461. *The Structures of Some Acylcytosines.*

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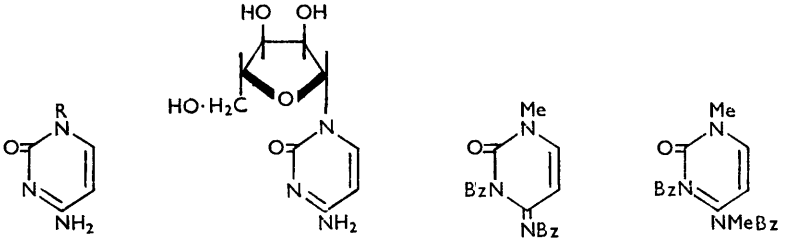
A number of new acyl derivatives of cytosine and 3-methylcytosine are described. Acylation normally yields N^6-monoacyl compounds which, in hot 80% acetic acid, yield large amounts of uracil derivatives. Exceptionally, benzoyl chloride can also yield a 1 : N^6-dibenzoate from 3-methylcytosine and a tribenzoate from cytosine. Similarly, benzoylation of cytidine yields, according to conditions, either a tetrabenzoyl or pentabenzoyl derivative.

The work which forms the subject of the present communication arose as a result of our interest in partially acylated nucleosides of known structure as intermediates in nucleotide syntheses, and was carried out simultaneously with that on the acylation of 3-methylcytosine reported by Kenner, Reese, and Todd.[1] The results confirm the conclusions reached by these authors and supplement them in certain directions; in addition, a number of new crystalline derivatives of cytosine (I; R = H), 3-methylcytosine (I; R = Me), and cytidine (II) were prepared and are placed on record as reference compounds.

Acetylation of 2′ : 3′-*O*-benzylidenecytidine [2,3] gave a diacetyl derivative, but attempts

[1] Kenner, Reese, and Todd, *J.*, 1955, 855.
[2] Gulland and Smith, *J.*, 1948, 1527.
[3] Brown, Haynes, and Todd, *J.*, 1950, 3299.

to remove the benzylidene group from the product with acetic acid led to partial deacetylation and deamination and gave complex mixtures. In the hope that it would resist deacylation the corresponding dibenzoyl derivative was prepared, but with hot acetic acid it, too, gave a mixture. Cytidine itself with benzoyl chloride in pyridine at room temperature gave a tetrabenzoyl and at 100° a pentabenzoyl derivative, both of which underwent partial debenzoylation and deamination with acetic acid. It was therefore decided to examine the simpler cases of the acyl derivatives of cytosine and 3-methylcytosine since the complications encountered in hydrolysing the cytidine derivatives were clearly associated with acylation of the cytosine residue present in them.



(I) (II) (III) (IV)

Cytosine with acetic anhydride in pyridine yields only a monoacetyl derivative;[4] on treatment with hot 80% acetic acid this product yielded a mixture of uracil and cytosine although cytosine itself was unaffected by the same treatment. When benzoylated at room temperature for 45 minutes, cytosine gave a monobenzoyl derivative which was partially converted by hot 80% acetic acid into uracil and cytosine; benzamide was also isolated. The conclusion from these results that the acyl derivatives were N^6-acetyl- and N^6-benzoyl-cytosine was confirmed by methylation with diazomethane which yielded N^6-acetyl- and N^6-benzoyl-1 : 3-dimethylcytosine respectively. Partial deamination with production of 3-methyluracil was also observed when N^6-acetyl- and N^6-benzoyl-3-methylcytosine were heated with 80% acetic acid at 100° for 1 hour. When 3-methylcytosine was allowed to react with excess of benzoyl chloride in pyridine during several hours it yielded a dibenzoyl derivative. This we formulate as 1 : N^6-dibenzoyl-3-methylcytosine (III) on the grounds that it undergoes ready hydrolysis to N^6-benzoyl-3-methylcytosine and that its infrared spectrum shows the band at 1656 cm.$^{-1}$ assigned to the 2-carbonyl group in a variety of pyrimidine derivatives,[5,6] together with a general similarity to that of N^6-benzoyl-1 : 3-dimethylcytosine and gross differences from N^6-benzoyl-3 : N^6-dimethylcytosine (IV) in the finger-print region (700—1300 cm.$^{-1}$). Cytosine, itself, on prolonged benzoylation gives a tribenzoyl derivative, presumably 1 : 3 : N^6-tribenzoylcytosine, which is easily hydrolysed to N^6-benzoylcytosine. It is of interest that in attempts to prepare mixed diacyl derivatives the monobenzoates of cytosine and 3-methylcytosine resisted further acylation with acetic anhydride or with acetyl, *p*-bromobenzoyl, *p*-nitrobenzoyl, *p*-toluoyl, or 3 : 4 : 5-trimethoxybenzoyl chloride and that, applied to the free bases, these reagents yielded only monoacyl derivatives. 1-Amino*iso*quinoline and 2-aminopyridine both yield dibenzoates,[7,8] the latter easily forming 2-benzamidopyridine with dilute acid.

