CCCXXVI.—*Digitalis Glucosides. Part II. Digoxigenin, the Aglucone of Digoxin.*

By SYDNEY SMITH.

THE isolation of digoxin, a new digitalis glucoside, was described in a recent communication (this vol., p. 508) in which it was also shown that the sole products of the hydrolysis of the glucoside were digoxigenin (1 mol.) and digitoxose (3 mols.).

Digoxigenin, $C_{23}H_{34}O_5$, when heated with dilute acids readily loses one molecule of water and since the resulting *anhydrodigoxigenin*, $C_{23}H_{32}O_4$, gives a *diacetate*, digoxigenin itself presumably contains three hydroxyl groups. Three of the five oxygen atoms in the empirical formula are thus accounted for. The remaining two oxygen atoms are present in a lactone group which can be estimated by hydrolysis and titration. Digoxigenin gives the red colour with alkaline sodium nitroprusside (Legal test) which Jacobs, Gustus, and Hoffmann (*J. Biol. Chem.*, 1926, **67**, 333; **70**, 1) consider typical of $\Delta^{\beta\gamma}$-unsaturated lactones. More direct proof of the unsaturated nature of digoxigenin is afforded by the formation of *dihydrodigoxigenin* on catalytic reduction. Digoxigenin is therefore a trihydroxy $\Delta^{\beta\gamma}$-unsaturated lactone. Jacobs and his co-workers have shown that the aglucones of the digitalis and strophanthus series when treated with alkali lose their unsaturated nature owing to the formation of an oxide ring. Digoxigenin also is typical in this respect. When treated with alkali it readily yields *iso*digoxigenin, which no longer gives the Legal reaction and is not reduced by palladium and hydrogen under the conditions successfully employed

for the reduction of digoxigenin. The recent work of Jacobs and Gustus (*J. Biol. Chem.*, 1930, **86**, 199) has given for the first time definite proof that gitoxigenin and digitoxigenin, the aglucones of gitoxin and digitoxin, have a common structure. It is probable that this structure is also common to digoxigenin, although there is as yet no direct proof of this assumption. Further work in this direction is in progress.

EXPERIMENTAL.

Digoxigenin (*loc. cit.*) reduces alkaline silver nitrate (Tollens's reagent) and gives a red colour with sodium nitroprusside (Legal test) under the conditions given by Jacobs and Hoffmann (*loc. cit.*). When dissolved in sulphuric acid containing ferric sulphate (Kiliani's reagent), it gives a yellow solution which on standing becomes red by transmitted light but greenish by reflected light. Digitoxigenin behaves in a similar way with Kiliani's reagent, but gitoxigenin gives a solution which rapidly becomes red and does not become dichroic even on prolonged standing. Digoxigenin possesses the bitter taste typical of the digitalis glucosides.

The lactone group was estimated by boiling under reflux for 3 hours about 20 mg. of the substance with 2 c.c. of neutralised alcohol and 0·100 c.c. of 2·7*N*-potassium hydroxide solution (measured in a Trevan micrometer syringe). After hydrolysis was complete the solution was titrated with 0·1*N*-sulphuric acid solution (phenolphthalein indicator). 20·97 Mg. required 0·52 c.c. of 0·1*N*-potassium hydroxide. Calc. for 1 equiv., 0·54 c.c.

Diacetyldigoxigenin was prepared by boiling digoxigenin with acetic anhydride and a trace of pyridine for 10 minutes. It crystallised readily from aqueous methyl alcohol in prisms, m. p. 221° (corr.), $[\alpha]_{5461}^{20^\circ}$ + 61·3° (*c* in methyl alcohol, 1·56) (Found for the substance dried at 100° in a vacuum : C, 68·3; H, 8·0. $C_{27}H_{38}O_7$ requires C, 68·3; H, 8·1%. The lactone and acetyl groups in this and the other acetyl compounds described in this paper were estimated together by hydrolysis and titration as described above. 20·78 Mg. required 1·36 c.c. of 0·1*N*-potassium hydroxide. Calc. for 3 equivs., 1·31 c.c.).

