II.—*Digitalis Glucosides. Part III. Glucosides of* Digitalis lanata.

By Sydney Smith.

In a recent communication (J., 1930, 508) it was shown that the leaves of *Digitalis lanata* contain a previously unknown physiologically active glucoside to which the name digoxin was given. A second crystalline glucoside has now been separated and found to be identical with gitoxin, a glucoside which has hitherto only been found in the leaves of *D. purpurea*.

Gitoxin was first prepared in an impure state by Kraft (*Arch. Pharm.*, 1912, **250**, 118) and described under the name anhydrogitalin. It was subsequently isolated by Windaus and Schwarte (*Ber.*, 1925, **58**, 1515) and named gitoxin; shortly afterwards it was described by Cloetta (*Arch. Exp. Path. Pharm.*, 1926, **112**, 261) under the name "Bigitalinum cristallisatum." The empirical formula $C_{41}H_{64}O_{14}$ was established by the work of Windaus, Westphal, and Stein (*Ber.*, 1928, **61**, 1847). The glucoside from *D. lanata* corresponds with the general description given by Windaus and Schwarte, and Cloetta, but differs in one respect—Cloetta states (*loc. cit.*, p. 270) that the glucoside is optically inactive. In pyridine solution the glucoside from *D. lanata* had a small but definite optical activity, but since a specimen of gitoxin prepared from *D. purpurea* had the same specific rotation, the glucosides from both sources are identical in this as in other respects. On hydrolysis the glucoside gave a practically quantitative yield of gitoxigenin (1 mol.) and digitoxose (3 mols.) as indicated by Windaus and Schwarte (*loc. cit.*). In addition to digoxin, which has now been isolated in considerable quantity, and gitoxin, which is present to a smaller

extent, the leaves of *D. lanata* contain other glucosides which are at present under examination.

EXPERIMENTAL.

The total glucosides were dissolved in acetone and kept until a mixture of the less soluble glucosides separated. After being washed with acetone, the sparingly soluble fraction was extracted with successive small quantities of hot 80% alcohol. The first extracts, which were rich in digoxin, were worked up by the process previously described (*loc. cit.*). The final alcoholic extract consisted chiefly of gitoxin. It was concentrated under reduced pressure until a finely crystalline solid separated. The more soluble glucosides were removed from this solid by boiling with small amounts of chloroform, in which gitoxin itself is but sparingly soluble. The residue was recrystallised by concentrating a solution in hot 80% alcohol or in a boiling mixture of methyl alcohol and chloroform. Purification was completed by repeating the extraction with chloroform and recrystallisation until the m. p. and specific rotation became constant.

Gitoxin separates from hot dilute alcohol, or on concentration of a solution in methyl alcohol and chloroform (equal volumes), in short stout prisms, m. p. 285° (corr.; decomp.). It can also be crystallised by diluting a solution in pyridine with water or ether. In pyridine solution it has $[\alpha]^{20°}_{5461} + 3{\cdot}5°$ ($c = 1{\cdot}02$) (Found: C, 63·1, 62·8; H, 8·1, 8·2; OMe, absent. Calc. for $C_{41}H_{64}O_{14}$: C, 63·0; H, 8·3%).

Hydrolysis of Gitoxin.—The glucoside (3·16 g.), finely powdered, was boiled under reflux with 80% alcohol (1500 c.c.) until solution was nearly complete. Hydrochloric acid (*d* 1·16) (6 c.c.), diluted with water to 60 c.c., was added to the hot cloudy solution, and heating continued. The remaining gitoxin soon went into solution and heating was continued for 15 minutes after addition of the acid. The solution was then cooled, neutralised with sodium hydroxide solution, concentrated in a vacuum to about 200 c.c., and kept in a refrigerator until crystallisation was complete. The genin (dried at 100° in a vacuum) weighed 1·305 g. (82·6% of the theoretical yield). The filtrate and washings were mixed with one-third of their volume of 0·3% hydrochloric acid and heated on a water-bath for 15 minutes. After being concentrated slightly under reduced pressure, the solution was cooled, neutralised with sodium hydroxide, and extracted with chloroform, which removed 0·274 g. of crude gitoxigenin (17·3% of the theoretical yield. Total, 99·9%). The aqueous liquid was evaporated to dryness under reduced pressure, the residue dried, and the sugar extracted with acetone. The

acetone extract was evaporated and gave a syrupy residue, which solidified completely and after drying at 75° in a vacuum weighed 1·70 g. (calc. for 3 mols., 1·798 g.), *i.e.*, 94·6% of the theoretical yield.

Gitoxigenin.—The crude genin, after drying at 100° in a vacuum, had $[\alpha]_{5461}^{20°}$ + 38·4° (*c* in methyl alcohol, 0·585). After crystallisation from dilute alcohol it had for the dry substance $[\alpha]_{5461}^{20°}$ + 38·5° (*c* in methyl alcohol, 0·681) and m. p. 234°. Cloetta (*loc. cit.*) gave m.p. 232° and $[\alpha]_D$ + 32·6° (methyl alcohol). There was no depression in the m. p. when the genin was mixed with gitoxigenin prepared by the hydrolysis of gitoxin from *D. purpurea*. It crystallised in the characteristic plates illustrated by Cloetta (*loc. cit.*). The crystals contained 1·5 mols. of water (Found for the air-dried crystals: C, 66·1, 66·4, 66·1; H, 8·6, 8·8, 8·6; loss at 100° in a vacuum, 6·6. Calc. for $C_{23}H_{34}O_5$,1·5H_2O: C, 66·2; H, 8·9; H_2O, 6·5%. Found for the substance dried at 100° in a vacuum: C, 70·7, 70·6; H, 8·6, 8·7. Calc. for $C_{23}H_{34}O_5$: C, 70·7; H, 8·8%).

Digitoxose.—The unpurified digitoxose had $[\alpha]_{5461}^{20°}$ + 55·3° (*c* in water, 3·16), m. p. 103—104°. When crystallised from ethyl acetate, it gave the following yields: 1·22 g., m. p. 106° (softening at 96°) (corr.), $[\alpha]_{5461}^{20°}$ + 55·7° (*c* in water, 2·19); 0·15 g., m. p. 103—104° (softening at 95°), $[\alpha]_{5461}^{20°}$ + 52·6° (*c* in water, 0·821). Total, 1·37 g. By recrystallisation the m. p. was raised to 112°. The m. p. was not lowered on admixture with digitoxose prepared from gitoxin from *D. purpurea* and the sugar gave the characteristic Keller reaction (Found: C, 48·8; H, 7·9. Calc. for $C_6H_{12}O_4$: C, 48·6; H, 8·2%).

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