Acylation of Nitrogen Heterocycles. II (1a). Carbobenzoxylation of Substituted Adenines under the Conditions of the Schotten-Baumann Reaction (1b).

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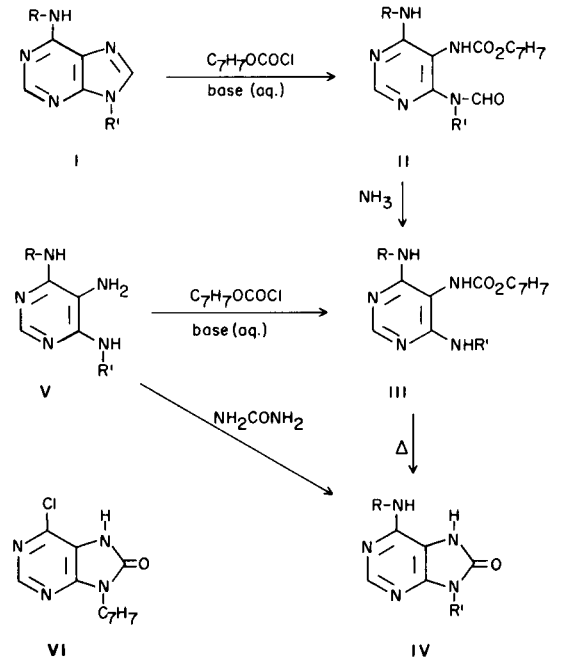
9-Benzyladenine, 6-benzylamino-9-benzyladenine, 6-piperidino-9-benzyladenine and 2′,3′-*O*-isopropylidene-5′-trityladenosine underwent a Bamberger fission of the imidazole ring when treated with carbobenzoxy chloride in aqueous base. Under similar experimental conditions adenine itself afforded two monoacyl derivatives.

Benzoylation of adenine with benzoic anhydride under anhydrous conditions has long been known to give 6-benzamidopurine (2,3). Fusion of adenine with other acid anhydrides or refluxing it with the anhydride in xylene or toluene readily yields the 6-acylaminopurine (2). In a number of cases diacyladenines were also obtained. It is assumed that the second acyl group is at position 7 or 9 of the imidazole ring (4,5). Adenosine derivatives afforded on benzoylation with excess benzoyl chloride in pyridine, benzoyl derivatives, the structures of which were not fully established (7,8). Treatment of adenine with *p*-nitrobenzenesulfonyl chloride gave a product which is probably a 7 (or 9) sulfonylpurine (2). All the reactions reported were carried out in non-aqueous media. In the present paper the acylation of five adenine derivatives in aqueous acetonitrile or in ethyl acetate-aqueous alkali is reported.

Benzoylation of 9-benzyladenine with benzoyl chloride in aqueous acetonitrile failed to give any benzoylation products; the 9-benzyladenine was recovered unchanged. Carbobenzoxy chloride, which is a more stable acid chloride than benzoyl chloride and therefore less susceptible to basic hydrolysis, was tried next. It was indeed found that carbobenzoxy chloride did react with 9-benzyladenine in aqueous acetonitrile in the presence of potassium acetate, to give 4-amino-5-carbobenzoxyamino-6-benzylformamidopyrimidine (II, R = H; R′ = C_7H_7) in 74% yield. No 6-acylaminopurine was detected in the reaction mixture. The pyrimidine derivative showed two carbonyl absorptions at 1730 and 1680 cm^{-1} and NH absorptions at 3550, 3440 and 1510 cm^{-1} in the infrared. The formyl group was removed from II by treatment with ethanolic ammonia to give 4-amino-5-carbobenzoxyamino-6-benzylaminopyrimidine (III, R = H; R′ = C_7H_7) which was found to be identical with a monocarbobenzoxy derivative obtained by the carbobenzoxylation of 4,5-diamino-6-benzylaminopyrimidine (V, R=H; R′ = C_7H_7). The pyrimidine derivative III was further cyclized on heating to 6-amino-9-benzyl-8-purinone (IV). The structure of the purinone was established by its synthesis from 4-chloro-5-amino-6-benzylaminopyrimidine and urea and subsequent amination of the intermediate 6-chloro-9-benzyl-8-purinone (VI).

