Stereochemistry of the Hexosulose in Cardenolide Glycosides of the Asclepiadaceae

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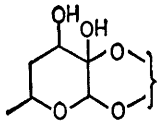
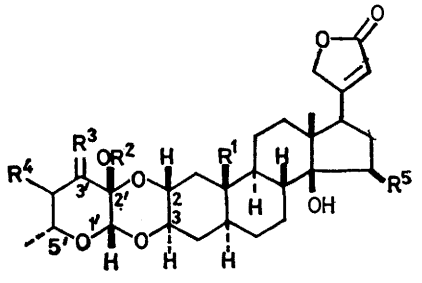
The 4,6-dideoxyhexosulose moiety of gomphoside (1) and afroside (2) has been shown to have the 1'*S*,2'*S*,3'*R*,-5'*R* configuration. Formation of 2',3'-*OO*-isopropylidene derivatives establishes that the hydroxy-group at C-3', which is axial, is *cis* to that at C-2'. The latter group is *cis* to the anomeric C-1' hydrogen, as shown by nuclear Overhauser enhancement measurements. Chemical and ^1H and ^{13}C n.m.r. data have been used to show that the same chirality at carbons 1', 2', and 5' is present in the following glycosides from plants of the Ascepiadaceae family: calactin (3), syriobioside (10), desglucosyrioside (11), syrioside (12), uscharidin (5), calotoxin (9), uscharin (7), and voruscharin (8).

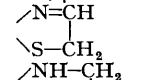
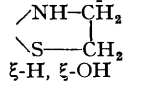
CERTAIN *Asclepias*, *Calotropis*, and *Pergularia* species (Asclepiadaceae) produce cardiac glycosides which are resistant to acid hydrolysis,[1-8] and some of which have cytotoxic properties.[3] The unusual stability to acids of



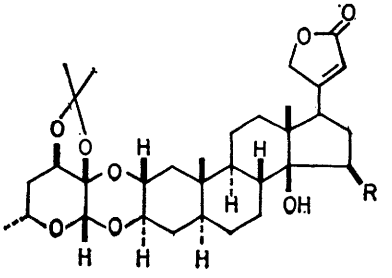
(A)

the carbohydrate group in these cardiac glycosides is due to its double attachment, through acetal and hemiacetal links, to positions 3β and 2α, respectively, of the cardenolide aglycone. The sugar structure (*A*), based on 4,6-dideoxyhexosulose, was first proposed[4]

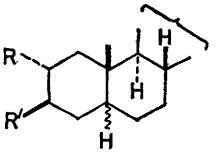


		R^1	R^2	R^3	R^4	R^5
Gomphoside	(1)	Me	H	α-H, β-OH	H	H
	(1a)	Me	Ac	α-H, β-OAc	H	H
	(1b)	Me	H	α-H, β-OAc	H	H
Afroside	(2)	Me	H	α-H, β-OH	H	OH
	(2a)	Me	Ac	α-H, β-OAc	H	OAc
	(2b)	Me	H	α-H, β-OAc	H	OAc
Calactin	(3)	CHO	H	α-H, β-OH	H	H
	(3a)	CHO	Ac	α-H, β-OAc	H	H
Calotropin	(4)	CHO	H	β-H, α-OH	H	H
	(4a)	CHO	Ac	β-H, α-OAc	H	H
Asclepin	(4b)	CHO	H	β-H, α-OAc	H	H
Uscharidin	(5)	CHO	H	O	H	H
	(6)	CH_2OAc	Ac	α-H, β-OAc	H	H
Uscharin	(7)	CHO	H		H	H
Voruscharin	(8)	CHO	H		H	H
Calotoxin	(9)	CHO	H	ξ-H, ξ-OH	ξ-OH	H

by one of us for gomphoside (1) from *Asclepias fruticosa*, and has since been shown [6,7] to be present also in calactin (3), calotropin (4), proceroside, syriobioside (10), and desglucosyrioside (11). The same carbohydrate in altered forms occurs also in uscharidin (5),[6] calotoxin (9),[9] uscharin (7),[6] voruscharin (8),[6] syrioside (12),[7] asclepin (4b),[9] and a number of other *Asclepias* cardenolides.[10,11] Below we present evidence on the stereochemistry of the carbohydrate group in gomphoside (1), and in afroside (2) (15β-hydroxygomphoside) † and extend the findings to the other glycosides of the Asclepiadaceae.



