702. *The Cardiac Glycosides of* Urechites suberecta.

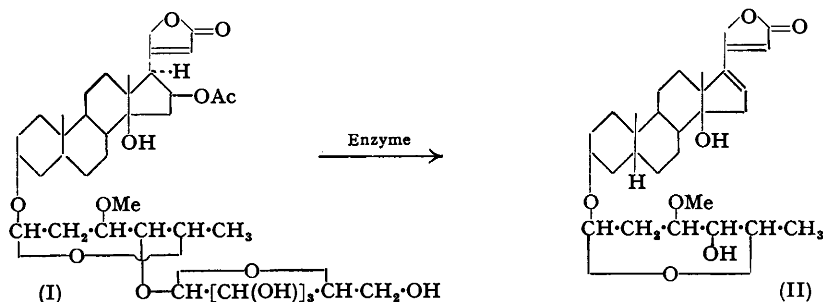
By C. H. Hassall.

Urechitoxin, $C_{38}H_{58}O_{14}$, isolated from leaves of *Urechites suberecta* in which enzymic hydrolysis is inhibited, is formulated as glucosido-oleandrin. When enzymic hydrolysis is permitted, deacetylanhydro-oleandrin and oleandrin are obtained.

The presence of toxic compounds in the shrub *Urechites suberecta* Muell. Arg. (family Apocynaceæ) has been recognised for some time. Dancer ("The Medical Assistant," 1801) records the use of the leaves as a poison. It is said to have been widely employed for this purpose in Obeah practices in Jamaica in the eighteenth and nineteenth centuries (Bowrey, *Proc. Roy. Soc.*, 1878, **27**, 309). Descourtilz ("Flore pittoresque et medicale des Antilles," 1829, Vol. VII, p. 68) described medicinal uses of the plant.

The first experiments on the isolation of active constituents were by Bowrey (*J.*, 1878, **33**, 252) who obtained two crystalline products, urechitoxin and urechitin. Minckiewiz (Dissertation, Dorpat, 1888), apparently without knowledge of Bowrey's work, also obtained two crystalline substances which were, however, incompletely characterised and had limited biological activity. The similarity of the toxic action of the constituents of the leaves to that of cardiac glycosides of the digitalis series has been confirmed by several investigators (Stockman, *Lab. Reports, Royal College of Physicians*, 1892, **4**, 64).

Although Bowrey did not obtain any direct evidence of the nature of urechitoxin and urechitin he made two observations which, when interpreted in terms of current knowledge of the cardiac glycoside series, suggested the possibility of a relation between gitoxin and these compounds. On hydrolysis of urechitoxin with concentrated hydrochloric acid a yellow, crystalline compound, termed urechitoxetin, was obtained. The properties of this product correspond to those of the yellow compound dianhydro-gitoxigenin, $C_{23}H_{30}O_3$, obtained on vigorous hydrolysis of oleandrin. When Bowrey heated urechitin at 200° a volatile acid was formed. This is reminiscent of Hesse's observation (*Ber.*, 1937, **70**, 2264) that oleandrin, when strongly heated, yields 16-deacetylanhydro-oleandrin and acetic acid.



The relationship of the glycosides to oleandrin has been established. When enzymic hydrolysis is permitted, a mixture of deacetylanhydro-oleandrin (II) and oleandrin can be isolated from an alcoholic extract of the leaves. When enzyme action is prevented by maceration of fresh leaves with absolute alcohol, urechitoxin is the principal product. Urechitoxin, $C_{38}H_{58}O_{14}$, $\lambda_{max.}$ 220 (log ε 4·24), contains one methoxyl group and gives no Keller–Kiliani reaction. It is hydrolysed by an enzyme preparation from the leaves to deacetylanhydro-oleandrin and glucose. This estalishes its identity as glucosido-oleandrin (I). Two similar glycosides, cryptograndoside B (Aebi and Reichstein, *Helv. Chim. Acta*, 1950, **33**, 1031) and hongheloside C (Hunger and Reichstein, *ibid.*, p. 76), have recently been isolated. In these compounds sarmentose and cymarose respectively replace the oleandrose present in urechitoxin (I).

