

The Cardiac Glycoside from *Urginea rubella* Baker. Part I.—Isolation and Properties of Rubellin.

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THE toxicity of *Urginea rubella* Baker (Fig. 1) was proved for the first time by Van der Walt and Steyn (1941).

From preliminary tests it was evident that the active principle was in all probability a cardiac glycoside. In due course a glycoside (Fig. 2) with melting point $261\text{--}3^{\circ}\text{C.}^*$ was isolated and proved to be the toxic principle with a specific cardiac action. The yield of the glycoside varied from 0.015 to 0.045 per cent.

A probable molecular formula of $\text{C}_{35}\text{H}_{48}\text{O}_{16}$ for the glycoside was arrived at from combustion analyses and molecular weight determinations. According to the available literature this is an entirely new cardiac glycoside.

The isolation of this glycoside from *Urginea rubella* by the author had already been mentioned by Sapeika (1944) and the name suggested for the glycoside at the time was "Rubellin".

THE TOXICITY OF RUBELLIN.

Sapeika (1944) compared the action of rubellin with ouabain and reported that rubellin has a digitalis action and is more potent than ouabain on the frog heart.

In a following publication Sapeika (1946) compared the actions of digitoxin, digoxin, lanatoside C, rubellin, and a solution of standard strophanthidin B.P. 1932 with international standard ouabain. In descending order the relative potencies were: rubellin, ouabain, standard strophanthidin B.P. 1932, lanatoside C, digoxin and digitoxin.

White rats were used to determine the toxicity of rubellin by intraperitoneal injection of aqueous solutions of rubellin, and the L.D. 50 was determined to be 0.692 mgm. /Kgm. rat.

Using rabbits, the m.l.d. of rubellin when dosed *per os*, was found to be approximately 10 mgm. per Kilogram rabbit.

* All the melting points were determined on the Kofler micro-melting-point apparatus and are therefore corrected.

THE LACTONE GROUP IN RUBELLIN.

The characteristic Legal test which is positive in the case of the digitalis-strophanthus group of cardiac glycosides, is not given by rubellin. In this respect it resembles the bufagins, the scilla glycosides, viz. scilliroside, scillaren A and its derivatives. This suggests that the lactone ring in rubellin corresponds with that of the squill-toad group of cardiac compounds in which a doubly unsaturated six-membered lactone ring is present.



FIG. 1

This was confirmed by the absorption spectrum of rubellin in alcohol.*
 (Fig. 3.) Maximum absorption occurs at $297\text{ m}\mu$ with $\log E \frac{1\text{ mol./ltr.}}{1\text{ cm}} = 3.80$.

The absorption curve is practically coincident with that of scilliroside and pro-scillaridin A in alcohol. (Fig. 3, Stoll and Renz, 1942). In contrast with this the glycosides of the digitalis-strophanthus group with mono-unsaturated five-membered lactone rings have maximum absorption at approximately $220\text{ m}\mu$. [Compare Stoll and Hoffmann (1935) and Elderfield *et al.* (1941).]

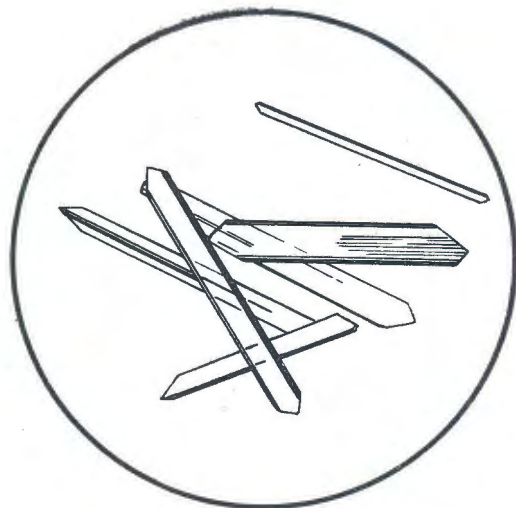


Fig. 2 : Rubellin crystallised
 from Absolute Alcohol.

Magnif. x 140

Rubellin can be titrated with alkali, but varying amounts of alkali are used depending on the reaction time. The reaction with alkali is being further investigated.

THE SUGAR MOIETY IN RUBELLIN.

Attempts to hydrolyse rubellin with α -glucosidase (maltase), β -glucosidase (emulsin) and an enzyme preparation from the fresh autolysed bulb *Urginea rubella*, proved unsuccessful. Recourse was therefore taken to the acid hydrolysis of the glycoside. This was easily achieved with hydrochloric acid in methanol, the hydrolysis yielding a crystalline product, pro-rubillidin, which still contained carbohydrate. The sugar that was hydrolysed off was an aldehyde sugar that could not be identified yet. The analysis of the hydrolysis product corresponded with the formula $C_{30}H_{38}O_{11}$.

* The absorption spectrum of rubellin was determined by Dr. H. M. Schwartz of the Department of Chemistry of the University of Cape Town, to whom the author is greatly indebted.