(1c) R = H
(2c) R = OH
(2d) R = OAc



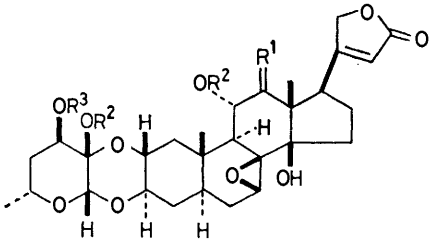
(2e) R = R' = OAc; 5α - H
(13) R = H, R' = OAc; 5α - H
(14) R = H, R' = OAc; 5β - H

RESULTS AND DISCUSSION

Gomphoside and Afroside.—Previous work from various research groups [1,2,4,6] on the structures of gomphoside (1), calactin (3), and calotropin (4) has been thoroughly reviewed by Reichstein and his co-workers.[6,7] While the stereochemistry of the steroid aglycones is known, uncertainty remains regarding the stereochemistry of the

† While the structural elucidation of afroside is presented in full elsewhere,[12] evidence is given (below) that the carbohydrate groups in gomphoside and afroside are identical.

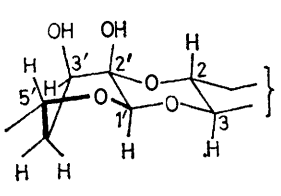
4,6-dideoxyhexosulose group. The following are some features of the stereochemistry of the carbohydrate in gomphoside (1) and afroside (2) which are readily discerned. (*a*) The chirality at C-5′ is *R*, established by isolation of D-(—)-butane-1,3-diol on degradation of gomphoside.[4] (*b*) The 3′-hydroxy-group is axial, since small vicinal couplings between H-3′ and the two protons at C-4′ are observed ($J_{4'\alpha,3'} + J_{4'\beta,3'}$ 5—6 Hz) * for the acetyl derivatives of gomphoside and afroside, *i.e.* (1a), (1b), (2a), and (2b). An additional and important feature is that the 3′- and 2′-hydroxy-groups are *cis*. Thus although gomphoside was shown to be inert to



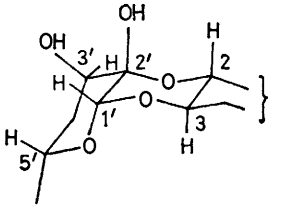
		R^1	R^2	R^3
Syriobioside	(10)	β-H, α-OH	H	H
	(10a)	β-H, α-OAc	Ac	Ac
Desglucosyrioside	(11)	O	H	H
	(11a)	O	Ac	Ac
Syrioside	(12)	O	H	β-D-glucosyl

acetone and anhydrous copper sulphate at room temperature,[4] it gives an excellent yield of the 2′,3′-isopropylidene derivative (1c) on being refluxed with acetone and acetone dimethyl acetal in the presence of toluene-*p*-sulphonic acid. Similarly afroside (2) was converted into 2′,3′-isopropylideneafroside (2c) which was then acetylated to produce the 15-acetate (2d). An *OO*-isopropylidene ring may be formed *only* if there is a *cis* relationship between the 3′-hydroxy-, which is axial, and the 2′-hydroxy-groups.†

Only two partial structures (*B*) and (*C*), with respectively *trans* and *cis* fusion of the pyranose and 1,4-dioxan rings, incorporate all the stereochemical features cited above. The *trans* system can possess an axial 3′-hydroxy-group only by adopting a twist-boat conformation for the pyranose ring [see (*B*)]. This structure,



(*B*)



(*C*)

with an α-glycosidic link, is among those rejected by Reichstein and his co-workers [6,7] on the basis of the early observation of Klyne [13] that all natural cardenolides with carbohydrates of the D-series were β-glycosides. We present below unambiguous evidence for the presence of a *cis*-fused chair–chair system (*C*) in gomphoside, which corresponds to 1′*S*,2′*S*,3′*R*,5′*R* chirality for the 4,6-dideoxyhexosulose moiety.