Information has recently been made available through the courtesy of Professor T. Reichstein of a preliminary study (A. Hunger, to be published) on the glycosides present in the related species *Urechites lutea*. Several crystalline products were isolated and the identity of the principal constituent as deacetylanhydro-oleandrin was established.

EXPERIMENTAL.

Isolation of Cardiac Glycosides after Preliminary Fermentation.—In a typical experiment, the leaves of mature plants (6 kg.) collected in Jamaica were macerated in a Waring blender and set aside, just covered by water and a supernatant layer of toluene, for 7 days. The solid residue obtained on filtration was set aside for 2 days with absolute ethanol (9 l.). The solid was filtered off and washed with alcohol (2 × 1·5 l.). The filtrate and washings were stirred with moist lead hydroxide (prepared from 190 g. of lead acetate and washed until washings were neutral). The yellow lead complex was filtered off, washed with alcohol (2 × 500 c.c.), and discarded. The filtrate and washings were concentrated *in vacuo* at a temperature not exceeding 40°. As the light green filtrate became concentrated, a dark green, tarry product separated. This was free from glycoside. The alcoholic solution was separated from tar by decantation until a stage was reached when no significant amount of tar separated. Further concentration until the bulk of the alcohol was removed left a suspension of light green, semicrystalline product in aqueous solution. The solid (36 g.) was filtered off, dried, and extracted with light petroleum (b. p. 60—80°; 3 × 75 c.c.) to remove wax and with ethyl acetate to remove tar, pigment, and further wax. The crude product remaining was recrystallised (charcoal) from methanol–water. The crude glycoside obtained in this way was mixed with further purified material isolated from the mother-liquors. The colourless, crystalline product (26·3 g.; yield on fresh leaves, 0·47%), m. p. 210—240°, $[\alpha]_D^{30}$ +4·0° (*c*, 0·7 in chloroform), was subjected to paper chromatography using ethyl acetate-water (Hassall and Martin, *J.*, 1951, 2766). This indicated the presence of two products, of R_{FF} 0·96 and 0·87 respectively at 35°.*

16-*Deacetylanhydro-oleandrin.*—When the mixture of glycosides (0·89 g.) was subjected to fractional elution chromatography on neutral activated alumina (30 g.) with, successively, 1 : 3 chloroform–benzene (10 × 100-c.c. fractions), 1 : 1 chloroform–benzene (6 × 100 c.c.), and chloroform (5 × 100 c.c.), amorphous material (18 mg.) separated in the first fraction. Fractions 2—10 (0·425 g.) consisted of a mixture with ultra-violet absorption characteristics similar in type to the original product, while material from later fractions (m. p. 230°) was found to consist of essentially pure 16-deacetylanhydro-glycoside. The latter fractions were recrystallised from methanol–water, to give plates, $[\alpha]_D^{30}$ +16·2° (*c*, 1·1 in methanol), $\lambda_{max.}$ 271 mμ. (log ε 4·21) (Found : C, 69·55; H, 8·6; OMe, 5·4. Calc. for $C_{30}H_{44}O_7$: C, 69·7; H, 8·6; OMe, 6·0%). Legal, Raymond (*Analyst*, 1939, **64**, 113), and Keller–Kiliani reactions were positive. The m. p. and mixed m. p. with authentic 16-deacetylanhydro-oleandrin (kindly provided by Professor T. Reichstein) was 232—234°.

