The Action of Chloroformates on Adenine (1a,b)

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Reactions of adenine with benzyl chloroformate were described by Altman and Ben-Ishai (2,3) and with ethyl chloroformate by Dyer, Reitz, and Farris (4). Observations in our laboratory that these reactions are more complex than originally reported led to further investigation. The present paper reports a reassignment of the structure of an isomer obtained from adenine and benzyl chloroformate, and in the ethyl chloroformate reaction, the formation of an additional isomer and of a new tricyclic compound.

Altman and Ben-Ishai (3) obtained two isomeric benzyl carboxylate derivatives from the reaction of benzyl chloroformate with adenine. One isomer, formed in ethanolic sodium ethylate, showed ultraviolet absorption at 254 nm and was postulated to be benzyl 6-aminopurine-9-carboxylate. This structure was confirmed in the present work by preparation of the compound **1** from the thallium salt of adenine, known to direct substitution to the 9-position (5).

The second isomer (**2**), formed in aqueous ethyl acetate with potassium acetate as the base, absorbed at 291 nm and was originally proposed (3) to be a 7-substitution product. However, the following spectral properties suggested that **2** is a 3-substituted adenine: a) the large difference (31 cps) in the shifts of the 2- and 8-protons in the nmr spectra, b) the relatively high maximum in the uv absorption, and c) the large negative value of -13 for the difference in uv absorption minima in acid and neutral solutions. These properties are analogous to those of 3-alkyladenines (6,7). Efforts to prepare a third isomeric benzyl carboxylate of adenine were unsuccessful. The use of sodium hydride or triethylamine in dimethylformamide as condensing agents gave mixtures of the 3- and 9-isomers, identified by the presence in the nmr spectra of two sets of 2- and 8-protons, one with a $\Delta\delta$ value of 15 cps (9-isomer) and the other with a $\Delta\delta$ value of 31 cps (3-isomer).

Of interest in connection with the formation of both isomers is the observation that the 3-carboxylate was converted to the 9-carboxylate by heating in dimethylsulfoxide solution at 60° for a short time. This is a somewhat more facile change than the N-3 to N-9 migrations of alkyl groups observed (8) with various adenine derivatives.

The reaction of ethyl chloroformate with adenine in the presence of aqueous base gave a product (**3**) that was assigned (4) the structure ethyl 6-aminopurine-9-carboxylate. Recently Chheda and Hong proved (9) that this structure was correct by the thallium synthesis. Meanwhile reinvestigation of the ethyl chloroformate-adenine reaction in our laboratory under various conditions showed that the product usually was a mixture of the 9-carboxylate with a small amount of another isomer, postulated to be the 3-carboxylate by the similarity of its spectral properties to those of benzyl 6-aminopurine-3-carboxylate (Table I).

TABLE I

Spectral Properties of Adenine Carboxylates

	$CO_2CH_2C_6H_5$		$CO_2C_2H_5$	
Position	9	3	9	3
Uv λ max, nm	257 (a)	291 (b)	254 (c)	288 (c,d)
Nmr $\Delta\delta$ (2H, 8H), cps	15	31	14 (e)	29 (d)

(a) In 95% ethanol. (b) In acetonitrile. (c) In water. (d) Observed only in mixtures. (e) Both this work and ref. (9).

Unlike the 3-benzyl carboxylate of adenine, the 3-ethyl analog could not be easily separated from these mixtures, nor obtained pure by varying the base in the reaction medium. The use of sodium hydride or triethylamine in dimethylformamide or sodium ethylate in ethanol gave mixtures of the 3- and 9-ethyl isomers. The absence of adenine in these purified mixtures was confirmed by nmr.

