzylpurine-6(1*H*)-thione (Ib)[2] and 254 mg (2.00 mmoles) of benzyl chloride in 25 ml of *N*,*N*-dimethylformamide containing a suspension of 69 mg (0.50 mmole) of anhydrous potassium carbonate was stirred at 100° for 24 hr. The insoluble solid was collected and the filtrate evaporated to dryness *in vacuo*. The residue was twice triturated with 25 ml of ether and then dissolved in 25 ml of chloroform. To remove unchanged starting material, the yellow, chloroform solution was washed first with 25 ml of 0.1 *N* NaOH and then with 25 ml of water, dried over magnesium sulfate, and evaporated to dryness *in vacuo:* yield, 172 mg of a mixture of **1,9-dibenzylpurine-6(1*H*)-thione (IIb)** and **1,7-dibenzylpurine-6(1*H*)-thione (IIIb)**.[2] Integration of the pmr spectrum of this mixture showed it to consist of one part of IIb and two parts of IIIb.

B. Without Base.—A solution of 242 mg (1.00 mmole) of 1-benzylpurine-6(1*H*)-thione (Ib)[2] and 378 mg (3.00 mmoles) of benzyl chloride in 10 ml of *N*,*N*-dimethylacetamide was heated at 100° for 20 hr and 140° for 16 hr. The solution was evaporated to dryness *in vacuo*. The residue was suspended in water and the suspension was evaporated *in vacuo*. An ethanol solution of this residue was then evaporated to dryness. The residue from this treatment was crystallized from 5 ml of absolute ethanol: yield, 280 mg (84%) of a mixture of II and IIIb. Integration of the pmr spectrum of this mixture showed that it consisted of two parts of IIb and one part of IIIb.

Benzylation of 3-Benzylhypoxanthine (IV). A. At 110°.—A solution of 226 mg (1.00 mmole) of 3-benzylhypoxanthine (IV) and 171 mg (1.00 mmole) of benzyl bromide in 25 ml of *N*,*N*-dimethylacetamide was heated at 110° for 18 hr. The solution was evaporated to dryness *in vacuo*. To remove any starting compound, the residue was dissolved in 25 ml of chloroform and this solution was washed twice with 25 ml of saturated sodium bicarbonate solution and once with water, dried over magnesium sulfate, and evaporated to dryness *in vacuo:* yield, 260 mg (82%) of a mixture of **1,9-dibenzylhypoxanthine (IIa)** and **1,7-dibenzylhypoxanthine (IIIa)**. Integration of the pmr spectrum of this mixture showed it to be a 1:1 mixture of IIa and IIIa.

B. At 80°.—A suspension of 452 mg (2.00 mmoles) of 3-benzylhypoxanthine (IV)[2] in 50 ml of dry acetonitrile containing 342 mg (2.00 mmoles) of benzyl bromide was stirred and refluxed for 16 hr. The precipitate that had formed was collected by filtration: yield, 438 mg. Evaporation of the filtrate to 25 ml gave a second crop of 96 mg: total yield of **1,3-dibenzylhypoxanthinium bromide (Va)**, 534 mg (67%).

The analytical sample was obtained by recrystallization from ethanol. It was dried at 78° (0.07 mm) over phosphorus pentoxide for 18 hr: mp 196–197°; λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—254 (10.2) and 280 (sh) (3.92), pH 7—245 (sh) (9.4) and 304 (sh) (0.51), pH 13—unstable; σ, cm^{-1}, 3100–2400 (CH, acidic H), 1730 (C=O), 1630 and 1545 (C=C, C=N).

Anal. Calcd for $C_{19}H_{16}N_4O \cdot HBr$: C, 57.44; H, 4.31; N, 14.10. Found: C, 57.33; H, 4.36; N, 14.18.

This reaction, when carried out in *N*,*N*-dimethylacetamide at 60° for 2.5 hr, gave a 74% yield of the same product (Va).

Reaction of 1,3-Dibenzylhypoxanthinium Bromide (Va) and Adenine in *N*,*N*-dimethylacetamide at 110°.—A solution of 246 mg (0.62 mmole) of 1,3-dibenzylhypoxanthinium bromide and 83.6 mg (0.62 mmole) of adenine in 50 ml of *N*,*N*-dimethylacetamide was stirred at 120° for 4 hr.

The reaction solution was evaporated to dryness *in vacuo*. The residue was dissolved in 2 ml of methanol and the solution was resolved by means of thin layer chromatography. It was found to contain 1,3-dibenzylhypoxanthine (Va), adenine, 3-benzyladenine, 1-benzylhypoxanthine (Ia), 1,7-dibenzylhypoxanthine (IIIa), and 1,9-dibenzylhypoxanthine (IIa).

