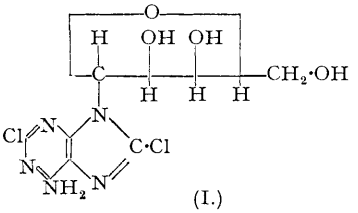
344. *Experiments on the Synthesis of Purine Nucleosides. Part XX. A Synthesis of Guanosine.*

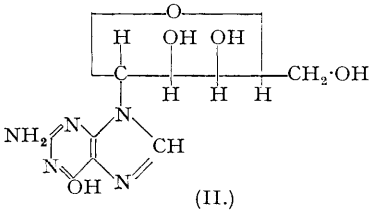
By J. Davoll, B. Lythgoe, and A. R. Todd.

2 : 8-Dichloro-9-β-D-ribofuranosidoadenine (Davoll, Lythgoe, and Todd, this vol., p. 967) has been converted into 9-β-D-*ribofuranosidoguanine*, identical with the natural nucleoside, guanosine. The structures accepted for guanosine and xanthosine are thereby confirmed, and they are shown to possess a β-type of glycosidic linkage.

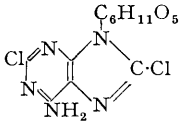
In Part XIX (this vol., p. 967) we described a synthesis of adenosine starting from 2 : 8-dichloro-9-β-D-ribofuranosidoadenine (I). Reactions designed to convert glycosides of 2 : 8-dichloroadenine into those of guanine had already been explored by Fischer and Helferich (*Ber.*, 1914, **47**, 210), so that the dichloro-compound (I) was clearly of potential value as a starting point for a synthesis of guanosine (II). Our experiences in effecting this synthesis are reported in the present paper.



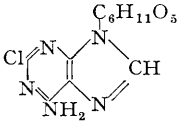
(I.)



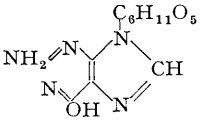
(II.)



(III.)



(IV.)



(V.)

Fischer and Helferich's experiments were carried out with " 2 : 8-dichloroadenine glucoside " (III) as starting material (for details of the structure of this compound, see Part XIX, *loc. cit.*). Treatment with zinc dust and water replaced one chlorine atom by hydrogen; the

resulting monochloroadenine glucoside was considered tentatively to retain the chlorine atom at position 2 (IV), a view based upon its behaviour in the following reactions. Treatment of (IV) with nitrous acid replaced the amino-group by a hydroxyl group, giving a chlorohypoxanthine glucoside which, without isolation in a state of purity, was heated with ethanolic ammonia to replace the chlorine atom by an amino-group. Owing to lack of material, the final product was not obtained quite pure, but it behaved as a guanine glucoside (V), giving on acidic hydrolysis a substance with the properties of guanine. The difficulties of characterising this purine satisfactorily are well known, and perhaps on this account Fischer and Helferich did not feel able to state with certainty that the reactions followed the course indicated above, and they promised a more detailed investigation at a later date; so far as we are aware, however, this was not carried out.

In order to gain experience with the reactions involved, it was thought desirable first to repeat the above work. No difficulty was experienced in obtaining the final glucoside in an analytically pure condition and with characteristics substantially the same as those originally reported. Its properties satisfied us that it was in all probability a derivative of guanine, and we were able to proceed with the application of similar methods to the dichlororibofuranoside (I). We had found in the course of work with the glucoside (III) that catalytic reduction offered a rapid and convenient alternative to treatment with zinc dust and water as a method for conversion into the monochloro-derivative (IV). The hydrogenation method was therefore applied to (I), and the product of reduction deaminated directly to the corresponding monochlorohypoxanthine glycoside, which, after a preliminary purification through the lead salt, was aminated by heating it with ethanolic ammonia. The crystalline 9-β-D-*ribofuranosidoguanine* so obtained was identical in m. p. behaviour and optical rotation with natural guanosine, and the *X*-ray powder photographs of the natural and synthetic materials were indistinguishable. Fischer and Helferich's views on the course of the reactions involved receive confirmation from this identity.

The above synthesis gives final verification of the structures now accepted for guanosine and its deamination product, xanthosine. Furthermore, in this synthesis guanosine has been prepared from the same 2 : 8-dichloro-9-D-ribofuranosidoadenine as was employed in the synthesis of adenosine (Part XIX, *loc. cit.*). It follows, therefore, that guanosine and consequently xanthosine have the same configuration at the glycosidic centre as adenosine; we regard it as virtually certain that this is of the β-type (cf. Part XII, *J.*, 1946, 833; Part XV, *ibid.*, p. 861). This feature of the guanosine molecule, although suspected from the similarity between its optical rotation and that of adenosine, we had previously been unable to establish by direct chemical methods. It may be noted that the conclusion is now warranted that all four purine and pyrimidine nucleosides present in yeast ribonucleic acid are β-D-ribofuranosides, a fact which may be significant in determining the structural pattern of the polynucleotide.

Experimental.