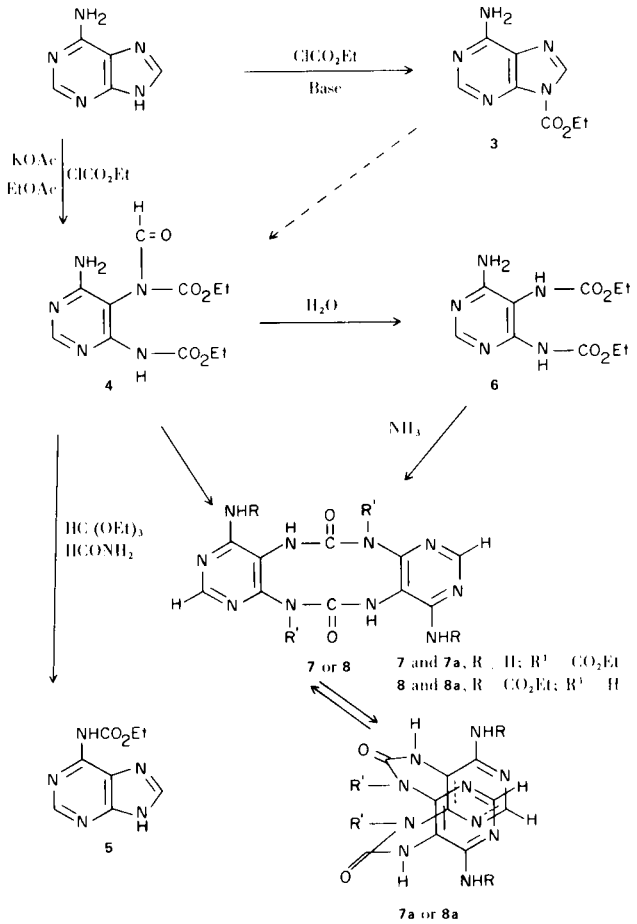
In one experiment, not duplicated, the pure ethyl 6-aminopurine-9-carboxylate (**3**) was isolated in 15% yield by using a Grignard reagent, followed by ethyl chloroformate, as done to prepare ethyl imidazole-*N*-carboxylate (10). The difficulty of obtaining pure **3** from adenine and ethyl chloroformate in the presence of bases is in contrast to the behavior of 6-dimethylaminopurine, which gave the corresponding 9-carboxylate in excellent yield (11).

When adenine was treated with ethyl chloroformate in mixtures of ethyl acetate and aqueous potassium

acetate, a medium used by Altman and Ben-Ishai for carbobenzyloxylation (2,3), a Bamberger reaction occurred, giving compound **4**. The nmr spectrum of **4** included signals of δ 9.35 ppm, assigned to the formyl group and at 7.60 ppm, assigned to the C-2 proton of the pyrimidine ring. The formyl group is postulated to be at the 5-position because of the formation of the known 6-ethoxycarbonylaminopurine **5** (12), by treatment of **4** with a mixture of formamide and ethyl orthoformate. If the formyl group were at the 6-position, as proposed (2) for a Bamberger product from 9-benzyladenine, the cyclization of **4** to **5** would be difficult to explain.

The mechanism of the Bamberger reaction probably involves the initial formation of **3**, followed by further substitution and quaternization of the imidazole ring, as suggested for benzimidazole (13). Evidence for the intermediate formation of **3** is the isolation of **3** together with **4** from a reaction done at a slightly higher *p*H.

The formyl group of **4** was easily removed by hot water to give 4-amino-5,6-di(ethoxycarbonylaminopyrimidine) **6**, identified by spectral properties and analysis.



The action of ammonia on either **4** or **6** gave a tricyclic compound of molecular formula $C_{16}H_{18}N_{10}O_6$, shown by analyses for four elements and the mass spectrum. Since **6** was converted to the tricyclic compound in 75% yield, **6** is probably the primary precursor. The presence of two carbonyl groups is indicated by the ir spectrum and the nmr spectrum shows only one ethyl group and one aromatic proton. These data require a symmetrical molecule formed by condensation of two units of **6**; either structure **7** or **8** (14) is possible. In structure **8**, but not in **7**, the 4-amino group is involved in the cyclization. Although the amino nitrogen would be expected to be more nucleophilic than the carbamate nitrogen, the reverse has been demonstrated in the intramolecular cyclization of 4-amino-5-carbobenzoxyamino-6-benzylaminopyrimidine (2). In the present problem the available evidence does not permit an unequivocal distinction between structures **7** and **8**.

The formation of this energetically unfavorable ten-membered ring in good yield may have been assisted by forces resulting from stacking of the pyrimidine rings as in structures **7a** and **8a**. Use of the Stuart-Briegleb Models showed that these formulas are sterically feasible. Base-stacking in pyrimidines has been used to explain a steric preference in other cases of ring formations (15).

EXPERIMENTAL

Melting points (corrected) were determined with a Fisher-Johns apparatus or a duPont 900 Differential Thermal Analyzer, uv spectra on a Perkin-Elmer 202 spectrophotometer, ir spectra on a Perkin-Elmer 137 spectrophotometer (in potassium bromide), nmr on a Varian A-60A spectrophotometer using dimethylsulfoxide-d_6 as solvent and TMS as internal standard, and mass spectra on a CEC 21-110B double-focus mass spectrometer.

