The Structure of Cotyledoside, a Novel Toxic Bufadienolide Glycoside from *Tylecodon wallichii* (Harv.) Toelken

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The structure of cotyledoside, the active toxic principle of *Tylecodon wallichii* (Harv.) Toelken subs. *wallichii* was assigned as 7β,8-epoxy-14-hydroxy-2α,3β-(tetrahydro-3,5-dihydroxy-4-methoxy-6-methyl-2*H*-pyran-2,4-diyldioxy)-5α-bufa-20,22-dienolide. The structure is based on a detailed study of the high-field ^1H and ^{13}C n.m.r. spectra of cotyledoside. The relative configuration of the metabolite was deduced from the observed proton–proton nuclear Overhauser effects and the magnitude of the proton–proton coupling constants.

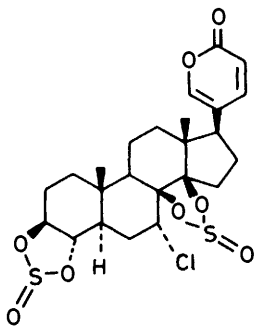
Members of the genera *Cotyledon*, *Tylecodon*, and *Kalanchoe* (Crassulacea) cause annual stock losses in South Africa through cotyledonosis, an intoxication affecting the nervous and muscular systems.[1] The active principle of *Tylecodon wallichii* (Harv.) Toelken subs. *wallichii*,[2] called cotyledoside, was first isolated by van Rooyen and Pieterse.[3] van Wyk[4] studied the structure of cotyledoside, $C_{31}H_{42}O_{10}$, and observed the formation of a chlorohydrin aglycone upon treatment of the substance with hydrogen chloride in dry chloroform. This compound was subsequently treated with thionyl chloride in pyridine to produce the disulphite (**1**). Structure (**2**) was proposed for the aglycone of cotyledoside.

We now report a revised structure for cotyledoside, including the nature, stereochemistry, and mode of attachment of the carbohydrate moiety to the aglycone. The structure of cotyledoside is based mainly on the analysis of the high-field ^1H and ^{13}C n.m.r. spectra. Extensive ^1H-{^1H} homonuclear decoupling experiments enabled us to assign the ^1H n.m.r. spectrum. The ^{13}C n.m.r. spectrum was assigned on the basis of broad-band proton-decoupled and single-frequency off-resonance proton-decoupled experiments as well as the reported ^{13}C n.m.r. chemical shifts of related compounds. The residual splittings observed in a series of off-resonance proton-decoupled ^{13}C n.m.r. experiments enabled us to correlate the signals of the proton-bearing carbon atoms with specific carbon resonances.[5] The ^1H and ^{13}C n.m.r. data of cotyledoside are collated in Tables 1 and 2, respectively.

Well-resolved signals at δ_H 7.399 (dd, *J* 2.7 and 0.9 Hz, 21-H), 7.848 (dd, *J* 9.8 and 2.6 Hz, 22-H), and 6.171 (dd, *J* 9.8 and 0.8 Hz, 23-H) were assigned to the protons constituting the pyrone ring. The presence of the epoxide moiety in cotyledoside was evident from the proton resonance, δ_H 3.300 (d, *J* 5.5 Hz), and the carbon resonances, δ_C 63.28 (s) and 51.34 p.p.m. [d, ^1J(CH) 175 Hz] which are characteristic of 7,8-epoxy steroids.[6] The observed directly bonded (C,H) coupling constant is characteristic of proton-bearing oxirane carbon atoms.[7]

An analysis of the splitting patterns of the protons belonging to the A-ring of cotyledoside prompted us to modify the proposed 3,4-diol arrangement to the 2α,3β-diol. Extensive proton–proton decoupling experiments enabled us to differentiate between 2-H (δ_H 4.963) and 3-H (δ_H 3.842). The 2-proton is coupled to two geminal protons [δ_H 1.985 (dd, *J* 12.8 and 4.8 Hz, 1-H$_\beta$) and δ_H 0.983 (dd, *J* 12.6 and 11.6 Hz, 1-H$_\alpha$)] which are not further coupled and can therefore be assigned as indicated.

