SYNTHESIS OF SOME ALKYLATED ADENINES AS POTENTIAL ANTIMETABOLITES

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ABSTRACT

Several substituted adenines and (or) their salts have been prepared by condensing adenine in dimethylformamide with the following halides: benzyl chloride, *p*-methylbenzyl chloride, *n*-propyl bromide, (3-chloropropyl) benzene, α-bromo-γ-butyrolactone, allyl bromide, and cinnamyl chloride. The reaction may be carried out in large quantities and the salts were isolated in high yields.

Characterization of these compounds indicated that they are pure 3-substituted adenines with the possible exception of the allyl and the *n*-propyl derivatives, which may contain little, if any, of the 7- or 9-isomers.

It is well known that derivatives of adenine, such as 6-mercaptopurine, have interesting antimetabolic activities. This paper reports the synthesis of N-alkylated purines to evaluate their biological activities.

Within the recent past, various workers have succeeded in alkylating purines on the basic nitrogens of the heterocyclic rings (1, 2, 3). Some of these compounds have also been isolated as natural products (4–12). Most of the laboratory alkylation studies of purine derivatives have, to date, been achieved in basic media. Recently, however, workers have reported that the alkylation of purines occurs in dimethylformamide or dimethylacetamide without the addition of a basic reagent (1, 2). However, there still exists confusion concerning the site of alkylation. In a recent paper, Pal (13) states that "no correlation between alkylation sites and theoretically predicted relative basicities of the ring nitrogen atoms of adenine was observed." Recently, however, it has been proved by several authors that of all the common purines, adenine is the only one which is alkylated in position 3 (1), in neutral medium.

We have prepared several of these substituted adenines by reacting adenine with excess of an alkyl halide in dimethylformamide. Various halides were chosen, to include a variety of reactivity ranging from highly reactive, such as allyl bromide, to weakly reactive, such as *n*-propyl bromide (see Table I). In all cases, the yields were very good and the compounds could be isolated in fairly large quantities. We have isolated all of our compounds directly from the reaction mixture as the corresponding ammonium salts; these were obtained by removal of most of the solvent and trituration of the residue with acetone. Thus 3-benzyl adenine hydrochloride, 3-(*p*-methylbenzyl) adenine hydrochloride, 3-cinnamyl adenine hydrochloride, 3-(3′-phenyl-*n*-propyl) adenine hydrochloride, 3-(γ-butyrolactonyl-α) adenine hydrobromide, allyl adenine hydrobromide, and *n*-propyl adenine hydrobromide were isolated and characterized. In four cases, we isolated the free bases by neutralizing the salts with sodium hydroxide solution. Leonard and Fujii (2) recently reported the isolation of the free bases 3-benzyl adenine and 3-allyl adenine. The 3-benzyl adenine had also been synthesized previously by Denayer (14).

All of the analyses (C, H, N, halogen, titration) agree with the theoretical values, thereby suggesting purity of the compounds. However, because of the numerous possibilities that alkylation can take place at positions 1, 3, 7, or 9, we cannot disregard the

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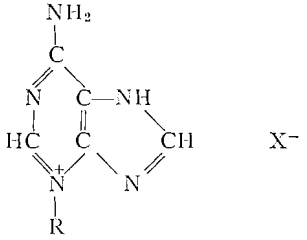
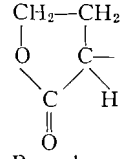
TABLE I

Preparation of 3-substituted adenine salts



X^-

	R	Adenine (g)	Halide (ml)	DMF (ml)	Reflux time (h)	Yield (g)	Yield (%)	M.p. (°C)
I		5.0	α-Bromo-γ-butyrolactone (60)	215	1/4	9.5	86	>300
II	Benzyl	2.0	Benzyl chloride (17)	100	1/4	2.7	70	261–267
III	*p*-Methylbenzyl	1.5	*p*-Methylbenzyl chloride (10)	70	1/4	1.5	49	277–282
IV	*n*-Propyl*	20.0	*n*-Propyl bromide (250)	1000	3	20.0	52	252–253
V	3′-Phenyl-*n*-propyl	20.0	(3-Chloropropyl) benzene (100)	1000	3	22.0	45	269–273
VI	Allyl*	40.0	Allyl bromide (340)	2000	1/4	50.0	66	196–197
VII	Cinnamyl	22.0	Cinnamyl chloride (100)	1000	1/4	21.0	45	237–244

