279. *Benzylidene Guanosine.*

By (the late) J. Masson Gulland and W. G. Overend.

Knowledge of the structure of benzylidene guanosine is important in connection with synthetic experiments. Acetyl guanosine, formed by acetylation of the unsubstituted hydroxyl of the sugar radical of benzylidene guanosine and removal of the benzylidene group, was shown by periodate titration to be the 2′- or 3′-, not the 5′-, derivative. The choice between these alternatives was made by combined deacetylation and methylation of acetyl benzylidene guanosine and subsequent hydrolysis to give a methyl ribitol which, being optically active and hence asymmetrically substituted, was 2-methyl ribitol. Benzylidene guanosine is thus the 3′ : 5′-derivative.

Benzylidene guanosine was prepared by Bredereck and Berger (*Ber.*, 1940, **73**, 1124) by condensation of guanosine with benzaldehyde in presence of zinc chloride, and its m. p. and nitrogen analysis, but no other details, were recorded. These authors quoted a failure to form a trityl derivative with triphenylmethyl chloride and a negative reaction with copper sulphate and alkali (Parnas and Klimek, *Z. physiol. Chem.*, 1933, **217**, 75; Klimek and Parnas, *ibid.*, 1933, **218**, 30) as evidence that the benzylidene group occupied the 3′ : 5′-position. It has, however, been shown (Hockett and Hudson, *J. Amer. Chem. Soc.*, 1934, **56**, 945; Jackson, Hockett, and Hudson, *ibid.*, p. 947) that reaction with trityl chloride is not a specific attribute of primary hydroxyl groups and that secondary hydroxyl groups may react. Since benzylidene guanosine was required as a starting material in certain syntheses and its constitution has implications in connection with the synthesis of cytidine-2′ phosphate (Gulland and Smith, in the press), proof of its constitution was necessary.

Anhydrous guanosine was converted into the benzylidene derivative by means of benzaldehyde and zinc chloride, and by reconversion of this derivative into guanosine through treatment with dilute acetic acid and phenylhydrazine it was demonstrated that the furanose structure of the ribose moiety had not been disturbed during the benzylidenation. Acetylation of benzylidene guanosine with acetic anhydride and sodium acetate yielded a *O*-monoacetyl benzylidene guanosine (Bredereck and Berger, *loc. cit.*) in which the amino-group did not carry an acetyl substituent, as shown by the solubility of the compound in dilute acid. Removal of the benzylidene radical by means of acetic acid and phenylhydrazine yielded acetyl guanosine (Bredereck and Berger, *loc. cit.*). This could have been 2′-, 3′-, or 5′-acetyl guanosine, according to whether the benzylidene group had substituted in the 3′ : 5′-, 2′ : 5′-, or 2′ : 3′-positions of the sugar; the 1 : 4-ring structure of the sugar radical is long established (Levene and Tipson, *J. Biol. Chem.*, 1932, **97**, 491; Lythgoe and Todd *J.*, 1944, 592), so that the 4′-position is excluded. Titrimetric investigation of the action of sodium metaperiodate on acetyl guanosine revealed that oxidative fission did not occur; thus acetyl guanosine did not contain adjacent hydroxyl groups

and is not the 5′-acetyl derivative. Consequently benzylidene guanosine was either the 3′ : 5′- or the 2′ : 5′-derivative, the acetyl group of acetyl benzylidene guanosine occupying position 2′ or 3′.

In order to distinguish between these alternatives, a simultaneous de-acetylation and methylation of acetyl benzylidene guanosine was effected with methyl sulphate and alkali in aqueous acetone. Hydrolysis of the product brought about simultaneously the removal of the benzylidene radical and the fission of the glycosidic linkage, and yielded, as a gum, a sugar which must have been either 2- or 3-methyl ribose. Reduction of this with hydrogen in presence of Raney nickel catalyst produced an optically active methyl ribitol. This must be *2-methyl ribitol* and not 3-methyl ribitol, since the latter is symmetrical and would not exhibit optical activity. It follows that the compounds from which this substance was prepared are 2-methyl ribose, 2′-acetyl 3′ : 5′-benzylidene guanosine, and 3′ : 5′-benzylidene guanosine, proof of the positions of the substituents in these compounds being thus provided.

Experimental.

3′ : 5′-*Benzylidene Guanosine.*—(*a*) A mixture of anhydrous guanosine (15 g.), freshly distilled, dry benzaldehyde (150 c.c.), and anhydrous zinc chloride (40 g.) was shaken until a clear solution formed, warmed on the water-bath for 1 hour, cooled, and poured into ether (1·5 l.). The precipitate of crude benzylidene guanosine was collected, washed with ether and then with boiling water, and crystallised from 70% alcohol. As it still contained traces of zinc compounds, it was dissolved in the minimum quantity of cold glacial acetic acid, and the solution was stirred into a slight excess of very dilute ammonia. The precipitated benzylidene guanosine was collected, washed, and crystallised from 70% alcohol, from which it separated in colourless clusters of minute needles, m. p. 296° (yield, 16 g.) (Found: C, 54·6; H, 4·5; N, 18·8. Calc. for $C_{17}H_{17}O_5N_5$: C, 54·9; H, 4·6; N, 18·9%). Bredereck and Berger (*loc. cit.*) give m. p. 295°.

(*b*) Guanosine (10 g.), benzaldehyde (140 c.c.), and zinc chloride (26 g.) were condensed as before; the solution was poured into water containing sodium carbonate (25 g.), and the benzaldehyde removed by steam distillation. The solvent was removed under diminished pressure, and the residue was dried by distillation of ethyl alcohol from it, and then extracted with boiling alcohol under reflux; the extract was evaporated to dryness; the residue crystallised from 70% alcohol in clusters of needles, m. p. 295—296° (yield, 11 g.).