The assignment of 2-H and 1-H$_\beta$ was confirmed by the observation of nuclear Overhauser effects (n.O.e.s) between these protons and the 19-methyl protons (δ_H 0.891).



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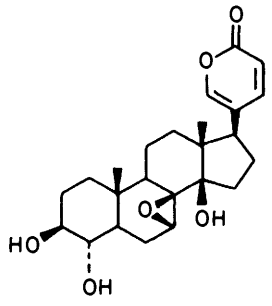


(2)

Table 1. ^1H N.m.r. (500 MHz) data for cotyledoside (**8**) and 2′,4′-di-*O*-acetylcotyledoside (**10**) (*J* values in Hz)

Proton	δ_H/p.p.m.[a] (**8**)	δ_H/p.p.m.[b] (**10**)
1α	0.983 (*J* 12.6, 11.6)	0.790 (*J* 12.7, 11.4)
1β	1.985 (*J* 12.7, 4.8)	2.028 (*J* 12.8, 4.8)
2β	4.963 (*J* 11.5, 8.6, 4.7)	4.668 (*J* 11.3, 8.9, 4.8)
3α	3.842 (*J* 10.6, 8.9, 5.3)	3.886 (*J* 10.6, 8.9, 5.3)
7	3.300 (*J* 5.6)	3.296 (*J* 6.0)
17	2.647 (*J* 9.4, 6.7)	2.514 (*J* 9.2, 7.0)
18	0.765	0.732
19	0.891	0.924
21	7.399 (*J* 2.7, 0.9)	7.194 (*J* 2.7, 0.9)
22	7.848 (*J* 9.8, 2.6)	7.725 (*J* 9.8, 2.7)
23	6.171 (*J* 9.8, 0.8)	6.241 (*J* 9.8, 0.9)
1′	5.023 (*J* 4.7)	5.477 (*J* 4.7)
2′	4.330 (*J* 4.7, 4.4)	5.278 (*J* 4.8)
4′	3.597 (*J* 4.4, 1.9)	5.104 (*J* 1.9)
5′	4.555 (*J* 6.4, 6.4, 6.4, 1.7)	4.699 (*J* 6.4, 6.4, 6.4, 1.9)
6′	1.151 (*J* 6.4)	1.109 (*J* 6.4)
OMe	3.268	3.181
2′-OH	4.204 (*J* 4.4)	
4′-OH	3.870 (*J* 4.4)	
OAc		2.151
		2.139

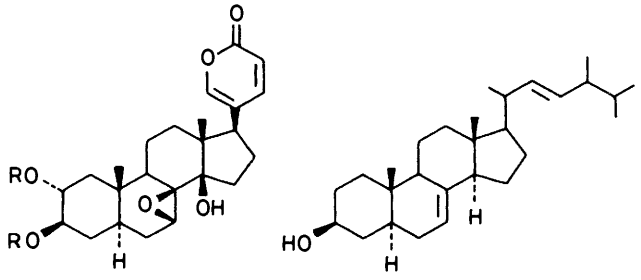
[a] For solutions in [$^2H_6$]acetone. [b] For solutions in $CDCl_3$.

Differentiation between the 18-methyl group (δ_H 0.765) and the 19-methyl group (δ_H 0.891) was based on the n.O.e. observed between δ_H 0.765 (18-H) and the pyrone protons.

Table 2. ^{13}C N.m.r. (125 MHz) data for cotyledoside (**8**) and 2′,4′-di-*O*-acetylcotyledoside (**10**)

Carbon	δ_C/p.p.m. (**8**)[a]	(**10**)[b]
1	44.68	44.97
2	72.19	73.71[c]
3	67.91	70.35[c]
4	34.38[c]	34.23[d]
5	37.74	38.67
6	34.13[c]	34.17[d]
7	51.34	52.42
8	63.28	63.36
9	44.68	45.77
10	34.99	35.82
11	20.47	20.68
12	40.16	41.05
13	50.09	50.56
14	80.00	80.44
15	29.73	30.07
16	26.98	27.23
17	49.84	51.05
18	17.40	17.19
19	13.71	13.82
20	122.11	122.04
21	149.32	148.41
22	147.02	146.24
23	114.26	115.48
24	161.20	162.02
1′	96.88	94.46
2′	80.21	80.38
3′	98.42	97.14
4′	69.95	71.12[c]
5′	71.73	71.55[c]
6′	17.51	17.50
Me	47.15	48.87
OCOMe		169.91, 169.38
OCO*Me*		21.11, 20.85

