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STRUCTURE OF ASCLEPIN AND SOME OBSERVATIONS ON THE NMR SPECTRA OF *CALOTROPIS* GLYCOSIDES*

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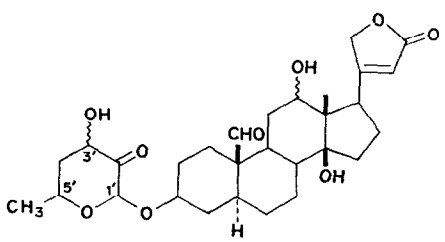
Abstract—Asclepin has been shown to be 3′-*O*-acetylcalotropin using various chemical and physical methods. The NMR data of all the *Calotropis* glycosides have been analysed in conjunction with that of asclepin, certain anomalies have been pointed out and revised structures have been proposed for the acetylated glycosides.

INTRODUCTION

FROM *Asclepias curassavica*, we have recently reported the isolation of a number of cardenolides.[1] One of them, substance B, now named as asclepin, is present in considerable amount in this plant and the present communication deals with its structure elucidation as 3′-*o*-acetylcalotropin.

Calotropin and calactin, two unusual cardiac glycosides, were first isolated by Hesse *et al.*[2] from the latex of *Calotropis procera* and *C. gigantea* and structures[3] were assigned to them, which were later modified by Hassall *et al.*[4] The structure (I) proposed by Hassall *et al.* involved the unusual sugar 2,4-dideoxy hexulose and it was suggested that calactin and calotropin were isomeric at C-3′.

Reichstein *et al.*[5] isolated calotropin from *Pergularia extensa* and Kupchan *et al.*[6] showed that it was also present in *A. curassavica* of Brazilian origin. We have isolated calotropin and calactin from the Indian variety of this plant.[1]



(I)

* Part II in the series "Chemical Investigation of *Asclepias curassavica*" and Communication No. 1632 from the Central Drug Research Institute, Lucknow.

[1] B. SINGH and R. P. RASTOGI, *Indian J. Chem.* 7, 1105 (1969).

[2] G. HESSE, H. EILBRACHT and F. REICHENEDER, *Ann* **546**, 233 (1941); G. HESSE, *Angew. Chem.* **61**, 339 (1949); G HESSE, L. J. HEUSER, E. HUTZ and F. REICHENEDER, *Ann.* **566**, 130 (1950); G. HESSE, *Naturwissenschaften* **9**, 227 (1956); G. HESSE and H. HERTEL, *Angew. Chem.*, **69**, 61 (1957); G. HESSE and G. LUDWIG, *Ann.* **632**, 158 (1960).

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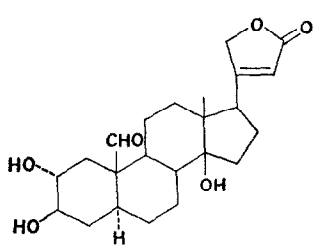
[6] S. M. KUPCHAN, J. R. KNOX and J. E. KELESEY, *Science* **146**, 1685 (1964).

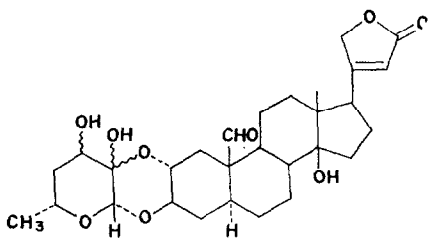
Reichstein *et al.*[7] have recently re-examined the structures of *Calotropis* cardenolides and have proposed new structures for calotropin, calactin and other glycosides of this series based on chemical and physical evidence. The structure of calotropagenin, the aglycone of all the *Calotropis* glycosides, has been revised to II and the structure of calotropin has been assigned as III which is similar to the structure of gomphoside proposed by Watson *et al.*[8] Calotropin is isomeric with calactin and they differ in the stereochemistry at C-2′ or C-3′.

