18. *Cardenolides. Part III.* The Constitution of Calotropagenin.*

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Calactin is converted through calactinic acid into calotropagenin. On degradative evidence the aglycone is considered to be 3β : 12ξ : 14β-trihydroxy-19-oxo-5α-card-20(22)-enolide (II).

THE shrub *Calotropia procera* (family Asclepiadaceae) occurs extensively in Asia and Africa. It has been used since antiquity as a galenical and for the preparation of arrow poison. Early attempts to isolate the biologically active constituents failed but pharmacological investigations showed that compounds with digitalis-like properties were present.[1,2] From the plant latex Hesse and his co-workers [2-4] separated calotropin ($C_{29}H_{40}O_9$),

* The communications in *J.*, 1951, 2766 and 3193, are regarded as Parts I and II, respectively.

[1] Lewin, "Die Pfeilgifte," J. A. Barth, Berlin, 1923; Wehmer, "Die Pflanzenstoffe," Gustav Fischer, Jena, 1931, **2**, 1001.

[2] Hesse and Reicheneder, *Annalen*, 1936, **526**, 252.

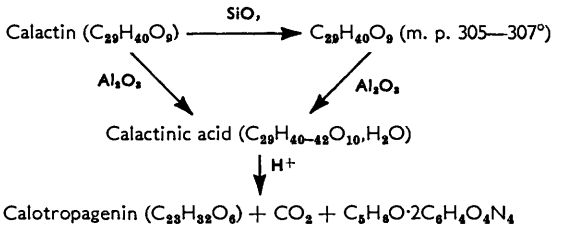
[3] Hesse, Heuser, Hütz, and Reicheneder, *ibid.*, 1950, **566**, 130.

[4] Hesse and Lettenbauer, *Angew. Chem.*, 1957, **69**, 392.

calactin ($C_{29}H_{40}O_9$), uscharidin ($C_{29}H_{38}O_9$), uscharin ($C_{31}H_{41}O_8NS$), calotoxin ($C_{29}H_{40}O_{10}$), and voruscharin ($C_{33}H_{47}O_9NS$), and suggested that these were new steroid glycosides. The seeds of *Calotropis procera* were shown by Reichstein's school [5] to contain frugoside, coroglaucigenin, and corotoxigenin but very little, if any, of the crystalline steroids isolated from the latex.

In 1936, Hesse and Reicheneder [2] proposed the partial structure (I) for calotropin. A later investigation [3] provided evidence that the aglycone is common to the first five latex glycosides which are listed above. In a preliminary communication [6] we suggested structure (II) for this aglycone, calotropagenin. There has been independent support [7] for the proposal. Our detailed evidence is now presented.

The glycosides of *Calotropis procera* resist mild hydrolysis with dilute acid. It was this that prompted the unusual use of pyrolysis or hydrolysis with alkali for degradation.[2] However, these conditions led to decomposition of the aglycone. We have found that calotropagenin may be obtained easily from calactin. This is converted by alumina, either directly or through an isomer formed by the action of silica gel, into the new compound calactinic acid, $C_{29}H_{40-42}O_{10},H_2O$. This acid is hydrolysed readily by dilute sulphuric acid to calotropagenin, carbon dioxide, and a fragment which has been isolated as a bis-2 : 4-dinitrophenylhydrazone ($C_5H_8O{\cdot}2C_6H_4O_4N_4$). The calotropagenin which is prepared in this way is identical with the aglycone obtained in low yield from plant material.[2]



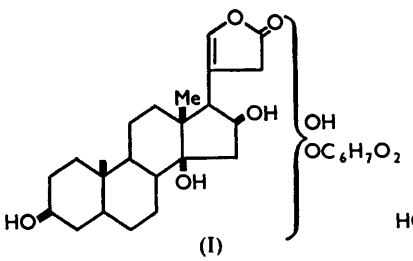
The ultraviolet and infrared absorption spectra of calotropagenin indicate that a but-20(22)-enolide ring is attached to the steroid portion. The maximum found at 310 mμ (log ε 1·4) is regularly observed in cardenolides with a 10-formyl substituent. This group also accounts for the reduction by sodium borohydride to a dihydrocalotropagenin which gives a positive Legal reaction, yields a triacetyl derivative, and is oxidised by chromic acid to a stable keto-acid, $C_{29}H_{28}O_7$.