6-Benzylamino-9-benzylpurine (I, R = R′ = C_7H_7) and 6-piperidino-9-benzylpurine were also found to undergo the Bamberger reaction (6) when treated with carbobenzoxy chloride in ethyl acetate-aqueous bicarbonate

Chart



mixture. The yields of the pyrimidine derivatives were 62 and 13%, respectively. The low yield in the case of the 6-piperidino derivative is due probably to steric effect. The structure of the products were also established by their infrared spectra and their conversion to a purinone derivative on deformylation and cyclization.

Carbobenzoxylation of 2′,3′-*O*-isopropylidene-5′-trityladenosine in aqueous acetonitrile and in the presence of bicarbonate gave a product in 38% yield which after removal of the sugar residue and the formyl group by acid hydrolysis afforded 4,6-diamino-5-carbobenzoxyaminopyrimidine (III, R = R′ = H). This product was identical through mixed melting point and infrared spectra with a monocarbobenzoxy derivative obtained by the carbobenzoxylation of 4,5,6-triaminopyrimidine (V, R = R′ = H) in aqueous potassium acetate.

Carbobenzoxylation of adenine in ethyl acetate-aqueous potassium acetate afforded a monocarbobenzoxy derivative that showed NH_2 absorptions at 3480 and 3360 cm^{-1} and a high carbonyl absorption at 1750 cm^{-1} in the infrared. It gave a positive hydroxamate test (active acyl group). If the carbobenzoxylation was carried out in absolute ethanol and in the presence of sodium ethoxide a different monocarbobenzoxy derivative was obtained with absorptions at 3510, 3400 cm^{-1} (NH_2) and 1770 cm^{-1} (CO) in the infrared. The two derivatives differ also in their U.V. spectra. Carbobenzoxylation of adenine in ethyl acetate-aqueous sodium hydroxide afforded a mixture of the two isomers. It seems from the infrared and U.V. spectra and from their chemical properties (hydroxamate test) that these are 7- and 9-carbobenzoxyadenines and that the acylation is *p*H dependent. In the first case (potassium acetate) the reacting species is the neutral adenine molecule while in the presence of the strong base (sodium ethylate) acylation occured on the adenine anion. Assuming that the site of attack of the acyl chloride on the unsubstituted adenine is the same as in the cases of the 9-substituted adenines discussed above, would lead to the assignment of a 7-carbobenzoxyadenine to the product obtained in ethyl acetate-aqueous potassium acetate reaction.

Carbethoxylation of adenine in aqueous sodium hydroxide with ethyl chloroformate was reported to give a carbethoxyadenine which is different from 6-*N*-(ethoxycarbamoyl)purine (9). The preferred structure assigned to the product on the basis of its U.V. is a 9-carbethoxyadenine (10).

The different behaviour of the adenines on acylation in aqueous or non-aqueous solutions can be explained assuming that the first site of attack of the electrophilic reagent (the acylating agent) is at position 7 of the purine system. The kinetically controlled product rearranges thermally under anhydrous conditions to the more stable 6-acylamino purine. In aqueous solutions an alternative path is possible which leads to the fission of the imidazole ring (1a).

EXPERIMENTAL

Melting points are corrected. Infrared spectra were measured in chloroform solution (unless otherwise indicated).

Carbobenzoxylation of 9-Benzyladenine.