In contrast to the formation of uracil derivatives during the acid hydrolysis of the N^6-benzoylcytosines, noted above, alkaline hydrolysis effected no deamination. Tribenzoylcytosine and dibenzoyl-3-methylcytosine were converted by ethanolic ammonia in the cold into the N^6-monobenzoyl compounds, dibenzoyl-3-methylcytosine also affording traces of 3-methylcytosine. Cytosine was formed quantitatively when N^6-benzoylcytosine was treated with dilute sodium hydroxide solution.

On the basis of the experiments with cytosine and 3-methylcytosine it is concluded that

[4] Wheeler and Johnson, *Amer. Chem. J.*, 1903, **29**, 500.
[5] Short and Thompson, *J.*, 1952, 168.
[6] Dr. C. L. Angell, personal communication.
[7] Craig and Cass, *J. Amer. Chem. Soc.*, 1942, **64**, 783.
[8] Tschitschibabin and Bylinkin, *Ber.*, 1922, **55**, 998.

the acyl derivatives prepared from 2′ : 3′-*O*-benzylidenecytidine are N^6 : 5′-diacetyl- and N^6 : 5′-dibenzoyl-2′ : 3′-*O*-benzylidenecytidine and that benzoylation of cytidine yields, according to conditions, N^6 : 2′ : 3′ : 5′-tetrabenzoyl- or 1 : N^6 : 2′ : 3′ : 5′-pentabenzoylcytidine. By analogy with the latter compound it is inferred that the pentabenzoyl derivatives of adenosine and cordycepin [9] are benzoylated on both $N_{(1)}$ and N^6 in the adenine nucelus.

Experimental

N^6 : 5′-*Diacetyl*-2′ : 3′-O-*benzylidenecytidine*.—2′ : 3′-*O*-Benzylidenecytidine was treated with acetic anhydride in pyridine at room temperature. After 6 hr. ethanol was added to remove excess of anhydride, the solution evaporated, and the residue recrystallised from ethanol. The *diacetyl derivative* formed colourless elongated rectangular prisms, m. p. 119—120° (Found : C, 58·1; H, 5·0; N, 9·7. $C_{20}H_{21}O_7N_3$ requires C, 57·8; H, 5·1; N, 10·1%). Light absorption in 95% EtOH : $\lambda_{max.}$ 300, 250 mμ (ε 6070, 16,960) ; $\lambda_{min.}$ 276, 227 mμ (ε 3690, 4730).

N^6 : 5′-*Dibenzoyl*-2′ : 3′-O-*benzylidenecytidine*.—A suspension of 2′ : 3′-*O*-benzylidenecytidine (1·74 g.) in dry pyridine (50 c.c.) was shaken with benzoyl chloride (4·35 c.c.) for 4 hr. at room temperature. The solution so obtained was poured into cold water and neutralised with hydrochloric acid. The sticky red precipitate was separated by decantation and treated with hot ethanol (20 c.c.). The red impurities dissolved, leaving a residue which was then recrystallised from ethanol. The *dibenzoate* formed colourless needles (2·65 g.), m. p. 211—212° (Found : C, 67·0; H, 5·0; N, 7·8. $C_{30}H_{25}O_7N_3$ requires C, 66·8; H, 4·7; N, 7·8%). Light absorption in 95% EtOH : $\lambda_{max.}$ 302, 262, 231 mμ (ε 8880, 26,500, 21,600) ; $\lambda_{min.}$ 292, 245, 221 mμ (ε 8120, 18,000, 20,400).

N^6 : 2′ : 3′ : 5′-*Tetrabenzoylcytidine*.—Benzoyl chloride (3 c.c.) was added to a suspension of anhydrous cytidine (0·5 g.) in dry pyridine (10 c.c.), and the mixture shaken at room temperature for 2 hr. Water and dilute hydrochloric acid were added to the clear solution, and the precipitate was collected and washed with a little hot ethanol. Recrystallised from ethyl acetate the *tetrabenzoate* (1·15 g.) formed colourless needles, m. p. 202—203·5° (Found : C, 67·6; H, 4·8; N, 6·1. $C_{37}H_{29}O_9N_3$ requires C, 67·4; H, 4·4; N, 6·4%). Light absorption in 95% EtOH : $\lambda_{max.}$ 302, 263, 231 mμ (ε 9700, 29,500, 45,400), $\lambda_{min.}$ 295, 250, 213 mμ (ε 9250, 24,000, 26,700).