The lactone ring, the 3 : 14-hydroxyl groups regularly found in cardenolides, and the 10-formyl group account for all but one of the oxygen atoms in calotropagenin. The last one is present in an acylatable hydroxyl group which is tentatively placed at position 12 to account for the formation of two *iso*-derivatives when calotropagenin reacts with mild alkali. One of these (III) evidently results from the well-known interaction of the αβ-unsaturated lactone with the hydroxyl group at position 14. The second could be formed by interaction with a hydroxyl group at positions 12, 15, or 16. Position 15 is unlikely as calotropagenin is not oxidised by periodic acid during 56 hours; and position 16 is excluded since vigorous treatment with hydrochloric acid does not lead, as in the case of gitoxigenin, to a $\Delta^{14:16}$-dianhydro-derivative with an absorption maximum near 337 mμ.[8]

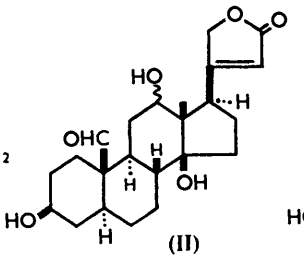
The stereochemistry of calotropagenin is suggested by analogy with other cardiac glycosides obtained from Asclepiadaceae. In particular, attention has been drawn to the fact that cardenolides isolated from species of this plant family have *trans*-A/B rings.

[5] Rajagopalan, Tamm, and Reichstein, *Helv. Chim. Acta*, 1955, **38**, 1809.
[6] Hassall and Reyle, *Chem. and Ind.*, 1956, 487.
[7] Geiger, Hesse, Lettenbauer, and Schildknecht, *Naturwiss.*, 1957, **44**, 328.
[8] Tschesche, *Chem. Ber.*, 1937, **70**, 1554.

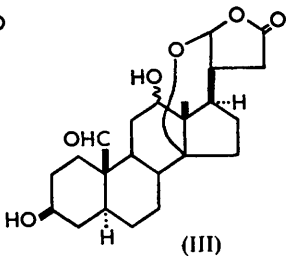
There is evidence which supports this in the case of calotropagenin. The difference in the molecular rotation of cardenolides with substituents CHO and CH_2·OH at position 10 is greater when rings A/B are *trans* than when they are *cis*. The difference in $[M_D]$ values



(I)



(II)



(III)

for calotropagenin and dihydrocalotropagenin is in reasonable agreement with that observed for *trans*-compounds (see Table)

Substance	A/B ring	10-Subst.	$[M]_D$ *	Δ
Gofruside } *a*	*trans*	CHO	−27°	65°
Frugoside } *a*	,,	CH_2·OH	−92	—
Corotoxigenin } *a, b*	,,	CHO	+163	73
Coroglaucigenin } *a, b*	,,	CH_2·OH	+90	—
Calotropagenin	—	CHO	+170	81
Dihydrocalotropagenin	—	CH_2·OH	+89	—
α-Antiarin } *c*	*cis*	CHO	−23	28
Dihydro-α-antiarin } *c*	,,	CH_2·OH	−51	—
Strophanthidin } *d*	,,	CHO	+175	16
Dihydrostrophanthidin } *d*	,,	CH_2·OH	+159	—

* Limits of error approx. ±8.[a]

[a] Hunger and Reichstein. *Helv. Chim. Acta*, 1952, **35**, 1073. [b] Stoll, Pereira, and Renz, *ibid.*, 1949, **32**, 293. [c] Doebel, Schlittler and Reichstein, *ibid.*, 1948, **31**, 688. [d] Rabald and Kraus, *Z. physiol. Chem.*, 1940, **265**, 39.

Experimental

M. p.s were determined by means of a Kofler block. Rotations refer to chloroform solutions at 28—30° except where other solvents are specified. Ultraviolet spectra were determined for ethanol solutions on a Beckman spectrophotometer, model D.U. Infrared spectra were measured with potassium bromide discs. We are grateful to Dr. S. M. Nagy, Massachusetts Institute of Technology, for the determinations of infrared absorption spectra and to Dr. F. Pascher, Bonn, for the microanalyses.