3-Benzyl-1-methylhypoxanthinium Iodide (Vb).—To a solution of 226 mg (1.00 mmole) of 3-benzylhypoxanthine in 10 ml of *N*,*N*-dimethylacetamide was added 1.0 ml (16 mmoles) of methyl iodide and the solution was stirred at 60° for 2.5 hr before it was evaporated to dryness *in vacuo*. The residue was crystallized from ethanol: yield, 296 mg (80.5%); mp 185–187° (Mel-Temp); λ_{max}, in mμ ($\epsilon \times 10^{-3}$); pH 1—254 (9.95) and 280 (sh) (3.26), pH 7—256 (sh) (6.68) and 295 (sh) (1.72), pH 13—unstable; σ, cm^{-1}, 3100–2600 (CH, acidic H), 1725 (C=O), 1635 and 1550 (C=C, C=N).

The analytical sample was obtained from a previous reaction using acetonitrile as the solvent.

Anal. Calcd for $C_{13}H_{12}N_4O \cdot HI$: C, 42.41; H, 3.56; N, 15.22. Found: C, 42.15; H, 3.27; N, 14.95.

***N*-Benzyl-5- (or 4-) (*N*-benzylformamido)imidazole-4- (or 5-) carboxamide (VIa).**—A solution of 136 mg (0.34 mmole) of 1,3-dibenzylhypoxanthinium bromide (Va) in 10 ml of water was obtained by gentle heating. The solution was made basic (pH 10–11) with concentrated ammonium hydroxide. The resulting mixture was extracted twice with chloroform (25 ml). The chloroform solution was dried over magnesium sulfate and evaporated to dryness *in vacuo*. Trituration of the residue with pentane gave a white solid that melted to a glass upon drying at 78° (0.07 mm) over phosphorus pentoxide: yield, 98 mg (85%); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—244 (sh) (8.61), pH 7—241 (9.75), pH 13—266 (12.2); σ, cm^{-1}, 3350, 3200, 3100, 3060, 3030 (NH, CH), 2940–2500 (CH, acidic NH), 1680 (sh) (formyl carbonyl), 1650 (amide carbonyl), 1620 (sh), 1590, 1530 (C=N, C=C); τ, in ppm, 5.57 d (CH_2 of benzylamide), 5.03 (CH_2 of benzyl formamido), 2.78 and 2.72 (phenyl), 2.32 and 1.53 (C^2-H and formyl), 1.73 t (amide NH), 0.90 (br) (ring NH). Upon the addition of D_2O the doublet at 5.57 ppm became a singlet and NH–D_2O exchange was evident.

Anal. Calcd for $C_{19}H_{18}N_4O_2$: C, 68.24; H, 5.42; N, 16.76. Found: C, 67.98; H, 5.35; N, 16.64.

***N*-Benzyl-5- (or 4-) benzylaminoimidazole-4- (or 5-) carboxamide (VIb) Hydrochloride. A.**—A solution of 400 mg (1.20 mmoles) of *N*-benzyl-5- (or 4-) (*N*-benzylformamido)imidazole-4- (or 5-) carboxamide (VIa) in 20 ml of methanol containing 1.2 ml of 1-*N* methanolic hydrogen chloride was refluxed for 1 hr and evaporated to dryness *in vacuo*. The residue crystallized from ethanol: yield 377 mg (92%); mp 200–202° dec (Mel-Temp); λ_{max}, in mμ (ϵ 10^{-3}), pH 1—247 (11.9) and 282 (13.6), pH 7—281 (15.3), pH 13—288 (15.5); σ, in cm^{-1}, 3310, 3255, 3110, 3060, 3030, 2980, 2930, 2860 (NH, CH), 2800–2400 (acidic H), 1655 (amide carbonyl), 1610, 1600, 1575, 1530 (C=H, C=C); τ, in ppm, 5.52 d (CH_2 of benzylamide), 5.40 (CH_2 of benzylamine), 2.67 (phenyl), 1.27 (C^2-H), 0.90 (amide NH). Upon addition of D_2O the doublet at 5.52 ppm became a singlet and NH–D_2O exchange was evident.

Anal. Calcd for $C_{18}H_{18}N_4O \cdot HCl$: C, 63.07; H, 5.59; N, 16.34. Found: C, 63.08; H, 5.61; N, 16.49.

B.—A solution of 304 mg (1.00 mmole) of *N*-benzyl-5- (or 4-) benzylideneaminoimidazole-4- (or 5-) carboxamide (IX) in 300 ml of ethanol was hydrogenated in the presence of 50 mg of platinum oxide (prereduced) at atmospheric pressure and 25°. The catalyst was removed by filtration, and the solution was evaporated to dryness. To obtain the hydrochloride salt, the residue was dissolved in 1 ml of 1 *N* methanolic hydrogen chloride and 25 ml of methanol and evaporated to dryness. This residue crystallized from ethanol: yield, 280 mg (82%); mp 200–202° dec (Mel-Temp).

This material was identical in all respects, melting point and infrared and ultraviolet spectra, with that obtained by deformylation of *N*-benzyl-5- (or 4-) (*N*-benzylformamidoimidazole-4- (or 5-) carboxamide (VIa).

