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Rhodexin A and rhodexoside in *Ornithogalum umbellatum*

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Two Kedde-positive compounds, isolated in crystalline form from bulbs of *Ornithogalum umbellatum*, were shown by their infrared and ultraviolet spectra to be cardenolides. Paper chromatographic analyses of the hydrolysates of these compounds indicated that both compounds contain sarmentogenin as the aglycone moiety, and that one is a rhamnoside while the other is the corresponding rhamnoside-glucoside. The identification of the monoglycoside as rhodexin A, i.e. sarmentogenin-α-L-rhamnoside, a glycoside previously isolated from *Rhodea* (*Rohdea*) *japonica*, was confirmed by comparison with an authentic sample. The diglycoside, an apparently new compound of composition sarmentogenin-rhamnose-glucose is designated rhodexoside.

AFTER the initial pharmacological examination of bulbs of *Ornithogalum umbellatum* (Waud, 1951; Waud & Boyd, 1954; Vogelsang, 1956), two cardenolide glycosides were isolated and identified as convallatoxin and convalloside (Mrozik, Waud, Schindler & Reichstein, 1959). Of the six remaining Kedde-positive spots noted on paper chromatograms developed in toluene–butanol (1 : 1) with water as the stationary phase, that of highest Rf value was noted by these authors to be approximately the same in position as the glycoside vallarotoxin which had been isolated from *Convallaria majalis*.

We have isolated from the bulbs of *Ornithogalum umbellatum*, two Kedde-positive compounds which we identified as sarmentogenin-α-L-rhamnoside, previously isolated by Nawa (1951, 1952) from *Rhodea* (*Rohdea*) *japonica*, and sarmentogenin-L-rhamnose-D-glucose. This apparently new compound is designated rhodexoside.

Experimental and results

MATERIALS AND GENERAL PROCEDURES

Chloroform U.S.P. dried over calcium chloride and distilled before use; ethanol prepared aldehyde-free (U.S.P. XVI). Alumina, neutral activity grade 2 (Woelm), magnesium silicate, activity grade 3 (Woelm), and silica gel, 60 to 200 mesh, reagent (Fisher Scientific).

Adsorption and partition column chromatography were according to Reichstein & Shoppee (1949) and Neher (1959, 1964) respectively. For paper chromatograms, Whatman No. 1 paper and the descending technique were used. Thin-layer plates were prepared with silica gel G (Merck, Darmstadt) according to Duncan (1962), using a slurry of 1 part gel with 2 parts ethanol–water (1 : 1) shaken for 15 min before application.

Reference materials included convallatoxin (California Corporation for Biochemical Research), digoxigenin (Aldrich Chemical Co.), strophanthidin and L-rhamnose (Mann Research Lab.), and D-glucose (British Drug Houses), as well as authentic samples of sarmentogenin and rhodexin A. All other chemicals were of reagent grade.

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Enzymatic hydrolysis was with *Suc d'Helix pomatia* (Industrie Biologique Française S.A.) in McIlvaine's pH 4·8 buffer (McIlvaine, 1921), prepared from 9·86 ml of 0·2M disodium hydrogen phosphate mixed with 10·14 ml of 0·1M citric acid.

For the detection of cardenolide glycosides and aglycones, Kedde reagent (Bush & Taylor, 1952) and 2,2',4,4'-tetranitrobiphenyl reagent (Mauli, Tamm & Reichstein, 1957) were used with paper and thin-layer chromatograms, respectively. 2,2',4,4'-Tetranitrobiphenyl was synthesized according to Ullman & Bielecki (1901). Aniline hydrogen phthalate reagent (Partridge, 1949) was used to detect sugars on paper chromatograms.

Melting ranges (Kofler block) are uncorrected. Infrared spectra, from potassium bromide micropellets, were determined with a Beckman IR-8 spectrophotometer. Ultraviolet spectra were determined in methanol using silica cells of 1 cm path length in a Beckman DK-2 spectrophotometer fitted with a hydrogen lamp.

