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CRYPTOSIN, A CARDENOLIDE FROM THE LEAVES OF *CRYPTOLEPIS BUCHANANI*

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Key Word Index—*Cryptolepis buchanani*, Asclepiadaceae; cardenolide, 3β-(D-deoxy glucose-oxy)-14β,11β-dihydroxy-7α,8α-epoxy-12-oxo-5β-card-20 (22)-enolide

Abstract—A new cardenolide named cryptosin was isolated from the leaves of *Cryptolepis buchanani*. By spectral studies and single crystal X-ray crystallography, cryptosin was found to possess a novel structure. The structure thus established was 3β-(D-deoxy glucose-oxy)-14β,11β-dihydroxy-7α,8α-epoxy-12-oxo-5β-card-20 (22)-enolide

INTRODUCTION

Several species of *Asclepiadaceae* are known sources of cytotoxic and cardiac glycosides [1, 2]. Notably, species of *Asclepias*, *Calotropis* and *Cynanchum* were shown to possess steroidal glycosides of the cardenolide type [3–6]. However, the genus *Cryptolepis* has not been investigated for its phytochemical constituents. The genus *Cryptolepis* R. Brown comprises 28 paleotropic species of woody twining shrubs. *Cryptolepis buchanani* Roem and Schult, a woody climbing shrub well adapted to the semi-arid areas throught India has been used in folk medicine [7]. The santals make a preparation from this plant which they give to children to cure them of rickets They also combine it with *Euphorbia microphylla* (Euphorbiaceae) and give to women when the supply of milk is deficient or fails [8]. In our search for cardenolides from other *Asclepiadaceae* members we have examined the leaves of Indian milkweed *C buchanani* and report the isolation spectral and single crystal X-ray crystallographic characterization of a novel cardenolide named cryptosin

RESULTS AND DISCUSSION

Cryptosin was obtained as a crystalline powder from the acetone–methanol elution fraction from silica gel chromatography. The IR absorption spectrum was characteristic of cardiac glycoside. The diagnostic vibrations were 1700 and 1690 cm^{-1} due to a carbonyl of an α,β-unsaturated lactone, 1060 cm^{-1} due to a glycosidic ether and 1020 cm^{-1} due to cyclic ether oxygen The EI mass spectrum showed neither a molecular ion nor characteristic glucose fragments (observed in EIMS spectra of all cardenolides with a normal glycosidic linkage between C-2 of the aglycone and C-1′ of the sugar moiety) but the ion at *m/z* 518 (70%) was most abundant and this fragment lost a ketone $[M-44]^+$ giving an ion at *m/z* 474 (25%). The *m/z* 401 fragment (45%) was characteristic and represents the cryptosin aglycone fragment. The fragment at *m/z* 401 lost a molecule of water $[M-18]^+$ and gave rise to an ion at *m/z* 383 (15%). Another characteristic ion was at *m/z* 113 (50%) typical of a cardenolide [3] A similar assignment of the mass spectral peaks has been reported recently for cardenolides from *Asclepias subulata* [4] and *Nerium oleander* [9].

The ^1H NMR spectrum of cryptosin lent further support to the structural features inferred from the IR and mass spectra. The ^1H NMR spectrum recorded in CD_3COCD_3 was typical of a steroidal glycoside. The steroidal ring protons were at δ 1.28 to 1 83 and the sugar protons were resolved at δ 3 to 4.5. However, the most characteristic was the sharp singlet at δ 3.35 of two protons at C-21 and the singlet at δ 6 of a proton at C-22. Moreover, the presence of a downfield doublet of a doublet ($J = 16$ 1 Hz) due to the proton at C-7 could be attributed to the epoxide. The C-7 proton showed clear cross peaks (COSY spectrum) with a doublet at δ 2 4 to 2.5 ($J = 13$ Hz) assigned to H-6α which means the presence of an α-epoxide at C-7/C-8 The H-6β peak showed cross peaks with ring protons in the region of δ 1.65 to 1 7 ($J = 16$ Hz). The coupling of the H-6α with the C-7 proton leads to its downfield appearance as against the H-6β. The presence of an α-epoxide at C-7/C-8 was confirmed by single crystal X-ray crystallography The multiplet at δ 4.92 ($J = 13.8$ Hz) of three protons corresponding H-1′, H-3 and H-11 showed cross peaks with protons at δ 1.4 ($J = 11$ Hz) corresponding to the ring protons. The proton at C-22 appeared downfield at δ 6.0 and did not show any cross peaks. The methyl singlets at δ 1 12 and 1.22 were assigned at C-18 and C-19, and the sugar was found to be devoid of methyl groups The positions of the hydroxyls were found to be at C-11, C-14, C-3′, C-4′, C-5′ and C-6′ For a detailed assignment of the protons see the experimental section.