The Benzylation of 6-Methoxypurine (VII). A.—A solution of 6-methoxypurine (VII, 1 g, 6.7 mmoles), benzyl bromide (2 ml, 12+ mmoles), and *N*,*N*-dimethylformamide (25 ml) was stirred at room temperature under anhydrous conditions for 18 hr before it was heated at 60–70° for 4 hr. After the reaction was complete, the solution was evaporated to dryness and the residue was triturated with ether. The insoluble solid that formed was collected by filtration, washed with ether, and dried, giving the crude product which was shown by thin layer chromatography to be a mixture of two major products. The crude product was dissolved in water containing enough ethanol to give complete solution. After neutralization (pH 6–7) of the solution with 1 *N* NaOH, the ethanol was removed *in vacuo* and the aqueous solution was extracted with chloroform. Evaporation of the chloroform extract to dryness gave an oil which was identified by its spectra and thin layer chromatographic behavior to be impure *N*-benzyl-5- (or 4-) benzylaminoimidazole-4- (or 5-) carboxamide (VIb): yield, 550 mg (25%); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—240 (sh), pH 7—246 (8.5), pH 13—265 (10.0); σ, in cm^{-1}, 3400–2900 (NH, CH), 1690 (sh), 1650 (NH, C=O), 1590 (C=C).

After concentration *in vacuo*, the water solution was allowed to stand until crystallization was complete. The crystals were collected, washed, and dried *in vacuo*, giving 195 mg (7.4%) of 7,9-dibenzylhypoxanthinium bromide. This crude product was dissolved in water (10 ml) and the solution was brought to

pH 9–10 with 1 *N* NaOH. The precipitate that formed was collected by filtration and recrystallized from boiling water (40 ml) to give **7,9-dibenzylhypoxanthine (XI)**: yield, 48 mg, mp >260° (sublimation). Thin layer chromatography using chloroform–methanol (9:1) as eluent showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—255 (10.5), pH 7—267 (9.4), pH 13—264 (7.5); σ, in cm^{-1}, 3000–2950 (CH), 1620, 1565, 1545, 1490 (C=C, C=N), 1295, 690 strong, unassigned.

Anal. Calcd for $C_{19}H_{16}N_4O$: C, 72.21; H, 5.10; N, 17.68. Found: C, 72.42; H, 4.82; N, 17.74.

B.—A solution of 6-methoxypurine (VII, 500 mg, 3.3 mmoles) and 1 ml of benzyl bromide in acetonitrile (25 ml) was refluxed for 4 hr before it was allowed to stand at room temperature overnight. The crystals that precipitated were collected by filtration, washed with ether, and dried to give 116 mg (25%) of hypoxanthine. The reaction filtrate was evaporated to dryness *in vacuo* and the residue was triturated with ether. The insoluble solid was collected by filtration and redissolved in acetone (15 ml); the solution was allowed to stand until crystallization was complete. The crystals were collected by filtration, washed with acetone, and dried *in vacuo* to give 142 mg (11%) of **1,3-dibenzylhypoxanthinium bromide (Va)**, which was identified by its infrared spectrum. Concentration of the acetone filtrate gave an additional 200 mg of solid which was shown by thin layer chromatography to be a 2:1 mixture of **7,9-dibenzylhypoxanthinium (XI) bromide** and **1,3-dibenzylhypoxanthinium bromide (Va)**. Thin layer chromatography using chloroform–methanol (9:1) as the eluent indicated that the oil obtained on evaporation of the acetone filtrate to dryness was primarily XI bromide.

***N*-Benzyl-5- (or 4-) benzylideneaminoimidazole-4 (or 5-) carboxamide (IX).**—A solution of 432 mg (2.00 mmoles) of *N*-benzyl-5 (or 4-) aminoimidazole-4- (or 5-) carboxamide[12] in 2.5 ml of 1 *N* hydrochloric acid and 50 ml of methanol was evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and the solution was evaporated to dryness. This residue was then dissolved in 20 ml of ethanol and 241 mg (2.25 mmoles) of benzaldehyde was added. A precipitate formed in the solution after about 5 min at room temperature. The mixture was chilled and and the solid was removed by filtration: yield, 500 mg (82%); mp 220–221° (Mel-Temp).

The analytical sample was obtained by recrystallization from ethanol: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—249 (22.4), pH 7—244 (20.8) and 333 (14.9), pH 13—248 (22.6) and 368 (14.7); σ, in cm^{-1}, 3250, 3160, 2980, 2920, and 2865 (NH, CH), 1650, 1605, 1595, 1585, 1570, and 1540 (amide carbonyl, C=N, C=C, and phenyl).

Anal. Calcd for $C_{18}H_{16}N_4O$: C, 71.04; H, 5.30; N, 18.41. Found: C, 71.20; H, 5.52; N, 18.51.

