315. *Digitalis Glucosides. Part V. On the Constitution of Digoxigenin.*

By Sydney Smith.

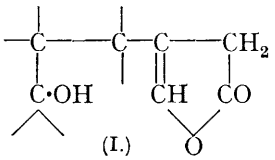
Digoxigenin is the aglucone of digoxin, a cardiotonic glucoside occurring in the leaves of *Digitalis lanata*. It has been shown (Part II; J., 1930, 2478) that of the five atoms of oxygen present in the molecule of digoxigenin, $C_{23}H_{34}O_5$, three are accounted for by the presence of hydroxyl groups, and that the remaining two occur in a lactone group. The nature of these substituent groups has now been investigated in greater detail.

Digoxigenin readily loses one hydroxyl group on treatment with dilute acids (*loc. cit.*). Two of the hydroxyl groups can be acetylated and on treatment with chromic acid two of the hydroxyl groups are oxidised to form the corresponding diketone. The latter readily loses the remaining hydroxyl group on treatment with acids. It is clear that two of the hydroxyl groups are secondary and the third is tertiary in character. Although the analytical data indicate the formation of a diketone in the above-mentioned oxidation, the product gives only a *monoxime* and a *monosemicarbazone*. Analyses of the corresponding oxidation products of *iso*digoxigenin and dihydrodigoxigenin also indicate the formation of the corresponding diketones, although here again in each case only one of the ketonic groups reacts with hydroxylamine or semicarbazide. The consistent series of analyses, however, leaves no doubt that two such groups must be present and confirmation of the presence of two ketonic groups is afforded by the formation of a *dioxime* from the oxidation product of

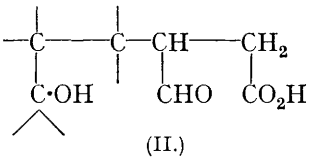
anhydrodigoxigenin. Analyses of the oxidation products of *methyl* iso*digoxigeninate*, iso*digoxigenic acid* and its *methyl* ester also agree with the figures required for the oxidation of two secondary alcoholic groups.

Digoxigenin has all the properties associated with the presence of the $\Delta^{\beta\gamma}$-lactone group (partial formula I) which is characteristic of the genins of the cardiac glucosides. It gives a red colour with alkaline sodium nitroprusside (Legal reaction). On catalytic reduction it takes up one molecule of hydrogen and then no longer gives the Legal reaction. Hydrolysis of digoxigenin gives rise to an aldehydo-acid (II) (digoxigeninic acid); this can be oxidised to the dicarboxylic acid (III), which is unstable and is isolated as the corresponding lactone acid (IV) (digoxigenic acid). Relactonisation of the acid (II) gives rise, not to the original lactone, but to the isomeric lactone (V) (*iso*digoxigenin). Since *iso*digoxigenin is not reducible by catalytic reduction and does not give the Legal reaction, it can no longer possess a double bond and must therefore have acquired an additional cyclic structure. In all these respects digoxigenin behaves similarly to digitoxigenin and the explanation of these reactions first put forward in the case of digitoxigenin by Jacobs and Gustus (*J. Biol. Chem.*, 1928, **78**, 576; cf. Windaus and Stein, *Ber.*, 1928, **61**, 2440) applies equally to digoxigenin. The original formulation has been modified slightly and an additional carbon atom has been inserted between the tertiary hydroxyl group and the lactone group as required by the present views on the structure of digitoxigenin.

The constitution of the cardiac aglucones has recently been discussed by Kon (*Chem. and Ind.*, 1934, 593, 956), Tschesche (*Angew. Chem.*, 1934, **47**, 729; *Z. physiol. Chem.*, 1934, **229**, 219), and Jacobs and Elderfield (*J. Biol. Chem.*, 1935, 497) and formula (VI) has been assigned to digitoxigenin. Although no direct correlation of digoxigenin with digitoxigenin has yet been effected, there can be little doubt, in view of the close similarity in properties of the two genins and their derivatives, that both have the same skeletal structure. It then remains necessary to determine the positions of the lactone group and the two secondary and one tertiary hydroxyl groups. The position of the lactone group at C17 has been established in the case of uzarigenin by degradation to *allo*aetiocholanic acid (Tschesche, *Z. physiol. Chem.*, 1935, **229**, 219) and for digitoxigenin by degradation to aetiocholanic acid (Jacobs and Elderfield, *J. Biol. Chem.*, 1935, **108**, 497). It is probable that in digoxigenin the lactone group is situated at the same position.



(I.)



