The Paper-Chromatographic Examination of the Cardiac Aglycones of *Strophanthus* Seeds

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The Medical Research Council Medicinal Plants Survey in its expedition to Africa collected many specimens of the seed of *Strophanthus hispidus* P.D.C. and *S. sarmentosus* P.D.C., mostly from individual plants, in order to investigate the occurrence of sarmentogenin, a substance which forms a possible starting material for the production of cortisone. The isolation of sarmentogenin from certain of these seeds has already been reported (Callow, Meikle & Taylor, 1951).

The present paper is a report on the preliminary investigation of all the samples collected, and reveals certain interesting features about the distribution of cardiac aglycones in various *Strophanthus* species.

As the examination of each seed sample by isolation of the constituents might take several years for completion, a method has been developed for the routine semi-micro investigation of the easily hydrolysable glycosides of the seeds. These easily hydrolysable glycosides (Reichstein, 1951), which

are completely hydrolysed by refluxing for half an hour in 50% aqueous methanol which has been made 0.1N with respect to sulphuric acid, are those in which the steroidal portion of the molecule is directly attached in a glycosidic linkage to a 2-deoxy sugar. Most of the best known glycosides belong to this group, which is much easier to investigate than the group in which the aglycones are directly attached to 'normal' sugars carrying a hydroxyl group at C₆, such as glucose or rhamnose, where isolation of the intact aglycone is usually difficult and has in several cases not yet been achieved. Investigation of the 'normal' glycosides in our seed samples has so far only been carried out in selected cases.

The paper-chromatographic method of Zaffaroni, Burton & Keutmann (1950) (cf. Burton, Zaffaroni & Keutmann, 1951) has been used by Schindler & Reichstein (1951) for separating this type of substance, but the procedure is in many ways complicated

Table 1. R_F values of some cardiac aglycones and derivatives in the system benzene/50% methanol at 37.5°

(Systematic names follow the rules given in *J. chem. Soc.* 1951, p. 3526.)

Compound	R_F
' β '-Anhydrodigitoxigenin (3 β -hydroxycarda-14:20(22)-dienolide)	0.91
' α '-Anhydrodigitoxigenin*	0.63
' β '-Anhydrodigoxigenin (3 α :12 β -dihydroxycarda-14:20(22)-dienolide)	0.73
' β '-Anhydrodigoxigenone (3:12-diketocarda-14:20(22)-dienolide)	0.59
' β '-Anhydrosarmentogenin (3 β :11 α -dihydroxycarda-14:20(22)-dienolide)	0.69
' β '-Anhydrozargarigenin (3 β -hydroxy-5 α -carda-14:20(22)-dienolide)	0.94
Digitoxigenin (3 β :14-dihydroxycard-20(22)-enolide)	0.76
Digitoxigenin acetate	0.89
Digoxigenin (3 α :12 β :14-trihydroxycard-20(22)-enolide)	0.075
Digoxigenin diacetate	0.70
Digoxigenone (3:12-diketo-14-hydroxycard-20(22)-enolide)	0.68
Gitoxigenin† (3 β :16 β :14-trihydroxycard-20(22)-enolide)	0.26
Gitoxigenin diacetate	0.88
Periplogenin (3 β :5:14-trihydroxycard-20(22)-enolide)	0.43
Sarmentogenin (3 β :11 α :14-trihydroxycard-20(22)-enolide)	0.10
Sarmentogenin diacetate	0.85
Sarnovide mono(?)acetate†	0.72
Sarverogenin*	0.40
Strophanthidin (3 β :5:14-trihydroxy-19-oxocard-20(22)-enolide)	0.16
Strophanthidol (3 β :5:14:19-tetrahydroxycard-20(22)-enolide)	0.05
'Substance H'*	0.28
'Substance K'*	0.56
Uzargenin (3 β :14-dihydroxy-5 α -card-20(22)-enolide)	0.83

* Compound of unknown structure.

† Reacts only weakly with the *m*-dinitrobenzene reagent.

‡ Callow & Taylor (1952).

by the treatment of the paper and the relatively long period of development required. The alternative systems developed to handle the steroid hormones (Bush, 1952) can easily be applied to the separation of cardiac aglycones, which are then detected by their colour reaction with 3:5-dinitrobenzoic acid and alkali. The advantage of these solvent systems is that no pretreatment of the paper is necessary; a continuous variation in the R_F values of a given set of compounds can be produced by slight variations in the solvent systems used; and the systems flow very quickly, allowing rapid and routine use of the method for analysing seed extracts or crystalline materials.

This method has been applied to a number of pure genins and to some simple derivatives still containing the $\alpha\beta$ -unsaturated lactone ring, and the R_F values found in the system benzene/50% aqueous methanol are listed in Table 1. This system proved to be satisfactory for general use, although when only the more polar genins were present, the use of 60% methanol was found to give rather better separations. For substances which proved to flow at inconvenient rates in these systems modifications were made as described by Bush (1952).