The Benzylation of Hypoxanthine (Xa).—A solution of hypoxanthine (Xa, 1 g, 7.4 mmoles) and benzylbromide (2 ml) in *N*,*N*-dimethylformamide (25 ml) was heated for 2 hr at 100° before it was evaporated to dryness. The residue was dissolved in chloroform and the solution was extracted with 1 *N* NaOH (6 ml). The resulting slightly acid (pH 4) aqueous layer was shown by thin layer chromatography to contain primarily unreacted hypoxanthine. When the chloroform layer was washed with fresh water and the mixture was allowed to stand at room temperature overnight, crystals were precipitated at the interface. The crystals were collected and recrystallized from water to give 1.0 g (34%) of **7,9-dibenzylhypoxanthinium (XI) bromide** which was shown by thin layer chromatography to be contaminated with a small amount of 3-benzylhypoxanthine (IV).

9-Benzylhypoxanthine (Xb).—A solution of 5-benzylamino-1-benzylimidazole-4-carboxamide (XXIIa, 200 mg, 0.7 mmole) in formic acid (10 ml) was refluxed for 3 days before it was evaporated to dryness; the residue was dissolved in ethanol, evaporated from ethanol solution twice, and finally triturated with ether. The insoluble solid that formed was collected by filtration, washed with ether, and dried to give 116 mg of Xb contaminated with XXIIa and b: mp 285° dec. The crude product was recrystallized from ethanol to give the pure material: yield, 50 mg (34%); mp 293° dec. Thin layer chromatography using chloroform–methanol (19:1) as eluent showed a single spot. The ultraviolet and infrared spectra were identical with those of an authentic sample of 9-benzylhypoxanthine.

7,9-Dibenzylhypoxanthinium (XI) Bromide. A.—A suspension of 1.31 g (5.00 mmoles) of 9-benzylhypoxanthine in 100 ml of acetonitrile containing 941 mg (5.50 mmoles) of benzyl bromide was stirred and refluxed for 16 hr. Since, at the end of this time, there was still undissolved starting compound, 50 ml more acetonitrile was added and reflux was continued for 4 hr. Since the reaction still contained starting compound, benzyl bromide was added in two portions of 941 mg each over a 2-day reflux period. Upon cooling, the solution deposited a crystalline solid that was collected by filtration: yield, 2.18 g (100%); mp 210–212° (Mel-Temp); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—256 (10.8), pH 7—268 (9.20), pH 13—unstable.

Stability of 7,9-Dibenzylhypoxanthinium Bromide (XI) in *N*,*N*-Dimethylacetamide. A. At 100°.—A solution of 397 mg (1.00 mmole) of 7,9-dibenzylhypoxanthinium (XI) bromide in 40 ml of *N*,*N*-dimethylacetamide was heated at 110° for 14 hr and evaporated to dryness *in vacuo*. The residue, which after ether trituration crystallized from ethanol, was found to be unchanged 7,9-dibenzylhypoxanthinium bromide (94 mg).

The ethanol filtrate was found by means of thin layer chromatography to consist of 9-benzylhypoxanthine (Xb, 16% conversion), **1,9-dibenzylhypoxanthine** (IIa, 2% conversion), and a trace of **7-benzylhypoxanthine (XII)**.

B. At 150°.—A solution of 397 mg (1.00 mmole) of 7,9-dibenzylhypoxanthinium (XI) bromide in 40 ml of *N*,*N*-dimethylacetamide was heated at 150° for 16 hr. The reaction solution was evaporated to dryness *in vacuo*. A methanol solution of the residue was resolved by means of thin layer chromatography. From the plate was obtained 66 mg (29%) of **9-benzylhypoxanthine (Xb)** and 107 mg (34%) of a 1:1 mixture of **1,7-** and **1,9-dibenzylhypoxanthine (II and IIIa)**.

The Reaction of 3,7-Dibenzylhypoxanthine with Hydrogen Bromide in *N*,*N*-Dimethylacetamide.—A solution of 100 mg (0.33 mmole) of 3,7-dibenzylhypoxanthine and 82 mg (0.33 mmole) of hydrogen bromide (32% solution in acetic acid) in 10 ml of *N*,*N*-dimethylacetamide was heated at 110° for 4 hr and evaporated to dryness *in vacuo*. The residue was resolved by means of thin layer chromatography. It consisted of **1,7-dibenzylhypoxanthine (IIIa, 30%)**, **1,9-dibenzylhypoxanthine** (IIa, 30%), **1,3,7-tribenzylhypoxanthinium bromide (XIV, 15%)**, **7,9-dibenzylhypoxanthinium (XI) bromide** (10%), and **7-benzylhypoxanthine (XII, 10%)**.

The Benzylation of 7-Benzylhypoxanthine (XII). A. With Base and 1 Equiv of Benzyl Bromide.—A solution of 226 mg (1.00 mmole) of 7-benzylhypoxanthine (XII) and 171 mg (1.00 mmole) of benzyl bromide in 20 ml of *N*,*N*-dimethylacetamide containing a suspension of 138 mg of anhydrous potassium carbonate was stirred at 110° for 16 hr. The insoluble solid was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with ether and then dissolved in 25 ml of chloroform. After washing the chloroform solution with water, with saturated sodium bicarbonate solution, and again with water, it was dried over magnesium sulfate and evaporated to dryness *in vacuo*. The residue crystallized from ethanol: yield of **1,7-dibenzylhypoxanthine (IIIa)**, 181 mg (57%); mp 108–110°.