Regeneration of Guanosine from Benzylidene Guanosine.—A mixture of benzylidene guanosine (0·5 g.) and 30% acetic acid (20 c.c.) containing phenylhydrazine (0·5 g.) was left at room temperature for 5 hours. The resulting solid was collected, and the filtrate poured into water in order to precipitate any guanosine which might have remained in solution; only an opalescence resulted. The solid was extracted with boiling water to dissolve out guanosine (extract *A*), then extracted with cold alcohol to remove benzaldehyde phenylhydrazone (extract *B*), and the remaining solid (0·2 g.) was shown to be unchanged benzylidene guanosine, m. p. 295—296°.

When extract *A* was cooled, guanosine (0·2 g.) separated; it recrystallised from water in needles, m. p. 238° alone or mixed with an authentic specimen. Extract *B* was evaporated to dryness, and, when the residue was crystallised from dilute alcohol, benzaldehyde phenylhydrazone was obtained, m. p. 154° alone or mixed with an authentic sample.

An attempt to regenerate guanosine from benzylidene guanosine (0·5 g.) in alcohol (150 c.c.) by hydrogenation at room temperature for 2 hours in presence of palladium black (Tausz and von Putnoky, *Ber.*, 1919, **52**, 1573) failed: no hydrogen was absorbed, and evaporation of the solvent after removal of the catalyst yielded unchanged benzylidene guanosine.

2′-*Acetyl* 3′ : 5′-*Benzylidene Guanosine.*—Benzylidene guanosine (0·5 g.), sodium acetate (0·5 g., freshly fused and powdered), and freshly distilled acetic anhydride (10 c.c.) were boiled under reflux for 1 hour, and all the solid dissolved. When cold, the liquid was poured into excess of sodium hydrogen carbonate solution, and the resulting oil was washed by decantation with water until it became solid. Acetyl benzylidene guanosine crystallised from dilute alcohol in small, colourless needles, m. p. 260—262° (yield, 0·4 g.) (Found: C, 55·2; H, 4·8. Calc. for $C_{19}H_{19}O_6N_5$: C, 55·2; H, 4·6%). It dissolved in dilute mineral acid and became sticky when left exposed to the atmosphere.

2′-*Acetyl Guanosine.*—Acetyl benzylidene guanosine (0·4 g.) and 30% aqueous acetic acid (15 c.c.) containing phenylhydrazine (0·4 g.) were warmed at 70° for 2 hours; benzaldehyde phenylhydrazone separated as yellow needles, m. p. 154°, and was collected. The filtrate was poured into water (600 c.c.), and 2 days later the precipitate of acetyl guanosine was collected and washed; it crystallised from water containing a little alcohol as colourless crystals, m. p. 176—179° (decomp.) (yield, 0·15 g.) (Found: C, 44·5; H, 4·6; Ac, 13·7. Calc. for $C_{12}H_{15}O_6N_5$: C, 44·3; H, 4·6; Ac, 13·2%). Bredereck and Berger give m. p. " about 180° ".

Periodate Titrations.—(a) *Guanosine.* A solution of guanosine (0·254 millimol.) in hot water (20 c.c.) was cooled to 25°, mixed with 0·2774 M-sodium metaperiodate solution (5 c.c.), diluted to 100 c.c. with water, and kept at 24°. At intervals aliquots were removed and the unchanged periodate determined iodometrically by means of 0·0993N-sodium arsenite (Barnebey, *J. Amer. Chem. Soc.*, 1916, **38**, 330); the experiment was prolonged for 30 hours until no further utilisation of periodate occurred and one molecular proportion had been consumed. No formic acid was produced as shown by titration with N/10-sodium hydroxide to a methyl-red end-point (Jackson and Hudson, *ibid.*, 1939, **61**, 1530). These observations agree with those of Lythgoe and Todd (*J.*, 1944, 593).

(b) *Guanosine regenerated from benzylidene guanosine.* One molecular proportion of sodium metaperiodate was consumed and no formic acid was produced.

(c) *Acetyl guanosine.* No sodium metaperiodate had been utilised after 70 hours.

2-Methyl Ribitol.—Methyl sulphate (5 c.c.) and 30% aqueous sodium hydroxide (39 c.c.) were added during 2 hours to acetyl benzylidene guanosine (3·2 g.) suspended in acetone (30 c.c.). The solution was heated at 85° for 2 hours, cooled, neutralised, and evaporated to dryness under reduced pressure, and the methylation process repeated on the residue. After this treatment the residue was heated with sulphuric acid at 80° for 5 hours, neutralised with excess of barium carbonate whilst hot, filtered, steam distilled to remove benzaldehyde, and extracted thoroughly with chloroform. The extract was dried ($MgSO_4$) and evaporated, leaving a slightly yellow gum (Found: OMe, 18·6. Calc. for $C_6H_{12}O_5$: OMe, 18·9%) which easily reduced Fehling's solution and could not be induced to crystallise; its detailed examination is reserved for a subsequent paper.

The gum, dissolved in alcohol (20 c.c.), was hydrogenated at room temperature using Raney nickel as catalyst. The solution was filtered and evaporated to dryness, finally under reduced pressure, leaving a glass-like material which did not reduce Fehling's solution and had $[\alpha]_D^{18°}$ + 8·3° (*c*, 0·104 in chloroform) (Found: C, 43·5; H, 8·6; OMe, 18·4. $C_6H_{14}O_5$ requires C, 43·3; H, 8·5; OMe, 18·7%). After being kept at room temperature for some weeks, this *2-methyl ribitol* crystallised, but when exposed to the atmosphere it very soon became syrupy.

UNIVERSITY COLLEGE, NOTTINGHAM.

[*Received, October 9th,* 1947.]