The proximity of H-1′ to one of the isopropylidene methyl groups in 2′,3′-isopropylidenegomphoside (1c) and to the 2′-hydroxy-group in 3′-acetylgomphoside (1b) is clearly demonstrated by the nuclear Overhauser enhancement (n.O.e.) data shown in Table 1. This is consistent with the partial structure (*C*), but not structure (*B*). The *cis* ring fusion is supported, furthermore, by a number of other n.O.e. measurements. In the *cis* structure (*C*), H-1′ has a 1,3-diaxial relationship to H-5′, but is relatively far from H-3α; the relationship is reversed in the *trans* structure (*B*). The enhancement of the H-1′ signal (relative to H-22) in 2′,3′-diacetylgomphoside (1a) was 30% when H-5′ (δ 3.97) was saturated, but it dropped to 16% when the irradiation frequency was adjusted slightly in order to centre it at δ 4.05, *i.e.* near the H-3α resonance. Similarly, for 3′,15-diacetylafroside (2b) and 2′,3′,15-triacetylafroside

TABLE 1

Nuclear Overhauser enhancements ($CDCl_3$ solution)

Compound	Signal irradiated (δ)	Signal observed (δ)	Integration standard	n.O.e. (%)
(1c)	isopropylidene Me (1.48)	H-1′ (4.69)	H-21	9
(1c)	isopropylidene Me (1.48)	H-1′ (4.69)	added CH_2Cl_2	21
(1b)	*both* OHs (1.49, 3.19)	H-1′ (4.74)	added CH_2Cl_2	11

(2a), the maximum n.O.e. was observed with irradiation centred at δ 3.92, *i.e.* near the H-5′ signal.

Before the n.O.e. measurements could be made it was necessary to identify the signals of H-2β, H-3α, and H-5′, all of which fall within the δ 3.8—4.1 region for gomphoside, afroside, and their acetyl derivatives. The assignments shown in Table 2 for these and other protons from the carbohydrate portion of the molecule are based on deconvoluted 270-MHz Fourier-transform spectra and n.O.e. data,‡ and are internally consistent (see later).

With the elucidation of the complete stereochemistry of the carbohydrate in gomphoside, it is possible to apply the n.m.r. data to cardenolides with related carbohydrate groups. Comparing pairs of compounds from the gomphoside and afroside series [*e.g.* 3′-acetylgomphoside (1b) *versus* 3′,15-diacetylafroside (2b)], there is complete correspondence § of ^1H and ^{13}C n.m.r. data for the carbohydrate region [see Tables 2 and 3: (1a) and (2a); (1b) and (2b); (1c), (2c), and (2d)]. This demonstrates that

* ABX system, since irradiation at δ 1.83 p.p.m. (AB) caused collapse of the H-3′ triplet signal (X) of 2′,3′-diacetylgomphoside (1a) to a singlet.

† This conclusion is valid only if it is shown that no epimerization at the acetal and hemiacetal positions 1′ and 2′ takes place during the formation of the isopropylidene derivative. Indeed, gomphoside was regenerated when 2′,3′-isopropylidenegomphoside (1c) was treated with toluene-*p*-sulphonic acid in methanol.

‡ For 3′,15-diacetylafroside (2b), distinction between signals of H-2β (δ 3.95) and H-3α (δ 4.07) was achieved by n.O.e. measurements on the signal of the C-10 methyl which is 1,3-diaxial to H-2β. Enhancement upon saturation at δ 3.85 was 8%; none was observed when the irradiation was at δ 4.11.

§ To ±0.03 p.p.m., and ±0.1 p.p.m. respectively for ^1H and ^{13}C chemical shifts in $CDCl_3$.

the stereochemistry of the carbohydrate in afroside is identical with that in gomphoside.

