

LXXII.—*Digoxin, a New Digitalis Glucoside.*

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IN recent years it has been shown that the leaves of *Digitalis lanata* have a greater physiological activity than those of *Digitalis purpurea*, the species used in medicine; e.g., Wokes (*Quart. J. Pharm. Pharmacol.*, 1929, 2, 292) found the potency of a specimen of *Digitalis lanata* to be 3.5—4 times that of the international standard leaf.

The total glucosides of the leaves of this species were therefore isolated and fractionated in order to separate the glucosides and compare them with those from the official species. This part of the work is not yet completed, but during the fractionation a glucoside was obtained in small amount differing from those hitherto known. This new glucoside, which has been named *digoxin*, resembles gitoxin in its sparing solubility in chloroform, but is readily distinguished from it by giving an olive-brown colour completely free from red when dissolved in acetic acid containing a trace of ferric chloride and treated with sulphuric acid (Keller reaction). In the latter respect it is similar to pure digitoxin. Digoxin is optically active. It is very easily hydrolysed and gives a crystalline genin and only one sugar, digitoxose. The results of combustion and the molecular-weight determinations of the genin suggest the empirical formulæ $C_{41}H_{64}O_{14}$ and $C_{23}H_{34}O_5$ for digoxin and *digoxigenin* respectively and the yields of the hydrolytic products are in agreement with the equation $C_{41}H_{64}O_{14} + 3H_2O = C_{23}H_{34}O_5 + 3C_6H_{12}O_4$.

Dr. J. W. Trevan, of the Wellcome Physiological Research Laboratories, has kindly estimated the activity of the glucoside and its genin by the Trevan and Boock frog method with the following results: 1 mg. of digoxin is equivalent to 0.28 mg. of standard ouabain and 1 mg. of digoxigenin is equivalent to 0.026 mg. of standard ouabain.

EXPERIMENTAL.

The total glucosides were dissolved in acetone and kept for some time until a mixture of the less soluble glucosides (fraction A) separated. The mother-liquor was diluted with water and allowed to stand. The precipitate (fraction B) was separated, and the filtrate saturated with sodium chloride (fraction C). Each of these three precipitates contained digoxin. The acetone crop (A) may be worked up by dissolving it in 80% alcohol and concentrating the solution under diminished pressure until solid separates; after filtering, the solution is concentrated and one or two further crops are obtained. By boiling each fraction with chloroform or ethyl acetate,

a sparingly soluble portion is obtained which is purified by solution in 80% alcohol and concentration as before. The purification is continued in this way until the specific rotation remains unchanged and the Keller reaction gives an olive-brown colour completely free from any trace of red. Fractions B and C may be worked up by dissolving them in alcohol and gradually diluting the solutions with water. The less soluble fractions are then purified by the process described for fraction A.

Digoxin crystallises from dilute alcohol or from pyridine and water in colourless, four- or five-sided plates free from solvent (Found in material dried at 100° in a vacuum : C, 63.1, 63.1, 63.0; H, 8.2, 8.3, 8.2. $C_{41}H_{64}O_{14}$ requires C, 63.0; H, 8.3%). The m. p. varies with the rate of heating, but when placed in a bath at 260° and slowly heated, the substance melts and decomposes at about 265° (corr.). It is almost insoluble in chloroform, ethyl acetate, and acetone, but dissolves in a mixture of chloroform and alcohol or in pyridine or dilute alcohol. It is more soluble in hot 80% alcohol than gitoxin. In pyridine solution the specific rotation $[\alpha]_{5461}^{20} = +13.3^\circ$ ($c = 1.5$). Like digitoxin and gitoxin, digoxin gives on heating an oily distillate which readily crystallises.

Hydrolysis of digoxin. Digoxin (2.903 g. dried at 100° in a vacuum) was boiled under reflux with a mixture of alcohol (290 c.c.) and water (290 c.c.) containing 2.25 c.c. of hydrochloric acid (d 1.16) for $\frac{1}{2}$ hour. The solution was neutralised with sodium hydroxide and concentrated under reduced pressure. The genin separated in well-defined prisms and after drying at 80° in a vacuum weighed 1.387 g. (47.8%). The filtrate and washings were diluted to 200 c.c. and after addition of 0.4 c.c. of concentrated hydrochloric acid the mixture was heated on a water-bath for 20 minutes. After cooling, the liquid was extracted with chloroform, which removed a trace of sticky material (0.037 g.; 1.3%). The aqueous liquid was neutralised with sodium hydroxide and evaporated to dryness under diminished pressure, the residue was thoroughly dried over sulphuric acid in a vacuum desiccator and extracted with dry acetone, and the extract was evaporated to dryness. The residual sugar rapidly crystallised and after drying at 80° in a vacuum weighed 1.541 g. (53.1%).

Digoxigenin. The anhydrous genin without purification had $[\alpha]_{5461}^{20} = +25.8^\circ$ (c in methyl alcohol, 1.04). After one crystallisation from ethyl acetate it had $[\alpha]_{5461}^{20} + 27.0^\circ$ (c in methyl alcohol, 1.77), and this value was not altered by further crystallisation. *Digoxigenin* separates from ethyl acetate in stout colourless prisms free from solvent, m. p. 222° (corr.). It crystallises from dilute alcohol in prismatic rods containing water of crystallisation. When dissolved

in glacial acetic acid containing a trace of ferric chloride and treated with sulphuric acid, it gives a greenish-yellow ring (Found for the air-dried crystals: C, 64.5; H, 8.8; loss at 80° in a vacuum, 8.6. $C_{23}H_{34}O_5 \cdot 2H_2O$ requires C, 64.7; H, 9.0; H_2O , 8.5%. Found for the substance dried in a vacuum at 80°: C, 70.7, 70.5, 70.7, 70.4; H, 8.6, 8.6, 8.7, 8.7; *M*, in camphor, 394, 366, 372. $C_{23}H_{34}O_5$ requires C, 70.7; H, 8.8%; *M*, 390.3).

Digitoxose. The sugar (1.541 g.) without purification had $[\alpha]_{5461}^{20} + 55.5^\circ$ (c in water, 1.6). After crystallisation from ethyl acetate it had $[\alpha]_{5461}^{20} + 55.6^\circ$ and melted at 104°. Yield of successive crops, 1.134 g., 0.145 g., and 0.084 g. (total, 88.5%). By recrystallisation the m. p. was raised to 112°. Cloetta (*Arch. Exp. Path. Pharm.*, 1926, **112**, 276) recorded 110°. The sugar gave the Keller reaction characteristic of digitoxose (Found: C, 48.9, 48.6; H, 8.2, 8.2. Calc. for $C_6H_{12}O_4$: C, 48.6; H, 8.2%).

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