1 : N^6 : 2′ : 3′ : 5′-*Pentabenzoylcytidine*.—Anhydrous cytidine (0·3 g.), benzoyl chloride (4 c.c.), and dry pyridine (6 c.c.) were heated together for 45 min. at 100°, cooled, and filtered from pyridine hydrochloride. Light petroleum (200 c.c. ; b. p. 40—60°) was added, and the sticky precipitate washed with water and then recrystallised from ethanol and finally from methanol, giving the *pentabenzoate* (0·45 g.) as colourless prisms, m. p. 148—150° after some shrinking at 100° (Found : C, 68·7; H, 4·7; N, 5·6. $C_{44}H_{33}O_{10}N_3$ requires C, 69·2; H, 4·4; N, 5·5%). Light absorption in 95% EtOH : $\lambda_{max.}$ 307, 232 mμ (ε 13,200, 56,300), $\lambda_{min.}$ 294, 214 mμ (ε 12,400, 34,900).

N^6-*Acetylcytosine*.—This was prepared by acetylation with hot acetic anhydride in pyridine.[4] Light absorption in 95% EtOH : $\lambda_{max.}$ 293, 244—245, 215 mμ (ε 4920, 14,200, 20,700), $\lambda_{min.}$ 270, 226 mμ (ε 2550, 5780). The same compound was obtained under all conditions of acetylation and the substance could not be further acylated with benzoyl chloride or benzoic anhydride. N^6-Acetylcytosine was heated with 80% acetic acid at 100° for 1 hr. and the products were submitted to paper chromatography in butan-1-ol–acetic acid–water (5 : 2 : 3). Only a trace of starting material remained, the ultraviolet-absorbing material consisting of uracil (R_F 0·53) and cytosine (R_F 0·43) in approximately equal amounts. Cytosine was recovered unchanged after similar treatment with 80% acetic acid.

On treatment with excess of ethereal diazomethane the above acetyl derivative yielded N^6-acetyl-1 : 3-dimethylcytosine (see below), m. p. and mixed m. p. 156—157°.

N^6-*Acetyl*-1 : 3-*dimethylcytosine*.—1 : 3-Dimethylcytosine [10] was acetylated with acetic anhydride in pyridine at room temperature in the usual way. Recrystallised from ethanol the *acetyl derivative* formed needles, m. p. 156—157° (Found : C, 53·5; H, 5·9; N, 23·3. $C_8H_{11}O_2N_3$ requires C, 53·0; H, 6·1; N, 23·2%).

N^6-*Benzoylcytosine*.—Anhydrous cytosine (0·1 g.), suspended in dry pyridine (10 c.c.), was shaken with benzoyl chloride (1·3 c.c.) at room temperature for 45 min. Dilute hydrochloric acid was now added and after 2 hr. the precipitate was collected and washed with hot ethanol. Recrystallised from pyridine or aqueous acetic acid the *benzoate* formed needles which darkened

[9] Bentley, Cunningham, and Spring, *J.*, 1951, 2301.
[10] Hilbert, *J. Amer. Chem. Soc.*, 1934, **56**, 190.

at 320° but did not melt below 350° (Found : C, 61·2; H, 4·0; N, 19·7. $C_{11}H_9O_2N_3$ requires C, 61·4; H, 4·2; N, 19·5%). Light absorption in 95% EtOH : $\lambda_{max.}$ 299—300, 258 mμ (ε 6600, 21,000), $\lambda_{min.}$ 289, 230 mμ (ε 6180, 8920). Methylation with ethereal diazomethane gave N^6-benzoyl-1 : 3-dimethylcytosine (see below), m. p. and mixed m. p. 155—156°.

Though stable to half-saturated ammonia at 0° for 15 hr., N^6-benzoylcytosine was completely converted into cytosine by 0·1N-sodium hydroxide at room temperature in 18 hr. Heating it with 85% acetic acid at 100° for 2 hr. left about one-third of the benzoate unaffected, the remainder being converted into uracil and cytosine in a ratio of *ca.* 33 : 1 (paper chromatography). Extraction of the basified hydrolysis solution with ether afforded benzamide (m. p. and mixed m. p. 129°).

N^6-*Benzoyl*-1 : 3-*dimethylcytosine*.—Prepared by heating 1 : 3-dimethylcytosine [10] with benzoyl chloride in pyridine for 30 min. at 100° and recrystallised from ethanol, the *benzoate* formed needles, m. p. 155—156° (Found : C, 64·1; H, 5·2; N, 17·2. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3%).

