# THE ISOLATION OF CYMARIN AND PERIPLOCYMARIN FROM THE SEEDS OF *CASTILLOA ELASTICA* CERV.

BY (MISS) GILLIAN R. ADAMS AND S. WILKINSON

*From the Chemical Research Laboratory, The Wellcome Research Laboratories, Beckenham, Kent*

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Two known cardiac glycosides, cymarin and periplocymarin have been isolated from the seeds of the South American rubber tree, *Castilloa elastica.*

CARDIAC glycosides have been found in a limited number of plants of the botanical family *Moraceae.* Thus, the latex of *Antiaropsis* K. Schum and *Ogcodeia ternstroemiiflora* (Bisset, 1958) and *Antiaris toxicaria* Lesch (Upas tree) (Dolder, Tamm and Reichstein, 1955), which are native arrow poisons, contain a complex series of glycosides including α- and β-antiarin. The presence of cymarin in the roots of *Cannabis indica,* Lam has been reported (Soldatova, 1957).

In the course of examining a number of botanical species and *materia medica* collected by the late Dr. David Hooper, and now housed in the Wellcome Historical Medical Museum, a small sample of the seeds of *Castilloa elastica* Cerv. (*syn. Castilla elastica* Cerv.)* was found to give the characteristic colour reactions of the cardenolides. Raffauf and Morris (1960) have recently drawn attention to the persistence of alkaloids in plant tissue after prolonged storage. Although the precise date when the seeds were collected is not known, they are at least 50 years old and this illustrates the stability of the cardenolides in the seeds over this period of time. Similar results have been found with old seeds of *Strophanthus kombé, sarmentosus, gratus* and *dichotomus* from the same collection.

As a result of this preliminary chemical screening, fresh seeds and latex of *C. elastica* were obtained. Although glycosides could not be detected in the latex, they were readily extracted from the defatted seeds with chloroform. Examination by paper chromatography (Whatman No. 1 paper impregnated with formamide (25 per cent) in acetone; solvent methylisobutylketone: diisopropyl ether 100:25, saturated with formamide) revealed a major component ($R_F$ 0·45), a minor component ($R_F$ 0·66) and a trace of a third glycoside ($R_F$ 0·25).

The glycosides could be crystallised directly from methanol as colourless mixed crystals but it was found more convenient to fractionate the crude mixture on acetic acid (10 per cent)-deactivated alumina, with benzene: chloroform (1 : 2 v/v) as the solvent. The first runnings, which contained only a yellow pigment, were followed by the glycoside ($R_F$ 0·66) and then by the major component.

The main glycoside (0·76 per cent of the seed) was identified as cymarin from its physical constants and by hydrolysis to strophanthidin and

* A brief history of *C. elastica* is given by Loomis, *Agriculture of the Americas,* September, 1942.

cymarose. The second glycoside (0·14 per cent) gave periplogenin and cymarose on hydrolysis and was found to be periplocymarin. The remaining glycoside could not be isolated in quantities sufficient for identification.

## EXPERIMENTAL

The seeds (3,550 g.), from a mixture of kernels and seeds (9 kg.) were finely ground and percolated with light petroleum (b.p. 60–80°). Evaporation of the extract gave a thick viscous oil (1,010 g.). The marc was dried in a current of air and percolated with chloroform until no further blue colour was obtained with alkaline *m*-dinitrobenzene (Raymond's reagent). The percolate was evaporated under reduced pressure and the gummy residue triturated with dry ether (750 ml.). The pale yellow solid was filtered, washed with ether and dried (67·5 g; 1·9 per cent). Percolation of the residual seed powder with methanol gave a thick gum (200 g.) which contained only a trace of cardenolides.

The crude glycosides (20 g.) in benzene:chloroform (1:2 v/v; 50 ml.) were absorbed on a column of deactivated alumina (7 × 32 cm.) and the chromatogram was developed with the same solvent mixture. The following fractions were detected by paper chromatographic analysis.