The ^{13}C NMR spectrum (Table 1) clearly showed substitution on C-11 (δ 79.4), C-12 (201.07) and C-14 (63.86)

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Table 1 ^{13}C NMR chemical shifts of cryptosin (63 79 MHz CD_3COCD_3) (ppm relative to TMS)

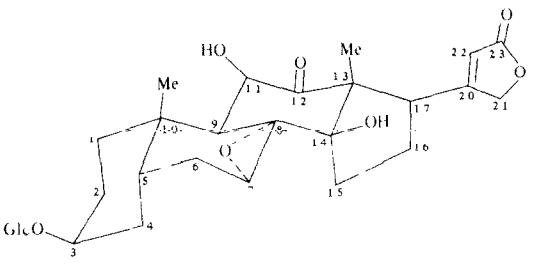
C	δ	C	δ	C	δ
1	23 81	13	57 13	1′	82 25
2	27 64	14	63 86	2′	77 50
3	74 22	15	25 31	3′	71 59
4	27 91	16	25 64	4′	69 04
5	36 17	17	36 93	5′	74 78
6	33 27	18	18 63	6′	33 72
7	52 86	19	18 26		
8	64 55	20	118 79		
9	36 67	21	35 59		
10	43 39	22	96 25		
11	79 39	23	213 69		
12	201 07				

whose positions were downfield with respect to other steroidal carbons The appearance of C-12 at δ 201 indicated the presence of a carbonyl carbon at C-12 which is a unique feature of cryptosin The appearance of an epoxide at C-7/C-8 was revealed by the downfield appearance of C-7 (52 8) and C-8 (64 65) A general examination of the ^{13}C NMR spectrum of cryptosin agreed with earlier proton assignments, particularly the characteristic α, β-unsaturated lactone carbons, C-20 and C-22 appearing downfield at δ 118 and 96 25, respectively The signal for C-21 appeared at 213, while the secondary carbon C-21 was at 35 59 The position of glucose carbons was as expected and the spectrum did not show the presence of methyl groups on sugar, indicating that the glycone is a normal hexose of the deoxy type which was further confirmed by a single crystal X-ray crystallography

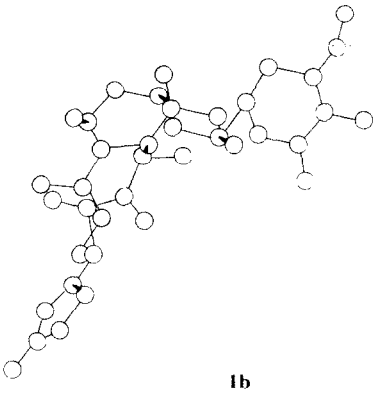
The X-ray analysis was undertaken in view of some uncertainity regarding the nature of oxygens present at C-12 and C-7/C-8 The chemical structure obtained from X-ray analysis (Fig 1a) confirmed our spectral data. Cryptosin contains a hexose sugar (deoxyglucose) commonly found in cardioactive glycosides [10–12] The anomeric $C_{1'}$–O_3 bond [1 418(11) Å] is much shorter than the $C_{1'}$–$O_{5'}$ bond [1 551(14) Å] Conformation about the exocyclic $C_{5'}$–$C_{6'}$ bond is *gauche–trans* with torsion angles $O_{6'}$–C_6–C_5–O_5 of 86 5° and $O_{6'}$–C_6–$C_{5'}$–$O_{4'}$ of −151 4° The bond angle between O_3–$C_{1'}$–$O_{5'}$ is 108 2° as found in β-sugars [13] The pyranose deoxyglucose ring assumes a chair conformation Interior angles of the epoxy group have an average value of 60 0 The presence of hydroxyl groups at C-11 and C-14 and a carbonyl oxygen at C-12 are features of the steroid which are characteristic of cryptosin, as these groups are not present in strophanthidin [14] and digitoxigenin [15] The A/B and C/D ring junctions are *cis*, similar to that found in other cardioactive compounds

Of the two endocyclic C–O bonds of the α,β-unsaturated lactone ring C_{23}–O'_{23} [1 354 (10) Å] is much shorter than C_{21} O'_{23} [1 451(10) Å] The torsion angle C_{13}–C_{17}–C_{20}–C_{21} is 120 9° in contrast to that found in strophanthidin (−110 9°) and digitoxigenin (76 2°).

There is a single water molecule per asymmetric unit Cryptosin packs in a network which contains three distinct hydrogen bonds in the range 2 85 to 3 1 Å The



1a



1b

Fig 1 Chemical structure (**1a**) of cryptosin and the stereo view of the cryptosin molecule (**1b**) viewed down the *b*-axis draw by the programme PLUTO

stereo view of the molecule as seen by X-ray crystallographic study is shown in Fig 1b Fractional co-ordinates of the atoms, bond lengths and bond angles are available from the Cambridge Crystallographic Centre

The structure elucidated for cryptosin by spectroscopic and single crystal X-ray crystallographic methods was 3β-(D-deoxyglucose-oxy)-14β,11β-dihydroxy-7α,8α-epoxy-12-oxo-5β-card-20(22)-enolide While fulfilling the general structural requirements of a cardenolide, the unique structural features of cryptosin are the presence of a carbonyl group at C-12 and a hydroxyl at C-11 The glycone was shown to be a pyranose deoxyglucose in the chair conformation Earlier studies on cardenolides from Asclepiadaceae showed the presence of glucosides and rhamnoglucosides which were either acetylated or methylated sugars [3–5, 9]. Recently, the presence of an epoxide at C-8/C-14 has been reported in kaneroside [9] The presence of epoxide groups in cardenolides has been reported at C-7/C-8 and C-11/C-12 [9, 16] but previously all the epoxides reported in naturally occurring cardenolides have the β-orientation We are reporting for the first time the presence of an α-epoxide at C-7/C-8. The unique structural features of cryptosin pose intriguing questions both on its biosynthesis and its cardioactive properties