Guanine Glucoside.—2 : 8-Dichloro-9-β-D-glucopyranosidoadenine (1 g., Part XII, *loc. cit.*) in dilute sodium hydroxide solution (135 c.c. of 0·037N) was shaken at room temperature with palladised barium sulphate (1·5 g. of material prepared from 22 g. of barium sulphate and 1·8 g. of palladous chloride) in an atmosphere of hydrogen. After 9 minutes the hydrogen uptake corresponded to removal of one chlorine atom, and the solution was filtered from catalyst and neutralised with dilute hydrochloric acid. Evaporation of the solution to small bulk (*ca.* 10 c.c.) gave 2-chloro-9-β-D-glucopyranosidoadenine (0·52 g.), separating from water as fine needles sintering at 190°, m. p. 223° (decomp.) (Found : Cl, 10·5. Calc. for $C_{11}H_{14}O_5N_5Cl$: Cl, 10·7%). This material was identical with that obtained by reduction with zinc dust as described by Fischer and Helferich (*loc. cit.*). Conversion into guanine glucoside was accomplished by the methods of these authors. From the above monochloroglucoside (1 g.) there was obtained a specimen of material (0·1 g.) with the following characteristics : m. p. 295—305°, $[\alpha]_D^{16°}$ − 44·6° (*c*, 0·68 in N-sodium hydroxide) (Found in material dried at 140°/0·1 mm. : C, 42·3; H, 5·3; N, 22·5. Calc. for $C_{11}H_{15}O_6N_5$: C, 42·2; H, 4·8; N, 22·4%). Fischer and Helferich record for guanine glucoside, m. p. 298°, $[\alpha]_D^{15}$ − 41·6° (*c*, 7·5 in N-sodium hydroxide). This material gave an intense colour with the phenol reagent described by Hitchings (*J. Biol. Chem.*, 1941, **139**, 845). Whilst not specific for guanine this reaction is known to fail when applied to purines containing an unsubstituted position 2 (adenine, hypoxanthine). The only possible alternative for the structure of the compound, *viz.*, 8-amino-6-hydroxypurine glucoside, would therefore be expected to give no colour when tested in this way.

Guanosine.—2 : 8-Dichloro-9-β-D-ribofuranosidoadenine (0·950 g., Part XIX, *loc. cit.*) in dilute sodium hydroxide solution (120 c.c. of 0·025N) containing palladised barium sulphate (0·7 g.) was shaken with hydrogen at room temperature until the volume corresponding to removal of one chlorine atom had been absorbed (21 minutes). After removal of catalyst and neutralisation of the solution (dilute hydrochloric acid), it was evaporated to dryness. A solution of the product in water (40 c.c.) was treated with sodium nitrite (1·37 g.) and glacial acetic acid (1·6 c.c.) and kept at 25° for 7 hours. After addition of more sodium nitrite (0·8 g.) and acetic acid (1 c.c.) the solution was allowed to stand

at 25° for a further 12 hours, and then evaporated to dryness under reduced pressure at 35°. The residue was dissolved in water and treated with lead acetate (2·57 g.) and excess of aqueous ammonia. The precipitate was collected, washed with water, dissolved in glacial acetic acid (40 c.c.), and the solution decomposed with hydrogen sulphide. After removal of lead sulphide the solution was evaporated to dryness under reduced pressure, and the residue heated at 150° for 5 hours with ethanolic ammonia (53 c.c. of ethanol containing 3·7 c.c. of water saturated with ammonia at 0°). The cooled solution was filtered, evaporated to dryness under reduced pressure, and the residue extracted with hot water (30 c.c.). The extract was treated with lead acetate (2·6 g.) and excess of aqueous ammonia, and the precipitate collected, washed, and dissolved in dilute acetic acid (40 c.c. of 4·5%). The solution was decomposed with hydrogen sulphide, the filtrate from the lead sulphide evaporated to dryness under reduced pressure, and the residue dissolved in hot water (7 c.c.). On cooling, 9-*β*-D-*ribofuranosidoguanine* separated and was purified by recrystallisation from water (5 c.c.). Yield, 72 mg.; m. p. 239° (decomp.) (rapid heating), alone or in admixture with guanosine obtained by hydrolysis of yeast ribonucleic acid. $[\alpha]_D^{13°}$ — 64° (*c*, 0·193 in 0·1N-sodium hydroxide) (Found in material dried over phosphoric oxide at room temperature/15 mm. for 48 hours : C, 37·5; H, 5·7; N, 21·8. $C_{10}H_{13}O_5N_5,2H_2O$ requires C, 37·6; H, 5·3; N, 21·9%. Found in material dried at 124°/1 mm. : N, 24·5. $C_{10}H_{13}O_5N_5$ requires N, 24·7%). A sample of natural guanosine crystallised and dried under the same conditions as above gave analytical values corresponding to the same dihydrate as recorded for the synthetic specimen (Found in material dried at room temperature/15 mm. for 48 hours over phosphoric oxide : C, 37·6; H, 5·2; N, 22·1%). Natural and synthetic samples of the dihydrate were examined by *X*-ray powder photography in the Department of Crystallography of this University and their identity confirmed.

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