Isolation of the Cardiac Glycosides.—The experiments were on plant material obtained from *Calotropis procera* shrubs growing on the Pallisadoes peninsular, near Kingston, Jamaica. In preliminary experiments latex, leaves, and stalks, or root bark were examined separately. The last proved to be the most convenient source of glycosides, the following procedure being the best found. The bark (43·3 kg.) from freshly collected roots was cut into small pieces and made into a thin slurry with toluene-saturated water by means of a Waring blendor. After 24 hr., the slurry was filtered and the solid residue was left for a further 24 hr. just covered with toluene-saturated water. The residue obtained on filtration did not give a Raymond reaction. Both the first (36 l.) and the second (22 l.) filtrate were extracted in 3 l. portions, each with 6 × 3 l. of chloroform–ethanol (2 : 1). The chloroform extracts were combined, washed with 5% sodium hydrogen carbonate solution and water, dried, and evaporated. The residue was taken up in chloroform (200 c.c.) and treated with light petroleum (1 l.) to separate the steroid glycosides from plant resins which remained in solution. After repetition of this process the amorphous precipitates were combined (28 g.) and crystallised from ethanol, to give plates (3·9 g.), m. p. 238—248° (decomp.). This crystalline product (A) and the amorphous residue (B) obtained on evaporation of the filtrate contained combined nitrogen and sulphur (Lassaigne test; sodium plumbite test). Extracting product A (7·58 g.) successively with hot chloroform (2 × 100 c.c.) and methanol (2 × 50 c.c.) gave a relatively insoluble fraction (2·54 g.). This material on fractional elution chromatography on a column of silica gel (125 g.) with successively

chloroform–dioxan (10 : 1; 1 l.), chloroform (6 × 1 l.), and ethyl acetate (1 l.) gave uniform material in the last four chloroform fractions and in the ethyl acetate fraction. These fractions were combined and recrystallised from ethanol, to give plates, m. p. 265—271°, $[\alpha]_D$ +66° ± 3° [*c* 0·77 in chloroform–methanol (2 : 1)], $\lambda_{max.}$ 309, 217 mμ (log ε 1·49, 4·22) (Found: C, 63·2; H, 7·7. Calc. for $C_{29}H_{40}O_{10}$: C, 63·5; H, 7·4%). It has the same crystalline form as, and did not depress the m. p. of a sample of, calotoxin, m. p. 250—256°, kindly supplied by Professor G. Hesse. The acetate and benzoate were amorphous.

Attempts to separate the constituents of the amorphous product B, and the mother-liquors from the crystallisation of fraction A, by crystallisation and chromatography were impeded by the formation of mixed crystals and the rearrangements that took place when at least one of the constituents came in contact with columns of alumina and silica gel (see below). Lassaigne and sodium plumbite tests showed that both fractions contained nitrogen and sulphur. To simplify the separation the crude mixtures were separately treated with mercuric chloride in acetone, to remove nitrogen and sulphur.[10] The amorphous product obtained in this way from fraction B (37·2 g.) yielded crystals (6·1 g.) from ethanol. Similar treatment of the chloroform-soluble portion (7·8 g.) from fraction A gave crystals (5·2 g.). The crystalline material from both sources was mixed and fractionated by means of chloroform. The less soluble portion had properties similar to those of calotoxin. The chloroform-soluble fraction was identified as calactin, rectangular plates, m. p. and mixed m. p. (with calactin kindly supplied by Professor G. Hesse) 215—218°, $[\alpha]_D$ +45° ± 2° (*c* 1·2 in methanol), $\lambda_{max.}$ 301, 219 mμ (log ε 1·65, 4·19) [Found (for material dried over P_2O_5 at 130° *in vacuo*): C, 64·9; H, 7·4. Calc. for $C_{29}H_{40}O_9$: C, 65·3; H, 7·6%]. The *oxime*, prepared both from calactin kindly supplied by Professor G. Hesse and from our preparation, by the interaction (1 hr.) at 30° of the glycoside, hydroxylamine hydrochloride, and sodium acetate in methanol, had m. p. 280—284° (decomp.) [Found (for material dried over P_2O_5 at 130° *in vacuo*): C, 61·3; H, 7·5; N, 2·2. $C_{29}H_{41}O_9N,H_2O$ requires C, 61·6; H, 7·7; N, 2·5%]. Crystalline material could not be obtained when the more vigorous conditions described by Hesse *et al.*[3] were employed.