RESULTS AND DISCUSSION

We have also isolated calotropagenin[1] and have found that it consumed exactly one mole of $NaIO_4$, confirming the presence of a glycol system in the molecule in conformity with the structure proposed by Reichstein *et al.*[7]



(II)



(III)

Asclepin, $C_{31}H_{42}O_{11}$, m.p. 308–9°, showed in its NMR spectrum, an acetyl function (2·13 ppm), an aldehyde proton (9·96 ppm), a secondary methyl (1·30 ppm, $J = 6$ c/s), a tertiary methyl (0·83 ppm), a singlet (4·55 ppm) for O—CH—O-grouping and the usual 3 protons of the butenolide ring.

On deactylation with potassium bicarbonate under mild conditions it gave a crystalline product which has been shown by its NMR, IR, mixed m.p. and co-paper chromatography as calotropin (III). In mass spectrum the deacetylasclepin did not show a molecular ion peak (*m/e* 532 M^+) but gave a peak at *m/e* 514 (M^+-18). The fragmentation[7,11] of the molecule is shown in the Fig. 1 which further confirmed its structure as calotropin.

It has been found that acetylation of calotropin gives a monoacetate, chromatographically identical to asclepin. Asclepin formed an amorphous acetyl derivative in about 50 per cent yield whose NMR spectrum exhibited two acetyl functions (2·01, 2·08 ppm), the singlet for —O—CH—O— was now shifted downfield to 5·53 ppm and a 2H multiplet appeared in region of 5·65–6·00 ppm which is assigned to —O—CH—OAc— and an olefinic proton. The

[7] F. BRUSCHWEILER, K. STOCKEL and T. REICHSTEIN, *Helv. Chim. Acta* **52**, 2276 (1969); A. LARDON K. STOCKEL and T. REICHSTEIN, *ibid.* **52**, 1940 (1969); *ibid.* **53**, 167 (1970).
[8] T. R. WATSON and S. E. WRIGHT, *Chem. Ind.* 1178 (1954); *ibid. Austral. J. Chem.* **9**, 497 (1956); *ibid.* **10**, 79 (1957); *ibid.* **17**, 92 (1964).
[9] R. G. COOMBE and T. R. WATSON, *Australian J. Chem.* **17**, 573 (1964).
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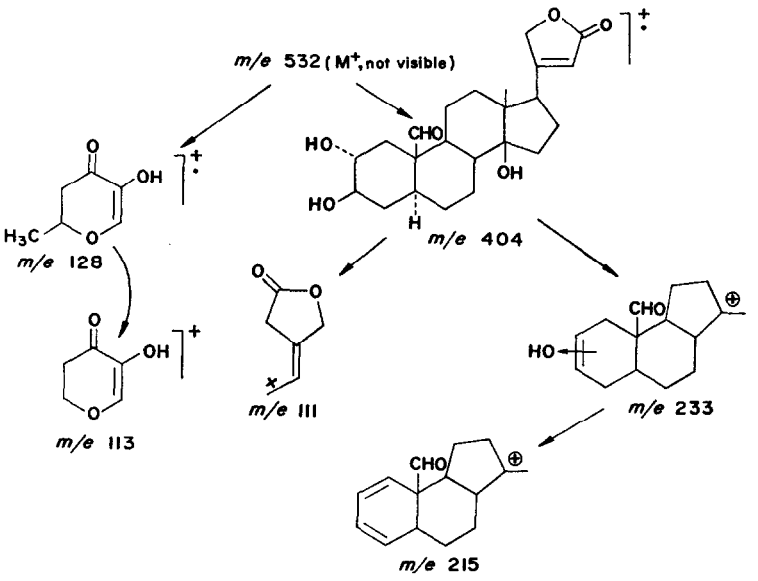