The filtrate from this recrystallization was shown to contain principally **3,7-dibenzylhypoxanthine (Va)**.

B. With Base and 3 Equiv of Benzyl Bromide.—A solution of 226 mg (1.00 mmole) of 7-benzylhypoxanthine (XII) and 513 mg (3.00 mmoles) of benzyl bromide in 20 ml of *N*,*N*-dimethylacetamide containing a suspension of 138 mg (1.00 mmole) of potassium carbonate was sitrred at 106° for 16 hr. The insoluble material was removed by filtration and the filtrate evaporated to dryness *in vacuo*. To remove unchanged starting material, a chloroform solution of the residue was washed with 1 *N* sodium hydroxide solution and then with water, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. The crude residue was further purified by thin layer chromatography using chloroform–methanol (19:1) as eluent. From the chromatographic plate was obtained the product as a light yellow glass: yield of **1-benzyl-4-benzylamino-5-(*N*-benzylformamido)pyrimidin-6(1*H*)-one (XIV)**: 120 mg (28%); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—229 (27.9) and 260 (6.90), pH 7—229 (28.1) and 260 (6.97), pH 13—229 (28.5) and 260 (7.25); σ, in cm^{-1}, 3400 and 3330 (NH), 3100–2840 (CH), 1675 and 1650 (C=O), 1610 and 1550 (pyrimidine ring).

C. Without Base and with 1 Equiv of Benzyl Bromide.—A suspension of 226 mg (1.00 mmole) of 7-benzylhypoxanthine in 40 ml of acetonitrile containing 171 mg (1.00 mmole) of benzyl bromide was refluxed for 18 hr. The solution was evaporated to dryness, and the residue was crystallized from 10 ml of ethanol: yield, 121 mg (30.5%). The ultraviolet and infrared spectra of this product were identical with those of authentic **7,9-dibenzylhypoxanthinium (XI) bromide**. The ethanol filtrate was found

to contain 174 mg of a mixture of **7,9-dibenzylhypoxanthine (XI) bromide, 7-benzylhypoxanthine (XII), 3,7-dibenzylhypoxanthine, 1,7-dibenzylhypoxanthine (IIIa), and 1,9-dibenzylhypoxanthine (IIa).**

1,7,9-Tribenzylhypoxanthinium Bromide (XIII). A.—A solution of 1.26 g (4.00 mmoles) of 1,7-dibenzylhypoxanthine (IIIa) and 2.05 g (12.00 mmoles) of benzyl bromide in 100 ml of acetonitrile was refluxed for 16 hr. The solution was evaporated to dryness *in vacuo* and the residue was triturated with ether. A glass that would not crystallize was obtained in quantitative yield.

The analytical sample was obtained by elution from a thick thin layer plate. It was dried for 16 hr at 78° (0.07 mm) over phosphorus pentoxide: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—257 (9.71), pH 7—257 (9.20), pH 13—262 (6.61) and 290 (sh) (4.14); σ, in cm^{-1}, 3100–2800 (CH), 1710 (C=O), 1630, 1600, 1590, 1565, 1540, and 1495 (purine and phenyl rings), 1455 (CH).

Anal. Calcd for $C_{26}H_{23}N_4OBr \cdot H_2O$: C, 61.79; H, 4.99; N, 11.09. Found: C, 61.48; H, 4.80; N, 10.93.

B.—A solution containing 316 mg (1.00 mmole) of 1,9-dibenzylhypoxanthine and 513 mg (3.00 mmoles) of benzyl bromide in 50 ml of acetonitrile was refluxed for 20 hr and then evaporated to dryness *in vacuo*. The product, a glass, was purified by thin layer chromatography: yield, 358 mg (75%). This material was found to be identical with that prepared from 1,7-dibenzylhypoxanthine (IIIa) as described in A above.

1,7,9-Tribenzylhypoxanthinium Formate.—A solution of 213 mg (0.5 mmole) of 1-benzyl-4-benzylamino-5-(*N*-benzylformamido)pyrimidin-6(1*H*)-one in 20 ml of 98% formic acid was refluxed for 3 days and evaporated to dryness *in vacuo*. The product was thus obtained as a nearly colorless glass: yield, 208 mg (92%); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—257 (10.8), pH 7—257 (10.8), pH 13—263 (7.35) and 284 (sh) (5.27). The infrared spectrum of the formate showed the expected similarities to the bromide described above.

1-Benzyl-4-benzylamino-5-(*N*-benzylformamido)pyrimidin-6-(1*H*)-one (XIV). A.—A solution of 2.17 g (4.45 mmole) of 1,7,9-tribenzylhypoxanthinium bromide in 25 ml of methanol was made basic (pH 10–11) with concentrated sodium hydroxide solution and evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of chloroform. This solution was washed with water, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. A yellow glass was obtained: yield, 1.18 g (62%), mp 98–105°; λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—229 (27.9) and 260 (6.90), pH 7—229 (28.1) and 260 (6.97), pH 13—229 (28.5) and 260 (7.25); σ, in cm^{-1}, 3400 and 3330 (NH), 3100–2840 (CH), 1675 and 1650 (amide C=O), 1610 and 1550 (pyrimidine ring).