Calactinic Acid.—Calactin (52 mg.) in chloroform was applied to a column of alumina (2 g.) and left for 12 hr. Elution with methanol and chloroform–methanol–water (5 : 5 : 2) gave material (12 mg.) which was not characterised. Elution with chloroform–methanol–water (5 : 5 : 2) to which 1% of acetic acid had been added gave *calactinic acid* which crystallised from ethanol–chloroform as prisms (30 mg.), m. p. 170—173° (decomp.), $[\alpha]_D$ −24° ± 2° [*c* 0·92 in chloroform–methanol (2 : 1)], $\lambda_{max.}$ 309, 217 mμ (log ε 1·39, 4·2) [Found (for material dried at 80° over P_2O_5 *in vacuo*): C, 61·2; H, 7·6. $C_{29}H_{40}O_{10},H_2O$ requires C, 61·5; H, 7·5. $C_{29}H_{42}O_{10},H_2O$ requires C, 61·3; H, 7·8. Found (for material dried at 120° over P_2O_5 *in vacuo*): C, 62·8; H, 7·4. $C_{29}H_{40}O_{10}$ requires C, 63·5; H, 7·4. $C_{29}H_{42}O_{10}$ requires C, 63·3; H, 7·7%]. There was evidence of decomposition with release of carbon dioxide on continued drying. The *acetate* crystallised as needles, m. p. 174—176° (from ethanol–water), $[\alpha]_D$ −61° ± 2° (*c* 1·07) [Found: C, 61·2; H, 7·3; O, 32·0; OAc, 13·7. $C_{29}H_{38}O_{10}(C_2H_3O)_2,H_2O$ requires C, 60·9; H, 7·1; O, 32·0; OAc, 13·2%. $C_{29}H_{40}O_{10},(C_2H_3O)_2H_2O$ requires C, 60·7; H, 7·4; O, 31·9; OAc, 13·2%].

Reaction of Calactin with Activated Silica Gel.—In attempts to purify crude calactin by chromatography it was modified by contact with silica gel. *E.g.*, calactin (261 mg.) in benzene was added to a column of silica gel (20 g.) which had been freshly activated *in vacuo* at 220° for 40 min. The column was eluted successively with benzene, benzene–chloroform (1 : 1), chloroform, chloroform–ethyl acetate (1 : 1), ethyl acetate, and methanol during 4—8 hr. Traces of unchanged calactin and amorphous impurities were obtained from the benzene–chloroform and the methanol eluate, respectively. The major *component* (230 mg.), which was isolated principally from the chloroform–ethyl acetate eluate, crystallised as needles, m. p. 305—307° (from dioxan–water), $[\alpha]_D$ +54° ± 1° [*c* 1·62 in chloroform–methanol (2 : 1)], $\lambda_{max.}$ 310, 218 mμ (log ε 1·49, 4·23). [Found (for material dried over P_2O_5 at 130° *in vacuo*: C, 65·7; H, 7·6. $C_{29}H_{40}O_9$ requires C, 65·4; H, 7·6%]. The *benzoate* crystallised only after chromatography on alumina (quick elution), as plates, m. p. 195—200°, from acetone–ether [Found (material dried at 80° *in vacuo*): C, 67·3; H, 6·7. $C_{29}H_{39}O_9,C_7H_5O$ requires C, 67·9; H, 7·0%].

When the product of m. p. 305—307° (38 mg.), in methanol–chloroform (2 : 1; 2 c.c.), was left in contact with acid-washed, activated alumina for 20 hr. and then filtered, neutral material

[9] Ref. *a* of Table.
[10] Hesse and Gampp, *Chem. Ber.*, 1952, **85**, 933.

(15 mg.) was obtained from the filtrate. The alumina gave calactinic acid (18 mg.) when it was eluted with chloroform–methanol–water (5 : 5 : 2) containing acetic acid (1%). Repetition of the process with the neutral fraction gave a further yield of calactinic acid.