Benzyl 6-Aminopurine-9-carboxylate (**1**).

A stirred suspension of 3.0 g. (0.009 mole) of the thallium salt of adenine (**5**) in 35 ml. of dimethylformamide was cooled to -35°, and 2.5 ml. (0.015 mole) of benzyl chloroformate was added dropwise from a syringe. After the mixture was kept at -10 to 0° for 1.5 hours, then at room temperature for 8 hours, the suspended solid was filtered off and discarded. The filtrate was poured into 200 ml. of cold water, a fine precipitate filtered, and the filtrate extracted with four 100 ml. portions of chloroform. Evaporation of the extracts gave 0.31 g. (16%) of **1**, m.p. 163-164° dec. (lit. (3) 162-163°); uv λ max (95% ethanol) 257 nm, (*p*H 1) 260 nm; ir 3300, 3050, 1750, 1665, 1160, 800, 770, 700 cm^{-1}; nmr δ 5.61 (s, 2, C*H*$_2$), 753 (m, 7, C_6H_5 and N*H*$_2$), 8.38 (s, 1, C*H*), 8.63 (s, 1, C*H*).

The same compound was prepared from benzyl chloroformate in dimethylformamide with adenine and sodium hydride in 36% yield.

Benzyl 6-Aminopurine-3-carboxylate (**2**).

This compound, obtained by the method of Altman and Ben-Ishai (3) was identical with theirs in melting point (149-150° dec.) and λ max (291 nm); λ min (*p*H 1) - λ min (*p*H 7),

-13; nmr δ 5.55 (s, 2, CH_2), 7.48 (m, 5, C_6H_5), 8.37 (s, 1, CH), 8.88 ppm (s, 1, CH); ir 3350, 3050, 1750, 1110, 800, 750, 695 cm^{-1}.

Compound **2** was isomerized by heating a stirred solution of 0.72 g. of **2** in 50 ml. of dimethylsulfoxide at 60° for 30 minutes. When the solution was poured into cold water, a precipitate of 0.58 g. (80%) of **1**, m.p. 163-164° dec. was obtained.

Ethyl 6-Aminopurine-9-carboxylate (**3**).

A suspension of 3.37 g. (0.025 mole) of adenine in tetrahydrofuran was slowly added to a solution of 6.2 g. (0.05 mole) of ethyl magnesium bromide in 15 ml. of tetrahydrofuran. The suspension was stirred at reflux until a clear solution was obtained, then cooled to 10°. A solution of 6.8 g. of ethyl chloroformate (0.63 mole) in 5 ml. of tetrahydrofuran was added dropwise, with stirring, after which the reaction was poured over ice. The crude product (2 g.) was recrystallized from 150 ml. of ethyl acetate to give 0.8 g. (15%) of pure **3**, m.p. 158-160° dec.; uv λ max (water) 255 nm. The ir and nmr spectra were the same as those reported (9). This substance was stable at room temperature for at least 4 years, but heating a sample in an nmr tube at 80° caused partial decomposition to ethanol and adenine.

Reinvestigation of the reaction product from adenine with ethyl chloroformate in aqueous base (4) showed that two isomers were present after recrystallization from tetrahydrofuran and from ethanol: the 9-isomer, nmr (2H, 8H) δ 8.50 and 8.27 and the 3-isomer, δ 8.78 and 8.30. Addition of adenine to the above mixture showed signals corresponding to the C-2 and C-8 protons of adenine at δ 8.15 and 8.10 ppm.

The same mixture of 9- and 3-isomers was obtained when the reaction media were dimethylformamide with sodium hydride or triethylamine, and ethanol with sodium ethoxide. In all cases, yields were low and separation of isomers practically impossible.

4-Amino-5-*N*-formylethoxycarbonylamino-6-ethoxycarbonylaminopyrimidine (**4**).

To 250 ml. of 0.2 *M* aqueous potassium acetate were added 5.0 g. (0.037 mole) of adenine and 200 ml. of ethyl acetate. The mixture was stirred, cooled to 14° and ethyl chloroformate (a total of 20.4 g., 0.19 mole) was added in three portions at 15 minute intervals. Thirty minutes after the last addition, the mixture was cooled to 0°, the precipitate filtered, washed with cold water, dried and recrystallized from 600 ml. of ethyl acetate to give 2.2 g. (20%) of **4**, m.p. 203° dec.; uv λ max (water) 205 nm; nmr δ 9.35 (s, 1, CHO), 7.60 (s, 1, CH), 4.18 (2q, 4, J = 6 Hz, 2 CH_2), 1.25 (2t, 6, J = 6 Hz, 2 CH_3).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.43; H, 5.09. Found: C, 44.66; H, 5.22.