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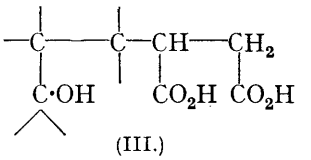

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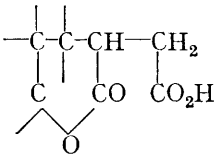

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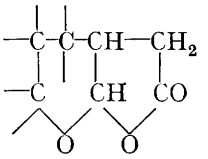

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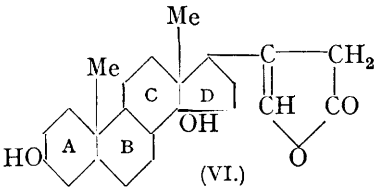

(VI.)

If this is so, it becomes possible to determine the position of the tertiary hydroxyl group, since the latter is concerned in the formation of *iso*digoxigenin and must therefore be in close proximity to the lactone group for easy ring formation to occur. The nearest available position for a tertiary hydroxyl group is at C14. That it is the tertiary hydroxyl group which takes part in the formation of *iso*digoxigenin is established experimentally by the conversion of *digoxigenone* into iso*digoxigenone*, identical with the *iso*-compound formed by the oxidation of *iso*digoxigenin. In digoxigenone the secondary alcoholic groups of digoxigenin have been oxidised to the corresponding ketone groups and it can only be the remaining unoxidised tertiary alcoholic group which takes part in the formation of the *iso*-compound. Additional evidence that the tertiary hydroxyl group takes part in the ring formation is afforded by the fact that *iso*digoxigenin forms a diacetate and hence

retains the two secondary alcoholic groups and that it does not form an anhydro-compound as it should if the tertiary hydroxyl group were still free. The positions at C14 and C17 are thus allocated to a tertiary hydroxyl group and a lactone group respectively. It then remains to determine the position of the two secondary alcoholic groups. One of these, on biogenetic grounds, is almost certainly situated at C3. The arrangement of a secondary alcoholic group at C3, a tertiary alcoholic group at C14, and a lactone group at C17 is that proposed for digitoxigenin by Tschesche and Jacobs and Elderfield (*locc. cit.*). The position of the additional secondary hydroxyl remains to be determined and the investigation is being continued with the object of locating it and of establishing a direct experimental correlation of digoxigenin with digitoxigenin.

Experimental.

Digoxigenone.—Digoxigenin (1 g.) was dissolved in 80% acetic acid (20 c.c.) and treated at laboratory temperature with Kiliani's chromic acid solution (5 c.c.). After 1 hour, the solution was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dilute sodium carbonate solution, and water, dried (magnesium sulphate), and evaporated; the residue crystallised from acetone in needles, m. p. 265° (decomp.), $[\alpha]_{5461}^{20°}$ + 130° (*c* in acetone 0·4), sparingly soluble in methyl and ethyl alcohol, benzene, and ethyl acetate, more soluble in acetone, and readily in pyridine (Found: C, 71·6; H, 7·9. $C_{23}H_{30}O_5$ requires C, 71·5; H, 7·8%. Lactone titration: 18·49 mg. required 0·51 c.c. 0·1*N*-NaOH. One equiv. requires 0·48 c.c.). Oxidation of digoxigenin with aqueous chromic acid under the conditions described above gave the same *digoxigenone*. The *oxime* was prepared by boiling the ketone (0·2 g.) with hydroxylamine hydrochloride (0·2 g.) in a little water and potassium acetate (0·4 g.) in methyl alcohol for 3 hours; when the solution was diluted with water and concentrated, the oxime separated in plates, m. p. 235° (decomp.) (Found: N, 3·6. $C_{23}H_{31}O_5N$ requires N, 3·5%). The *semicarbazone*, prepared from the ketone (0·2 g.), semicarbazide hydrochloride (0·2 g.) in a little water, and potassium acetate (0·3 g.) in methyl alcohol, the solution being diluted with water and concentrated after 24 hours, separated in granular aggregates of needles, m. p. 268° (decomp.) (Found: N, 9·2. $C_{24}H_{33}O_5N_3$ requires N, 9·5%).

iso*Digoxigenone.*—*iso*Digoxigenin (0·2 g.) was dissolved in a mixture of acetic acid (4 c.c.) and water (1 c.c.) and treated with 1 c.c. of 20% aqueous chromic acid. Crystals separated in a few minutes and after ¾ hour the *ketone* was collected (0·18 g.). It crystallised from chloroform in fine needles, m. p. 335°, sparingly soluble in the common organic solvents (Found: C, 71·5; H, 7·9. $C_{23}H_{30}O_5$ requires C, 71·5; H, 7·8%. Lactone titration: 20·06 mg. required 0·60 c.c. 0·1*N*-NaOH. One equiv. requires 0·52 c.c.).