Anal. Calcd for $C_{26}H_{25}N_4O_2$: C, 73.30; H, 5.92; N, 13.15. Found: C, 73.57; H, 5.82; N, 13.17.

B.—A solution of 1.26 g (4.00 mmoles) of 1,7-dibenzylhypoxanthine and 2.05 g (12.00 mmoles) of benzyl bromide in 80 ml of *N*,*N*-dimethylacetamide containing 552 mg (4.00 mmoles) of anhydrous potassium carbonate was stirred at 110° for 16 hr. The reaction solution was filtered and evaporated to dryness *in vacuo*. The residue was dissolved in chloroform. This solution was washed with water, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. A light yellow glass was obtained in quantitative yield. This material was identical in its ultraviolet and infrared spectra with those obtained as described in A above.

1,*N*-Dibenzyl-4-(*N*-benzylformamido)imidazole-5-carboxamide (XVI).—A solution of 316 mg (1.00 mmole) of 3,7-dibenzylhypoxanthine and 0.12 ml (1.00 mmole) of benzyl bromide in 25 ml of acetonitrile was refluxed for 5 hr and then evaporated to dryness *in vacuo*. Purification of the residue by thin layer chromatography gave a glass as the major product: yield, 216 mg (51%); λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—250 (sh) (5.67) and 257 (sh) (4.80), pH 7—250 (sh) (6.37) and 257 (sh) (5.53), pH 13—250 (sh) (6.43) and 257 (sh) (5.58); σ, in cm^{-1}, 3500–3300 (H_2O and NH), 3110, 3085, 3060, 3030, 2930, and 2870 (CH), 1660 (amide C=O), 1600 and 1590 (phenyl), 1560, 1525, and 1500 (imidazole ring), 1455 (CH); τ, in ppm, 5.70 d (CH_2 of benzylamide), 5.25 (CH_2 of benzyl formamido), 4.63 (CH_2 of 1-benzyl), 2.80 m (phenyl), 2.12 and 1.57 (C^2-H and formyl), 1.73 (amide NH). Upon the addition of D_2O the doublet at 3.70 ppm became a singlet and NH·D_2O exchange was evident.

Anal. Calcd for $C_{26}H_{24}N_4O_2 \cdot {}^7/_8H_2O$: C, 70.94; H, 5.90; N, 12.73. Found: C, 71.00; H, 5.62; N, 12.59.

In addition to the major product 55 mg (11.5%) of 1,7,9-tribenzylhypoxanthinium bromide was obtained.

1-Benzyl-2′,3′-isopropylideninosine (XVIIa).—Perchloric acid (13.6 ml of 70–72% acid) was added to a solution of 2,2-dimethoxypropane (10 ml) in anhydrous acetone (370 ml) and the mixture was stirred under anhydrous conditions for 5 min before 1-benzylinosine (10 g, 28 mmoles) was added rapidly to the solution. The resulting reaction solution was stirred at room temperature under anhydrous conditions for 40 min before it was neutralized with pyridine (15 ml). The mixture was evaporated to dryness and the residue was partitioned between chloroform (140 ml) and water (100 ml). The chloroform layer was washed with water, dried over magnesium sulfate, and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with boiling cyclohexane (four 50-ml portions) before it was solidified by trituration with ether. This solid was collected by filtration, washed with ether, and dried to give 9.5 g (85%), mp 156°, of blocked riboside suitable for use as an intermediate. The analytical sample was obtained by recrystallization of a sample (1.5 g) of the isolated product from benzene (20 ml): yield, 880 mg (58%); mp 157°. Thin layer chromatography using chloroform–methanol (19:1) as eluent showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—245 (sh), 252 (9.8), pH 7—245 (sh), 252 (9.6), pH 13—246 (sh), 252 (9.3); σ, in cm^{-1}, 3350 (OH), 3120, 3000, 2940 (CH), 1700, 1680, 1580, and 1545 (C=O, C=C, C=N), 1210 (COC), 1100 and 1080 (CO).

Anal. Calcd for $C_{20}H_{22}N_4O_5$: C, 60.35; H, 5.57; N, 14.08. Found: C, 60.27; H, 5.42; N, 14.09.