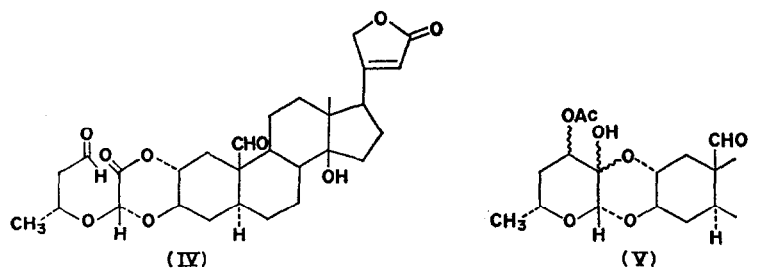
FIG. 1.

rest of the peaks in the spectrum maintained their original positions. The structure of acetylasclepin is discussed below.

Deacetylasclepin (calotropin) on periodate oxidation consumed one mole of periodate as expected from its structure (III). The major product was obtained pure by chromatography on silica gel and elution with $CHCl_3$–EtOAc (1:1). Its NMR showed two aldehyde groups (10·10 ppm a ssinglet and 9·86 ppm as triplet, $J = 2$ c/s). The singlet for —O—CH—O— was now shifted to 5·25 ppm and rest of the peaks maintained their original positions.

Consequently the oxidation product must have the structure (IV) and asclepin would, therefore, be 3′-*O*-acetylcalotropin (V).

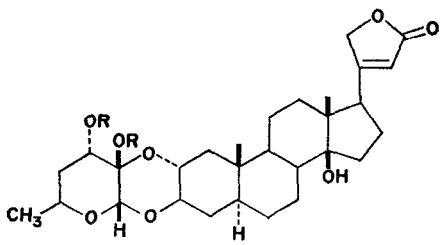
This is the first report of the isolation of this unusual cardenolide as the acetate.



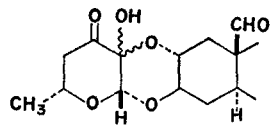
(IV) (V)

The NMR Spectra of Calotropis *glycosides*

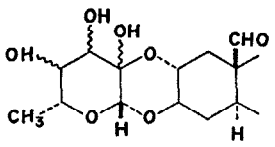
Gomphoside (VI) obtained from *Gomphocarpus fruticosus* by Watson *et al.*[8] has been used by Reichstein *et al.*[7] as the model compound for basing the structures of *Calotropis* cardenolides-calotropin and calactin (III), uscharidin (VII), calotoxin (VIII) and proceroside (X). The NMR assignments of C-1′ and C-3′ protons are summarized in Table 1.



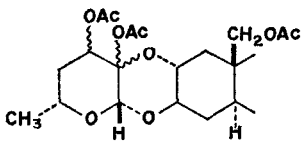
(VI) R = H
(VIa) R = Ac



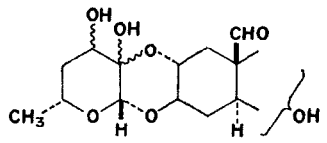
(VII)



(VIII)



(IX)



(X)

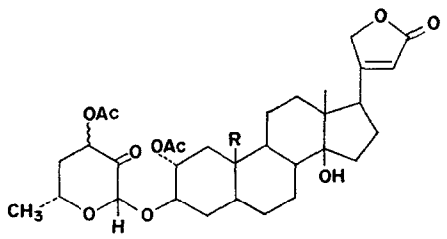
The NMR data of calotropin and its acetyl derivatives show that on monoacetylation the proton on C-3′ bearing secondary hydroxyl shifts 1·00 ppm downfield as expected, but C-1′ H keeps to its position as in calotropin. On the formation of diacetate, the C-3′ H again shifts downfield by 1 ppm along with a simultaneous similar shift of C-1′ H singlet to 5·53 ppm which is also the position assigned to this proton in gomphoside diacetate (VIa). This proton on the basis of the proposed structure, should have remained unaffected on acetylation.