9-Benzyladenine (225 mg.) was dissolved in a hot solution of acetonitrile (30 ml.) and water (3 ml.). The solution was cooled in an ice-water bath and aqueous potassium acetate (6 ml. of a 10% solution) was added followed by carbobenzoxy chloride (0.45 g.). The stirring was continued for 1 hour at the ice-water bath temperature and 6 hours at room temperature. Water (100 ml.) was added and the solution extracted with three portions of ethyl acetate (100 ml. each). The combined ethyl acetate solution was dried over sodium sulfate and evaporated to dryness. The residue was dissolved in ether, filtered from the unreacted benzyladenine (44 mg.) and the 4-amino-5-carbobenzoxyamino-6-benzylformamidopyrimidine (II, R = H; R′ = C_7H_7) was chromatographed over acid washed alumina (13 g.). The product was eluted with benzene-chloroform (2:1). It melted at 30° after crystallization from carbon tetrachloride-pentane in the cold. The yield was 280 mg. (74%). The infrared spectrum showed NH_2 absorption at 3500 and 3380 cm^{-1}, and CO absorptions at 1710 and 1680 cm^{-1}; λ max (ethanol) 232 mμ (log ε 4.27).

Anal. Calcd. for $C_{20}H_{19}N_5O_3$: C, 63.65; H, 5.07; N, 18.56. Found: C, 63.60; H, 5.05; N, 18.59.

4-Amino-5-carbobenzoxyamino-6-benzylaminopyrimidine (III, R = H; R′ = C_7H_7).

To a solution of 4-amino-5-carbobenzoxyamino-6-benzylformamidopyrimidine (II, R = H; R′ = C_7H_7; 38 mg.) in methanol (4 ml.) there was added a saturated methanolic ammonia solution (4 ml.). The mixture was left at room temperature for 48 hours and then concentrated to a small volume (3 ml.). The precipitate was filtered off and crystallized from ethanol. The yield was 32 mg. (92%), m.p. 201°. It showed NH absorptions at 3450 and 3360 cm^{-1} and CO absorption at 1680 cm^{-1} in the infrared (potassium bromide); λ max (ethanol), 225 (log ε 4.68) and 263 mμ (log ε 3.94).

Anal. Calcd. for $C_{19}H_{19}N_5O_2$: C, 65.31; H, 5.48; N, 20.05. Found: C, 65.40; H, 5.45; N, 20.26.

This compound (III, R = H; R′ = C_7H_7) was found to be identical through mixed m.p. and infrared spectra with a product obtained by the carbobenzoxylation of 4,5-diamino-6-benzylaminopyrimidine in ethyl acetate-aqueous potassium acetate. The yield of the carbobenzoxylation product was only 15%.

6-Amino-9-benzyl-8-purinone (IV, R = H; R′ = C_7H_7).

The product described above (50 mg.) was heated in an oil bath (240°) for 15 minutes. The residue was crystallized from ethanol-benzene and melted at 270° dec., yield 25 mg. (62%). The purinone showed a carbonyl absorption at 1710 cm^{-1} (potassium bromide); λ max (ethanol), 214 (log ε 4.59) and 272 mμ (log ε 4.01).

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.88; H, 4.69; N, 28.90.

6-Chloro-5-amino-4-benzylaminopyrimidine.

A suspension of 5-amino-4,6-dichloropyrimidine (11) (685 mg.)

in water (21 ml.) containing benzylamine (1.12 ml.) was refluxed for 0.5 hour. The solution was cooled and the solid was filtered and crystallized from aqueous ethanol, m.p. 202°, yield 810 mg. (80%).

Anal. Calcd. for $C_{11}H_{11}ClN_4$: C, 56.27; H, 4.69; N, 23.88; Cl, 15.12. Found: C, 56.23; H, 4.48; N, 23.61; Cl, 14.81.

6-Chloro-9-benzyl-8-purinone (VI).

A mixture of 4-chloro-5-amino-6-benzylaminopyrimidine (234 mg.) and urea (240 mg.) was heated in an oil bath (180°) for 30 minutes. The product was crystallized from aqueous ethanol and melted at 187°. The yield was 190 mg. (73%).

Anal. Calcd. for $C_{12}H_9ClN_4O$: C, 55.26; H, 3.48; N, 21.51; Cl, 13.59. Found: C, 56.09; H, 3.71; N, 21.70; Cl, 13.88.

6-Amino-9-benzyl-8-purinone.

