Structure Elucidation and Absolute Configuration of the Tyledosides, Bufadienolide Glycosides from *Tylecodon grandiflorus*

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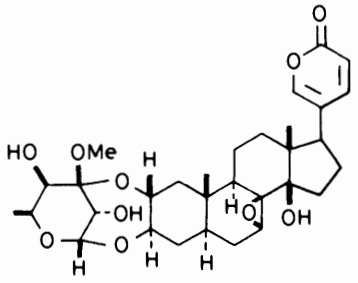
The isolation and characterization of six related bufadienolide glycosides, tyledosides A, B, C, D, F, and G from *Tylecodon grandiflorus* (Burm. F) Toelken are reported. The structure elucidation of these metabolites is based on a detailed study of their highfield ^1H and ^{13}C n.m.r. spectra. Extensive use was made of two-dimensional homonuclear and heteronuclear correlation experiments in the assignment of the n.m.r. spectra. The results obtained upon chemical derivatization of tyledosides A, C, and D are in agreement with the assigned structures.

The poisoning of animals by plants of the Crassulaceae, Iridaceae, Liliaceae, and Melianthaceae families is of considerable economic importance to agriculture in Southern Africa. The toxicity of these plants is frequently associated with the presence of bufadienolides.[1-5] Members of the genera *Cotyledon*, *Tylecodon*, and *Kalanchoe* (Crassulaceae) are associated with cotyledonosis or 'krimpsiekte', an intoxication which differs from the typical cardiac glycoside intoxication and affects the nervous and muscular systems of animals.[6,7] These succulents grow in the more arid regions and are usually consumed during periods of drought and shortage of feed. Earlier investigations into the toxic principles of such plants resulted in the isolation and structure elucidation of cotyledoside (**1**) from *Tylecodon wallichii*[1] and lanceotoxin A and B from *Kalanchoe lanceolata*.[2] We now present our results on a study of the toxins from *Tylecodon grandiflorus* (Burm. F) Toelken.

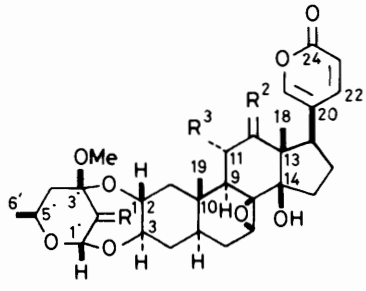


(1)

Extraction of fresh *T. grandiflorus* plants with ethyl acetate, followed by solvent-solvent partitioning of the crude extract and subsequent column chromatography on silica gel led to the isolation of seven novel bufadienolide glycosides, called tyledosides A–G.†

The structures of the tyledosides are based mainly on a detailed study of their highfield ^1H and ^{13}C n.m.r. spectra. The resonances in the 500 MHz ^1H n.m.r. spectra exhibited extensive fine structure. First-order analysis of these multiplets yielded the values of the proton chemical shifts and proton-proton coupling constants (see Table 1 for the ^1H n.m.r. data of the carbohydrate protons, and Experimental section). From the values of the coupling constants and by application of two-dimensional homonuclear ($^1H,^1H$) correlation spectroscopy[9,10] using the COSY-45 sequence, the proton connectivity pattern of the tyledosides could be constituted. The ^{13}C n.m.r. data for the tyledosides, as collated in Table 2, were obtained from broad-band proton-decoupled and single frequency nuclear Overhauser effect (n.O.e.) spectra. The multiplicities of the different ^{13}C resonances were determined by generating the proton-decoupled CH, CH_2, and CH_3 subspectra using the DEPT sequence.[11] The signals of all the proton-bearing carbon atoms were correlated in turn with specific proton resonances in two-dimensional ($^{13}C,^1H$) chemical shift correlation experiments.[9,12] In the assignment of the different ^{13}C n.m.r. resonances use was made of the two- and three-bond (C,H) connectivity pattern as determined by heteronuclear $^{13}C\{^1H\}$ selective population inversion (SPI)[13] experiments and the reported ^{13}C chemical shifts and (C,H) coupling constants of related compounds.[14-17]

Tyledoside A (**2**) crystallized from acetone–hexane as colourless prisms, m.p. 263 °C. Mass spectrometry (M^+ 586) established the molecular formula as $C_{31}H_{38}O_{11}$. In steroids the ^{13}C chemical shift of C-19 is strongly influenced by the stereochemistry at C-5,[18] and the observed value for C-19 (δ_C 13.37 p.p.m.) establishes the 5α stereochemistry and thus the *trans* AB ring junction. An evaluation of the coupling constants and ^1H chemical shift values of the protons of the C(1)–C(7)



	R¹	R²	R³
Tyledoside A (**2**)	O	O	OH
Tyledoside B (**9**)	O	H,H	OH
Tyledoside D (**7**)	β-OH,H	O	OH
Tyledoside F (**12**)	β-OH,H	H,H	OH
Tyledoside G (**13**)	β-OH,H	β-OH,H	H
(**10**)	β-OAc,H	O	OAc

† These compounds were referred to as tyl A—F in a preliminary toxicological report (ref. 8).

Table 1. ^{1}H N.m.r. data of the carbohydrate protons of tyledosides A (**2**), B (**9**), C (**14**), D (**7**), F (**12**), and G (**13**)

Proton	(2)[a]	(9)[b]	(14)[a]	(7)[a]	(12)[a]	(13)[a]
2β	3.530 ddd	3.649 ddd	3.897 ddd	4.192 ddd	3.994 ddd	4.047 ddd
	J 11.0, 8.3, 4.8	*J* 11.2, 8.4, 4.9	*J* 12.2, 10.0, 4.1	*J* 11.3, 8.7, 4.6	*J* 11.0, 9.2, 4.7	*J* 11.0, 8.6, 4.8
3α	3.997 ddd	4.039 ddd	3.719 ddd	3.576 ddd	3.583 ddd	3.607 ddd
	J 11.0, 8.2, 5.5	*J* 11.2, 8.4, 5.6	*J* 11.8, 9.9, 4.6	*J* 11.0, 8.7, 5.1	*J* 10.7, 9.2, 5.2	*J* 10.9, 8.6, 5.1
1′	5.071 s	5.033 s	4.417 s	4.914 d	4.912 d	4.922 d
				J 0.9	*J* 1.1	*J* 1.1
2′	—	—	—	4.132 br d	4.118 br d	4.131 br d
				J 4.6	*J* 4.4	*J* 4.8
2′-OH	—	—	6.223 s	5.512 d	5.145 d	4.727 d
				J 4.6	*J* 4.4	*J* 5.0
3′	—	—	4.846 dd	—	—	—
			J 12.0, 4.8			
4′α	2.288 dd	2.269 dd	1.426 ddd	1.376 ddd	1.375 ddd	1.409 ddd
	J 14.1, 2.5	*J* 13.8, 2.3	*J* 12.0, 12.0, 11.4	*J* 12.6, 2.8, 1.7	*J* 12.7, 2.8, 1.7	*J* 12.7, 2.8, 1.7
4′β	1.763 dd	1.769 dd	1.652 ddd	1.689 dd	1.717 dd	1.701 dd
	J 14.1, 10.6	*J* 14.0, 10.8	*J* 12.0, 4.7, 2.0	*J* 12.6, 11.3	*J* 12.7, 11.3	*J* 12.7, 11.3
5′	4.629 qdd	4.718 qdd	3.665 qdd	4.19 qdd	4.215 qdd	4.215 qdd
	J 6.1, 10.6, 2.3	*J* 6.1, 10.9, 2.6	*J* 6.1, 11.5, 1.8	*J* 6.1, 11.3, 2.8	*J* 6.3, 11.4, 3.0	*J* 6.3, 1.2, 2.8
6′	1.152 d	1.186 d	1.125 d	1.085 d	1.093 d	1.097 d
	J 6.1	*J* 6.1	*J* 6.1	*J* 6.1	*J* 6.3	*J* 6.3
OMe	3.276 s	3.339 s	—	3.312 s	3.123 s	3.148 s
OAc	—	—	2.017 s			