*Isomers present, therefore not all 3-substitution.

presence of two or several isomers. From the evidence accumulated by several authors (1, 2, 3, 13, 14, 15), we have assigned the alkylated chain on the 3-position of the adenine nucleus. The benzyl adenine which we have obtained has a melting point of 284–287 °C compared to 278–280 °C for 3-benzyl adenine reported by Leonard and Fujii (2). We were able to note a slight difference between the ultraviolet data for the free bases and their respective hydrohalides—in general, the free bases absorbed at a slightly lower wavelength than their corresponding hydrohalides. The pK_a' values of cinnamyl adenine and (3′-phenyl-*n*-propyl) adenine were 5.13 and 5.23 respectively, indicating that in both cases, the substitution on the adenine nucleus is in position 3 (3, 15). The allyl adenine and *n*-propyl adenine likewise showed pK_a' values of 5.43 and 5.45 respectively.

From the recent literature (1, 2, 3, 13, 14, 15) and our own work, it would seem that all of our compounds are 3-substituted adenines. To verify the presence or absence of isomers, all the compounds were analyzed by paper chromatography and electrophoresis in different buffers. This last method has proved to be very useful in observing the isomers which have different pK_a' values (see Fig. 1). All of the compounds as isolated showed

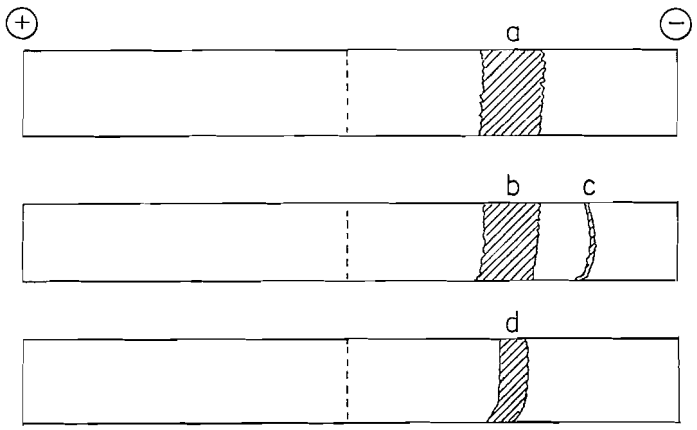


FIG. 1. Electrophoretic migration of adenine and alkylated adenines in buffer pH 1.9, 2 h at 10 mA. The spots are located by means of an ultraviolet lamp: (*a*) pure adenine, (*b*) 3-allyl adenine hydrobromide, (*c*) an uncharacterized allyl adenine isomer, and (*d*) 3-benzyl adenine hydrochloride.

one spotting except the following two: allyl adenine hydrobromide and *n*-propyl adenine hydrobromide. These two compounds showed two spots with both paper chromatography and electrophoresis. Since the elemental analyses and the titration data agreed for purity, the second spot which is somewhat weaker in intensity is obviously due to the presence of a position isomer. No attempts were made to identify the second isomers in question. For both the allyl and the *n*-propyl compounds, the ultraviolet data (λ_{max} = 277 mμ for both) and the pK_a' data (5.43 and 5.45 respectively) point to the fact that these compounds are both primarily 3-substituted.

Before realizing that we were dealing with a mixture of isomers, we prepared derivatives of the allyl compound. Treatment of this compound in acetic acid solution with bromine furnished the perbromide of (2,3-dibromopropyl) adenine hydrobromide (VIII). On heating, the bromine in the perbromide bonding was eliminated and (2,3-dibromopropyl) adenine hydrobromide (IX) resulted. Neutralization with aqueous sodium hydroxide yielded the free base (X). In all of these compounds the ultraviolet absorption peak remained virtually unchanged, indicating that the purine nucleus was not disrupted.

Elemental analyses and titration data also all agreed. As with the starting material, paper chromatography and electrophoresis showed the presence of a major compound and a minor one (obviously a position isomer).

It is logical that allyl adenine hydrobromide should add bromide on the exocyclic olefinic bond and thus compounds IX and X are easily understood. Evidence for the existence of the heat labile perbromide (VIII) lies in the fact that the compound separates from solution as beautiful orange-colored crystals for which analyses of nitrogen and bromine agreed; the ultraviolet absorption spectra are also similar to that of IX, X, and the starting material. The heat lability of two bromine atoms further suggests that this is really a perbromide. It has similar properties with the perbromide of 2 (or 8)-bromoadenine first reported by Bruhns and Kossel (16).