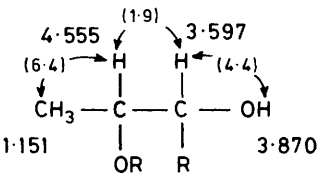
[a] In $(CD_3)_2SO$. [b] In $CDCl_3$. [c,d] Assignments in each column may be interchanged.



(3) (4)

The ^{13}C n.m.r. data (Table 2) are in agreement with the proposed structure (**3**) for the aglycone of cotyledoside and define the stereochemistry at C-5, C-7, and C-8. The chemical shifts of C-9 and C-19 in steroids are strongly influenced by the stereochemistry at C-5.[7] The observed shifts for C-9 (δ_C 44.68 p.p.m.) and C-19 (δ_C 13.71 p.p.m.) established the stereochemistry at C-5 as α and thereby the *trans* AB-ring junction.

According to Tori and co-workers[8] the configuration of an epoxide in a steroid may be determined by comparison of the ^{13}C n.m.r. data with those of the corresponding unsaturated steroid. A comparison of the chemical shift of C-5 of



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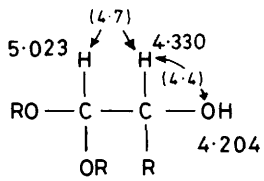

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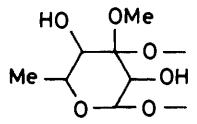

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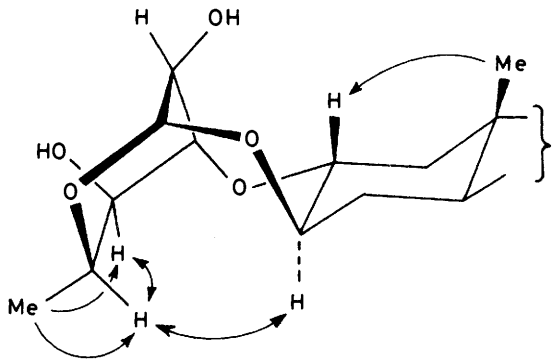


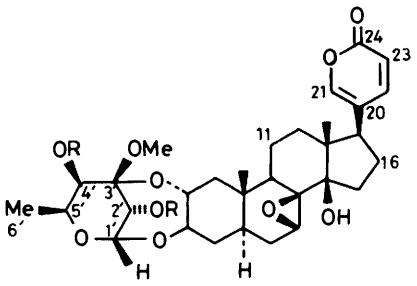
Figure. The n.O.e. connectivity pattern observed for cotyledoside

cotyledoside (δ_C 37.74 p.p.m.) with that of ergosta-7,22-dien-3β-ol (**4**) (δ_C 40.20 p.p.m.) indicated a *trans* relationship between 5-H_α and the 7,8-epoxide group. The β-configuration of the 7,8-epoxide is also evident from the formation of the 8,14-sulphite of the chlorohydrin aglycone of cotyledoside reported by van Wyk.[4]

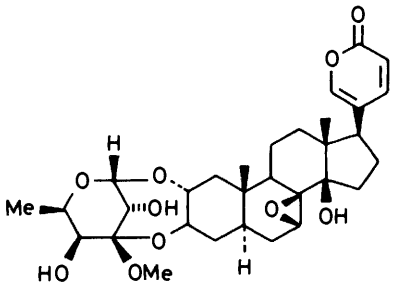
Cotyledoside forms a diacetate $C_{35}H_{46}O_{12}$ upon acetylation and that indicated the presence of two secondary hydroxy groups in the carbohydrate moiety. Substantial evidence was gained from the ^1H n.m.r. spectral data on the nature of the carbohydrate and its attachment to the aglycone. Two highly oxygenated fragments, (**5**) and (**6**), could be constituted. The ^1H and ^{13}C n.m.r. chemical shift data of the methoxy group (δ_H 3.26, δ_C 47.15 p.p.m.) are similar to those observed for the methyl *ortho*-ether of austalide D[9] (δ_H 3.40, δ_C 48.43 p.p.m.) and indicated its location on an oxygen-bearing sp^3 carbon atom bearing at least two oxygen groupings. The foregoing structural requirements are satisfied by a 6-deoxy sugar moiety (**7**), which is linked at C-1′ and C-3′ to the bufadienolide aglycone.