[a] δ_H/p.p.m. for solutions in [$^2H_6$]DMSO. [b] δ_H/p.p.m. for solutions in [$^2H_6$]acetone

Table 2. ^{13}C N.m.r. data (for [$^2H_6$]DMSO solutions) of tyledosides A (**2**), B (**9**), C (**14**), D (**7**), F (**12**), and G (**13**)

	(2)		(9)	(14)	(7)	(12)	(13)
Carbon	δ_C/p.p.m.	^1J(CH/Hz)	δ_C/p.p.m.	δ_C/p.p.m.	δ_C/p.p.m.	δ_C/p.p.m.	δ_C/p.p.m.
1	44.97 t	130.0	45.58 t	43.44 t	45.51 t	44.14 t	44.45 t
2	78.75 d	145.2	78.99 d	67.16 d	72.36 d	72.62 d	72.27 d
3	79.47 d	145.6	79.62 d	71.09 d	78.55 d	78.78 d	79.00 d
4	33.53 t	129.3	33.55 t	31.38 t	33.65 t	33.70 t	33.67 t
5	38.28 d	125.5	37.98 d	39.35 d	38.55 d	38.31 d	37.88 d
6	26.23 t	127.3	26.66 t	26.54 t	26.28 t	26.77 t	26.87 t
7	52.48 d	177.1	51.00 d	52.71 d	52.49 d	51.11 d	51.29 d
8	62.37 s		63.52 s	62.44 s	62.30 s	63.55 s	64.35 s
9	46.52 d	125.6	49.78 d	46.84 d	46.73 d	49.77 d	41.43 d
10	36.27 s		35.82 s	36.89 s	36.10 s	35.64 s	34.93 s
11	73.35 d	144.7	67.01 d	73.55 d	73.29 d	67.05 d	29.75 t
12	211.87 s		50.60 t	211.94 s	208.53 s	50.06 t	74.31 d
13	63.14 s		50.06 s	63.10 s	62.98 s	50.64 s	55.90 s
14	80.12 s		79.70 s	80.15 s	80.05 s	79.70 s	79.99 s
15	35.41 t	132.8	34.57 t	35.39 t	35.35 t	34.58 t	34.43 t
16	28.92 t	128.9	29.55 t	28.92 t	28.77 t	29.50 t	28.99 t
17	41.08 d	130.7	49.78 d	41.18 d	41.15 d	50.00 d	45.74 d
18	18.53 q	126.9	18.68 q	18.45 q	18.38 q	18.66 q	10.57 q
19	13.37 q	125.0	12.74 q	13.21 q	12.47 q	12.92 q	13.66 q
20	120.07 s		121.69 s	120.07 s	119.93 s	121.67 s	122.22 s
21	150.10 d	199.6	149.44 d	150.08 d	149.98 d	149.39 d	149.32 d
22	146.06 d	163.3	146.86 d	146.67 d	146.97 d	146.84 d	147.14 d
23	114.68 d	171.1	114.22 d	114.65 d	114.51 d	114.17 d	114.17 d
24	160.95 s		161.13 s	160.95 s	160.77 s	161.15 s	161.10 s
1′	98.42 d	177.3	98.40 d	95.28 d	99.25 d	99.35 d	99.34 d
2′	198.24 s		198.35 s	90.17 s	64.32 d	64.27 d	62.70 d
3′	99.62 s		99.59 s	73.37 d	99.30 s	99.29 s	99.34 s
4′	46.72 t	130.7	46.78 t	35.25 t	37.10 t	37.23 t	37.05 t
5′	69.90 d	147.0	69.85 d	66.72 d	69.80 d	69.86 d	69.93 d
6′	20.65 q	129.2	20.68 q	20.71 q	21.57 q	21.68 q	21.67 q
OMe	49.52 q	142.7	49.50 q	—	46.89 q	46.92 q	46.92 q
$OCOCH_3$	—		—	170.06 s	—	—	—
$OCOCH_3$	—		—	21.07 q	—	—	—

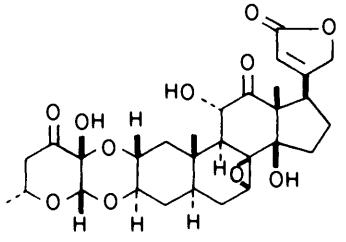
fragment in conjunction with the known stereochemistry at C-5, located the oxygen substituents at the 2α and 3β positions of the aglycone. The substitution pattern and stereochemistry of the A-ring is similar to that of closely related cardenolides[14–17] and the bufadienolide, cotyledoside (**1**).[1]

The presence of a 7β,8β-oxirane was inferred from the ^{13}C n.m.r. data (see Table 2). The value of 177.1 Hz for ^1J(CH) observed for the resonance centred at δ_C 52.48 p.p.m., and which has been correlated with the resonance at δ_H 3.380 p.p.m. (7-H), is diagnostic for a proton-bearing oxirane carbon atom.[19]

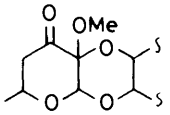
The coupling constant of 12.5 Hz for 11-H (δ_H 4.629 p.p.m.) and 9-H (δ_H 1.843 p.p.m.) points to a *trans* configuration for these protons and establishes the 11α configuration for the C-11 hydroxy group. This configuration at C-11 is supported by the pronounced deshielding effect experienced by the C-1β proton (δ_H 2.866 p.p.m.) as a result of the presence of a 11α-hydroxy group. The corresponding proton in cotyledoside (**1**) resonates at 1.985 p.p.m.[1]

The linkage between the different (^{1}H,^{1}H) spin-spin systems, as determined by COSY experiments (see earlier), followed from selective population inversion (SPI)[13] experiments. Selective irradiation of the C-18 proton transition in a SPI experiment proved that 18-H (δ_H 0.815 p.p.m.) is coupled to those carbon atoms two- and three-bonds removed which resonate at δ_C 211.87 (C-12), 63.14 (C-13), 80.12 (C-14), and 41.08 (C-17) p.p.m.

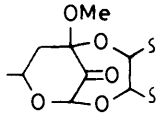
The ^{13}C n.m.r. assignments for the carbon atoms (C-1 to C-19) of the aglycone of tyledoside A (**2**) are in agreement with those reported for the carbon atoms of the related cardenolide labriformidin (**3**).[15] Cheung *et al.*[15] assigned C-6, C-15, and C-16 to signals at δ_C 35.9, 28.4, and 26.7 p.p.m. Our assignments, however, show unambiguously that C-6, C-15, and C-16 in labriformidin should be assigned to the signals at δ_C 26.7, 35.9, and 28.4 p.p.m., respectively.