From analytical data of rubellin and pro-rubillidin it is probable that rubellin, in which two sugar moieties are present, has the following structure:—

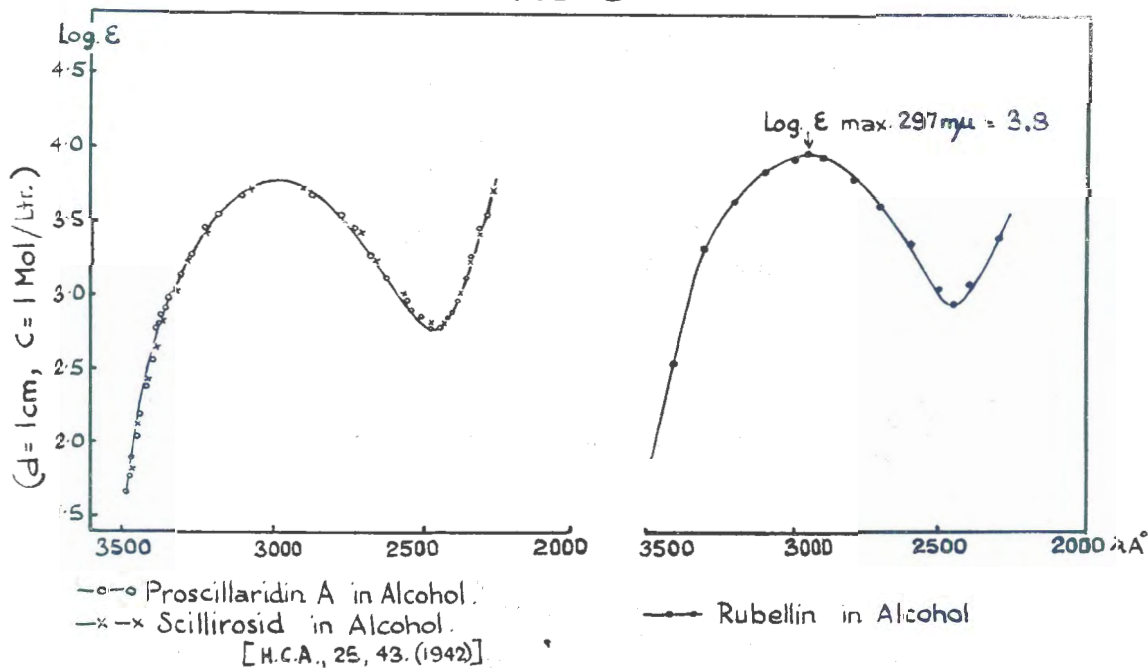


EXPERIMENTAL.

Isolation of the Glycoside.

The fresh bulbs, containing up to 90 per cent. moisture and free of grit, roots and stems, were minced and then pressed out immediately or dried in front of a fan for future use, in which case the dried material may be mixed with water and then pressed for five successive times. The pressed liquid was filtered and then shaken three times with ethyl acetate which removed the glycoside almost quantitatively. The ethyl acetate shakings were concentrated under reduced pressure and the concentrate allowed to evaporate slowly when the glycoside crystallised out. After crystallisation it was obtained pure with melting point 261–3° C. (It became opaque at 200° C. and melted with decomposition). The name that was previously suggested for the glycoside is “rubellin”.

FIG. 3.



The yield of glycoside varied with different samples of plant. Yields varying between 0.015 and 0.045 per cent. of glycoside on the basis of the fresh material were obtained.

The glycoside may also be easily obtained by extracting the dried and powdered bulbs with 96 per cent. alcohol or ethyl acetate.

Properties of Rubellin.

From ethyl acetate rubellin crystallises in prismatic needles as a colourless and odourless compound. It has an intensely bitter taste. It is practically insoluble in chloroform, ether and benzene, but is fairly soluble in water, methyl alcohol, ethyl alcohol, acetone and ethyl acetate. It is very soluble in cold pyridine.

Legal Test.

When rubellin is dissolved in pyridine and a solution of sodium nitroprusside is added to the alkaline solution according to the directions of Jacobs and Hoffmann (1926) a yellow colouration only is obtained. Rubellin therefore gives a negative Legal Test as in the case of the bufagins, scilliroside, scillaren A and its derivatives.

Liebermann Test.

On adding 3 ml. of a mixture of acetic anhydride and sulphuric acid (50:1) to a solution of 1 mgm. rubellin in a few drops of ethyl acetate, a red colour is produced. The red changes to purple, blue and finally to light brown. This display of colour is typical of steroid compounds.

Molisch Test.

When sulphuric acid is added to a dilute solution of rubellin in water to which a few drops of an alcoholic solution of α -naphthol has been added, a deep violet colour is obtained, indicating the presence of carbohydrate.

*Micro-analysis.**

(a) 3.840 mgm. rubellin: 8.310 mgm. CO_2 : 2.250 mgm. H_2O .

(b) 4.009 mgm. rubellin: 8.680 mgm. CO_2 : 2.380 mgm. H_2O .

(c) 3.691 mgm. rubellin: 7.976 mgm. CO_2 : 2.120 mgm. H_2O .

(d) 3.719 mgm. rubellin: 7.996 mgm. CO_2 : 2.136 mgm. H_2O .