The *oxime*, prepared as above, the reaction mixture becoming clear after 15 minutes' boiling, separated in long rectangular plates, m. p. 305° (decomp.) (Found: N, 3·5. $C_{23}H_{31}O_5N$ requires N, 3·5%). The *semicarbazone*, prepared as above (2 hours' heating), separated in needles, m. p. 295° (decomp.) (Found: N, 9·4. $C_{24}H_{33}O_5N_3$ requires N, 9·5%).

Dihydrodigoxigenone.—Dihydrodigoxigenin (0·5 g.), dissolved in 80% acetic acid (7 c.c.), was treated with Kiliani's chromic acid solution (2·5 c.c.) at laboratory temperature. On addition of water the *diketone* separated. It crystallised from alcohol in needles, m. p. 243°, $[\alpha]_{5461}^{20°}$ + 119·6° (*c* in chloroform, 0·90), sparingly soluble in methyl and ethyl alcohol, acetone, and ethyl acetate, readily soluble in chloroform (Found: C, 71·3; H, 8·3. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%. Lactone titration: 20·703 mg. required 0·60 c.c. 0·1*N*-NaOH. One equiv. requires 0·53 c.c.). The *monoxime*, prepared as above, crystallised from dilute methyl alcohol and had m. p. 250° (Found: N, 3·5. $C_{23}H_{33}O_5N$ requires N, 3·5%). The *monosemicarbazone*, prepared in the cold, formed granular aggregates of plates, m. p. 260° (Found: C, 64·8; H, 8·2; N, 9·4. $C_{24}H_{35}O_5N_3$ requires C, 64·7; H, 7·9; N, 9·4%).

Anhydrodigoxigenone.—Anhydrodigoxigenin (J., 1930, 2479) (0·5 g.), dissolved in 80% acetic acid (12·5 c.c.), was treated with Kiliani's chromic acid solution (2·5 c.c.) at 15°, the mixture being diluted with water after ½ hour. The precipitate obtained crystallised from alcohol in needles, m. p. 260°, $[\alpha]_{5461}^{20°}$ + 87·9° (*c* in chloroform, 0·65) (Found: C, 74·9; H, 7·7. $C_{23}H_{28}O_4$ requires C, 74·9; H, 7·7%. Lactone titration: 12·121 mg. required 0·40 c.c. 0·1*N*-NaOH. One equiv. requires 0·33 c.c.). The *dioxime*, prepared as above, separated on the addition of water to the concentrated solution in needles, m. p. 270° (decomp.) after recrystallisation from dilute methyl alcohol (Found: N, 6·9. $C_{23}H_{30}O_4N_2$ requires N, 7·0%). The semicarbazone, prepared by boiling with the above reaction mixture for 1 hour, was obtained in amorphous

granules, decomp. *ca.* 260°, which appeared to consist of a mixture of the mono- and the di-semicarbazone (Found: N, 14·3. $C_{24}H_{31}O_4N_3$ requires N, 9·9%. $C_{25}H_{34}O_4N_6$ requires N, 17·4%).

Methyl iso*Digoxigeninate*.—The acid (J., 1930, 2481), suspended in acetone, dissolved on treatment with ethereal diazomethane and the *methyl* ester soon separated in brilliant prisms, m. p. 145°, $[\alpha]_{5461}^{20°}$ + 17·8° (*c* in methyl alcohol, 0·59), fairly soluble in methyl alcohol, somewhat sparingly soluble in ethyl alcohol, acetone, chloroform, and ethyl acetate (Found: C, 67·9; H, 9·2; OMe, 7·4. $C_{24}H_{38}O_6$ requires C, 68·2; H, 9·1; OMe, 7·3%). It crystallised slowly from methyl alcohol in needles, m. p. 156° (Found: C, 66·6; H, 9·3; OMe, 12·9. $C_{24}H_{38}O_6$,MeOH requires C, 66·0; H, 9·3; 2OMe, 13·7%), apparently of an *acetal*; no change in methoxyl content occurred on drying at 100° in a vacuum.

Methyl iso*Digoxigenonate*.—Methyl *iso*digoxigeninate (0·2 g.), dissolved in acetic acid (4 c.c.) and water (1 c.c.), was treated with 1 c.c. of Kiliani's chromic acid reagent at laboratory temperature and after 5 minutes water was added until crystallisation began. The *ester* (0·15 g.) crystallised from dilute methyl alcohol in thin broad plates, m. p. 248°, $[\alpha]_{5461}^{20°}$ + 37·3° (*c* in methyl alcohol, 0·5) (Found: C, 69·1; H, 8·0; OMe, 7·2. $C_{24}H_{34}O_6$ requires C, 68·9; H, 8·2; OMe, 7·4%. Lactone titration: 14·00 mg. required 0·68 c.c. 0·1*N*-NaOH. Two equivs. require 0·65 c.c.).