Calactin, Syriobioside, Desglucosyrioside, and Syrioside.—A number of other cardiac glycosides of the Asclepiadaceae have the same carbohydrate portion (*A*) as in gomphoside and afroside. Of these, calactin (3), syriobioside (10), and desglucosyrioside (11) each has an axial 3′-hydroxy-group,[7] and we propose that for these glycosides the stereochemistry of the sugar is also identical with that in gomphoside. This is shown by the close similarity of the relevant signals (for nuclei in the carbohydrate region) in the published ^1H and ^{13}C n.m.r. spectra [7] of their acetyl derivatives (3a), (10a), and (11a) with those of 2′,3′-diacetylgomphoside (1a) and 2′,3′,15-triacetylafroside (2a) [see Table 2, items 1—5; and (1a), (2a), (3a) in Table 3; also ref. 7]. Syrioside (12) which is the 3′-β-D-glucosyl derivative of desglucosyrioside (11) [7] will also have the same stereochemistry.

Uscharidin, Uscharin, Voruscharin, and Calotoxin.—The glycoside uscharidin (5) has a 19-aldehyde function, and differs from calactin (3) in having a carbonyl group at C-3′ instead of an axial hydroxy-group.[6] From either uscharidin or calactin, upon reduction with sodium borohydride followed by acetylation, an identical 2′,3′,19-triacetyl product (6) (triacetyltetrahydrouscharidin) was obtained.[6] Comparing the ^1H n.m.r. spectra of triacetyltetrahydrouscharidin (6) and of 2′,3′-diacetylcalactin (3a), there is near identity of the signals of protons in the carbohydrate region (see items 6 and 5 in Table 2). Thus it is likely that in the borohydride reductions the configuration at positions 1′ and 2′ has not been altered. Uscharidin (5), we propose, has the same chirality at carbons 1′, 2′, and 5′ as in calactin (3) and in gomphoside (1). N.O.e measurements confirmed that the 2′-hydroxy-group in uscharidin is *cis* to H-1′. The enhancement of the H-1′ signal (relative to that of added dibromomethane) was 13% when both the 2′- and the 14-hydroxy-signals were saturated.*

The stereochemistry of the carbohydrate moiety in three other cardiac glycosides may be inferred, since these substances have been converted into the 3′-ketone uscharidin (5) of known stereochemistry (see above). Uscharin (7) and voruscharin (8), having reduced 2-thiazole groups attached to C-3′, were hydrolysed to uscharidin (5) (by acids or aqueous mercuric salts), and were also partially synthesized from it.[14] It is highly unlikely that epimerization at positions 1′ and 2′ occurred under all of these reaction conditions. We propose therefore that uscharin (7) and voruscharin (8) have the same *cis*-fusion of pyranose and dioxan rings as in uscharidin (5) (and hence as in gomphoside). The 2′,3′,4′,14β-tetra-ol calotoxin (9), which was dehydrated by alumina to uscharidin (5),[2] is also likely to have the same ring fusion. However, the chirality at C-3′ and at C-4′ is as yet unknown.

It is of interest that the 4,6-dideoxyhexosulose group, with *cis*-fused pyranose and 1,4-dioxan rings, as found in these cardiac glycosides of the Asclepiadaceae, has the same stereochemistry at the ring junction as the 4-deoxypentosulose moiety (*D*) in the tumour inhibitor elaeodendroside A (from the family Celastraceae), the structure of which has recently been determined by single-crystal *X*-ray analysis.[15] (See note added in proof at end of paper.)

Calotropin, Asclepin, and Proceroside.—Among the acid-stable cardiac glycosides of the Asclepiadaceae, calotropin is unusual in possessing an equatorial 3′-hydroxy-group, and it is generally accepted that it is the