Preparation of Calotropagenin from Calactinic Acid.—Calactinic acid (4·17 g.) was refluxed for 11 hr. with 0·05N-sulphuric acid (250 c.c.) and methanol (250 c.c.). Pure nitrogen was passed through the mixture to sweep carbon dioxide formed into 0·1N-barium hydroxide. Titration indicated 0·6 mol. of carbon dioxide. Methanol was then removed by evaporation *in vacuo*. The aqueous solution was extracted with chloroform–ethanol (9 : 1), and the combined extracts were washed with water, 10% aqueous sodium carbonate solution, and water. A neutral product (3·0 g.) was obtained by evaporation of the chloroform extract. A small quantity (0·35 g.) of unchanged calactinic acid was obtained from the sodium carbonate washings. The aqueous solution and washings were mixed and treated with 2 : 4-dinitrophenylhydrazine (2·0 g.) in N-sulphuric acid (600 c.c.). After 24 hr. the precipitate was filtered off and dried (0·86 g.). Chromatography on alumina separated a variety of 2 : 4-dinitrophenylhydrazones. A major *component* had m. p. 257—259° (Found: C, 42·7; H, 3·3; N, 23·7. $C_{17}H_{16}O_9N_8$ requires C, 42·8; H, 3·4; N, 23·5%).

The neutral fraction from the hydrolysis was introduced in chloroform (300 c.c.) on to a column (2·5 × 25 cm.) of acid-washed alumina (Spence, Type H), which had been activated at 110° for 20 hr. The column was eluted with 500 c.c. portions of chloroform–ethyl acetate (1 : 1), ethyl acetate (8 portions), ethyl acetate–ethanol (5 : 1), ethyl acetate–ethanol (1 : 1), ethanol (2 portions), and chloroform–methanol–water (2 : 2 : 1). The ethyl acetate fractions contained mixtures. The eluates containing ethanol were evaporated, to yield *calotropagenin* (1·87 g.) which recrystallised from ethanol as conglomerates, m. p. 238—250°, $[\alpha]_D$ +43° ± 2° [*c* 1·21 in chloroform–methanol (2 : 1)], $\lambda_{max.}$ 218, 309 mμ (log ε 4·24, 1·38) (Found: C, 68·2; H, 8·1. Calc. for $C_{23}H_{32}O_6$: C, 68·3; H, 8·0%). Hesse and Reicheneder [2] gave m. p. 240° and $[\alpha]_D$ +42°. Calotropagenin did not react with periodic acid during 56 hr. The amorphous product obtained when calotropagenin was heated with concentrated hydrochloric acid did not have an absorption maximum at 315—250 mμ.

Reduction of Calotropagenin with Sodium Borohydride.—Calotropagenin (385 mg.) was reduced with sodium borohydride (120 mg.) in 40% aqueous tetrahydrofuran during 5 hr. The mixture was worked up in the usual way, to give a crude product (318 mg.) which on recrystallisation from ethanol gave pure *dihydrocalotropagenin* as plates (240 mg.), m. p. 268—273°, $[\alpha]_D$ +21° ± 3° (Found: C, 67·9; H, 8·4. $C_{23}H_{34}O_6$ requires C, 68·0; H, 8·4%). The Legal and the Raymond reaction were positive. The *triacetate*, prepared by acetic anhydride in pyridine, crystallised from acetone–ether as needles, m. p. 257—258°, $[\alpha]_D$ −19° ± 2° (*c* 1·18) (Found: C, 65·7; H, 7·6; OAc, 22·9. $C_{29}H_{40}O_9$ requires C, 65·4; H, 7·2; 3OAc, 26%). The *tribenzoate* crystallised from acetone as plates, m. p. 258—262°, $[\alpha]_D$ −15° ± 2° (*c* 1·21) (Found: C, 73·1; H, 6·5. $C_{44}H_{46}O_9$ requires C, 73·5; H, 6·5%).

Oxidation of Dihydrocalotropagenin with Chromium Trioxide.—The dihydrogenin (84 mg.) in acetic acid (5 c.c.) was treated with five portions of chromium trioxide (total 70 mg.) at intervals of 15 min. The mixture was kept for 12 hr., then worked up in the usual way to remove chromium compounds. The crude product was separated into acidic and neutral fractions with saturated aqueous sodium hydrogen carbonate. The acidic *product* crystallised from acetone as rhombohedra, m. p. 277—180° (Found: C, 65·0; H, 7·3. $C_{23}H_{28}O_7$ requires C, 66·3; H, 6·8%).

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