EXPERIMENTAL

C buchanani is a large shrub with terate branches widely distributed in the tropics [17] and it is a common weed in India,

Sri Lanka, Burma and China [18]. Leaves, which exude latex [19], were collected from Sept to April Only fully matured leaves were selected for extraction The leaves were washed in running water for 3 hr to remove the latex and adhearing sand and dust and were processed directly

Isolation of cryptosin A detailed description of the isolation of cryptosin has been reported [20] The aq homogenate of the leaves was clarified by filtration, and the filtrate evapd and extracted repeatedly in EtOAc and Me_2CO The Me_2CO extract gave a crystalline residue (yield 0.05%) which upon repeated crystallization in Me_2CO gave colourless crystals (50 to 60% of the residue crystallized) The crystals were found to be pure by chromatographic methods for cardenolides [20, 21], mp 220° Elemental analysis yielded the empirical formula $C_{29}O_{12}H_{42}$ ($C_{,}$ 59.79, $O_{,}$ 32.99; $H_{,}$ 7.22. Found $C_{,}$ 60.76, $O_{,}$ 32.11; $H_{,}$ 7.13%). IR ν_{max}^{Nujol} cm^{-1} 3500 (−OH nonhydrogen bonded), 3350 (−OH hydrogen bonded), 2990, 2910, 1700, 1690 (−C=O lactone) 1630 (−CH conjugated double bonds), 1448, 1380, 1288 (−CH deformation CH_3, CH_2, C–Me, CH) 1150, 1060 (C–O–glycosidic ether), 1020 (–C–O–cyclic ether oxygen) EIMS *m/z* (rel int) 582 $[M]^+$ not observed 518 (70), 474 (25), 445 (30), 401 (45) (cryptosin aglycone), 383 $[401 - H_2O]^+$ (15), 355 $[383 - CHO]^+$ (10), 337 $[355 - H_2O]^+$ (8), 309 $[337 - CO]^+$ (10), 219 $[337 - C_7H_{13}O]^+$ (15), 145 (50), 113 $[337 - C_{15}H_{15}O_3]$, 74 (100) ^{1}H NMR (270 MHz, CD_3COCD_3) δ 1.12 (*s*, 3H, H_3-18), 1.22 (*s*, 3H, H_3-19), 1.28–1.83 (*m*, 13H, H-1, H-2, H-4, H-6, H-15, H_2-17), 2.10–2.15 (*dd*, 2H, J = 16.1 Hz, H_2-6'), 2.17–2.24 (*d*, 1H, J = 13.8 Hz, H-9), 3.35 (*s*, 2H, H_2-21), 3.59–3.65 (*m*, 1H, J = 16.1 Hz, H-2'), 3.98 (*m*, 1H, J = 16.1 Hz, H-4'), 4.01–4.12 (*t*, 1H, J = 16.1 Hz, H-3'), 4.13–4.15 (*d*, 1H, J = 6.9 Hz, H-5'), 4.68–4.73 (*dd*, 1H, J = 16.1 Hz, H-7), 4.92 (*m*, 3H, J = 13.8 Hz, H-1', H-3, H-11) and 6.0 (*s*, 1H, H-22) The hydroxyls were identified C-11, C-14, C-3', C-4', C-5' and C-6' by deuterium exchange

^{13}C NMR (67.89 MHz, CD_3COCD_3) complete assignment is given in Table 1. Spectral assignments were made partly through comparison of chemical shifts with the data published for similar compounds [22]

Crystal data of cryptosin. Cryptosin crystals were grown by straight evapn of an Me_2CO The crystals were orthorhombic with space group $P2_12_12_1$ having the following unit cell dimensions. a = 9.660 (5) Å, b = 11.723 (1) Å and c = 25.626 (3) Å The cell volume was calculated to be 2909 Å^3 The number of molecules in unit cell (z) is 4. The calculated density was 1.33 g/cm^3 Three dimensional intensity data up to a θ limit of 70 were collected on a CAD-4 diffractometer using CuKα radiation in ω-20 scan mode 2273 reflections out of 3162 were considered observed ($|F_o|/3\sigma|F_o|$) and unique Lorentz and polarization corrections were applied to all the reflections Structure was solved by direct methods (MULTAN 80) [23, 24] and refined by block-diogonal matrix least-squares calculations (SFLS) [25] The final R with Cruickshan weighting schme [26] was 0.077 The function minimized was ω $(|F_o|/K - |F_c|)^2$, where $\omega = (a + b|F_o| + c|F_o|^2)$ Scattering factors for non-H atoms were computed from ref. [27] and for H atoms from ref [28]

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