EXTRACTION

Bulbs of *Ornithogalum umbellatum* collected in Southern Ontario were air-dried at 40° (approximately 8 weeks), and ground to a coarse powder. The extraction procedure was similar to that of Mrozik & others (1959). The dry bulb material was extracted once with ethanol–water (1 : 1) or (2 : 1) and subsequently with ethanol until the marc was no longer bitter. Each extract was filtered through Celite 535. The combined extracts were evaporated under reduced pressure at 40° to an aqueous suspension which was subsequently diluted 1 : 4 with ethanol. The decanted supernatant extract, combined with ethanol–water (3 : 1) washings of the Kedde-negative, amber mucilaginous precipitate was evaporated to an aqueous suspension and extracted with chloroform and chloroform–ethanol (2 : 1) and (3 : 2) until one portion was negative to Kedde reagent. The chloroform–ethanol (2 : 1) extracts were washed with distilled water, sodium carbonate solution (M), and again with distilled water, before being dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure.

ISOLATION OF GLYCOSIDE I

From 1 kg and 2 kg weights of dry bulb material were obtained respectively 2·13 g and 4·86 g dry extract which were chromatographed by adsorption on silica gel and alumina respectively. Elution was with chloroform, followed by chloroform containing increasing concentrations of methanol.

The foam-like, Kedde-positive fractions from both columns were combined. This product (1·6 g) was re-chromatographed by partition using the solvent system toluene–butanol (1 : 1) with water as stationary phase using silica gel (1·25 kg) equilibrated with water (625 g). Fractions (150 ml) were collected at 0·6 ml/min.

Fraction 7 from this column yielded crude crystals (50·5 mg) from

ether-methanol which after recrystallization four times from this solvent, gave light buff-coloured platelets (19 mg); this product is designated glycoside I.

ISOLATION OF GLYCOSIDE II

The dry extract (2·48 g) from 2 kg dry powdered bulbs was chromatographed by adsorption on silica gel with elution by chloroform followed by chloroform containing increasing concentrations of methanol.

The foam-like, Kedde-positive fractions were combined (1·49 g) and re-chromatographed on silica gel (750 g) equilibrated with water (375 g), using toluene–butanol (1 : 1)/water. Fractions (90 ml) were collected at 0·5 to 0·6 ml/min. Paper chromatography of the resulting fractions in chloroform–tetrahydrofuran–formamide (50 : 50 : 6·5)/formamide (Kaiser, 1955) indicated in fractions Nos 26 to 51, three compounds of lower Rf value than convallatoxin. These fractions were combined and the product (379 mg) was chromatographed on silica gel as above. On paper chromatography, a single Kedde-positive substance was found in fractions Nos 42 to 60, the average Rr values of which, relative to convallatoxin, were 0·77 in toluene-butanol (1 : 1)/water (Schenker, Hunger & Reichstein, 1954) and 0·17 in chloroform–tetrahydrofuran–formamide (50 : 50 : 6·5)/formamide.

Fraction Nos 48–60 were combined and the product (75 mg) was chromatographed on magnesium silicate using chloroform–methanol. Fractions from this column yielded a white crystalline product (30 mg) from ether–methanol; this product was designated glycoside II.

CHARACTERIZATION OF THE ISOLATED GLYCOSIDES

Paper chromatography of isolated glycoside I (50 μg samples) in the solvent systems toluene–butanol (1 : 1)/water, benzene–n-amyl alcohol (1 : 1)/water (Kubelka & Wichtl, 1963), and chloroform–tetrahydrofuran–formamide (50 : 50 : 6·5)/formamide, and thin-layer chromatography (20 μg samples) in ethyl acetate–methanol–water (16 : 3 : 1) indicated that glycoside I was identical in Rf values with rhodexin A.

Microhydrolyses of 0·8 mg glycoside I, 1·1 mg glycoside II, and 0·6 mg rhodexin A were made according to Mannich & Siewert (1942). Paper chromatographic analyses of 5 μl aliquots of the hydrolysates were made after 11 and 14 days using the solvent systems chloroform/formamide (Schindler & Reichstein, 1951), xylene–methyl ethyl ketone (1 : 1)/formamide (Kaiser, 1955), and benzene–chloroform (3 : 7)/formamide (Schindler & Reichstein, 1951). In addition, 2 μl aliquots of the hydrolysate of glycoside I were chromatographed on thin-layer plates developed with ethyl acetate using sarmentogenin, strophanthidin, and digoxigenin as reference compounds. In each solvent system the hydrolysates of glycoside I and authentic rhodexin A gave rise to identical spots and the major Kedde-positive spot noted in each of the glycoside hydrolysates was identical in Rf value with sarmentogenin.