Calculated for $\text{C}_{36}\text{H}_{18}\text{O}_{16}$: C = 58.69%; H = 6.56%

Found: (a) C = 59.06%; H = 6.55%

(b) C = 59.09%; H = 6.64%

(c) C = 58.95%; H = 6.43%

(d) C = 58.65%; H = 6.43%

Molecular Weight.

The determination of the molecular weight of rubellin was accompanied by various difficulties. The determination by the depression of the freezing point of water was unsatisfactory because rubellin is not soluble enough to give an appreciable depression. The same holds for the ebulliscope methods using alcohol and acetone. Neither is rubellin readily soluble in camphor.

* C, H determinations by Drs. G. Weiler, and F. B. Strauss, Oxford.

Mol. Wt. Determination (Rast.).

(a) 2.958 mgm. rubellin: 109.115 mgm. camphor; $\Delta = 1.65^\circ$
 \therefore Mol. wt. = 656.

(b) 1.975 mgm. rubellin: 81.07 mgm. camphor; $\Delta = 1.60^\circ$
 \therefore Mol. wt. = 609.1.

Calculated for $C_{36}H_{48}O_{16}$: 736.7

Found: (a) 656

(b) 609.1

Optical Activity.

126.5 mgm. rubellin were dissolved in 25 ml. methyl alcohol and the rotation using a 2 dm. tube, determined.

$$\begin{aligned} \theta &= +0.16^\circ \\ \therefore [\alpha]_D^{27} &= \frac{+0.16 \times 1000 \times 25}{126.5 \times 2} \\ &= +15.81^\circ \text{ (MeOH)} \end{aligned}$$

The solution does not show an alteration in the rotation when left overnight.

Hydrolysis of Rubellin.

To 2 gm. rubellin dissolved in 200 ml. 50 per cent. methanol, 1 ml. HCl ($d = 1.16$) was added and the mixture heated in a bath at $70-80^\circ$ C. for 3 hours. The methanol was then distilled off under vacuum and the aqueous residue extracted with ethyl acetate yielding 1.2 gm. of a product which when recrystallised from ethyl acetate, melted at $267-8^\circ$ C. with decomposition. This product still contained carbohydrate as it reduced Fehling's solution and gave an intense violet colour with the Molisch test. The new compound, pro-rubillidin presumably contained one sugar less than rubellin.

The neutralised aqueous solution was strongly reducing towards Fehling's solution and formed an oily osazone which could not be induced to crystallise. The systematic identification of this sugar is continued.

Properties of Pro-Rubillidin.

Pro-rubillidin is obtained crystalline from ethyl acetate in small transparent plates M.P. $267-8^\circ$ C. (decomp.). It is very little soluble in ether, chloroform and benzene, sparingly soluble in ethyl acetate, acetone and water, but dissolves easily in absolute alcohol, methanol and dioxane.

50 mgm. pro-rubillidin dissolved in 10 ml. MeOH shows a rotation of 0.12° with a 1 dm. tube, giving—

$$[\alpha]_D^{20} = +24.0^\circ$$

Analysis.

Found C = 62.12%; H = 6.57%

Calculated for $C_{30}H_{38}O_{11}$: C = 62.70%; H = 6.66%

Isolation of Succinic Acid

On working up the mother liquor from which crude rubellin had been crystallised, succinic acid was isolated in small yield. Crystallised from ethyl acetate the acid sublimed at 150-160° C. and melted at 190-2° C.

Analysis.

(a)	3.652 mgm. acid:	5.286 mgm. CO ₂ :	1.601 mgm. H ₂ O
(b)	3.556 mgm. acid:	5.335 mgm. CO ₂ :	1.601 mgm. H ₂ O
	Calculated C ₄ H ₆ O ₄ :	C = 40.67 %;	H = 5.12 %
	Found:	(a) C = 40.47 %;	H = 5.03 %
		(b) C = 40.92 %;	H = 5.04 %

Molecular Weight.

20 mgm. of the acid was dissolved in water and titrated with $\frac{N}{10}$ KOH.

ml. $\frac{N}{10}$ KOH used = 3.40

Calculated C₄H₆O₄: 118

Found (for dibasic acid): 117.6

Mixed with authentic succinic acid the acid isolated showed no depression of melting point.

SUMMARY.

1. The active cardiac principle of *Urginea rubella* Baker has been isolated. Analysis points to the empirical formula C₃₆H₄₈O₁₆. The name suggested for this compound is "rubellin". Rubellin melts at 261-3° C. with decomposition and has $[\alpha]_D^{25} = -15.81^\circ$ (MeOH).

2. Hydrolysis of rubellin yields a sugar and pro-rubillidin, having M.P. 267-8° C. (decomp.) and $[\alpha]_D^{25} = -24^\circ$ (MeOH).

3. Succinic acid was isolated in small yield from the plant.

4. The toxicity of rubellin towards white rats was determined by intraperitoneal injection, the L.D.50 being 0.692 mgm./Kg. Dosed to rabbits *per os*, the toxicity of rubellin is about 10 mgm. per Kilogram rabbit.

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