TABLE 2

Chemical shifts [a] of protons in the carbohydrate and ring-A regions

Item	Compound	Substituents 2′	3′	10	H-1′	H-3′	H-5′	H-2β	H-3α	5′-Me [d] (H-6′)	10-Me	($CHCl_3$ as ref.)	Reference
1	(1a)				4.82 [f]	5.73 [f,g]	*ca.* 3.97	3.85 [k]	4.04 [k]	1.24	0.84	7.27	This work and ref. 7
2	(2a) [b]	OAc	β-OAc	Me	4.81	5.71 [g]	*ca.* 3.95	3.82	*ca.* 4.05	1.23	0.83	7.26	This work
3	(11a) [b]				4.82	5.74 [e]	3.6 —— 4.2			1.22	1.01	7.26	7
4	(10a)				4.83	5.75 [g]	*ca.* 3.95 [g]	3.74 [k]	*ca.* 4.0 [k]	1.25	1.05 [l]	7.25	7
5	(3a)	OAc	β-OAc	CHO	4.81	5.74 [j]	*ca.* 3.95	3.70 [k]	4.04 [k]	1.22			7
6	(6) [b]	OAc	β-OAc	CH_2OAc	4.75	5.67 [g]	3.6 —— 4.1			1.20		7.23	6
7	(4a) [b]	OAc	α-OAc	CHO	5.54	5.80 [i]	3.6 —— 4.2			1.26			7
8	(1b) [b]	OH	β-OAc	Me	4.74	4.97 [e,h]	*ca.* 4.0	3.97	*ca.* 4.1	1.25	0.86		This work
9	(2b)	OH	β-OAc	Me	4.75	4.96 [e]	3.99	3.95	4.07	1.26	0.86	7.29	This work
10	(4b) [b]	OH	α-OAc	CHO	4.55	4.6—5.1	3.4 —— 4.2			1.30			9
11	(1) [b,c]	OH	β-OH	Me	4.79	3.68	3.9 —— 4.2			1.24	0.88	7.47	This work
12	(2) [c]	OH	β-OH	Me	4.78	3.69 [g]	*ca.* 4.07	3.94	4.07	1.24	0.87	7.38	This work
13	(4) [b]	OH	α-OH	CHO	4.52		3.4 —— 4.2			1.30			9
14	Proceroside [b]	OH	ξ-OH	CHO	4.52	*ca.* 3.6	*ca.* 3.6	*ca.* 3.9	*ca.* 3.9	1.25		7.23	6
15	(1c) [b,m]	$-O-CMe_2-O-$		Me	4.63	4.08 [j]	3.8 —— 3.9			1.27	0.87	7.25	This work
16	(2c) [b,m]	$-O-CMe_2-O-$		Me	4.63	4.08 [j]	3.8 —— 3.9			1.28	0.88	7.25	This work
17	(2d) [b,m]	$-O-CMe_2-O-$		Me	4.63	4.08 [j]	3.8 —— 3.9			1.27	0.87	7.26	This work

[a] Measured relative to $SiMe_4$ in $CDCl_3$ at 270 MHz, unless otherwise stated. [b] At 100 MHz [or 60 MHz for items 10 and 13 (ref. 9)]. [c] 10% CD_3SOCD_3 added. [d] Doublet, *J* 6 Hz. [e] Masked by other signals. [f] Assignment of H-1′ and H-3′ were reversed in ref. 5. [g] Apparent triplet, *J′* 3 Hz. [h] Signal is triplet, *J* 2.5, in the Eu(fod)$_3$-shifted spectrum. [i] X of ABX, $J_{AX} + J_{BX}$ 16 Hz. [j] X of ABX, $J_{AX} + J_{BX} = 5.5$—6.5 Hz. [k] Our assignment of H-2β and H-3α is the reverse of that of Brown *et al.* (ref. 7). [l] Reassignment of data of Brown *et al.* (ref. 7). [m] Isopropylidene Me, δ 1.48.

* Further confirmation of the stereochemistry comes indirectly from our ^1H and ^{13}C n.m.r. studies on labriformidin [11] which has the same carbohydrate moiety as uscharidin.

3′-epimer of calactin (3).[6,9] Calotropin was obtained in 51% yield on partial reduction (by sodium borohydride) of the 3′-ketone uscharidin (5).[16] It is likely that calotropin and asclepin (3′-acetylcalotropin of natural origin) [9] have the same configuration at carbons 1′, 2′, and 5′ as in uscharidin (5), and this has been written into their structures (4) and (4b) respectively. However this afroside (see Table 2, items 11, 12, and 14; and Table 4).