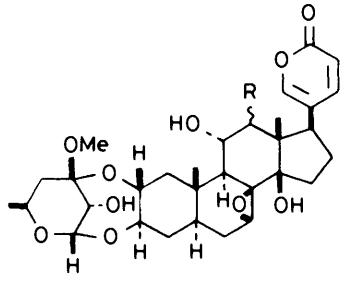
(**3**)



(**4a**)



(**4b**)



(**5**) R = α-OH

(**6**) R = β-OH

Consideration of the molecular formulae and a comparison of the n.m.r. data of tyledoside A (**2**) with those of cotyledoside (**1**)[1] and labriformidin (**3**)[15] suggested the presence of a carbohydrate moiety doubly-linked to the aglycone *via* acetal and hemiacetal bonds. Evaluation of the ^{1}H and ^{13}C n.m.r. data defines the carbohydrate as a 4,6-dideoxyhexose containing both a carbonyl (δ_C 198.24 p.p.m.) and a methoxy group (δ_C 49.52, δ_H 3.276 p.p.m.). Two part structures, (**4a**) and (**4b**), which meet all the above requirements, can be constructed. The chemical shifts of the acetal and hemiacetal carbon atoms (δ_C 98.42 and 99.62 p.p.m., respectively) correspond with those reported for the corresponding carbon atoms of cotyledoside (**1**)[1] (δ_C 96.88 and 98.42 p.p.m., respectively) rather than with those reported for labriformidin (**3**)[15] (δ_C 97.2 and 91.1 p.p.m., respectively) and structure (**4b**) is therefore favoured.

The products obtained from the reaction of tyledoside A (**2**) with sodium borohydride enabled us to differentiate unambiguously between the part structures (**4a**) and (**4b**). No (^{1}H,^{1}H) coupling should be observed between the C-1′ and the newly formed methine proton in the ^{1}H n.m.r. spectrum of the reduction product of (**4a**), whereas reduction of the carbonyl group in (**4b**) should lead to a compound which exhibits a three-bond coupling between 1′-H and the methine proton. In the event two products were obtained upon sodium borohydride reduction of tyledoside A (**2**) and identified as the 2′α,12α- (**5**) and 2′α,12β-dihydroxy (**6**) derivatives. The appearance of 1′-H as a doublet in both (**5**) and (**6**) [δ_H 5.257 (*J* 4.6 Hz) and 5.255 p.p.m. (*J* 4.6 Hz), respectively] unambiguously established the structure of the carbohydrate moiety of tyledoside A as (**4b**). The configuration at C-2′ in (**5**) and (**6**) is defined by the magnitude of the coupling constant (*J* 4.6 Hz) observed for the C-1′ and C-2′ protons which is in agreement with a 2′α hydroxy group [*cf.* cotyledoside (**1**), *J* 4.7 Hz][1] rather than a 2′β hydroxy substituent as in tyledoside D (**7**) (*J* 1.1 Hz) (see later). The stereochemistry at C-12 was determined by the magnitude of the coupling constant between 11-H and 12-H.

The relative and absolute configuration of tyledoside A (**2**) was deduced from the proton-proton coupling constants as well as the proton-proton n.O.e.s. The ring fusion of six- and seven-membered bridged rings as in (**4b**) is through necessity *cis*. Irradiation of the 3α-H resonance (δ_H 3.872 p.p.m.) in an n.O.e. experiment, followed by measurement of the resultant n.O.e.s in the difference mode resulted in an n.O.e. enhancement of the 5′-H resonance (δ_H 4.562 p.p.m.). Both these protons must therefore be on the α-side of the molecule. In order to place 5′-H and 3α-H in close proximity, the C-1′ proton and C-3′ methoxy group must both be β-orientated, as indicated in structure (**2**). Similarly, an n.O.e. was observed for 3-H upon irradiation of the C-5′ proton. The n.O.e. connectivity pattern for the carbohydrate moiety of tyledoside A (**2**) is shown in the Figure. The stereochemical assignments for tyledoside A (**2**) were corroborated by the chemical shift of the C-2 proton in compounds (**5**) (δ_H 4.784 p.p.m.) and (**6**) (δ_H 4.789 p.p.m.). The deshielding effect (Δδ 1.25 p.p.m.) experienced by 2-H is ascribed to the oxygen atom of the C-2′ hydroxy group and is only possible if the stereochemistry at both C-1′ and C-3′ is β.

Structure (**8**) was considered as an alternative for tyledoside A but this possibility is discounted on biogenetic grounds, as glycosidation occurs at the C-3 hydroxy group in all known bufadienolide and cardenolide glycosides.

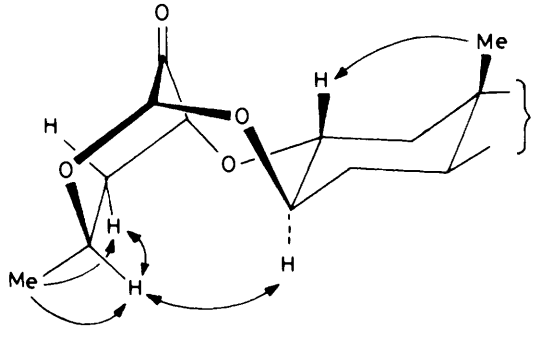
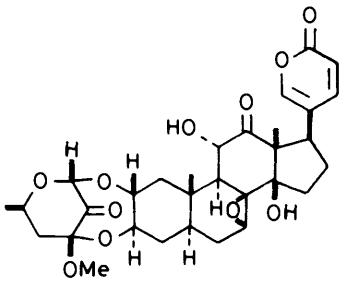


Figure. The n.O.e. connectivity pattern observed for tyledoside A.



(8)

Tyledoside B (**9**), $C_{31}H_{40}O_{10}$, contains one oxygen atom less than tyledoside A. A comparison of the ^1H and ^{13}C n.m.r. data of tyledoside A and B revealed that both compounds share a common carbohydrate moiety but that the steroid aglycone in tyledoside B lacks the 12-keto function as indicated by the absence of the resonance present at δ_C 211.87 p.p.m. in tyledoside A and the presence of a resonance at δ_C 50.60 p.p.m. in tyledoside B. The change of the C-12 keto function to a methylene group has a distinct influence on the ^{13}C chemical shifts of the C-11 ($\Delta\delta$ 6.34 p.p.m.), C-13 ($\Delta\delta$ 13.08 p.p.m.), and C-17 ($\Delta\delta$ −8.70 p.p.m.) resonances.

Tyledoside D (**7**) analyses for $C_{31}H_{40}O_{11}$ and differs in molecular weight by 2 mass units from tyledoside A (**2**). This difference corresponds to the reduction of the C-2′ carbonyl group present in tyledoside A, as the resonance assigned to C-2′ (δ_C 198.24 p.p.m.) in the proton-decoupled ^{13}C n.m.r. spectrum of (**2**) is absent in that of tyledoside D (**7**) and replaced by a resonance at δ_C 64.32 p.p.m. (see Table 2). Acetylation of (**7**) results in the formation of a diacetate derivate (**10**).

The small vicinal proton-proton coupling of 1.1 Hz observed for the C-1′ and C-2′ protons in the ^1H n.m.r. spectrum of tyledoside D corresponds to a dihedral angle of close to 90°, an arrangement possible only when the C-2′ hydroxy group has the β-configuration. This configuration would also explain the observed proton-proton coupling of 1.7 Hz over four bonds along an approximately planar W-path,[20] between 2′-H and the equatorial proton at C-4′. The proton n.O.e. connectivity pattern obtained for tyledoside D is similar to that observed for tyledoside A (see Table 3), indicating that the stereochemistry at C-1′, 3′, and 5′ of tyledosides A and D must be identical.

Treatment of tyledoside D (**7**) with 0.1M-hydrochloric acid resulted in the formation of the chlorohydrin derivative of the aglycone (**11**).

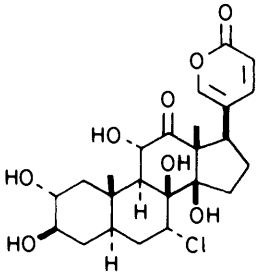
The compound previously described as tyl F,[8] is in fact a mixture of two structural isomers, $C_{31}H_{42}O_{10}$, which we now name tyledoside F and G. A study of the ^1H and ^{13}C n.m.r. spectral data for tyledoside D (**7**), F (**12**), and G (**13**) indicated that these three compounds contain the same carbohydrate moiety, doubly-linked to the aglycone *via* acetal and hemiacetal bonds, and differ from each other only in the substitution pattern of the aglycone. A comparison of the n.m.r. data for tyledoside B (**9**) and F (**12**) confirmed that both compounds lack the C-12 carbonyl function and structure (**12**) is therefore assigned to tyledoside F.