A suspension of 4-chloro-9-benzyl-8-purinone (100 mg.) in aqueous ammonia (12 ml. of a 25% solution) was heated in a sealed tube at 180° for 18 hours. The product obtained after the evaporation of the aqueous ammonia was crystallized from aqueous ethanol. It melted at 270° dec., and was found to be identical through mixed m.p. and infrared spectra with the 8-purinone described above.

6-Benzylamino-9-benzylpurine (I, R = R′ = C_7H_7).

This compound was prepared from 5-amino-4,6-dibenzylamino pyrimidine according to the procedure of Goldner and Carstens (12).

Carbobenzoxylation of 6-Benzylamino-9-benzylpurine (II, R = R′ = C_7H_7).

The purine derivative (315 mg., 1 mmole) was dissolved in a mixture of ethyl acetate (30 ml.) and aqueous bicarbonate (12 ml. of a 0.5 *N* solution). To the cooled (ice-water bath) and well stirred solution there was added dropwise, carbobenzoxy chloride (855 mg., 5 eq.). The mixture was stirred for 1 hour at the ice-bath temperature and 3 hours at room temperature. The ethyl acetate layer was separated, dried over sodium sulfate, and evaporated. Trituration with benzene removed most of the starting material. The benzene solution was evaporated and the residue chromatographed over basic alumina (30 g.). The product which came off the column with chloroform was rechromatographed over silica (20 g.). Elution with benzene-chloroform (4:1) afforded a product which melted at 103-104° after crystallization from hexane. The yield was 292 mg. (62.5%). The pyrimidine derivative (II, R = R′ = C_7H_7) showed NH absorptions at 3550 and 3440 cm^{-1} and CO absorptions at 1750 and 1680 cm^{-1} in the infrared.

Anal. Calcd. for $C_{27}H_{25}N_5O_3$: C, 69.36; H, 5.39; N, 14.98. Found: C, 69.18; H, 5.51; N, 15.18.

5-Carbobenzoxyamino-4,6-dibenzylaminopyrimidine (III, R = R′ = C_7H_7).

The product described above (100 mg.) was treated with a saturated ethanolic ammonia solution (8 ml.) for 24 hours at room temperature. The solution was evaporated to dryness and the product was dissolved in ethyl acetate. The solution was washed with water, dried and evaporated. The residue was crystallized from methylcyclohexane and melted at 141°. The yield was 71 mg. (76%). The pyrimidine derivative showed NH absorptions at 3530 and 3480 cm^{-1} and CO absorption at 1750 cm^{-1}.

Anal. Calcd. for $C_{26}H_{25}N_5O_2$: C, 71.05; H, 5.73; N, 15.94. Found: C, 70.93; H, 5.55; N, 15.67.

The 5-carbobenzoxyamino-4,6-dibenzylaminopyrimidine (III, R = R′ = C_7H_7) described above was found to be identical through mixed melting point and infrared spectra with a monocarbobenzoxy derivative obtained by the carbobenzoxylation of 5-amino-4,6-dibenzylaminopyrimidine (V, R = R′ = C_7H_7) in ethyl acetate-aqueous potassium acetate mixture. The yield of the product was 51%. The monocarbobenzoxyaminodibenzylaminopyrimidine gives a stable hydrochloride which melts with decomposition at 240°.

6-Benzylamino-9-benzyl-8-purinone (IV, R = R′ = C_7H_7).

The pyrimidine derivative described above (40 mg.) was heated in an oil bath at 175° for 0.5 hour. The product obtained after cooling to room temperature was triturated with benzene, filtered and crystallized from aqueous ethanol. The yield was 21 mg. (65%), m.p. 227-228°. It showed NH absorptions at 3550 and 3300 cm^{-1} and a CO absorption at 1700 cm^{-1} in the infrared (potassium bromide).

Anal. Calcd. for $C_{19}H_{17}N_5O$: C, 68.86; H, 5.17; N, 21.14. Found: C, 69.06; H, 5.40; N, 21.30.