*Column I: Fraction* (25 ml.):

| | | |
|---|---|---|
| (*a*) | 1–54: pigment | |
| (*b*) | 55–77: $R_F$ 0·66 | (0·685 g.) |
| (*c*) | 78–136: trace $R_F$ 0·66 + $R_F$ 0·45 | (3·5 g.) |
| (*d*) | 137–269: $R_F$ 0·45 | (6 g.) |

Fraction (*c*) was concentrated and the solid fractionated further on a second alumina column (4 × 30 cm.), using the same solvent mixture.

*Column II: Fraction* (10 ml.)

| | | |
|---|---|---|
| (*a*) | 1–30: $R_F$ 0·66 | (0·8 g.) |
| (*b*) | 31–71: trace $R_F$ 0·66 | (0·4 g.) |
| (*c*) | 71–90: $R_F$ 0·45 | (2·0 g.) |

*Cymarin.* Fractions (*d*) (Column I) and (*c*) (Column II) were combined and evaporated. The residue (8·0 g.) crystallised from methanol and ether in needles, m.p. 141–143° (after sintering at 138°) (Found: C, 64·1; H, 8·3 per cent. Calculated for $C_{30}H_{44}O_9$,MeOH: C, 64·1; H, 8·3 per cent). Recrystallisation from dilute ethanol gave hexagonal plates of the sesquihydrate, m.p. 184–185° (Found: C, 62·5; H, 8·2 per cent. Calculated for $C_{30}H_{44}O_9$,$1\frac{1}{2}H_2O$: C, 62·6; H, 8·2 per cent) which lost water on drying at 120°/0·01 mm. to give anhydrous cymarin $[\alpha]_D^{22} = +39{\cdot}0°$ ($c$ = 1·7 in $CHCl_3$) (Found: C, 65·8; H, 8·0; OMe, 5·75 per cent. Calculated for $C_{30}H_{44}O_9$: C, 65·7; H, 8·0; OMe, 5·7 per cent) (Authentic cymarin: m.p. 184°, $[\alpha]_D^{20} = +39{\cdot}3°$ (MeOH)) (Stoll, Renz and Kreis, 1937). Acetylcymarin crystallised from aqueous methanol in long needles, m.p. 175–176°, $[\alpha]_D^{22} = +45{\cdot}1°$ ($c$ = 1·0 in EtOH) (Found: C, 64·6; H, 7·4; OMe, 4·8 per cent. Calculated for C, 65·0; H, 7·85; OMe, 5·3 per cent).

Hydrolysis of the cymarin with hydrochloric acid (0·1N) in aqueous methanol (50 per cent) gave strophanthidin, m.p. 230–233° $[\alpha]_D^{22} = +43{\cdot}5°$ ($c = 0{\cdot}4$ in MeOH) (Found: C, 67·6; H, 8·1 per cent. Calculated for $C_{23}H_{32}O_6$: C, 68·3; H, 7·95 per cent). The $R_F$ (0·48) with the system described previously was identical with that of authentic strophanthidin prepared from k-strophanthin. Further proof of identity was obtained by preparing the oxime, m.p. 270° (decomp.) (Found: C, 66·3; H, 8·1; N, 3·0 per cent. Calculated for $C_{23}H_{38}NO_6$: C, 65·9; H, 7·9; N, 3·3 per cent) and the 3-acetyl derivative, m.p. 243–244° $[\alpha]_D^{22} = +57{\cdot}7$ ($c = 0{\cdot}45$ in $CHCl_3$) (Found: C, 67·0; H, 7·65 per cent. Calculated for $C_{25}H_{34}O_7$: 67·3; H, 7·6 per cent).