Tyledoside G (**13**) contains a 12-hydroxy substituent but lacks the 11-hydroxy group present in the other tyledosides. The absence of the 11-hydroxy group can also be deduced from the observed upfield shift ($\Delta\delta$ 1.039 p.p.m.) for 1β-H (δ_H 1.875 p.p.m.) of tyledoside G (**13**). The configuration at C-12 was deduced from the ^{13}C chemical shifts of the C-ring carbons. A 12α-hydroxy group has the axial orientation and the γ-carbon atoms C-9 and C-14 will be *gauche*, which should result in a more pronounced upfield shift than that observed for an equatorial 12β-hydroxy group with *anti* γ-carbon atoms.[18] The

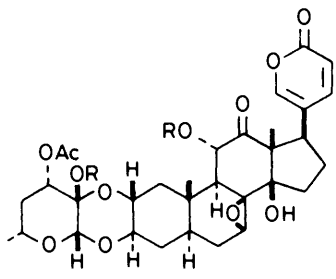
Table 3. Nuclear Overhauser effects ($CDCl_3$–10% [$^2H_6$]DMSO solutions)

Compd.	Signal irradiated (δ_H/p.p.m.)	Signals affected* (δ_H/p.p.m.)
(**2**)	5.053 (1′-H)	No effects
	4.562 (5′, 11-H)	3.872 (3-H), 2.233 (4′α-H), 1.126 (6′-H), 0.963 (19-H), 0.818 (18-H)
	3.872 (3, 17-H)	7.269 (21-H), 4.562 (5′-H)
	3.537 (2-H)	5.053 (1′-H), 2.894 (1β-H), 0.963 (19-Me)
	1.126 (6′-H)	5.053 (1′-H), 4.562 (5′-H)
(**7**)	5.143 (1′-H)	4.071 (2′-H)
	4.247 (5′-H)	3.680 (3-H), 1.185 (6′-H)
	3.680 (3-H)	4.247 (5′-H)
	1.185 (6′-H)	4.247 (5′-H)
(**14**)	4.738 (3′, 11-H)	4.509 (1′-H), 3.742 (11-OH), 1.129 (19-H), 0.875 (18-H)
	4.509 (1′-H)	4.959 (2′-OH), 3.622 (5′-H)
	4.018 (2, 17-H)	7.327 (21-H), 4.959 (2′-OH), 4.509 (1′-H), 1.129 (19-H)
	3.622 (5′-H)	4.738 (3′-H), 4.509 (1′-H), 1.209 (6′-H)
	1.209 (6′-H)	3.622 (5′-H)

* Effects in the region δ_H 1.0—2.0 p.p.m. were not rationalized due to the complexity of the spectra in these parts.

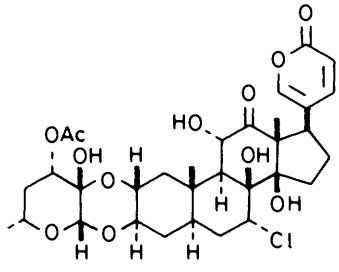


(11)



Tyledoside C (**14**) R = H

(**15**) R = Ac



(16)

difference in chemical shifts observed for the C-9 ($\Delta\delta$ 1.06 p.p.m.) and C-14 ($\Delta\delta$ −0.01 p.p.m.) resonances of tyledoside G when compared with cotyledoside (**1**) are in agreement with a 12β-hydroxy substituent for tyledoside G (**13**).

A comparison of the ^{13}C and ^1H n.m.r. data for tyledosides A (**2**) and C (**14**) shows that the same aglycone is present in both glycosides. Resonances at δ_H 2.017 p.p.m., and δ_C 170.06 and 20.71 p.p.m. in the n.m.r. spectra of tyledoside C confirmed the presence of an acetate group in the compound. Acetylation results in the introduction of a further two acetate groups, one of which must be located in the carbohydrate moiety. The

anomeric proton, identified by correlation of the doublet at δ_C 95.28 p.p.m. appears as a singlet at δ_H 4.417 p.p.m. whereas a methine proton (δ_H 4.846 p.p.m. dd) is coupled to both the C-4′ axial (*J* 12.0 Hz) and equatorial (*J* 4.8 Hz) protons. This evidence would suggest that the carbohydrate moiety of tyledoside C is linked to the aglycone at the C-1′ and C-2′ positions. This arrangement is corroborated by the ^{13}C chemical shifts of C-1′ (δ_C 95.28 p.p.m.) and C-2′ (δ_C 90.17 p.p.m.) which is in agreement with the chemical shifts of the corresponding carbon atoms in labriformidin (**3**)[15] (δ_C 97.2 and 91.1 p.p.m., respectively).

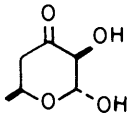
The proton of the hydroxy group present in the carbohydrate moiety of tyledoside C appears as a singlet (δ_H 6.223 p.p.m.) in the ^1H n.m.r. spectrum, in contrast to that of the C-11 hydroxy group which couples (*J* 5.1 Hz) with 11-H (δ_H 4.625 p.p.m.). This result suggests the location of the hydroxy group at C-2′ and thus an *O*-acetate function at C-3′. The assignment was confirmed by the deuterium isotope shift of 0.084 p.p.m. observed for C-2′ (δ_C 90.16 p.p.m.) upon addition of a mixture of H_2O–D_2O (1:1 v/v) to the sample.

The configuration of the carbohydrate moiety was deduced from the magnitude of the proton-proton coupling constants as well as the proton-proton n.O.e.s (Table 3). The n.O.e. observed for the proton of the C-2′ hydroxy group upon irradiation of either the C-1′ or C-2′ proton resonances indicates a *cis* relationship between these protons. Similarly an appreciable n.O.e. effect is observed for 3′-H, 1′-H, and 6′-H when the C-5′ proton resonance is irradiated. The *cis* 1,3-diaxial relationship between 5′-H and 3-H follows from the value of the coupling constant for these protons (*J* 11.4 and 12.0 Hz, respectively) with the C-4 axial proton which shows that both these protons are antiperiplanar with respect to 4′ax-H. These results established the configuration of tyledoside C as indicated in (**14**).

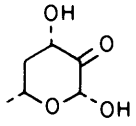
Attempts to hydrolyse tyledoside C failed and instead a chlorohydrin derivative (**16**) was formed when tyledoside C was treated with 0.1M-hydrochloric acid in ethanol. The resistance of the glycoside towards hydrolysis is in agreement with results reported for other 1′,2′-attached glycosides.[14–17]

The structure of tyledoside E will be reported in another publication. Preliminary studies indicated that this bufadienolide glycoside is not related to the other tyledosides as the characteristic substitution pattern of the aglycone is absent and it also lacks the double linkage between the aglycone and the sugar moiety.

The stereochemistry of the carbohydrate moieties indicates that tyledosides A, B, D, F, and G are derived from a 4,6-dideoxy-3-oxo-L-hexose (**17**), while tyledoside C is derived from a 4,6-dideoxy-2-oxo-D-hexose (**18**). The absolute stereochemistry of the carbohydrate moiety of tyledoside C is in agreement with related cardenolide glycosides which are also derived from 2-oxo-D-hexoses.[14–17] The occurrence of D and L forms of closely related sugars in the same plant, as well as in the same compound (*e.g.* L-cymarose and D-oleandrose in glaucoside D[21]) has been well established.