The acetate, needles from acetone–ether, had m. p. and mixed m. p. with authentic material 209—211°. Hydrolysis of the glycoside (50 mg.) in methanol (5 c.c.) and 0·1N-sulphuric acid (2 c.c.) gave, on working up in the usual way, aglycone corresponding to 16-anhydrogitoxigenin (Aebi and Reichstein, *Helv. Chim. Acta*, 1950, **33**, 1028; Hesse, *Ber.*, 1937, **70**, 2264), m. p. 244—245°, $[\alpha]_D^{30}$ +95·5 (*c*, 1·52 in methanol) (Found : C, 73·95; H, 9·0. Calc. for $C_{23}H_{32}O_4$: C, 74·2; H, 8·7%), and a sugar which on paper chromatography in which 3 : 3 : 1 ethyl acetate–water–acetic acid was used (Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402) with aniline hydrogen phthalate as indicator (Partridge, *Nature*, 1949, **164**, 443), gave a spot corresponding to that obtained with authentic oleandrose, R_{FC} 0·53 at 35°.

Oleandrin.—Attempts to separate other constituents of the crude glycoside mixture by chromatography on alumina or talc–Celite (1 : 2) columns were not successful. However, when a large quantity of the mixture (3·0 g.) was subjected to fractional crystallisation from methanol–water a more soluble fraction (80 mg.) was obtained. This was characterised as oleandrin (Neumann, *Ber.*, 1937, **70**, 1547), m. p. and mixed m. p. with authentic material (Prof. T. Reichstein) 250°, $[\alpha]_D^{25}$ −48·0° (*c*, 1·3 in methanol), $\lambda_{max.}$ 220 mμ. (log ε, 4·20). Hydrolysis with 0·1N-sulphuric acid in methyl alcohol and working up in the usual way gave results on paper chromatography of the aglycone and the sugar identical with those obtained with authentic oleandrin : aglycone (oleandrigenin), by use of water–butyl alcohol, R_{FF} 0·62 at 34°; sugar (oleandrose), by use of 3 : 3 : 1 ethyl acetate–water–acetic acid, R_{FC}, 0·55 at 34°.

Isolation of Cardiac Glycosides without Preliminary Fermentation.—The leaves (2 kg.) of mature plants were macerated with absolute ethanol (1·5 l.) in a Waring blender within 2 hours of being picked. The mixture was treated with further alcohol (2 l.), set aside for 24 hours in a refrigerator, and filtered, and the solid material washed with alcohol (2 × 500 c.c.). The mixed extract and washings were treated with lead hydroxide (prepared from 90 g. of acetate and washed until washings were neutral) then evaporated *in vacuo* at $<40°$ until all the alcohol was removed. The aqueous solution was decanted from a resinous product that separated and was extracted with light petroleum (b. p. 60—80°; 3 × 100 c.c.) to remove green pigment, and then with chloroform (2 × 100 c.c.). The aqueous solution, which no longer gave a positive Legal reaction and was not bitter, was discarded. This chloroform extract was mixed with a chloroform extract of the resinous product, which contained the cardiac glycoside present in this fraction, dried (Na_2SO_4), and evaporated. The amorphous residue (10·0 g.) was extracted with light petroleum (b. p. 60—80°; 5 × 100 c.c.) which removed a small amount of pigment and wax. The remaining resin was recrystallised from 30% methanol–water, to give a crude, crystalline product, m. p. 150° (4·3 g.). A further small yield (0·21 g.) of similar material was isolated from the mother-liquors on concentration. No further crystalline material could be separated by repeated recrystallisation of the amorphous product.