iso*Digoxigenic Acid*.—*iso*Digoxigenin (2 g.), aqueous sodium hydroxide (10%, 6 c.c.), and alcohol (50 c.c.) were boiled together under reflux for 15 minutes. After removal of the alcohol by dilution with water and concentration under diminished pressure to 60 c.c., the solution was treated with 20 c.c. of sodium hypobromite solution (prepared from bromine, 1 c.c., and *N*-sodium hydroxide, 50 c.c., at 0°). The mixture was kept for 1 hour at laboratory temperature and then acidified with acetic acid. iso*Digoxigenic acid* crystallised slowly in prisms or thin hexagonal plates, m. p. 235° (frothing), soluble in acetone and alcohol, less soluble in chloroform; $[\alpha]_{5461}^{20°}$ − 36·5° (*c* in pyridine, 1·03) [Found: C, 67·9; H, 8·5. $C_{23}H_{34}O_6$ requires C, 67·9; H, 8·4%. Lactone titration: 13·08 mg. required 0·50 c.c. 0·1*N*-NaOH. Two equivs. require 0·64 c.c. Carboxyl titration: 5·099 mg. required 1·28 c.c. 0·01*N*-$Ba(OH)_2$. One carboxyl group requires 1·26 c.c.].

*iso*Digoxigenic acid formed a sparingly soluble *pyridine* salt, which crystallised in prisms, m. p. 260° (Found for material dried at 100° in a vacuum: C, 69·2; H, 8·2; N, 3·0. $C_{28}H_{39}O_6N$ requires C, 69·2; H, 8·1; N, 2·9%). It was hydrolysed to the free acid, m. p. 235°, by crystallisation from hot dilute alcohol.

Methyl iso*digoxigenate*, prepared by the action of diazomethane on the acid suspended in acetone, crystallised in fine needles, m. p. 208°, from ethyl acetate; $[\alpha]_{5461}^{20°}$ − 45·6° (*c* in methyl alcohol, 0·60) (Found: C, 68·7; H, 8·7; OMe, 7·2. $C_{24}H_{36}O_6$ requires C, 68·5; H, 8·6; OMe, 7·4%. Lactone titration: 17·50 mg. required 0·80 c.c. 0·1*N*-NaOH. Two equivs. require 0·84 c.c.).

iso*Digoxigonic Acid*.—*iso*Digoxigenic acid (0·15 g.), dissolved in 5 c.c. of 80% acetic acid, was treated with Kiliani's chromic acid solution at laboratory temperature. After 20 minutes, the solution was diluted with water. The *acid* separated in needles, m. p. 260° after recrystallisation from dilute methyl alcohol; $[\alpha]_{5461}^{20°}$ + 56·6° (*c* in acetone, 1·12) (Found: C, 68·6; H, 7·5. $C_{23}H_{30}O_6$ requires C, 68·6; H, 7·5%).

Methyl iso*digoxigonate*, prepared by the action of diazomethane on a solution of the acid in acetone or by chromic acid oxidation of methyl *iso*digoxigenate, crystallised from methyl alcohol in needles or plates, m. p. 253°, $[\alpha]_{5461}^{20°}$ + 48·0° (*c* in acetone, 0·5), rather sparingly soluble in methyl and ethyl alcohol, more soluble in acetone, ethyl acetate, and benzene, readily soluble in chloroform (Found: C, 69·2; H, 7·7; OMe, 8·0. $C_{24}H_{32}O_6$ requires C, 69·2; H, 7·7; OMe, 7·5%).

Conversion of Digoxigenone into Anhydrodigoxigenone.—Digoxigenone (0·5 g.) was dissolved in concentrated hydrochloric acid (3 c.c.) by gentle heating. After a few minutes crystals separated (0·35 g.), identical in m. p. and rotation with the anhydrodigoxigenone formed by the oxidation of anhydrodigoxigenin, m. p. 182° (Found: C, 74·7; H, 7·6. Calc. for $C_{23}H_{28}O_4$: C, 74·9; H, 7·7%).

Conversion of Digoxigenone into iso*Digoxigenone*.—Digoxigenone (0·5 g.) was dissolved in a mixture of 10% methyl-alcoholic potassium hydroxide (3·75 c.c.) and water (1·25 c.c.) with the aid of heat. The solution was kept for ½ hour, diluted with water, warmed, made acid to Congo-paper with hydrochloric acid, and kept for ½ hour; lactonisation was then nearly complete. The product was sparingly soluble in the common organic solvents and after crystallisation from chloroform melted at 335° (decomp.) (Found: C, 71·3; H, 7·8%). The m. p. was not lowered by

admixture with *iso*digoxigenone prepared by the oxidation of *iso*digoxigenin. The identity with *iso*digoxigenone was confirmed by the preparation of the oxime, m. p. 305°.

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