(17)



(18)

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. U.v. absorptions were measured for solutions in methanol on a Unicam SP 8-100 spectrometer while i.r. spectra were recorded for KBr discs on a Perkin-Elmer 237 spectrometer. Mass spectra were taken on a Varian MAT 212 double focussing spectrometer.

N.m.r. spectra were recorded on a Bruker WM-500 spectrometer operating at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C nuclei. N.O.e. experiments were performed on a Bruker AM-300 spectrometer. Chemical shifts are reported in p.p.m. relative to tetramethylsilane (δ = 0.000). The abbreviations s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br d = broadened doublet are used in connection with ^1H n.m.r. data.

C.d. spectra were measured for solutions in methanol on a JASCO J-20 spectropolarimeter. Merck silica gel 60 (particle size 0.063—0.200 nm) was used for column chromatography.

The toxicity of the plant extracts was monitored in guinea pigs as described earlier.[8]

Isolation of Toxins.—Fresh *T. grandiflorus* plants (405 kg) were minced and extracted in a Waring blender with ethyl acetate (3 times). The solvent was evaporated under reduced pressure and the resultant syrup was partitioned between 95% aqueous methanol (5 l) and light petroleum. Both extracts were evaporated to dryness. Only the residue from the methanolic extract (711 g) was toxic. This residue was agitated with methanol (3 l) and centrifuged at 4 000 rev/min for 10 min. The supernatant fluid was decanted and evaporated to yield a toxic syrup (408 g). The precipitate was not toxic.

The syrup was chromatographed on a silica gel column (4.5 kg). Elution with benzene (12 l) yielded non-toxic material (118 g). Elution with benzene–ethyl acetate (1:1 v/v), followed by ethyl acetate and ethyl acetate–methanol (80:20 v/v), yielded toxic fractions (216 g), while elution with methanol yielded non-toxic material. The combined toxic fractions were evaporated to dryness to yield an amorphous residue (216 g).

Repeated chromatography of the toxic residue on silica gel using benzene–acetone (70:30) and chloroform–acetone–methanol (70:30:1, v/v) yielded five crystalline compounds, tyledoside A (392 mg), tyledoside B (60 mg), tyledoside C (1.35 g), tyledoside E (137 mg), and tyledoside F (650 mg), as well as two amorphous components tyledoside D (720 mg) and tyledoside G (130 mg).

Tyledoside A (**2**) crystallized from chloroform–ether as white crystals, m.p. 263—264 °C, $\lambda_{max.}$ 297 (log ε 3.74); $\nu_{max.}$ 1 755, 1 740, 1 720, and 1 640 cm^{-1}; $\Delta\varepsilon_{360}$ −0.25, $\Delta\varepsilon_{325}$ −2.56, $\Delta\varepsilon_{303}$ 0, $\Delta\varepsilon_{288}$ 2.04, $\Delta\varepsilon_{256}$ 0, and $\Delta\varepsilon_{234}$ −1.20; M^+ 586 (FD); δ_H ([$^2H_6$]DMSO) 7.657 (dd, *J* 9.7 and 2.7 Hz, 22-H), 7.566 (d, *J* 2.8 Hz, 21-H), 6.295 (d, *J* 9.7 Hz, 23-H), 5.071 (s, 1′-H), 4.629 (d, *J* 12.5 Hz, 11-H), 4.629 (ddq, *J* 10.6, 2.3, and 6.1 Hz, 5′-H), 3.997 (ddd, *J* 11.0, 8.2, and 5.5 Hz, 3-H), 3.910 (t, *J* 8.4 Hz, 17-H), 3.530 (ddd, *J* 11.0, 8.3, and 4.8 Hz, 2-H), 3.380 (d, *J* 6.0 Hz, 7-H), 3.276 (s, OMe), 2.866 (dd, *J* 13.6 and 5.0 Hz, 1β-H), 2.288 (dd, *J* 14.1 and 2.5 Hz, 4′α-H), 1.96 (m, 16-H), 1.848 (ddd, *J* 14.0, 6.0, and 4.6 Hz, 6α-H), 1.843 (d, *J* 12.5 Hz, 9-H), 1.762 (dd, *J* 14.1 and 10.7 Hz, 4′β-H), 1.730 (ddd, *J* 13.5, 5.5, and 3.5 Hz, 4α-H), 1.96 (m, 16-H), 1.65 (m, 15-H), 1.595 (m, 15-H), 1.463 (dd, *J* 14.8 and 12.7 Hz, 6β-H), 1.377 (dddd, *J* 12.7, 12.7, 4.4, and 3.8 Hz, 5-H), 1.207 (dd, *J* 13.6 and 11.1 Hz, 1α-H), 1.156 (ddd, *J* 13.5, 12.8, and 11.0 Hz, 4β-H), 1.152 (d, *J* 6.2 Hz, 6′-H), 0.969 (s, 19-H), and 0.815 (s, 18-H); R_F = 0.43 (benzene–acetone, 7:3); R_F = 0.51 (chloroform–acetone–methanol, 70:30:1).

Tyledoside B (**9**) was obtained as white crystals ($CHCl_3$–ether), m.p. 291 °C, $\lambda_{max.}$ 298 (log ε 3.70); $\nu_{max.}$ 1 755, 1 740, 1 720, and 1 640 cm^{-1}; M^+, 572 (FD); δ_H([$^2H_6$]acetone) 7.809 (dd, *J* 9.8 and 2.6 Hz, 22-H), 7.426 (dd, *J* 2.6 and 0.7 Hz, 21-H), 6.160 (dd, *J* 9.8 and 0.8 Hz, 23-H), 5.033 (s, 1′-H), 4.718 (qdd, *J* 6.1, 10.9, and 2.6 Hz, 5′-H), 4.066 (dddd, *J* 10.8, 8.6, 5.7, and 4.1 Hz, 1β-H), 4.039 (ddd, *J* 11.2, 8.4, and 5.6 Hz, 3-H), 3.782 (d, *J* 5.7

Hz, 11-OH), 3.649 (ddd, *J* 11.2, 8.4, and 4.9 Hz, 2-H), 3.339 (s, OMe), 3.317 (d, *J* 6.1 Hz, 7-H), 3.170 (dd, *J* 13.6 and 4.9 Hz, 1β-H), 2.703 (dd, *J* 9.4 and 7.0 Hz, 17-H), 2.392 (ddd, *J* 13.1, 9.3, 9.3, and 2.6 Hz, 16-H), 2.269 (dd, *J* 13.8 and 2.3 Hz, 4α-H), 2.2 (m, 16-H), 1.914 (dd, *J* 13.2 and 4.0 Hz, 12β-H), 1.861 (d, *J* 10.8 Hz, 9α-H), 1.850 (ddd, *J* 14.8, 6.3, and 4.8 Hz, 6α-H), 1.8 (m, 2 × 16-H), 1.769 (dd, *J* 14.0 and 10.8 Hz, 4β-H), 1.761 (ddd, *J* 13.5, 5.6, and 3.2 Hz, 4α-H), 1.678 (ddd, *J* 13.3, 8.6, and 1.2 Hz, 12α-H), 1.499 (dd, *J* 14.9 and 13.1 Hz, 6β-H), 1.276 (*J* 13.6 and 11.2 Hz, 1α-H), 1.225 (ddd, *J* 13.3, 13.3, and 11.3 Hz, 4β-H), 1.186 (d, *J* 6.1 Hz, 6′-H), 1.015 (s, 19-H), and 0.806 (s, 18-H); R_F = 0.31 (benzene–acetone, 7:3); R_F = 0.41 (chloroform–acetone–methanol, 70:30:1).