1-Benzyl-2′,3′-*O*-isopropylidine-5′-*O*-mesylinosine (XVIIb).—Methane sulfonyl chloride (1.6 ml) was added dropwise over a 15-min period to a cold (5°) continuously stirred solution of 1-benzyl-2′,3′-*O*-isopropylidininosine (XVIIa, 8 g, 20 mmoles) in anhydrous pyridine (90 ml). The cold reaction mixture was stirred continuously for 4 hr and refrigerated overnight before it was poured into water plus ice (1 l.). The gum that precipitated was triturated until it solidified. The solid was collected by filtration washed with water before it was dissolved in acetone, and filtered through dry Celite, and the filtrate was evaporated to dryness *in vacuo*. The residue was solidified by ether trituration to give the crude product which was further purified by magnetically stirring an ether (100 ml) suspension of it for several hours at room temperature. The resulting, finely divided, insoluble solid was collected by filtration and dried *in vacuo:* yield, 4.6 g (48%); mp <100°, indefinite. Thin layer chromatography using chloroform–methanol (15:1) as eluent showed a trace of starting compound as the only contaminant. The presence of a band at 1165 cm^{-1} in the infrared spectrum of this compound indicates that the sulfonate is covalent.[22]

The Anhydronucleoside from *N*-Benzyl-5-formamido-1-β-D-ribofuranosyl)imidazole-4-carboxamide (XIX).—An anhydrous mixture of 1-benzyl-2′,3′-*O*-isopropylidine-5′-*O*-mesylinosine (XVb, 2.38 g, 5 mmoles), sodium iodide (0.76 g, 5 mmoles), and *N*′,*N*′-dimethylformamide (15 ml) was heated with continuous stirring to 105° over a 15-min period. The resulting reaction solution was sitrred at 100–110° for 30 min before it was cooled to room temperature. The solid that precipitated on cooling was removed by filtration and the filtrate was concentrated to one-third volume before it was poured into water plus ice (50 ml). The crude product that precipitated was collected by filtration, washed with water, and recrystallized from ethanol to give the pure anhydronucleoside: yield, 440 mg (22%); mp 204°. Thin layer chromatography using chloroform–methanol (32:1) as the eluent showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—250 (br, sh) (10.3), pH 7—240–252 (plateau) (10.4), pH 13—262 (unstable) (11.1); σ, in cm^{-1}, 3380, 3110, 2990, 2940 (NH, CH), 1685 (C=O), 1655 (NH), 1600, 1520, 1210 (unassigned).

Anal. Calcd for $C_{20}H_{21}N_4O_5$: C, 60.50; H, 5.33; N, 14.11. Found: C, 60.36; H, 5.29; N, 14.45.

3,9-Dibenzyl-*N*,*N*-dimethyladeninium Bromide (XXI).—A solution of 9-benzyl-*N*,*N*-dimethyladenine (XX, 2 g, 7.9 mmoles), benzyl bromide (2 ml), and acetonitrile (75 ml) was refluxed for 3 days. The reaction solution was filtered through dry Celite and the filtrate was evaporated to dryness. The residue was triturated with ethanol–ether (1:9) and the solid that formed was collected by filtration and resuspended in ether (75 ml). After trituration of the ether suspension, the finely divided solid was collected by filtration, washed with ether, and dried to give 1.7 g (51%) of product suitable for use as an intermediate. Thin layer chromatography using ethyl acetate as the eluent showed a trace of starting compound as the only contaminant.

The analytical sample was prepared by dissolving a sample of the purified product (85 mg) in acetone, decolorizing the resulting solution with Norit, and crystallizing the pure product from the filtrate by the addition of ether: yield, 59 mg (70%); mp 220°; λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—274 (sh), 287 (18.5), pH 7—274 (sh), 286 (19.0), pH 13—unstable; σ, in cm^{-1}, 3030, 2960, 2920 (CH), 1625, 1550 (NH, C=C, C=N).

Anal. Calcd for $C_{21}H_{22}BrN_5$: C, 59.44; H, 5.23; N, 16.51. Found: C, 59.51; H, 5.27; N, 16.58.

1-Benzyl-5-benzylaminoimidazole-4-carboxamide (XXIIa).—A suspension of 3,9-dibenzyl-6-dimethylaminopurine bromide (XXI, 1.5 g, 3.5 mmoles) in 0.15 *N* NaOH (4 ml) was refluxed for 6 hr before it was allowed to stand at room temperature overnight. The solid that precipitated was collected by filtration, washed with water, and dried to give 765 mg (71%) of product suitable for use as an intermediate: mp 152°.

The analytical sample was obtained by recrystallizing a sample of the purified product (110 mg) from 50% aqueous ethanol (10 ml): yield, 60 mg (55%); mp 153°. A Bratton–Marshall test for diazotizable amino groups was negative. Thin layer chromatography using chloroform–methanol (15:1) showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—254 (7.3), pH 7, 13—269 (8.7); σ, in cm^{-1}, 3320, 3140, 3060, 3030 (NH, CH), 2960, 2920, 2860 (aliphatic CH), 1670, 1600, 1590 (C=O, NH); τ, in ppm, 5.74 d (CH_2 of benzylamino), 4.84 (CH_2 of 1-benzyl), 3.83 t (NH of amine), 3.13 (NH_2 of amide), 2.80 (phenyl), and 2.7 (C^2–H). Upon addition of D_2O the doublet at 5.74 ppm became a singlet and NH–D_2O exchange was evident.

Anal. Calcd for $C_{18}H_{18}N_4O$: C, 70.54; H, 5.92; N, 18.28. Found: C, 70.37; H, 5.76; N, 18.35.