We are presently doing extensive biological investigation of all the pure compounds.

EXPERIMENTAL

The carbon and hydrogen analyses were done by C. Daessle, Micro Analyses, Montreal. The nitrogen analyses were performed in our laboratory by the Kjeldahl procedure. The acid titrations were done by merely dissolving the compound in water and titrating with 0.1 *M* aqueous sodium hydroxide using phenolphthalein as indicator. The ultraviolet spectra were obtained in ethyl alcohol solution using a Cary Model 15 spectrophotometer. The melting points were determined on a Fisher–Johns melting point apparatus and were not corrected. All paper chromatograms were developed with the organic layer formed from a mixture of 1-butanol, water, and glacial acetic acid (75:20:5). Development was performed by the ascending method in a chamber saturated with solvent vapor. Comparison of samples were made on the same sheet and not by R_f values. Spots were detected by means of ultraviolet absorbance.

All electrophoreses were carried out using a Spinco Duostat apparatus and employing 10 mA for a period of 2 h. Two buffers were used, one at pH 1.9 (98–100% formic acid, glacial acetic acid, and water, 1:4:28) and one at pH 8 (0.667 *M* Na_2HPO_4 – 0.05 *M* borate – KCl – NaOH, 9:10). The pK_a' data for 3-cinnamyl adenine and 3-(3′-phenyl-*n*-propyl) adenine was obtained in dimethyl formamide (DMF), water solution (1:1). In the case of the allyl and *n*-propyl compounds, the salts were dissolved in water, titrated to neutralization with aqueous sodium hydroxide, and, since the free base did not precipitate, the solution was then used as such for pK_a' investigation.

Condensation of Adenine with Alkyl Halides

The halogen compound was added to a stirred solution of adenine in dimethyl formamide solvent at the reflux temperature. The resulting solution was refluxed for some time, then the solvent was removed by vacuum and gentle heat. Trituration of the residue with acetone yielded the ammonium salt in high purity. Table I gives the results obtained with seven different alkyl halides.

The products were analyzed for C, H, N, and either HCl or HBr. Ultraviolet spectral analysis was carried out on each compound; a 1% solution employing ethyl alcohol as solvent was used. Table II contains this data. Paper chromatography and electrophoresis were carried out on all compounds (salts and bases); only in the cases of the allyl and propyl compounds were two spots present. The pK_a' values of four of the compounds were determined as previously mentioned. The free bases 3-benzyladenine, 3-(*p*-methylbenzyl) adenine, 3-(3′-phenyl-*n*-propyl) adenine, and 3-cinnamyl adenine were obtained on neutralization in water of the acid salts. Table III lists the data obtained.

Reaction of Allyl Adenine Hydrobromide with Bromine

Two grams of allyl adenine hydrobromide were dissolved in 80 ml of boiling glacial acetic acid. After the solution was cooled to room temperature, bromine in glacial acetic acid was added. At first, the bromine was decolorized; the bromine solution was added until a slight excess was present. The total resultant solution was about 100 ml. Slight cooling caused precipitation. A 2.7 g yield of orange-colored crystals (perbromide of N-(2′,3′-dibromopropyl) adenine hydrobromide) (VIII) was obtained. These crystals began to change color from orange to white at about 130° and the white crystals subsequently melted with decomposition at 200–207°. Anal. Calc. for $C_8H_{10}N_5Br_3$: N, 12.15; Br, 69.28. Found: N, 12.40; Br, 68.68. The ultraviolet spectrum of this compound in ethyl alcohol showed a λ_{max} at 278 mμ with an ϵ of 19 100.

The above perbromide (800 mg) was dissolved in 25 ml of hot glacial acetic acid. This was heated and distilled. The distillate passed over at 118°; it was reddish in color and probably consisted of bromine and acetic acid. The residue in the distillation flask gradually decolorized from red to colorless. The distillation was stopped when the distillate was completely colorless (about 10 ml remained in the residue). This residue was cooled whereupon a white precipitate formed. This was filtered, washed with ether, and dried. A 550 mg yield of a product melting at 200–208° was obtained (N-(2′,3′-dibromopropyl) adenine hydrobromide) (IX).