Tyledoside C (**14**) was isolated as white crystals ($CHCl_3$–ether), m.p. 210—213 °C, $\lambda_{max.}$ 298 (log ε 3.69); $\nu_{max.}$ 1 740, 1 720, and 1 640 cm^{-1}; c.d. $\Delta\varepsilon_{360}$ −0.12, $\Delta\varepsilon_{325}$ −0.48, $\Delta\varepsilon_{309}$ 0, $\Delta\varepsilon_{288}$ 1.97, $\Delta\varepsilon_{252}$ 0, and $\Delta\varepsilon_{230}$ −0.81; M^+, 616; δ_H([$^2H_6$]-DMSO) 7.654 (dd, *J* 9.7 and 2.7 Hz, 22-H), 7.568 (d, *J* 2.7 Hz, 21-H), 6.297 (d, *J* 9.7 Hz, 23-H), 6.223 (s, 2′-OH), 4.975 (d, *J* 5.7 Hz, 11-OH), 4.846 (dd, *J* 12.0 and 4.8 Hz, 3′-H), 4.625 (dd, *J* 12.6 and 5.7 Hz, 11-H), 6.563 (s, 14-OH), 4.417 (s, 1′-H), 3.899 (t, *J* 8.6 Hz, 17-H), 3.897 (ddd, *J* 12.2, 10.0, and 4.1 Hz, 2-H), 3.719 (ddd, *J* 11.8, 9.8, and 4.6 Hz, 3-H), 3.665 (qdd, *J* 6.1, 11.4, and 1.8 Hz, 5′-H), 3.365 (d, *J* 6.2 Hz, 7-H), 2.538 (dd, *J* 13.0 and 4.1 Hz, 1β-H), 2.017 (s, OAc), 1.91 (m, 16-H), 1.899 (d, *J* 12.6 Hz, 9-H), 1.830 (ddd, *J* 15.1, 6.2, and 4.7 Hz, 6α-H), 1.71 (m, 15, 16-H), 1.652 (ddd, *J* 12.0, 4.7, and 2.0 Hz, 4′-H), 1.57 (m, 15-H), 1.529 (dd, *J* 15.1 and 13.1 Hz, 6β-H), 1.489 (ddd, *J* 12.5, 4.6, and 3.5 Hz, 4α-H), 1.425 (ddd, *J* 12.0, 12.0, and 11.4 Hz, 4′β-H), 1.387 (dddd, *J* 13.1, 12.2, 4.7, and 3.5 Hz, 5-H), 1.210 (ddd, *J* 12.5, 12.2, and 11.8 Hz, 4β-H), 1.125 (d, *J* 6.1 Hz, 6′-H), 1.056 (s, 19-H), 1.046 (dd, *J* 13.0 and 12.2 Hz, 1α-H), and 0.811 (s, 18-H); R_F = 0.27 (benzene–acetone, 7:3); R_F = 0.36 (chloroform–acetone–methanol, 70:30:1).

Tyledoside D (**7**) was obtained as a white amorphous solid, $\lambda_{max.}$ 298 (log ε 3.69); $\nu_{max.}$ 1 740, 1 720, and 1 640 cm^{-1}; c.d. $\Delta\varepsilon_{350}$ −0.09, $\Delta\varepsilon_{321}$ −0.34, $\Delta\varepsilon_{311}$ 0, $\Delta\varepsilon_{288}$ 2.15, $\Delta\varepsilon_{252}$ 0, and $\Delta\varepsilon_{235}$ −0.40; M^+ 588; δ_H([$^2H_6$]DMSO) 7.652 (dd, *J* 9.7 and 2.7 Hz, 22-H), 7.565 (*J* 2.7 Hz, 21-H), 6.296 (d, *J* 9.7 Hz, 23-H), 5.152 (d, *J* 4.6 Hz, 2′-OH), 4.954 (d, *J* 5.1 Hz, 11-OH), 4.914 (d, *J* 0.9 Hz, 1′-H), 4.624 (dd, *J* 12.6 and 5.1 Hz, 11-H), 4.582 (s, 14-OH), 4.192 (qdd, *J* 6.2, 11.3, and 2.8 Hz, 5′-H), 4.132 (br d, *J* 4.6 Hz, 2′-H), 4.012 (ddd, *J* 11.4, 8.6, and 4.6 Hz, 2-H), 3.902 (t, *J* 8.3 Hz, 17-H), 3.580 (ddd, *J* 11.0, 8.6, and 5.2 Hz, 3-H), 3.366 (d, *J* 6.0 Hz, 7-H), 3.312 (s, OMe), 2.697 (dd, *J* 13.4 and 4.6 Hz, 1β-H), 1.90 (m, 16-H), 1.817 (ddd, *J* 15.3, 6.0, and 5.1 Hz, 6α-H), 1.800 (d, *J* 12.6 Hz, 9-H), 1.71 (m, 16-H), 1.689 (ddd, *J* 12.6 and 11.3 Hz, 4′β-H), 1.68 (m, 15-H), 1.581 (ddd, *J* 13.3, 5.1, and 3.3 Hz, 4α-H), 1.58 (m, 15-H), 1.444 (dd, *J* 15.3 and 13.0 Hz, 6β-H), 1.376 (ddd, *J* 12.6, 2.8, and 1.7 Hz, 4′α-H), 1.284 (dddd, *J* 12.8, 12.8, 5.1, and 3.3 Hz, 5-H), 1.132 (ddd, *J* 13.0, 13.0, and 11.3 Hz, 4β-H), 1.085 (d, *J* 6.1 Hz, 6′-H), 1.014 (s, 19-H), 0.988 (dd, *J* 13.5 and 11.4 Hz, 1α-H), and 0.813 (s, 18-H); R_F = 0.20 (benzene–acetone, 7:3); R_F = 0.29 (chloroform–acetone–methanol, 70:30:1).

Tyledoside F (**12**) crystallized from chloroform–acetone as white crystals, m.p. 206—208 °C, $\lambda_{max.}$ 298 (log ε 3.69); $\nu_{max.}$ 1 710 and 1 630 cm^{-1}; δ_H([$^2H_6$]DMSO) 7.761 (dd, *J* 9.8 and 2.8 Hz, 22-H), 7.530 (d, *J* 2.8 Hz, 21-H), 6.242 (d, *J* 9.8 Hz, 23-H), 5.145 (d, *J* 4.4 Hz, 2′-OH), 4.912 (d, *J* 1.1 Hz, 1-H), 4.547 (d, *J* 6.3 Hz, 11-OH), 4.215 (ddq, *J* 11.4, 3.0, and 6.3 Hz, 5′-H), 4.118 (br d, *J* 4.4 Hz, 2′-H), 3.994 (ddd, *J* 11.0, 9.2, and 4.7 Hz, 2-H), 3.828 (dddd, *J* 10.5, 10.5, 5.4, and 5.0 Hz, 11-H), 3.733 (s, 14-OH), 3.583 (ddd, *J* 10.7, 9.2, and 5.2 Hz, 3-H), 3.176 (d, *J* 6.1 Hz, 7-H), 3.123 (s, OMe), 2.814 (dd, *J* 13.5 and 6.7 Hz, 1-H), 2.583 (dd, *J* 9.3 and 7.9 Hz, 17-H), 2.23 (m, 16-H), 2.153 (dd, *J* 12.9 and 9.2 Hz, 12α-H), 1.736 (ddd, *J* 15.3, 6.1, and 5.1 Hz, 6α-H), 1.717 (dd, *J* 12.7 and 11.3 Hz, 4′β-H), 1.655 (d, *J* 10.9 Hz, 9-H), 1.547 (ddd, *J* 12.9, 5.2, and 3.1 Hz, 4α-H), 1.530 (dd, *J* 12.9 and 2.8 Hz, 12β-H), 1.375 (ddd, *J* 12.7, 2.8, and 1.7 Hz, 4′α-H), 1.359 (dd, *J* 15.3 and 12.8 Hz, 6β-H), 1.193 (dddd, *J* 12.8, 12.8, 5.1, and 3.1 Hz, 5-H), 1.086 (ddd, *J* 12.9, 12.9, and 11.0 Hz, 4β-H), 1.093 (d, *J* 6.1 Hz, 6′-H), 0.989 (dd, *J* 13.7 and 11.0 Hz, 1α-H), 0.941 (s, 19-H), and 0.663 (s, 18-H); R_F = 0.11 (benzene–acetone, 7:3); R_F = 0.18 (chloroform–acetone–methanol, 70:30:1).