The attachment of two oxygen atoms to both C-1′ and C-3′ is evident from the ^{13}C n.m.r. chemical shifts [δ_C 96.88 (d) and 98.42 p.p.m. (s), respectively], whereas the rather low-field chemical shift of C-2′ (δ_C 80.21 p.p.m.) is in agreement with the proximity of the four α-oxygen substituents.

Two possible modes of attachment of the carbohydrate moiety to the aglycone are possible. C-1′ and C-3′ may be attached to O-3 and O-2, respectively, of the aglycone or *vice versa*, as is indicated in structures (**8**) and (**9**). It is not possible to distinguish between these two structures by n.m.r. methods, but we assigned structure (**8**) to cotyledoside as in all the known bufadienolide and cardenolide glycosides the carbohydrate moieties are attached to C-3.



(8) R = H
(10) R = Ac



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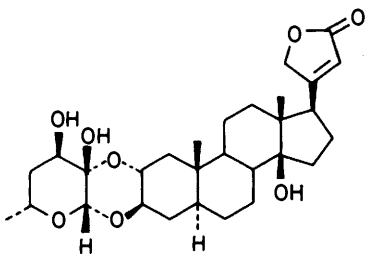

(11)

The assignment of the stereochemistry of the carbohydrate moiety in structure (**8**) results from the measurement of n.O.e.s. The n.O.e. connectivity pattern is shown in the Figure. The ring fusion of the six- and seven-membered bridged rings must be *cis* through necessity. An appreciable n.O.e. observed between 5′-H and 3-H defines the stereochemistry at C-1′, C-3′, and C-5′ as β. The *cis* relationship between 4′-H and 5′-H followed from an observed n.O.e. between these two protons as well as a coupling constant of 1.9 Hz between them. No n.O.e. is observed between 2-H and 2′-H which suggested that the hydroxy group is attached at the 2α-position. This supposition is corroborated by the ^{1}H n.m.r. data of di-*O*-acetylcotyledoside (**10**) (Table 1). The only aglycone proton which exhibited a chemical shift difference upon acetylation of cotyledoside was 2-H, which indicated the presence of an acetate group nearby.

A number of cardenolide glycosides[6,10] are known in which the carbohydrate group is doubly linked to the aglycone through the 2α- and 3β-hydroxy groups, *e.g.* gomphoside[10] (**11**). Cotyledoside (**8**) represents the first example of a bufadienolide with a doubly linked sugar. The mode of attachment of cotyledoside (**8**), however differs from that of the corresponding cardenolides in that the carbohydrate moiety is linked at C-1′ and C-3′ to the aglycone in contrast to attachment at C-1′ and C-2′ as in the cardenolides. The difference in the mode of attachment is also clearly reflected by the ^{13}C n.m.r. chemical shifts of the two acetal carbon atoms. In gomphoside[10] (**11**), C-1′ and C-2′ resonate at δ_C 93.9 and 90.1 p.p.m., respectively, whereas C-1′ and C-3′ of cotyledoside (**8**) resonate δ_C 96.88 and 98.42 p.p.m., respectively.

A recent report[11] on the clinical effects of cotyledoside demonstrated that the administration of relatively large doses of cotyledoside produced typical cardiac glycoside intoxication, whereas the paralytic syndrome, cotyledonosis, occurred only after the administration of repeated small doses.

Experimental

Cotyledoside (**8**) was isolated from *Tylecodon wallichii* (Harv.) Toelken as described in the literature.[3,4] ^{1}H (500 MHz) and ^{13}C (125 MHz) N.m.r. spectra were recorded on a Bruker WM-500 spectrometer.

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