The 6-benzylamino-9-benzyl-8-purinone was identical with the product obtained by fusion of 5-amino-4,6-dibenzylaminopyrimide (V, R = R′ = C_7H_7, 303 mg.) with urea (3 g.) at 170° for 1 hour. The yield of the purinone derivative was 229 mg. (70%).

5-Amino-4-benzylamino-6-piperidinopyrimidine (V, RNH = $C_5H_{10}N$).

A mixture of 5-amino-4-benzylamino-6-chloropyrimidine (800 mg.), piperidine (4 ml.) and absolute ethanol (4 ml.) was heated in a sealed tube at 180° for 7 hours. The cold reaction mixture was diluted with water (25 ml.) and was left overnight. The brown precipitate was filtered and crystallized from aqueous ethanol with charcoal treatment. The yield was 650 mg. (64%), m.p. 151°.

Anal. Calcd. for $C_{16}H_{21}N_5$: C, 67.81; H, 7.47; N, 24.72. Found: C, 68.03; H, 7.55; N, 24.67.

6-Piperidino-9-benzylpurine.

A solution of the pyrimidine derivative described above (200 mg.) in formamide (2 ml.) was refluxed for 1 hour. The solution was diluted with water (8 ml.) and extracted into ethyl acetate. The ethyl acetate solution was dried and evaporated to dryness. The yellow solid residue was crystallized from hexane and melted at 76-77°, yield 132 mg. (63.5%).

Anal. Calcd. for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53; N, 23.88. Found: C, 69.79; H, 6.54; N, 23.97.

Carbobenzoxylation of 6-piperidino-9-benzylpurine.

Carbobenzoxylation of the piperidinopurine in ethyl acetate-aqueous potassium acetate as described above for the 6-benzylamino-9-benzylpurine afforded an oily product. The unstable oil (decomposes on standing) which was obtained in 13% yield after chromatography over neutral alumina showed only one carbonyl absorption at 1740 cm^{-1} in the infrared. The same oily product (identical infrared) was also obtained in 70% yield by the carbobenzoxylation in ethyl acetate aqueous bicarbonate of 5-amino-4-benzylamino-6-piperidinopyrimidine.

6-Piperidino-9-benzyl-8-purinone.

The oily product described above (70 mg.) was heated in an oil bath at 190° for 5 minutes. The solid residue was crystallized from aqueous ethanol and melted at 224°; yield 41 mg. (79%). It showed NH absorption at 3500 and CO absorption at 1700 cm^{-1} (potassium bromide).

Anal. Calcd. for $C_{17}H_{19}N_5O$: C, 66.00; H, 6.19; N, 22.64. Found: C, 65.91; H, 6.20; N, 22.47.

The same purinone (mixed melting point and infrared spectra)

was obtained by heating a mixture of 5-amino-4-benzylamino-6-piperidinopyrimidine (105 mg.) and urea (1 g.) at 180° (oil bath) for 1 hour. The reaction mixture was cooled, triturated with water and filtered. The piperidinopurinone was crystallized from aqueous ethanol and melted at 224°, yield 84 mg. (73.5%).

2′,3′-*O*-Isopropylidene-5′-trityladenosine.

This compound was prepared by the tritylation of 2′,3′-*O*-isopropylideneadenosine (1.55 g.) with trityl chloride (1.54 g.) in pyridine (35 ml.) at room temperature for 7 days. The solution was diluted with cold water and left overnight in the refrigerator. The solid precipitate was filtered and the solution was extracted with benzene. The benzene was dried and evaporated to dryness. The combined solids were dried in a dessicator and chromatographed over basic alumina (50 g.). The product was eluted with benzene-chloroform (3:2) and melted at 100-101° after crystallization from benzene-hexane; yield 1.127 g. (40%). It showed NH absorptions at 3500 and 3400 in the infrared.

Anal. Calcd. for $C_{32}H_{31}N_5O_4$: C, 69.93; H, 5.69; N, 12.74. Found: C, 69.78; H, 5.57; N, 12.90.