1 : 3 : N^6-*Tribenzoylcytosine*.—Anhydrous cytosine (0·2 g.), suspended in dry pyridine (20 c.c.) and benzoyl chloride (2·6 c.c.), was shaken at room temperature, the solid slowly dissolving to a deep red solution. After 3 days light petroleum (400 c.c.; b. p. 60—80°) was added, the mixture set aside for 1 day, and the colourless crystals were collected and recrystallised from ethanol. 1 : 3 : N^6-*Tribenzoylcytosine* was thus obtained as prisms (0·3 g.), m. p. 140—142° (Found : C, 70·4; H, 4·2; N, 10·1. $C_{25}H_{17}O_4N_3$ requires C, 70·9; H, 4·1; N, 9·9%). Light absorption in 95% EtOH : $\lambda_{max.}$ 240 mμ (ε 35,600), $\lambda_{min.}$ 218 mμ (ε 20,200).

When set aside in half-saturated ethanolic ammonia at 0° overnight the tribenzoyl derivative was smoothly hydrolysed to N^6-benzoylcytosine. The same product was obtained in rather low yield by 1 hour's heating at 100° with 80% acetic acid, large amounts of uracil and benzamide together with a little cytosine being also produced. The N^6-benzoylcytosine obtained in these hydrolyses was identified by analysis and absorption spectrum.

N^6-(3 : 4 : 5-*Trimethoxybenzoyl*)*cytosine*.—Prepared by heating anhydrous cytosine with excess of 3 : 4 : 5-trimethoxybenzoyl chloride in pyridine, the *product* crystallised from acetic acid as rectangular prisms, m. p. 296° (decomp.); it resisted further acylation with benzoyl chloride (Found : C, 52·5; H, 5·2; N, 11·5. $C_{14}H_{15}O_5N_3,C_2H_4O_2$ requires C, 52·6; H, 5·2; N, 11·5. Found, in material dried at 120°/1 mm. for 12 hr.: C, 54·4; H, 4·7; N, 13·6. $C_{14}H_{15}O_5N_3$ requires C, 55·1; H, 4·9; N, 13·8%).

1 : N -*Dibenzoyl*-3-*methylcytosine*.—When a suspension of anhydrous 3-methylcytosine [1,11] (0·17 g.) in a mixture of dry pyridine (20 c.c.) and benzoyl chloride (2·1 c.c.) was vigorously shaken, the solid dissolved giving a clear solution. After 4 hr. crushed ice was added and the precipitate collected and recrystallised from ethanol–ethyl acetate. The *dibenzoate* formed hexagonal prisms (0·21 g.), m. p. 213—215° (Found : C, 68·5; H, 4·5; N, 12·7. $C_{19}H_{15}O_3N_3$ requires C, 68·5; H, 4·5; N, 12·6%). Light absorption in 95% EtOH : $\lambda_{max.}$ 314, 250 mμ (ε 10,100, 26,400), $\lambda_{min.}$ 286, 228 mμ (ε 7580, 17,800). The infrared absorption (Nujol mull) showed a strong band at 1656 cm.$^{-1}$.

When treated for 15 min. at 100° with 80% acetic acid the dibenzoate gave N^6-benzoyl-3-methylcytosine, m. p. and mixed m. p. 222°. The same product accompanied by traces of 3-methylcytosine was obtained by treatment with half-saturated ethanolic ammonia at 0° overnight.

Other N^6-*Acyl Derivatives of* 3-*Methylcytosine*.—The following derivatives were prepared by heating 3-methylcytosine with an excess of the appropriate acyl halide in pyridine : N^6-p-*bromobenzoyl*-3-*methylcytosine*, prisms (from ethanol), m. p. 251—252° (Found : C, 46·5; H, 3·1; N, 13·5. $C_{12}H_{10}O_2N_3Br$ requires C, 46·8; H, 3·3; N, 13·6%); 3-*methyl*-N^6-p-*nitrobenzoylcytosine*, pale yellow needles (from ethanol–ethyl acetate), m. p. 272—274° (Found : C, 52·9; H, 4·0; N, 20·2. $C_{12}H_{10}O_4N_4$ requires C, 52·6; H, 3·7; N, 20·4%); N^6-(3 : 4 : 5-*trimethoxybenzoyl*)-3-*methylcytosine*, colourless plates (from ethanol), m. p. 221—223° (Found : C, 56·7; H, 5·6; N, 12·5. $C_{15}H_{17}O_5N_3$ requires C, 56·4; H, 5·4; N, 13·2%); and 3-*methyl*-N^6-*toluene*-p-*sulphonylcytosine*, yellow prisms (from ethyl acetate or acetic acid), m. p. 236—237° (Found : C, 51·5; H, 4·9; N, 15·0. $C_{12}H_{13}O_3N_3S$ requires C, 51·6; H, 4·7; N, 15·0%).

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[11] Hilbert and Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2001.