Tyledoside G (**13**) was obtained as a white amorphous solid, $\lambda_{max.}$ 298 (log ε 3.67); $\nu_{max.}$ 1 710 and 1 630 cm^{-1}; δ_H ([$^2H_6$]DMSO) 7.733 (dd, *J* 9.7 and 2.6 Hz, 22-H), 7.441 (dd, *J* 2.6 and 1.1 Hz, 21-H), 6.248 (dd, *J* 9.7 and 1.1 Hz, 23-H), 5.170 (d, *J* 4.6 Hz, 12-OH), 4.922 (d, *J* 1.1 Hz, 1′-H), 4.727 (d, *J* 5.0 Hz, 2′-OH), 4.215 (qdd, *J* 6.3, 14.2, and 2.8 Hz, 5′-H), 4.131 (br d, *J* 4.8 Hz, 2′-H), 4.047 (ddd, *J* 11.0, 8.6, and 4.8 Hz, 2-H), 3.705 (d, *J* 0.4 Hz, 14-OH), 3.607 (ddd, *J* 10.9, 8.6, and 5.1 Hz, 3-H), 3.444 (ddd, *J* 11.7, 4.6, and 4.3 Hz, 12α-H), 3.148 (s, OMe), 3.146 (d, *J* 5.9 Hz, 7-H), 3.025 (t, *J* 8.0 Hz, 17-H), 2.082 (m, 16-H), 1.875 (dd, *J* 12.8 and 4.7 Hz, 1β-H), 1.793 (dt, *J* 15.2 and 5.6 Hz, 6α-H), 1.701 (dd, *J* 12.7 and 11.3 Hz, 4′β-H), 1.565 (ddd, *J* 13.2, 5.0, and 3.1 Hz, 4α-H), 1.409 (ddd, *J* 12.7, 2.8, and 1.7 Hz, 4′α-H), 1.403 (dd, *J* 15.2 and 12.7 Hz, 6β-H), 1.225 (dddd, *J* 12.7, 12.7, 5.1, and 3.1 Hz, 5-H), 1.1322 (s, 18-H), 1.097 (d, *J* 6.1 Hz, 6′-H), 1.070 (ddd, *J* 12.9, 12.9, and 11.0 Hz, 1α-H), 1.053 (dd, *J* 12.8 and 11.0 Hz, 1α-H), and 0.813 (s, 19-H).

Reduction of Tyledoside A (**2**).—A solution of tyledoside A (10 mg) and $NaBH_4$ (10 mg) in 2 ml EtOH was stirred at room temperature for 2 h. After the additon of water (10 ml), the solution was acidified with 3M HCl and extracted with chloroform. Evaporation of chloroform and chromatography of the extract on t.l.c. plates (benzene–acetone, 7:3) yielded the 2′α,12α-dihydroxy A (**5**) (3 mg, R_F 0.23) and 2′α,12β-dihydroxy (**6**) (3 mg, R_F = 0.13) derivatives of tyledoside A as amorphous solids.

7β,8-*Epoxy*-11α,12α,14-*trihydroxy*-2α,3β-(*tetrahydro*-3α-*hydroxy*-4β-*methoxy*-6β-*methyl*-2H-*pyran*-4α,2α-*diyldioxy*)-5α,8β,14β-*bufa*-20,22-*dienolide* (**5**) has M^+, 590; δ_H($CDCl_3$) 7.730 (dd, *J* 9.7 and 2.6 Hz, 22-H), 7.221 (d, *J* 2.6 Hz, 21-H), 6.246 (d, *J* 9.7 Hz, 23-H), 5.257 (d, *J* 4.6 Hz, 1′-H), 4.784 (ddd, *J* 11.6, 8.7, and 4.8 Hz, 2-H), 4.447 (qdd, *J* 6.0, 11.1, and 2.8 Hz, 5′-H), 4.242 (ddd, *J* 11.0, 5.0, and 2.3 Hz, 11-H), 4.000 (dd, *J* 4.6 and 1.8 Hz, 2′-H), 3.854 (ddd, *J* 10.9, 8.6, and 5.3 Hz, 3-H), 3.594 (t, *J* 2 Hz, 12β-H), 3.298 (s, OMe), 3.287 (d, *J* 5.8 Hz, 7-H), 3.281 (t, *J* 8.8 Hz, 17-H), 1.221 (d, *J* 6.2 Hz, 6′-H), 1.041 (s, 19-H), and 0.712 (s, 18-H).

7β,8-*Epoxy*-11α,12β,14-*trihydroxy*-2α,3β-(*tetrahydro*-3α-*hydroxy*-4β-*methoxy*-6β-*methyl*-2H-*pyran*-4α,2α-*diyldioxy*)-5α,8β,14β-*bufa*-20,22-*dienolide* (**6**) has M^+, 590; δ_H 7.642 (dd, *J* 9.7 and 2.6 Hz, 22-H), 7.283 (d, *J* 2.7 Hz, 21-H), 6.238 (d, *J* 9.7 Hz, 23-H), 5.255 (d, *J* 4.6 Hz, 1′-H), 4.789 (ddd, *J* 11.5, 8.7, and 4.8 Hz, 2-H), 4.434 (qdd, *J* 6.0, 11.0, and 2.7 Hz, 5′-H), 3.998 (dd, *J* 4.6 and 2.7 Hz, 2′-H), 3.874 (ddd, *J* 11.0, 9.6, and 3.0 Hz, 11β-H), 3.839 (ddd, *J* 11.1, 8.8, and 5.1 Hz, 3-H), 3.310 (dd, *J* 9.1 and 3.6 Hz, 12α-H), 3.298 (d, *J* 6.0 Hz, 7-H), 3.281 (s, OMe), 3.069 (dd, *J* 9.2 and 7.8 Hz, 17-H), 1.219 (d, *J* 6.1 Hz, 6′-H), 1.043 (s, 19-H), and 0.693 (s, 18-H).

2′,11-*Di*-O-*acetyltyledoside C* (**15**).—Acetylation of tyledoside C (**14**) (20 mg) with acetic anhydride–pyridine yielded the diacetate as a white amorphous solid (18 mg); M^+, 700; δ_H([$^2H_6$]DMSO) 7.642 (dd, *J* 9.7 and 2.6 Hz, 22-H), 7.566 (d, *J* 2.8 Hz, 21-H), 6.303 (d, *J* 9.8 Hz, 23-H), 5.643 (dd, *J* 11.8 and 5.3 Hz, 3′-H), 5.570 (d, *J* 13.3 Hz, 11-H), 5.398 (s, 14-OH), 4.758 (s, 1′-H), 3.882 (ddd, *J* 11.8, 10.6, and 4.3 Hz, 3-H), 3.845 (t, *J* 8.6 Hz, 17-H), 3.815 (ddd, *J* 11.8, 10.0, and 4.0 Hz, 2-H), 3.732 (qdd, *J* 6.2, 11.8, and 1.6 Hz, 5′-H), 3.461 (d, *J* 6.2 Hz, 7-H), 2.399 (d, *J* 13.3 Hz, 9-H), 2.158 (s, OAc), 2.112 (dd, *J* 12.3 and 4.3 Hz,

1β-H), 2.016 (s, OAc), 1.992 (s, OAc), 1.169 (d, *J* 6.1 Hz, 6′-H), 0.939 (s, 19-H), and 0.862 (s, 18-H).