Anhydrodigoxigenin.—A solution of digoxigenin (1 g.) in 50% alcohol (100 c.c.) containing sulphuric acid (5 g.) was boiled under reflux for 2 hours. The solution was diluted with 100 c.c. of water, and the alcohol removed by evaporation under reduced pressure. After standing for some time the semi-crystalline deposit (0·8 g.) was separated and crystallised first from aqueous acetone and then from ethyl acetate, from which it separated in brilliant prisms, m. p. 182° (corr.), $[\alpha]_{5461}^{20^\circ}$ + 16·3° (*c* in methyl alcohol, 1·6). It

crystallised from aqueous alcohol in rectangular plates containing water of crystallisation (Found for the air-dried crystals : C, 71·0; H, 8·7; loss at 100° in a vacuum, 4·4. $C_{23}H_{32}O_4,H_2O$ requires C, 70·7; H, 8·8; H_2O, 4·6%. Found for the substance dried at 100° in a vacuum : C, 74·1; H, 8·7. $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·7%. 17·22 Mg. required 0·49 c.c. of 0·1*N*-potassium hydroxide. Calc. for 1 equiv., 0·46 c.c.). Gitoxigenin under the same conditions gave dianhydrogitoxigenin, m. p. 212°, $[\alpha]_{5461}^{20°}$ + 740° (*c* in methyl alcohol, 0·47). Cloetta (*Arch. Exp. Path. Pharm.*, 1926, **112**, 261) gave m. p. 211°, $[\alpha]_D$ + 576° (methyl alcohol). Digitoxigenin similarly gave anhydrodigitoxigenin, m. p. 183—184°, $[\alpha]_{5461}^{20°}$ − 0·7° (*c* in methyl alcohol, 1·89). Cloetta (*ibid.*, 1920, **88**, 113) gave m. p. 183—185° and Windaus and Stein (*Ber.*, 1928, **61**, 2436) gave m. p. 193° and $[\alpha]_D^{18°}$ − 4·68° (methyl alcohol).

Diacetylanhydrodigoxigenin was prepared by boiling the substance with acetic anhydride containing a trace of pyridine under reflux for 15 minutes. It crystallised readily from dilute alcohol in solvent-free needles, m. p. 199° (corr.), $[\alpha]_{5461}^{20°}$ + 38·6° (*c* in methyl alcohol, 0·5) (Found for the substance dried at 100° in a vacuum : C, 71·2; H, 7·9. $C_{27}H_{36}O_5$ requires C, 71·0; H, 8·0%. 8·911 Mg. required 0·60 c.c. of 0·1*N*-potassium hydroxide. Calc. for 3 equivs., 0·59 c.c.).

Dihydrodigoxigenin.—0·5 G. of digoxigenin dissolved in 25 c.c. of 80% alcohol was shaken with palladium-black and hydrogen until no more gas was absorbed. Reduction proceeded slowly for 2 hours and was complete after about 12 hours. After the catalyst had been removed, the solution was concentrated, until solid separated, and kept. The solid (0·4 g.) after crystallisation from ethyl acetate melted at 215° (corr.) and had $[\alpha]_{5461}^{20°}$ + 20·5° (*c* in methyl alcohol, 0·5). It crystallised from dilute alcohol in needles which melted with frothing at 170° and on further heating solidified a few degrees higher and finally melted at 214°. *Dihydrodigoxigenin* is soluble in most organic solvents but like digoxigenin is sparingly soluble in benzene, light petroleum, and ether. Its colour reactions are similar to those of digoxigenin and it possesses the bitter taste of the latter. It does not give the Legal reaction (Found in material dried at 100° in a vacuum : C, 70·3; H, 9·4. $C_{23}H_{36}O_5$ requires C, 70·4; H, 9·3%. 19·78 Mg. required 0·47 c.c. of 0·1*N*-potassium hydroxide. Calc. for 1 equiv., 0·50 c.c.).