In general it has been found that the R_F value decreases the larger the number of polar groups in the molecule, where these are widely separated; but where two or more polar groups occur close together, as in periplogenin, some form of vicinal action occurs which greatly increases the rate of flow.

The limitations of the method should be noted. Thus, whilst substances of only slightly different structure, such as digitoxigenin and uzarigenin which differ only in the configuration at C_{10} , can be separated quite adequately, other pairs of substances which differ widely in structure may have almost identical R_F values. An example of this is provided by ' β '-anhydrosarmentogenin and the partially acetylated sarnovide derivative (Callow & Taylor, 1952) which were for some time thought by us to be identical.

EXPERIMENTAL

A sample (1 g.) of the seed to be investigated was ground in a coffee mill and then extracted in a Soxhlet apparatus with methanol (150 ml.) for 2 hr. The extract was then evaporated to 25 ml., diluted with water (5 ml.) and extracted with light petroleum (3×25 ml.). The aqueous solution was then diluted with 0.1 N- H_2SO_4 (30 ml.) and refluxed for 30 min. After cooling, the solution was extracted twice with $CHCl_3$ (2×50 ml.) and the extract evaporated to 5 ml. One drop of this extract was sufficient for paper chromatography in the case of a seed with an average glycoside content. The chromatography itself was carried out as described by Bush (1952) in a system employing aqueous methanol as the stationary phase. For the routine analysis of large numbers

of samples it was found convenient to employ an ascending chromatogram, although in this case the R_F values varied rather widely from one chromatogram to the next. The values given in Table 1 were obtained by employing a descending system, and are reproducible within the limits described by Bush provided that the appropriate precautions are taken.

For development of the spots a 1% solution of 3:5-dinitrobenzoic acid in 0.5 N 50% (v/v) aqueous methanolic KOH was found very suitable. This reagent gave a purple colour which had the advantage of being reasonably permanent, unlike the blue colour given by *m*-dinitrobenzene, which fades very quickly, in some cases even before the spots can be marked.

Fig. 1. Chromatogram given by typical seed extracts in an ascending benzene/60% methanol system. A, *S. hispidus*; B, *S. sarmentosus* ('savannah' form); C, *S. sarmentosus* ('forest' form). Spots: 1, periplogenin; 2, strophanthidin; 3, strophanthidol; 4, sarmentogenin; 5, sarverogenin.

RESULTS

The two species, *S. hispidus* and *S. sarmentosus*, have been found to give very different and readily recognizable chromatograms, differences between varieties of the same species being subordinate to the general pattern; typical chromatograms of each species are shown in Fig. 1.

The twenty samples of *S. hispidus* investigated have all been found to contain strophanthidin as their main constituent, together with a smaller amount of strophanthidol and a trace of peri-

plogenin, which has always been found to be present. Some specimens have also contained a fourth substance, 'substance H', which in one sample (CB/67; RDM/1032) gathered from a single tree at Kaboji near Kontagora, in Northern Nigeria, was present in

as well. These are respectively the 'savannah' and 'forest' forms of Callow *et al.* (1951) and are, in the extreme cases, distinguishable botanically. The seed of the two forms has also a fairly characteristic appearance to the practised eye.

Fig. 2. Map showing places where seeds of *S. hispidus* and *S. sarmentosus* were collected. ■, indicates a plant of *S. hispidus*; ▲, a plant of the 'savannah' form of *S. sarmentosus*; and ●, a plant of the 'forest' type of this species.

roughly the same amount as strophanthidol. Another plant (CB/68) in the same village was of the standard type and contained none of this substance. As yet it has not been possible to identify this 'substance H', but work in this direction is proceeding.

The 38 samples of *S. sarmentosus* investigated proved more interesting, being divided fairly sharply into two types, one containing sarmentogenin as the only genin present linked to a deoxy sugar, and the other type containing sarverogenin

The accompanying map (Fig. 2) shows the distribution of the two types. It should be noted that the collections in the neighbourhood of Katsina were made by local labour and represent hundreds of individual plants. The relative amounts of sarverogenin and sarmentogenin in the seeds of the 'forest' type vary, but in the typical form are roughly equal; so far we have found no sample of *S. sarmentosus* in which sarmentogenin is completely absent. One or two samples have contained only very small amounts of glycoside, but these

Table 2. *Cardiac aglycones of Strophanthus species*

(Where possible the quantities of aglycones contained in the seeds are indicated as a percentage of the weight of the seed. Where no quantitative data are available, the following abbreviations are used. *M*, main constituent; *S*, secondary constituent; *T*, trace only present.)