1-Benzyl-5-(*N*-benzylformamido)imidazole-4-carboxamide (XXIIb). A.—A solution of 1-benzyl-5-benzylaminoimidazole-4-carboxamide (XXIIa, 100 mg, 0.33 mmole) in formic acid (3 ml) was refluxed for 6 hr before it was evaporated to dryness *in vacuo*. The residue was triturated with ethanol and ether and the insoluble solid was collected by filtration to give 85 mg (78%) of essentially pure product: mp 208°. Thin layer chromatography showed the presence of a small amount of 9-benzylhypoxanthine. Recrystallization of a sample of the isolated product (20 mg) from ethanol with Norit treatment gave the pure product: yield, 14 mg (70%); mp 208°. Thin layer chromatography using chloroform–methanol (15:1) as the eluent showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1,7,13—245 (sh); σ, in cm^{-1}, 3350, 3160, 3100, 3060, 3010, 2930 (NH, CH), 1690, 1670 (amide I), 1600, 1580 (C=C, C=N); τ, in ppm, 5.27 (CH_2 of both benzyl groups), 3.00 (NH), 2.77 (phenyl), 2.29 and 2.17 (C^2–H and formyl).

Anal. Calcd for $C_{19}H_{18}N_4O_2$: C, 68.24; H, 5.43; N, 16.76. Found: C, 67.99; H, 5.54; N, 16.82.

B.—A suspension of 1-benzyl-5-benzylaminoimidazole-4-carboxamide (XXIIa, 100 mg, 0.33 mmole) in diethoxymethyl acetate was stirred at room temperature for 2 days. Thin layer chromatography using chloroform–methanol (19:1) as eluent indicated the presence of two products and the absence of starting compound. The reaction mixture was evaporated to dryness and the residue triturated with ethanol–ether. The insoluble solid was collected by filtration, washed with ethanol–ether and dried *in vacuo* to give the crude product, which was purified by preparative thin layer chromatography on silica gel using chloroform–methanol (19:1) as eluent. The two major products that separated were extracted from the chromatographic layer with methanol. Evaporation of the methanol extracts to dryness gave the purified products which were identified as **1-benzyl-5-(*N*-benzylformamido)imidazole-4-carboxamide** (60% of the crude product) and **9-benzylhypoxanthine** (20% of crude product) by comparison of their spectra with those of known compounds.

5-Amino-1,*N*-dibenzylimidazole-4-carboxamide (XXIII).—A solution of 1,9-dibenzylhypoxanthine (IIa, 250 mg, 0.79 mmole), 6 *N* NaOH (1.8 ml), and ethanol (100 ml) was refluxed for 3 hr. The solid that precipitated from the reaction mixture after partial neutralization (to pH 8 with concentrated HCl) was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. Trituration of the residue with water gave the crude product (235 mg, 97%). The crude product was recrystallized from ethanol (5 ml) to give the pure material: yield, 167 mg (69%); mp 161°. A Bratton–Marshall test for a diazotizable amino group was positive. Thin layer chromatography using chloroform–methanol (19:1) as the eluent showed a single spot: λ_{max}, in mμ ($\epsilon \times 10^{-3}$), pH 1—243 (10.7), 268 (13.3), pH 7—268 (16.8), pH 13—267.5 (16.6); σ, in cm^{-1}, 3405, 3270, 3210, 3150 (NH), 3015, 2895, 2850 (CH), 1615, 1610, 1580 (NH, C=C, C=N), τ, in ppm, 5.61 d (CH_2 of benzylamide), 4.92 (CH_2 of 1-benzyl), 4.20 (NH_2), 2.83 (C^2–H), 2.75 (phenyl), 2.12 t (amide NH). Upon the addition of D_2O the doublet at 5.61 ppm became a singlet and NH–D_2O exchange was evident.

Anal. Calcd for $C_{18}H_{18}H_4O$: C, 70.56; H, 5.93; N, 18.29. Found: C, 70.28; H, 5.97; N, 18.36.

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Studies on the Azidoazomethine–Tetrazole Equilibrium. V. 2- and 6-Azidopurines[1]

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The azidoazomethine–tetrazole equilibrium in seven new 2- and 6-azidopurine systems is examined. Results indicate that this equilibrium is solvent dependent, that the tetrazolo tautomer is stabilized by electron-donating groups, and that the azido tautomer is stabilized by electron-withdrawing groups. In addition, hydrolysis of the azido group or cleavage of the pyrimidine ring were the major reactions resulting from the treatment of the 6-azidopurine systems with aqueous acid or base.

In a recent paper we reported some observations on the azidoazomethine–tetrazole equilibrium for the systems involving 2- and 6-azidopurine.[2] Some substituted systems have now been prepared to examine the effect of solvent and of certain electron-donating and electron-attracting groups on this equilibrium. The results indicate that the azidoazomethine–tetrazole equilibrium is quite mobile, and furthermore that the use of chemical methods to assign either the azido or tetrazolo structure may not always be valid.[3] As in

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