Diacetyldihydrodigoxigenin was prepared by acetylating the dihydrogenin with acetic anhydride in pyridine solution. It crystallised readily from dilute alcohol in needles, m. p. 222° (corr.), $[\alpha]_{5461}^{20°}$ + 29·8° (*c* in methyl alcohol, 0·9), and possessed the bitter taste of the parent substance (Found in material dried at 100° in a vacuum:

C, 68·0; H, 8·4. $C_{27}H_{40}O_7$ requires C, 68·0; H, 8·5%. 18·43 Mg. required 1·07 c.c. of 0·1*N*-potassium hydroxide. Calc. for 3 equivs., 1·16 c.c.).

iso*Digoxigenin*.—Digoxigenin (2 g.), dissolved in a 10% solution of potassium hydroxide in methyl alcohol (20 c.c.), was kept for 30 minutes.. The solution was diluted with water (20 c.c.) and alcohol (20 c.c.) and acidified with 10% hydrochloric acid (25 c.c.). After standing for ½ hour, the solution was further diluted and then concentrated under diminished pressure. The solid which separated was either filtered off and purified by crystallisation or extracted with chloroform. In the latter case the chloroform solution was freed from a trace of the unlactonised acid by washing with dilute sodium carbonate solution, dried by magnesium sulphate, and evaporated. The chloroform residue was dissolved in ethyl acetate, and the solution concentrated on a water-bath until crystallisation began; the yields of successive crops were 0·45 g. (m. p. 245°) and 0·42 g. (m. p. 255°). After repeated crystallisation iso*digoxigenin* melts and decomposes at 260° (corr.). It separates from ethyl acetate in prisms and from dilute alcohol in needles. It is readily soluble in methyl and ethyl alcohol and acetone, somewhat sparingly soluble in chloroform, and practically insoluble in benzene and light petroleum. The specific rotation $[\alpha]_{5461}^{20°}$ is + 13·6° (*c* in methyl alcohol, 1). The substance retains the bitter taste of digoxigenin but no longer gives the Legal reaction, nor can it be reduced with palladium and hydrogen. The colour reaction with Kiliani's reagent is identical with that of digoxigenin (Found in material dried at 100° in a vacuum : C, 70·8, 70·9; H, 8·6, 8·6. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%). *iso*Digoxigenin dissolves readily in pyridine and crystallises from concentrated solution in long, stout, six-sided plates (m. p. 280° corr.) containing 1 mol. of pyridine (Found : loss at 100° in a vacuum, 13·5. $C_{23}H_{34}O_5,C_5H_5N$ requires loss, 16·8%).

*Diacetyl*iso*digoxigenin* was prepared with acetic anhydride in pyridine solution. It crystallised from aqueous alcohol in needles, m. p. 280° (Found : C, 68·6; H, 7·8. $C_{27}H_{38}O_7$ requires C, 68·3; H, 8·1%. 23·25 Mg. required 1·42 c.c. of 0·1*N*-potassium hydroxide. Calc. for 3 equivs., 1·47 c.c.).

iso*Digoxigeninic Acid*.—A solution of *iso*digoxigenin (0·6 g.) in alcohol (12·5 c.c.) and 10% sodium hydroxide solution (1·5 c.c.) was heated on a water-bath for 15 minutes, cooled, diluted with water, and acidified with acetic acid. After the alcohol had been evaporated under reduced pressure at a low temperature, the *acid* separated in fine needles, m. p. 228° (corr.; decomp.). The acid is very readily relactonised, but it can be recrystallised without

lactonisation by dissolving it in alcohol and freely diluting the solution with water or by adding dilute aqueous ammonia to a suspension of the acid in water until it has dissolved and acidifying the solution with acetic acid. For analysis the substance was dried over sulphuric acid in a vacuum desiccator (Found : C, 67·6; H, 8·4. $C_{23}H_{36}O_6$ requires C, 67·6; H, 8·9%).

The author is indebted to Mr. A. Bennett and Mr. H. C. Clarke for the analyses.

WELLCOME CHEMICAL WORKS,
DARTFORD.

[*Received, October 6th,* 1930.]