TABLE 1. NMR ASSIGNMENTS OF *Calotropis* CARDENOLIDES

Name of glycoside	Position of C-1′H (ppm)		Position of C-3′H (ppm)		Acetyl group
	Pyridine	$CDCl_3$	Pyridine	$CDCl_3$	
Gomphoside[9] (VI)	5·47	—	3·8–4·0	—	
Gomphoside diacetate (VIa)	5·27	5·68	—	4·8–5·0	2·10(2)
Uscharidin[7] (VII)	5·05	4·58			
Tetrahydrouscharidin triacetate (IX)	—	4·75	—	5·63	2·01(3)
Proceroside[10] (X)	—	4·52	—	—	
Deacetylasclepin (=calotropin) (III)	—	4·59	—	—	
Asclepin (V) (=3′-*O*-acetylcalotropin)	—	4·55	—	4·6–5·1	2·13(1)
Acetylasclepin (XI)	—	5·53	—	5·65–6·0	2·01(1) 2·08(1)

The above observations indicate that during acetylation the ketal ring is opened up to generate a carbonyl at C-2′ and a secondary OH at C-2 which is acetylated. The carbonyl being α to both the affected protons at C-1′ and C-3′ shift them downfield in the region of 5·6 and 4·8 ppm respectively. This seems to be a common feature in the acetylated products of this class having a proton singlet at 5·6 ppm. In the case of tetrahydrouscharidin and its triacetate, the data would be in accordance with the above if the proposed assignments of C-1′ and C-3′ in the triacetate (Table 1) are reversed and this seems to be justifiable as well.

Thus, the structure of acetylasclepin would be (XI), gomphoside diacetate (XII) and β-tetrahydroushcaridin triacetate as (XIII).



(XI) R = CHO
(XII) R = CH_3
(XIII) R = CH_2OAc

EXPERIMENTAL

All m.ps are uncorrected and were taken on a Kofler block. NMR spectra were recorded in $CDCl_3$ with TMS as internal standard. Whatman No. 1 filter paper was used for paper chromatography in benzene–formamide (S_1); benzene-$CHCl_3$ (7:5)/formamide (S_2) and $CHCl_3$/formamide (S_3). Kedde reagent[1] was used as spray reagent.

Isolation of asclepin. From fresh plant material (16 kg), asclepin (= substance B) was isolated according to the procedure already described,[1] in a yield of about 0·011%. It was crystallized from methanol or acetone-hexane (3:1), m.p. 308–309°, R_f 0·22 (S_2). $[\alpha]_D$ +10° (1% in $CHCl_3$). NMR (in $CDCl_3$ immediately after recrystallization with methanol): ppm 0·83 (3H, s, CH_3), 1·30 (3H, d, J = 6 c/s, —O—CH—CH_3), 2·13 (3H, s, $OCOCH_3$), 3·40, 4·20 (3H, m, C–2H, C–3H, C–5′H), 4·55 (1H, S, C–1′-H), 4·60–5·10 (1H, m, C–3′–H and 2H, broad, C–21 H_2), 5·86 (1H, S, olefinic), 9·96 (1H, S, –CHO). (Found: C, 62·79; H, 7·25. $C_{31}H_{42}O_{11}$ requires C, 63·05; H, 7·11%).

Deacetylation of asclepin. Asclepin (50 mg) in MeOH (2 ml) and aqueous $KHCO_3$ (50 mg, 1 ml) was allowed to stand at room temp. The progress of deacetylation was followed by chromatography in (S_2) and (S_3). After 4 days, the reaction mixture was diluted with water, MeOH was removed *in vacuo* and it was then extracted with $CHCl_3$ (5 ml × 3). The $CHCl_3$ layer gave a residue (45 mg) which crystallized from $CHCl_3$-ether as colourless needles (III), m.p. 208–210°, R_f 0·52 (S_3). NMR: ppm 0·86 (3H, S, CH_3), 1·30 (3H, d, J = 6 c/s, —O—CH—CH_3), 3·40–4·20 (4H, m, broad, C–2H, C–3H, C–3′H and C–5′H), 4·59 (1H, S, C–1′H), 4·93 (2H, broad, C–21 H_2), 5·90 (1H, s, olefinic), 9·98 (1H, s, –CHO). On chromatography (S_3) the deacetylated product gave a spot identical with that of calotropin (III). Its identity with calotropin was further confirmed by m.p. 207–209°, superimposable IR and mass spectrum *m/e* 514 (M^+-18), 404, 386, 357, 233 (base peak), 215, 192, 187, 128, 113, 91, 85, 79.