Samples of each of the isolated glycosides (1·0 mg) and rhodexin A (0·6 mg) were hydrolysed according to Kiliani (1930). Paper chromatographic analyses of 5 μl aliquots were made with the solvent systems butanol/water (Kaiser, 1955) and methyl ethyl ketone–butanol (1 : 1)/borate buffer (Kraus, Jäger, Schindler & Reichstein, 1960), with D-glucose and L-rhamnose as reference sugars. A single spot, identical in Rf value with L-rhamnose, was detected in the hydrolysates of glycoside I and rhodexin A. The hydrolysate of glycoside II gave two spots of Rf values identical with those of D-glucose and L-rhamnose. These two spots appeared of equal intensity, indicating equimolar quantities of the two sugars in the glycoside.

Glycoside I had melting range 248–252° (rhodexin A, 240–246°); no significant depression was noted on admixture. The melting range of glycoside II was 180–188°. Glycosides I and II had $[\alpha]_D^{22}$ — 23·1° and $[\alpha]_D^{20}$ — 23·9° (methanol) respectively [Nawa, 1951, 1952a, gives $[\alpha]_D$ — 20° (ethanol) for rhodexin A].

The ultraviolet spectrum of glycoside I showed a single absorption maximum at 218 mμ (log ϵ^* = 4·21); for glycoside II it was at 216 mμ (log ϵ^* = 4·16). The infrared spectra of glycoside I and rhodexin A showed identical absorption bands throughout the 2·5 to 15 μ region. The series of bands noted at 5·56 μ, 5·76 μ, and 6·18 μ for glycoside I and at 5·62 μ, 5·76 μ, and 6·16 μ for glycoside II are considered to confirm their cardenolide character.

The colour changes for glycoside I and rhodexin A on addition of 84% sulphuric acid (von Euw & Reichstein, 1948) were identical, the sequence being yellow → yellow-green → green → green-blue → green over 2 hr.

Combined chloroform–ethanol extracts of a sample (0·7 mg) of glycoside II, incubated with *Suc d'Helix pomatia* (0·3 ml) in pH 4·8 buffer (4·7 ml) at 37° for 21 hr, after paper chromatography in the toluene–butanol and chloroform–tetrahydrofuran–formamide systems showed a single Kedde-positive compound identical in Rf values with rhodexin A.

Discussion

It is concluded that glycoside I is the cardenolide glycoside rhodexin A, i.e. sarmentogenin-α-L-rhamnoside. Although the rhamnoside convallatoxin (strophanthidin-α-L-rhamnoside) had been isolated previously from *Ornithogalum umbellatum*, this is the first evidence for the presence in the plant of a glycoside of sarmentogenin.

Glycoside II appeared, on the basis of the paper chromatographic analysis of the acid-catalysed hydrolysates, to contain the sugars D-glucose and L-rhamnose, together with the aglycone sarmentogenin. Since the Kedde-positive product of enzymatic hydrolysis appeared to be identical chromatographically with rhodexin A, glycoside II is concluded to be a glucoside of rhodexin A, and is designated rhodexoside. In view of the

* The calculations of ϵ for glycosides I and II were based on the molecular weight of rhodexin A, i.e. 536·6, and on the molecular weight of $C_{35}H_{52}O_{14}$, i.e. 696·8, respectively.

general observation (Klyne, 1950) that in naturally-occurring cardenolide glycosides D-sugars are usually present in β-glycosidic linkage, rhodexoside is likely to be sarmentogenin-α-L-rhamnosido-β-D-glucoside.

The presence in this plant of rhodexin A and rhodexoside is therefore analogous with the presence of convallatoxin and the glucoside of convallatoxin, convalloside. Further, it is of interest that Nawa isolated from the plant *Rhodea* (*Rohdea*) *japonica*, in addition to rhodexin A, the glycosides rhodexin B, i.e. gitoxigenin-α-L-rhamnoside (Nawa, 1952a) and rhodexin C, a glucoside of rhodexin B (Nawa, 1952b, c).

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