Conformational Preferences deduced from ^1H *and* ^{13}C *N.M.R.*—The subtle changes in ^1H and ^{13}C shieldings accompanying structural variations among the Asclepiadaceae glycosides corroborate the sugar stereochemistry shown in (*C*), and in addition provide insight into the con-

TABLE 3

^{13}C Chemical shifts (in p.p.m. downfield from $SiMe_4$)

Carbon	(3a) (ref. 7)	(1a) [a]	(2a) [a]	(1b) [a]	(2b) [a]	(1) [b]	(2) [b]	(1c) [a]	(2c) [a]	(2d) [c]	(13)
C-1′	93.2	93.2	93.2	94.2	94.1	93.9 [f]	93.9 [f]	97.9	97.9	97.8	
C-2′	95.6	95.6	95.7	90.1	90.0	90.1	90.1	96.3	96.3	96.2	
C-3′	70.5	70.3 [d]	70.4 [d]	72.8	72.8 [d,f]	69.8 [d,f]	69.8 [d,f]	77.3	77.3	77.1	
C-4′	35.0	34.8	34.9	35.3	35.3	39.7	39.7	33.8	33.8	33.6	
C-5′	66.6	66.4	66.5	66.5	66.7	65.1	65.1	67.9	68.0	67.8	
C-6′	20.8	20.9	20.8	20.8	20.8	20.9	20.9	20.9	20.9	20.8	
C-1	35.7	41.7	41.8	42.1	42.1	41.9	42.1	42.3	42.3	42.0	36.8
C-2	70.8	71.1 [e,*]	71.1 *	69.4	69.3 [e]	68.1 [e]	68.1	72.1 *	72.0 *	71.8 *	27.3
C-3	71.2	71.8 [e*]	71.7 *	72.4	72.2 [e]	71.8 [e]	71.8 [e]	72.5 *	72.6 *	72.2 *	73.5
C-4	32.4	32.0	31.9	32.0	32.0	32.0	32.0	32.0	32.0	31.9	33.8
C-5	43.6	44.8	44.6	44.8	44.7	44.3	44.3	44.6	44.6	44.3	44.2
C-6	27.7	27.7	27.7	27.7	27.8	27.6	27.6	27.7	27.9	*ca.* 28	28.4
C-7	27.4	27.2	26.0	27.3	26.1	27.1	25.8	27.3	26.6	*ca.* 26	27.3
C-8	42.6	40.8	40.3	40.9	40.4	40.0	40.1	40.8	40.5	40.3	41.5
C-9	48.6	49.6	48.8 **	49.7	48.8 *	49.0 [f]	47.8 *	49.6 [f]	48.9**	48.7	49.6
C-10	52.8	38.0	38.1	38.0	38.0	37.3	37.4	37.8	37.9	37.9	35.7
C-11	22.0	21.3	21.0	*ca.* 21	*ca.* 21	20.9	20.7	21.3	21.0	*ca.* 21	21.1
C-12	39.5	39.6	38.4	39.7	38.5	38.8	37.8	38.8	38.5	38.4	39.7
C-13	49.4	49.6	48.2	49.6	48.2	49.3	48.1	49.6	48.8	48.0	49.6
C-14	85.0	85.1	81.8	85.4	81.9	83.5	80.7	85.1	81.6	81.7	85.1
C-15	33.1	32.9	75.6	33.0	75.6 [d,f]	32.0	72.0	32.9	73.3	75.4	33.0
C-16	26.9	26.9	34.1 [i]	26.9	34.0	26.4	37.3	26.9	37.1	33.9	26.9
C-17	50.7	50.8	48.6 **	50.8	48.6 *	50.1 [f]	48.3 *	50.8	49.3 **	48.7	50.9
C-18	15.6	15.7	16.0	15.8	16.1	15.6	16.4	15.7	16.5	16.0	15.8
C-19	206.4	13.7	13.9	13.8	14.0	13.5	13.7	13.6	13.8	13.8	12.1
C-20	174.2 *	174.9	172.9	174.6	173.0	176.1	175.4	175.1	173.9	172.8	175.1
C-21	73.5	73.5	73.3	73.5	73.3 [f]	73.1 [f]	73.1 [f]	73.6	73.5	73.2	73.5
C-22	118.0	117.5	118.5	117.7	118.5 [f]	116.2 [f]	116.6 [f]	117.5 [j]	118.1 [j]	118.3	117.4
C-23	173.9 *	174.6 [g]	174.1 [g]	174.6	174.2 [g]	173.7 [g]	173.6 [g]	174.8 [g]	174.5	174.0	174.7 [g]
CH_3CO	20.8	20.7	20.7	21.2	21.1						21.4
	21.6	21.7	21.7								
			21.3		21.2					21.2	
CH_3CO	168.5	168.7 [h]	168.7 [h]	171.9	171.1 [h]						170.6 [h]
	168.8	168.8 [h]	168.9 [h]								
			169.7 [h]		169.8 [h]					169.6	
$(CH_3)_2C$								26.1	26.1	26.1	
								27.9	27.9	27.9	
								109.3	109.3	109.1	