Acetylation of asclepin. Asclepin (50 mg) was acetylated with Ac_2O-pyridine at room temp. (48 hr). The product gave a spot R_f 0·85 (S_1) along with that of asclepin (7 mg). It was chromatographed over neutral alumina and the $CHCl_3$ and $CHCl_3$–EtOAc (1:1) eluates yielded acetylasclepin (XI) as an amorphous powder (25 mg). ν_{max} (KBr): 3410 (broad–OH), 1780, 1738, 1630 (butenolide ring), 1742, 1260 cm^{-1} (acetyl). NMR: ppm 0·83 (3H, S, CH_3), 1·25 (3H, d, J = 6 c/s, –O–CH–CH_3), 2·01, 2·08 (3H each, S, $OCOCH_3$), 3·50, 4·20 (3H, m, broad, C–2H, C–3H, C–5′H), 4·85 (2H, broad, C–21 H_2), 5·53 (1H, S, C–1′H), 5·65–6·00 (1H, s, olefinic and 1H m, C–3′H) and 10·00 (1H, S, –CHO).

Acetylation of calotropin. Calotropin (III, 20 mg) was acetylated with Ac_2O and pyridine overnight at room temp. The product (18 mg) on chromatography. (S_2) showed 3 spots. It was chromatographed over silica gel (2 g) when the major component (10 mg) was obtained from EtOAc eluate and was shown to be identical with asclepin (V) by mixed m.p. 307–310° and co-chromatography, R_f 0·22 (S_2). The least polar spot was found to be acetylasclepin (4 mg) (diacetylcalotropin, XI) prepared above.

Sodium periodate oxidations. (i) *Oxidation of calotropagenin.* Calotropagenin (II, 4·6 mg) was dissolved in $CHCl_3$–EtOH (1:3) (2 ml) and $NaIO_4$ (10 mg) in water (1 ml) was added. After 18 hr at room temp, $NaIO_4$ consumed = 2·75 mg; required for 1 mole = 2·43 mg. The reaction mixture was worked up and the product showed a major spot R_f 0·79 (S_2) along with some streaking.

(ii) *Oxidation of calotropin.* The deacetylated asclepin (III 44·2 mg) was treated with $NaIO_4$ in EtOH–H_2O (1:1) (5 ml) for 18 hr. $NaIO_4$ consumed = 23·5 mg; required for 1 mole = 18·0 mg.

The oxidation product (35 mg) was isolated from the reaction mixture by chromatography on silica gel (2 g). $CHCl_3$ and $CHCl_3$–EtOAc (1:1) eluates yielded an amorphous powder (15 mg, IV), R_f 6·76 (S_3). NMR: ppm 0·83 (3H, S, CH_3), 1·33 (3H, d, J = 6 c/s, –CH–C$\underline{H}_3$), 2·6–2·85 (2H, broad, –CH_2CHO), 5·90 (2H, broad, s, C–21 H_2), 5·25 (1H, S, C–1′H), 5·95 (1H, S, olefinic), 10·10 (1H, S, –CHO) and 9·86 (1H, t, J = 2 c/s, –CH_2–C$\underline{H}$O).

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Key Word Index—Calotropis; *Asclepias curassavica*; Asclepiadaceae; cardenolides; asclepin; NMR spectra.