TABLE II

Analyses of 3-substituted adenine salts

Product	% C		% H		% N		Acid titration (equivalent NaOH)	Ultraviolet	
	Calc.	Found	Calc.	Found	Calc.	Found		λ_{max} (mμ)	$10^{-4}\epsilon$
I	35.98	35.78	3.36	3.58	23.33	23.12	1.99	261	2.26
II	55.07	54.80	4.62	4.62	26.76	26.63	1.01	278	1.74
III	56.62	56.86	5.12	5.36	25.40	25.22	1.00	278	1.97
IV	37.22	36.88	5.04	4.69	27.13	26.86	1.00	277	1.33
V	58.02	58.26	5.55	5.78	24.17	23.79	1.00	277	1.64
VI	37.51	37.73	3.94	3.97	27.35	26.68	0.95	277	1.64
VII	58.43	58.38	4.90	4.98	24.34	24.15	1.00	256	2.21
								276	2.24

TABLE III

3-Substituted adenine free bases

Product	Yield (%)	M.p. (°C)	% N		Ultraviolet		pK_a'
			Calc.	Found	λ_{max} (mμ)	$10^{-4}\epsilon$	
3-Benzyl adenine*	59	284–287	31.09	30.84	274	1.25	—
3-(*p*-Methylbenzyl) adenine	61	284–286	29.27	29.00	273	1.35	—
3-(3′-Phenyl-*n*-propyl) adenine	88	248–253	27.13	26.84	275	1.22	5.23
3-Cinnamyl adenine	57	260–263	27.87	27.67	256	2.48	5.13
					276 shoulder		

*This compound has already been reported by: Leonard and Fujii (2) m.p. 278–280°, Denayer (14) m.p. 278–279°.

Anal. Calc. for $C_8H_{10}N_5Br_3$: N, 16.84; Found: N, 16.64. Titrations: 1 equivalent of NaOH needed for neutralization. The ultraviolet spectrum of this compound in ethyl alcohol also showed a λ_{max} at 277 mμ with an ϵ of 16 700. On neutralization of the above compound with aqueous sodium hydroxide, a white precipitate formed (N-(2′,3′-dibromopropyl) adenine) (X). This was obtained in 50% yield and had a melting point of >300°. Anal. Calc. for $C_8H_9N_5Br_2$: C, 28.67; H, 2.71; N, 20.91; Br, 49.79. Found: C, 28.85; H, 2.50; N, 20.73; Br, 49.36. The ultraviolet spectrum again had a λ_{max} of 280 mμ and an ϵ value of 12 500.

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REFERENCES

1. J. W. Jones and R. K. Robins. J. Am. Chem. Soc. **84**, 1914 (1962).
2. N. J. Leonard and T. Fujii. J. Am. Chem. Soc. **85**, 3719 (1963).
3. N. J. Leonard and J. A. Deyrup. J. Am. Chem. Soc. **84**, 2148 (1962).
4. B. Weissman, P. A. Bromberg, and A. B. Gutman. J. Biol. Chem. **224**, 407 (1957).
5. M. Adler, B. Weissman, and A. B. Gutman. J. Biol. Chem. **230**, 435 (1958).
6. J. D. Smith and D. B. Dunn. Biochem. J. **72**, 294 (1959).
7. J. D. Dunn and D. B. Smith. Biochem. J. **68**, 627 (1958).
8. J. W. Littlefield and D. B. Dunn. Biochem. J. **70**, 642 (1958).
9. D. Ackermann and P. H. List. Z. Physiol. Chem. **309**, 286 (1957).
10. D. Ackermann and P. H. List. Z. Physiol. Chem. **318**, 281 (1960).
11. D. B. Dunn. Biochim. Biophys. Acta, **46**, 198 (1961).
12. D. Ackerman and P. H. List. Naturwissenschaften, **48**, 74 (1961).
13. B. C. Pal. Biochemistry, **1**, 558 (1962).
14. R. Denayer. Bull. Soc. Chim. France, 1358 (1962).
15. R. Denayer, A. Cave, and R. Goutarel. Compt. Rend. **253**, 2994 (1961).
16. G. Bruhns and A. Kossel. Z. Physiol. Chem. **16**, 5 (1891).