*Cymarose* was isolated and purified by molecular distillation (120°/0·0001 mm.). Crystallisation from ether and light petroleum gave prisms, m.p. 93–94°, $[\alpha]_D^{20} = +54{\cdot}7°$ ($c = 3{\cdot}2$ in $H_2O$ after 24 hr.). Cymaronic acid phenylhydrazide, prepared according to the procedure of Shoppee and Reichstein (1940), crystallised from methanol and ether in needles, m.p. 152–152·5°, $[\alpha]_D^{20} = +0{\cdot}25°$ ($c = 4{\cdot}3$ in MeOH) (Found: C, 57·8; H, 7·6; N, 10·5; OMe, 12·3 per cent. Calculated for $C_{13}H_{20}N_2O_4$ requires C, 58·2; H, 7·5; N, 10·4; OMe, 11·6 per cent) (Authentic cymaronic acid phenylhydrazide, m.p. 151–152°, $[\alpha]_D^{19} = +0{\cdot}5 \pm 3°$ (MeOH) (Hunger and Reichstein, 1950).

*Periplocymarin.* Concentration of fraction (*b*) (Column I) and fraction (*a*) (Column II) gave a solid (1·48 g.) which crystallised from methanol and ether in prisms, m.p. 146–147° (after sintering at 140°) and which, after drying at 120°/0·001 mm., had m.p. 138–140°, $[\alpha]_D^{22} = +27{\cdot}8°$ ($c = 1{\cdot}1$ in MeOH) (Found: C, 66·9; H, 8·5; OMe, 6·3 per cent. Calculated for $C_{30}H_{46}O_8$: C, 67·4; H, 8·6; OMe, 5·8 per cent) (Authentic periplocymarin, m.p. 138–139·5°, $[\alpha]_D^{20} = +28{\cdot}3 \pm 2°$ ($c = 1{\cdot}2$ in MeOH) (Ruppol and Trukovic, 1955). Acetylperiplocymarin crystallised from aqueous ethanol in hexagonal plates, m.p. 129–130°, after drying at 60°/0·01 mm., $[\alpha]_D^{22} = +45{\cdot}25°$ ($c = 0{\cdot}44$ in $CHCl_3$) (Found: C, 64·6; H, 8·4 per cent. Calculated for $C_{32}H_{48}O_9,H_2O$: C, 64·6; H, 8·45 per cent).

Hydrolysis of periplocymarin gave cymarose ($R_F$ 0·79; Whatman No. 1, butanol: pyridine: water, 3:2:1·5; identical with that of authentic cymarose) and periplogenin, which crystallised from aqueous ethanol in prisms which sintered at *ca.* 140° and then melted at 220–222°. Recrystallisation from aqueous methanol gave prisms, m.p. 233–235° (Found: C, 67·2; H, 8·7; $H_2O$, 4·5 per cent. Calculated for $C_{23}H_{34}O_5,H_2O$: C, 67·6; H, 8·9; $H_2O$, 4·4 per cent). After drying at 120°/0·001 mm., $[\alpha]_D^{22} = +31{\cdot}1°$, $[\alpha]_{5461}^{22} = +38{\cdot}8°$ ($c = 0{\cdot}39$ in EtOH) (Authentic periplogenin, m.p. 142–150° and then 238–245°, from chloroform and ether, $[\alpha]_D^{23} = +29{\cdot}48°$ (MeOH), $[\alpha]_D^{19} = +30{\cdot}0°$ ($CHCl_3$) (Ruppol and Trukovic, 1955), $[\alpha]_D^{27} = +31{\cdot}5°$ ($c = 1{\cdot}04$ in EtOH) (Lehmann, 1897).

For further identification, 3-acetylperiplogenin, m.p. 242–244°, $[\alpha]_D^{22} = +46{\cdot}9°$ ($c = 0{\cdot}32$ in $CHCl_3$) was prepared (Found: C, 68·9; H, 8·2 per cent. Calculated for $C_{25}H_{36}O_6$: C, 69·4; H, 8·35 per cent).

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