Species	Aglycones present						References
	Strophanthidin (II)	Strophanthidol (III)	Periplogenin (IV)	Sarverogenin (V)	Sarmentogenin (VI)	Ouabagenin (I)	
Group I							
<i>S. gardeniiflorus</i>	—	—	—	—	—	<i>M</i>	—
<i>S. gratus</i>	—	—	—	—	—	3·6 <i>a</i>	(1)
<i>S. tholloni</i>	—	—	—	—	—	<i>M</i>	(2)
Group II							
<i>S. arnoldianus</i>	—	<i>T</i>	—	—	—	—	(2)
<i>S. barteri</i>	<i>M</i>	—	—	—	—	—	—
<i>S. eminii</i>	0·4 <i>b</i>	0·18 <i>c</i>	0·14 <i>d</i>	—	—	—	(3)
<i>S. gracilis</i>	<i>S</i>	<i>S</i>	<i>M</i>	—	—	—	(4)
<i>S. hispidus</i>	1·6	<i>S</i>	<i>T</i>	—	—	—	(5)
<i>S. hypoleucus</i>	?	<i>S</i>	<i>M</i>	—	—	—	(6)
<i>S. kombé</i>	2·4 <i>e</i>	<i>S</i>	<i>T</i>	—	—	—	(7)
<i>S. ledienii</i>	<i>M</i>	<i>S</i>	<i>S</i>	—	—	—	(8)
<i>S. mirabilis</i>	0·48	<i>S</i>	<i>S</i>	—	—	—	(9)
<i>S. mortehanii</i>	?	0·15	?	—	—	—	(2)
<i>S. nicholsonii</i>	0·2	<i>S</i>	<i>S</i>	—	—	—	(10)
<i>S. preussii</i>	<i>M</i>	—	—	—	—	—	—
Group III							
<i>S. amboënsis</i>	—	—	—	<i>M</i>	<i>T</i>	—	(11)
<i>S. congoensis</i>	—	—	—	1·02	—	—	(2)
<i>S. courmontii</i>	—	—	—	0·22	0·1	—	(12)
<i>S. gerrardi</i>	—	—	—	0·7 <i>f</i>	0·13 <i>g</i>	—	(13)
<i>S. intermedius</i>	—	—	—	1·46 <i>h</i>	—	—	(14, 15)
<i>S. petersianus</i>	—	—	—	—	0·1 <i>g</i>	—	(16)
<i>S. sarmentosus</i>	—	—	—	0·1	0·1	—	(17)
‘forest’ form	—	—	—	—	—	—	—
<i>S. sarmentosus</i>	—	—	—	—	0·26	—	(18)
‘savannah’ form	—	—	—	—	—	—	—
<i>S. schuchardii</i>	—	—	—	<i>M</i>	—	—	(14)
<i>S. welwitschii</i>	—	—	—	0·09 <i>h</i>	—	—	(19)

Notes

a Content of ouabain.

b Content of cymarín.

c Content of cymarol.

d Content of periplocymarin. A considerable quantity of emicymarin, a difficultly hydrolysable glycoside of periplogenin, is also present.

e Crude, contained some strophanthidol.

f Content of sarveroside. A considerable quantity of other sarverogenin glycosides is also present.

g Content of sarmentocymarin.

h Content of intermedioside. A considerable quantity of other sarverogenin glycoside is also present.

References

- (1) Thoms (1904).
- (2) Rothrock, Howe, Florey & Tishler (1950).
- (3) Lardon (1950).
- (4) Aebi & Reichstein (1951).
- (5) Cf. v. Euw & Reichstein (1950*a*).
- (6) v. Euw & Reichstein (1950*b*).
- (7) Koechlin & Reichstein (1947).
- (8) Hess, Speiser, Schindler & Reichstein (1951).
- (9) Cf. Bally, Schindler & Reichstein (1952).
- (10) Cf. v. Euw & Reichstein (1948).
- (11) v. Euw, Hess, Speiser & Reichstein (1951).
- (12) Figures for an average specimen of *S. courmontii* investigated by us. Cf. v. Euw & Reichstein (1950*c*).
- (13) v. Euw & Reichstein (1950*d*).
- (14) v. Euw, Hess *et al.* (1951).
- (15) Salmon, Smith & Bywater (1951).
- (16) v. Euw & Reichstein (1950*e*).
- (17) Cf. Buzas, v. Euw & Reichstein (1950).
- (18) Cf. v. Euw, Reber & Reichstein (1951).
- (19) v. Euw, Heitz, Hess, Speiser & Reichstein (1952).

were gathered in an extremely unripe condition, so the observation is probably not significant. Two large samples of seed collected in Sierra Leone and the Gambia proved to be similar to the Nigerian 'savannah' type.