A two-fold scale-up of the above reaction in a 2-l. Morton flask gave a yield of 25% plus 10% of compound **5** from the filtrate. At a *p*H of 9.3, the reaction mixture gave a product containing 15% of **4** and 20% of **3** (estimated from nmr).

Conversion of **4** to 6-Ethoxycarbonylaminopurine (**5**).

A mixture of 30 ml. of ethyl orthoformate and 30 ml. of formamide was heated with stirring until only one phase was present. After 1.5 g. of **4** was added, the mixture was refluxed at 80° for 20 minutes, concentrated *in vacuo* to one-half its volume and cooled. The product, 0.6 g. (55%) of **5**, was recrystallized four times from ethyl acetate to give 0.3 g. (27%) of pure **5**, identical in physical properties with **5** obtained by other methods (9,12). From the mother liquor 0.05 g. of **7** was obtained; the total products accounted for 85% of the starting material.

4-Amino-5,6-di(ethoxycarbonylamino)pyrimidine (**6**).

A suspension of 2 g. of **4** in 300 ml. of boiling water was heated for about 5 minutes. The nearly clear solution was filtered hot and cooled. The precipitate of **6**, which needed no further purification, weighed 0.65 g. (36%), m.p. 205° dec.; uv λ max (water) 235, 302 nm; nmr δ 7.18 (s, 1, CH), 4.18 (2q, 4, J = 6 Hz, 2 CH_2), 1.25 (2 t, 6, J = 6 Hz, 2 CH_3); mass spectrum (70 ev) m/e 269, 223, 177, 151, 135, 108, 81.

Anal. Calcd. for $C_{10}H_{15}N_5O_4$: C, 44.56; H, 5.61; N, 26.09. Found: C, 44.31; H, 5.97; N, 26.26.

Tricyclic Compound: 5*H*,12*H*-4,11-Diamino-7,14-di(ethoxycarbonyl)bispyrimido[4,5-*d*][4,5-*i*]-1,3,6,8-tetrazecine-6,13-dione (**7**) or 5*H*,7*H*,12*H*,14*H*-4,11-di(ethoxycarbonylamino)bispyrimido[4,5-*d*][4,5-*i*]-1,3,6,8-tetrazecine-6,13-dione (**8**).

A mixture of 2 g. of **4**, 200 ml. of water and 20 ml. of 6 *N* ammonium hydroxide was refluxed for 15 minutes. Concentration to a volume of 75 ml. by flash evaporation gave a somewhat colloidal precipitate, which was coagulated by heating on a steam bath for 15 minutes. The product, 1.09 g., was recrystallized from water (0.1 g./100 ml.) to give a 60% yield of the tricyclic compound, m.p. 230° dec.; uv λ max (water) 309 nm (ε, 5,800); ir 1700, 1650 (C=O); nmr δ 8.08 (s, 1, CH), 4.15 (q, 2, J = 6 Hz, CH_2), 1.25 (t, 3, J = 6 Hz, CH_3); mass spectrum (70 ev) m/e (rel. intensity) 446 (10), 413 (10), 399 (8), 307 (12), 280 (20), 223 (30), 177 (100), 151 (40), 135 (50), 120 (50), 109 (30).

Anal. Calcd. for $C_{16}H_{18}N_{10}O_6$: C, 43.06; H, 4.04; N, 31.39; O, 21.51. Found: C, 43.05; H, 4.12; N, 31.59; O, 21.76.

The tricyclic compound was also obtained in 75% yield by treating **6** in the same way.

An effort was made to distinguish structure **7** from **8** by nmr spectra. Although the -NH and $-NH_2$ protons of adenine are identifiable by their nmr peaks, there was no signal for these protons in the nmr spectrum of the tricyclic compound. Even in the spectrum of the simpler compound **6**, the signals for the -NH and $-NH_2$ protons were incomplete and too poorly differentiated to report.

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(1a) This work was partially supported by Public Health Service Research Grant R 01-CA03477 from the National Cancer Institute. (b) Taken from the M.S. Theses of David B. Smith (1968), George C. Emmett (1969), and Deborah Meeder (1971), University of Delaware. Certain erroneous conclusions in the earlier theses were corrected by D. M.

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