[a] In $CDCl_3$ with δ 77.15 ± 0.10 p.p.m. [b] In CD_3SOCD_3 with δ 39.5 p.p.m. [c] In 20 : 1 v/v $CDCl_3$–CD_3SOCD_3 with δ($CDCl_3$) 77.15 p.p.m. [d] Sharp doublet in single-frequency off-resonance (s.f.o.r.d.) spectra, showing that the attached proton is loosely coupled to vicinal proton(s). [e] Virtual coupling observed in s.f.o.r.d. spectra, showing that the attached proton is tightly coupled to vicinal proton(s). [f] Assignments of ^{13}C and ^1H inter-related by Birdsall-type plots (ref. 18). [g] Doublet in s.f.o.r.d. spectra due to J_{CCH}. [h] Quartet in s.f.o.r.d. spectra due to J_{CCH}. [i] Doublet of doublets in s.f.o r.d. spectra confirming the observation (ref. 11) that one of the C-16 protons is more deshielded than the other by more than 0.5 p.p.m. [j] Doublet of doublets in s.f.o.r.d. spectra. From the magnitude of the splitting, J_{CCCH} between H-17α and C-22 is estimated to be 13 Hz.

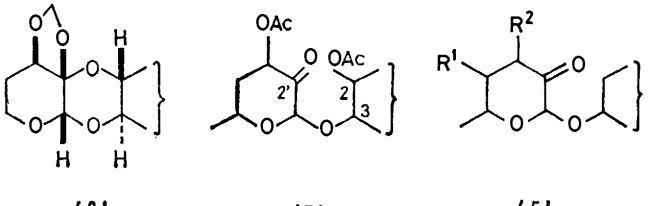
*, ** Values within a vertical column may be interchanged.

conclusion has to be considered as tentative until ^{13}C n.m.r. data are available for compounds of the calotropin series.

The glycoside proceroside was postulated to have the gross structure of calactin (3) or calotropin (4), but with an extra hydroxy-group in rings B or C.[6] We consider that it is more likely to be of the calotropin series than the calactin series, since n.m.r. signals of its carbohydrate protons resemble those of calotropin (4) (see Table 2, items 13 and 14) but not those of gomphoside or formation adopted by the acetoxy-groups in the acetylated derivatives. First, as H-2β on the steroid nucleus bears a 1,3-diaxial relationship with substituents at C-10 and C-2′, small but significant changes are observed in the shielding of H-2β upon conversion of the C-10 methyl into an aldehyde group (Δδ −0.13 p.p.m. for items 1 and 2 *versus* item 5 in Table 2), and on acetylation at C-2′ (Δδ +0.13 p.p.m. for items 8 and 9 *versus* 1 and 2 in Table 2). In contrast, such structural changes on the β-side of the fused ring system do not affect the shielding

of H-3α (δ 4.07 ± 0.03 p.p.m. for items 1, 2, 4, 5, 8, and 9 in Table 2).

Acetylation of 3′-acetylgomphoside (1b) and 3′,15-diacetylafroside (2b) at C-2′ results in a pronounced deshielding (+0.77 p.p.m.) of the equatorial α-proton at C-3′, but causes only small chemical-shift changes on H-1′ (−0.07 p.p.m.) and H-2β (+0.13 p.p.m.) (see item 8 *versus* 1, item 9 *versus* 2 in Table 2). Clearly the 2′-acetoxy-group in the fully acetylated sugar adopts a conformation in which the carbonyl function is directed



towards H-3′α. ^{13}C N.m.r. data support this conclusion. The *γ-gauche* effect of the 2′-acetoxy-carbonyl carbon on C-3′ is −2.5 p.p.m., but only −1.0 p.p.m. on C-1′ [Table 3, (1a) *versus* (1b), (2a) *versus* (2b)].*