Urechitoxin.—The crude, crystalline product was employed for paper chromatography, ethyl acetate–water and water–butanol mixtures being used. Single spots R_{FF} 0·82 and 0·00 respectively at 35° were obtained in each case. Fractional elution chromatography, with Celite–talc (2 : 1) and increasing concentrations of chloroform in benzene gave no indication of a second component. The product was

* The ultra-violet absorption spectra, $\lambda_{max.}$ 220 mμ (log ε 4·10) and 262 mμ (log ε 3·85) suggested a mixture containing a 16-deacetylanhydroglycoside and a glycoside with no unsaturation at $C_{(16)}$.

recrystallised from 30% methanol as stout prisms, m. p. 157—159° (Found: C, 62·2; H, 8·2; OMe, 4·6. $C_{38}H_{58}O_{14}$ requires C, 61·8; H, 7·9; 1OMe, 4·2%). The Legal and Raymond reaction were positive (red; blue), the Keller-Kiliani reaction negative. $\lambda_{max.}$ was 220 mμ. (log ε, 4·24), and $[\alpha]_D^{27}$ −58·4° (*c*, 0·87 in methanol); the colour with 85% sulphuric acid was yellow (immediate), carmine red (1 minute), grey (60 minutes). The acetyl derivative was amorphous.

Enzymatic Hydrolysis of Urechitoxin.—An enzyme preparation was obtained by macerating the fresh leaves (200 g.) of *Urechites suberecta* with water in a Waring blender, and precipitating the crude enzyme with ethanol (1 l.). The precipitate was dissolved in water (100 c.c.), reprecipitated with ethanol (500 c.c.), filtered off, and dried (weight, 1·4 g.). The crude enzyme (100 mg.), urechitoxin (100 mg.), and water (5 c.c.) were set aside at room temperature for 7 days with a supernatant layer of toluene. The suspension was filtered, washed with water, dried, and extracted with methanol. The aqueous solution was concentrated and examined for sugar constituents by paper chromatography. The methanolic solution was evaporated to yield a white, crystalline solid, m. p. 210°, which, recrystallised from 40% ethanol, had m. p. and mixed m. p. with authentic 16-deacetylanhydro-oleandrin 232—234°. The crude product, $\lambda_{max.}$ 220 (log ε, 3·7) and 270 mμ. (log ε, 4·1), appeared still to contain a small proportion of a 16-acetyl-glycoside (cf. Aebi and Reichstein, *Helv. Chim. Acta*, 1950, **33**, 1013).

Paper chromatography of the aqueous solution at 35° with ethyl acetate–water–acetic acid (3 : 3 : 1) for 30 hours according to Jermyn and Isherwood (*loc. cit.*) and Partridge (*loc. cit.*) gave a single spot (with centre 5·0 cm. from starting line). Mannose, glucose, and fructose, used for comparison in the same experiment, gave spots 6·0, 5·0, and 7·6 cm. distant respectively. This indicates the identity of the unknown hexose sugar with glucose (Hirst and Jones, "Chromatographic Analysis," *Faraday Soc. Disc.*, 1949, **7**, 268).

Acid Hydrolysis of Urechitoxin.—Urechitoxin (180 mg.) in methanol (5 c.c.) was refluxed for 25 minutes with 0·1N-sulphuric acid (5 c.c.). The methyl alcohol was evaporated *in vacuo* and the aglycone separated by filtration. The aqueous solution was extracted with chloroform (6 × 200 c.c.), neutralised with barium carbonate, filtered, and evaporated *in vacuo* at room temperature. The recrystallised aglycone (from methanol–ether), m. p. 241—243°, $[\alpha]_D^{30}$ −6·2° (*c*, 0·91), R_{PF} (water–*n*-butyl alcohol) 0·52 at 35°, was identified as oleandrigenin by direct comparison with an authentic sample.

The water-soluble residue was subjected to paper chromatography for the sugar constituents. In a typical experiment (22 hours; 35°) the mixture gave three spots at distances 22·6, 2·8, and 0·8 cm., respectively, from the starting point. In the same experiment glucose and oleandrose gave spots 0·8 and 22·6 cm., respectively, from the starting point. When hydrolysis of urechitoxin was carried out for a longer period (1½ hours), the centre spot, which was the most intense in the first experiment, became less concentrated while the two spots corresponding to glucose and oleandrose increased in intensity. This suggests that the centre spot corresponds to glucosido-oleandrose. No further evidence was obtained on this point.

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