2′,11-*Di*-O-*acetyltyledoside D* (**10**) (19 mg) was obtained as a white amorphous solid by the acetylation of tyledoside D (**7**) (20 mg) with acetic anhydride–pyridine at room temperature; M^+, 672; $\delta_H([^2H_6]DMSO)$ 7.643 (dd, *J* 9.7 and 2.7 Hz, 22-H), 7.564 (d, *J* 2.9 Hz, 21-H), 6.302 (d, *J* 9.7 Hz, 23-H), 5.562 (d, *J* 13.3 Hz, 11-H), 5.420 (br s, 2′-H), 4.960 (br s, 1′-H), 4.300 (qd, *J* 6.1 and 14.7 Hz, 5′-H), 4.105 (ddd, *J* 11.2, 8.6, and 4.9 Hz, 2-H), 3.845 (t, *J* 8.1 Hz, 17-H), 3.717 (ddd, *J* 11.0, 8.6, and 5.2 Hz, 3-H), 3.457 (d, *J* 6.0 Hz, 7-H), 3.115 (s, OMe), 2.349 (dd, *J* 13.4 and 4.8 Hz, 1β-H), 2.114 (s, OAc), 2.045 (s, OAc), 1.146 (d, *J* 6.2 Hz, 6′-H), 0.095 (s, 19-H), and 0.865 (s, 18-H).

Hydrolysis of Tyledoside D (**7**).—Tyledoside D (**7**) (150 mg) was dissolved in a mixture of ethanol (10 ml) and 1M-hydrochloric acid (1 ml). The reaction mixture was heated at 50 °C for 30 min and the solvent was then removed under reduced pressure (50 °C). Crystallization of the residue from methanol–ether yielded 7α-chloro-2α,3β,8,11α,14-pentahydroxy-12-oxo-5α,8β,14β-bufa-20,22-dienolide (**11**) (62 mg) as white needles, m.p. 234 °C; M^+, 482 (FD); $\delta_H([^2H_6]DMSO)$ 7.422 (dd, *J* 9.3 and 2.6 Hz, 22-H), 7.413 (d, *J* 2.6 Hz, 21-H), 6.294 (d, *J* 9.3 Hz, 23-H), 5.651 (s, OH), 4.992 (s, OH), 4.819 (t, *J* 2.5 Hz, 8-OH), 4.388 (t, *J* 2.5 Hz, 7-H), 4.186 (d, *J* 5.8 Hz, 11-H), 3.697 (dd, *J* 11.2 and 6.9 Hz, 17-H), 3.340 (ddd, *J* 11.3, 8.8, and 4.7 Hz, 3-H), 3.340 (s, OH), 3.153 (ddd, *J* 10.9, 8.8, and 5.3 Hz, 2-H), 2.322 (dd, *J* 12.8 and 5.5 Hz, 1β-H), 2.129 (ddd, *J* 14.1, 12.9, and 2.9 Hz, 6β-H), 1.875 (tt, *J* 12.7 and 2.6 Hz, 5-H), 1.802 (dd, *J* 6.0 and 2.5 Hz, 9-H), 1.421 (ddd, *J* 12.9, 5.3, and 3.0 Hz, 4α-H), 1.359 (td, *J* 2.7 and 14.1 Hz, 6α-H), 1.303 (td, *J* 12.8 and 11.1 Hz, 4β-H), 0.986 (s, 19-H), 0.906 (dd, *J* 12.8 and 11.0 Hz, 1α-H), 0.788 (s, 18-H); $\delta_C([^2H_6]DMSO)$ 210.74 (s, C-12), 160.96 (s, C-24), 149.72 (d, C-21), 146.21 (d, C-22), 118.86 (s, C-20), 114.30 (d, C-23), 87.13 (s, C-14), 78.54 (s, C-8), 75.09 (d, C-2), 72.92 (d, C-11), 70.84 (d, C-3), 61.66 (s, C-13), 60.59 (d, C-7), 49.43 (d, C-9), 46.57 (t, C-1), 43.86 (d, C-17), 38.26 (s, C-10), 38.08 (t, C-6), 36.88 (d, C-5), 38.15 (t, C-15), 33.12 (t, C-4), 28.06 (t, C-16), 17.68 (q, C-18), and 14.75 (q, C-19).

2α,3β-(4α-*Acetoxytetrahydro*-3β-*hydroxy*-6α-*methyl*-2H-*pyran*-3α,2α-*diyldioxy*)-7α-*chloro*-8,11α,14-*trihydroxy*-12-*oxo*-5α,8β,14β-*bufa*-20,22-*dienolide* (**16**).—A solution of tyledoside C (**14**) (150 mg) in ethanol (10 ml) and 1M-hydrochloric acid (1 ml) was refluxed for 30 min (50 °C). Evaporation of the solvent and crystallization from methanol–ether of the residue yielded compound (**16**) as white needles, m.p. 216 °C, M^+, 652 (FD); $\delta_H([^2H_6]DMSO)$ 7.449 (dd, *J* 9.6 and 2.6 Hz, 22-H), 7.424 (d, *J* 2.6 Hz, 21-H), 6.286 (s, 2′-OH), 6.296 (dd, *J* 9.6 Hz, 23-H), 5.662 (d, *J* 5.1 Hz, 11-OH), 5.063 (s, OH), 4.446 (s, 1′-H), 4.719 (dd, *J* 12.0 and 4.7 Hz, 3′-H), 4.446 (s, OH), 4.402 (t, *J* 2.8 Hz, 7-H), 4.238 (dd, *J* 6.6 and 5.1 Hz, 11-H), 4.032 (ddd, *J* 11.8, 9.8, and 4.2 Hz, 3-H), 3.827 (ddd, *J* 11.8, 9.8, and 5.1 Hz, 2-H), 3.696 (qdd, *J* 6.0, 10.8, and 2.0 Hz, 5′-H), 3.696 (dd, *J* 10.7 and 6.4 Hz, 17-H), 2.347 (dd, *J* 12.2 and 5.3 Hz, 1β-H), 2.028 (s, OAc), 1.143 (d, *J* 6.0 Hz, 6′-H), 1.091 (s, 19-H), and 0.809 (s, 18-H); $\delta_C([^2H_6]DMSO)$ 210.61 (s, C-12), 170.05 (s, $OCOCH_3$), 160.93 (s, C-24), 149.77 (d, C-27), 146.21 (d, C-22), 118.97 (s, C-20), 114.3 (d, C-23), 95.31 (d, C-1′), 90.28 (s, C-2′), 86.98 (s, C-14), 78.57 (s, C-8), 73.52 (d, C-11), 72.74 (d, C-3′), 71.93 (d, C-3), 67.24 (d, C-2), 66.79 (d, C-5′), 61.92 (s, C-13), 60.53 (d, C-7), 49.40 (d, C-9), 43.51 (d, C-17), 42.89 (t, C-1), 38.75 (s, C-10), 37.93 (t, C-6), 37.47 (d, C-5), 35.20 (t, C-4′), 32.96 (t, C-15), 30.99 (t, C-5), 28.02 (t, C-16), 21.10 (q, $OCOCH_3$), 20.73 (q, C-6′), 17.79 (q, C-18), and 15.05 (q, C-19).

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