Certain other species of *Strophanthus* have also been investigated by this method, including *S. cour-*

interesting, containing about 3% of sarverogenin, no sarmentogenin or only very slight traces, and small amounts of three unidentified substances. The authentic specimen of *S. intermedius*, which occurs very commonly in Angola, gave a chromatogram indistinguishable from that given by the unidentified specimen.

montii (two specimens), *S. mirabilis*, *S. petersianus*, *S. kombé* (six specimens), *S. amboënsis*, *S. nicholsonii*, *S. barteri*, *S. preussii*, a specimen of *S. intermedius* kindly provided by Prof. Reichstein, and a specimen from Angola which has not been satisfactorily identified, but is probably also *S. intermedius*.

Of the two specimens of *S. courmontii*, one contained sarverogenin as the only genin attached to a 2-deoxy sugar, whilst the other was very similar to the 'forest' form of *S. sarmentosus* except that it was rather richer in total genin content. The *S. amboënsis* was again similar to the 'forest' form of *S. sarmentosus*, but was considerably richer in total genin, while the *S. petersianus* proved similar to the 'savannah' form of *S. sarmentosus*.

The specimens of *S. kombé* proved to be similar to *S. hispidus*, a trace of periplogenin always being present, together with a fourth substance, 'substance K'. *S. mirabilis* and *S. nicholsonii* were also of the *S. hispidus* type, but here the periplogenin content was greatly increased, being roughly equal to the strophanthidin. *S. barteri* and *S. preussii* proved to be generally similar to the *S. hispidus* type, but contained rather small amounts of glycoside.

The specimen from Angola was particularly

DISCUSSION

From our results and those reported by other workers, especially Prof. T. Reichstein and his colleagues, in numerous papers in the last 12 years, it would appear that the species of the genus *Strophanthus* which have so far been investigated, fall into three well defined groups:

(1) *S. gratus* and the two closely related species, *S. tholloni* and *S. gardeniiflorus*, of which the principal glycoside is ouabain (I). Mr R. D. Meikle of the Royal Botanic Gardens, Kew, tells us that this group has been separated from *Strophanthus* to form the genus *Roupellia*, although this division has apparently not been accepted by all authorities (cf. Baillon, 1891).

(2) *S. arnoldianus*, *S. barteri*, *S. eminii*, *S. gracilis*, *S. hispidus*, *S. hypoleucus*, *S. kombé*, *S. ledienii*, *S. mirabilis*, *S. mortehanii*, *S. nicholsonii* and *S. preussii*. The typical genins of this group are strophanthidin (II) and periplogenin (III), the relative amounts of these two genins varying rather widely from *S. hypoleucus*, which is reported to contain only a little strophanthidin and mainly periplogenin (v. Euw & Reichstein, 1950b), to *S. kombé* which contains much strophanthidin and no more than a

minute trace of periplogenin. Strophanthidol (IV) is always present also as a minor constituent. It has been reported that periplogenin does not occur in *S. hispidus* and *S. kombé*, but we have always found traces of it in these plants.

(3) *S. amboënsis*, *S. courmontii*, *S. congoensis*, *S. gerrardi*, *S. intermedius*, *S. petersianus* and its variety *grandiflorus* (syn. *verrucosus*), *S. sarmentosus*, *S. schuchardti* and *S. welwitschii*. In this group the typical genins are sarverogenin (V) and sarmentogenin (VI), although once again the relative proportions of the two substances are variable, *S. intermedius* containing no sarmentogenin, and the 'savannah' form of *S. sarmentosus* and *S. petersianus* containing no sarverogenin.

Although the differences between the two types of *S. sarmentosus* are rather larger than might be expected within a species, Mr Meikle informs us that he can find no botanical justification for a division of the species as at present defined, and it has yet to be proved whether these differences are due to genetic variations or merely to the conditions under which the plants are growing.

Pichon (1949) states that *S. speciosus* and *S. boivinii*, which have been reported by Schindler & Reichstein (1952) as containing gitoxigenin digitaloside and other unidentified substances are not *Strophanthus* species, *S. speciosus* having been placed in the monotypic genus *Christya*, and *S. boivinii*, together with the closely related *S. grevei*, in the genus *Roupellina*.

No *Strophanthus* species has yet been found to

contain so much as 1% of sarmentogenin, in contrast to strophanthidin and sarverogenin which both occur in quantities up to 3%.

It is suggested that this extremely simple and rapid method of investigation of *Strophanthus* seeds may well be found suitable as a pharmacognostic test for seed samples to replace the sulphuric acid colour test at present employed (Mathiesen, 1928; Gilg & Schuster, 1919), which has been found to be extremely unreliable, and largely depends on substances other than cardiac glycosides present in the seed.

SUMMARY

1. A paper-chromatographic method for the identification of members of the group of cardiac aglycones has been devised.

2. This method has been applied as a routine analytical method to samples of *Strophanthus* seeds from many species.

3. The results are discussed, and a classification of the genus *Strophanthus*, on purely chemical grounds, is proposed.

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