Carbobenzoxylation of 2′,3′-*O*-Isopropylidene-5′-trityladenosine.

The adenosine derivative (500 mg.) was carbobenzoxylated in acetonitrile (35 ml.) and in the presence of aqueous bicarbonate (15 ml. 1 *N*) with carbobenzoxy chloride (598 mg.) as described above for the 9-benzyladenine. The product was chromatographed over basic alumina (30 g.). The product which was eluted with benzene-chloroform (2:3) melted at 117° after crystallization from benzene-hexane, yield 231 mg. (38%). It analyzed for a $C_{39}H_{39}N_5O_6$ compound and showed NH absorptions at 3520 and 3420 cm^{-1} and CO absorption at 1730 cm^{-1}.

4,6-Diamino-5-carbobenzoxyaminopyrimidine (III, R = R′ = H).

The product described above (70 mg.) was hydrolyzed in ethanol (10 ml.), hydrochloric acid (5 ml., 1 *N*) mixture. After refluxing for 1 hour the ethanol was removed *in vacuo* and water (10 ml.) added. The acid solution was extracted with ethyl acetate to remove the trityl alcohol and then neutralized with concentrated aqueous ammonia. The solid precipitate was filtered and dried *in vacuo*. It melted at 300° after crystallization from ethanol, yield 26 mg. (96%). It showed NH absorptions at 3580 and 3400 cm^{-1} and CO absorption at 1700 cm^{-1} in the infrared.

Anal. Calcd. for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.02. Found: C, 55.76; H, 5.22; N, 26.75.

This product was identical through mixed melting point and infrared spectra with a carbobenzoxy derivative obtained in 43% yield by the carbobenzoxylation of 4,5,6-triaminopyrimidine (250 mg.) in aqueous potassium acetate (25 ml. of a 8% solution) with carbobenzoxy chloride (1.4 g.).

Carbobenzoxylation of Adenine.

(a).

Adenine (270 mg.) was suspended in a mixture of ethyl acetate (20 ml.) and aqueous potassium acetate (20 ml., 0.2 *N*). To the cold and well stirred suspension there was added carbobenzoxy chloride (700 mg.). Stirring was continued for 0.5 hour at the ice-bath temperature and 1 hour at room temperature. The solid which separated was filtered, dried at room temperature and crystallized from benzene. It melted at 158° dec., yield 218 mg., (40%); ν max 3480, 3360, 1750, 1640, 1560, 1105 cm^{-1}; λ max (acetonitrile), 291 mμ (log ϵ 3.79).

Anal. Calcd. for $C_{13}H_{11}N_5O_2$: C, 57.98; H, 4.12; N, 26.01. Found: C, 58.01; H, 4.21; N, 25.88.

(b).

Adenine (270 mg.) was dissolved in absolute ethanol containing sodium ethylate (46 mg. sodium in 15 ml. ethanol). The solution was cooled to 0° and carbobenzoxy chloride added slowly (0.8 ml.). After 1 hour the ethanol was removed *in vacuo* at room temperature and the residue was divided between water and ethyl acetate (300 ml.). The ethyl acetate solution was washed with water, dried and evaporated at room temperature. The residue was crystallized from benzene and melted at 162° dec., yield 245 mg. (45%); ν max 3510, 3400, 1770, 1640, 1590, 1130 cm^{-1}; λ max (acetonitrile) 254 mμ (log ϵ 4.31).

Anal. Calcd. for $C_{13}H_{11}N_5O_2$: C, 57.98; H, 4.12; N, 26.01. Found: C, 58.17; H, 4.08; N, 25.75.

(c).

Adenine (135 mg.) was carbobenzoxylated in aqueous sodium hydroxide (4 ml., 0.5 *N*)-ethyl acetate (10 ml.) mixture at 0° (ice-water bath) with carbobenzoxy chloride (350 mg.) as described above (procedure a). According to the infrared spectrum the solid obtained was a 1:1 mixture of the two isomers described. Fractional crystallization from benzene afforded pure samples of the two isomers.

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