Singh and Rastogi [9] observed a strong deshielding (1.0 p.p.m.) of H-1′ upon acetylation of 3′-acetylcalotropin to a diacetate (δ 5.5 p.p.m.). This was attributed to deshielding by an adjacent C-2′ carbonyl group present in the postulated product [partial structure (*E*)] upon the opening of the 2′-hemiacetal.[9] However, in known glycosides with sugars [see (*F*)] closely similar to that in the postulated product, the anomeric H-1′ is not unusually deshielded (δ 4.65—4.70 p.p.m.) [17] We consider it unlikely that ring opening occurred during acetylation by acetic anhydride in pyridine, and offer the following alternative explanation of the deshielding of H-1′. If the diacetate of calotropin exists in a ring-closed form (4a), it is possible that with the 3′-acetoxy-group equatorial, the 2′-acetoxy-group will take up a conformation in which the deshielding effect of its carbonyl group is directed towards the axial protons at C-1′ and C-3′. This will cause strong deshielding of both protons to about the same extent (see items 10 and 7 in Table 2).

^{13}C *N.M.R. Assignments.*—The ^{13}C n.m.r. shieldings collated in Table 3 are based on observed multiplicities, chemical-shift theory, internal consistency, and correlation with chemical shift(s) of attached proton(s) by 'Birdsall plots.' [18] The analysis is initiated by assigning the carbon shifts of 3-acetyluzarigenin (13). As listed in Table 3, last column, the shieldings of ring-A carbons (and C-6) agree with those of 3β-acetoxy-5α-androstane,[19] while shifts of carbons in rings D and C (excluding C-9) are the same as those of the 5-epimer, 3-acetyldigitoxigenin.[13] To eliminate solvent effects, the shieldings compared above, as well as those to be quoted below, refer to $CDCl_3$ solutions.† Comments on the non-trivial assignments of the signals of the glycosides shown in Table 3 are given below.

To differentiate between the 4—5 methine signals in the 65—78 p.p.m. range due to carbons attached to oxygen, line-shape analysis of single-frequency off-resonance spectra becomes useful. Each of carbons 2, 3, and 5′ bears a proton which is tightly coupled to neighbouring ones. Unlike methine carbons 3′ and 15, which give rise to sharp doublets, carbons 2, 3, and 5′ yield doublets broadened by second-order coupling. The signal near 67 p.p.m., which is not affected by structural changes at carbons 2′ and 15, is assigned to C-5′. Distinction between C-2 and C-3 is based on a study of 2,3,15-triacetylafrogenin (2e). In the genin, C-2 and C-3 are assigned shifts of 71.6 and 74.4 p.p.m. respectively,[11] since the former carbon is *γ-gauche* to the C-10 methyl. Comparing the glycosides (1b) and (2b) with the genin (2e), increased *γ-gauche* shielding is expected *both* at C-2 (by 2′-hydroxy) and at C-3 (by pyranose oxygen).

TABLE 4

^{1}H Chemical shifts in [$^2H_5$]pyridine [a]

	H-1′	5′-Me
Gomphoside (1) [b]	5.36	1.36
Afroside (2) [b]	5.37	1.37
Proceroside [c]	4.92	1.33

[a] Relative to $SiMe_4$, measured at 60 or 100 MHz. [b] Ref. 5. [c] Ref 6.

TABLE 5

^{13}C Chemical shifts of methylene C atoms in the range δ 25—30 p.p.m. for solutions in $CDCl_3$ and (in parentheses) [a] [$^2H_5$]pyridine

	3β-Acetoxy-5α-androstane [b]	3-Acetyldigitoxigenin (14)	3-Acetyluzarigenin (13)	3-Acetyl-15α-hydroxyuzarigenin [a]
C-2	27.6	24.8	27.3 (27.8)	(27.7)
C-6	28.7	26.2	28.4 (28.8)	(29.4)
C-7			27.3 (27.8)	(26.9)
C-16		26.7	26.9 (27.3)	

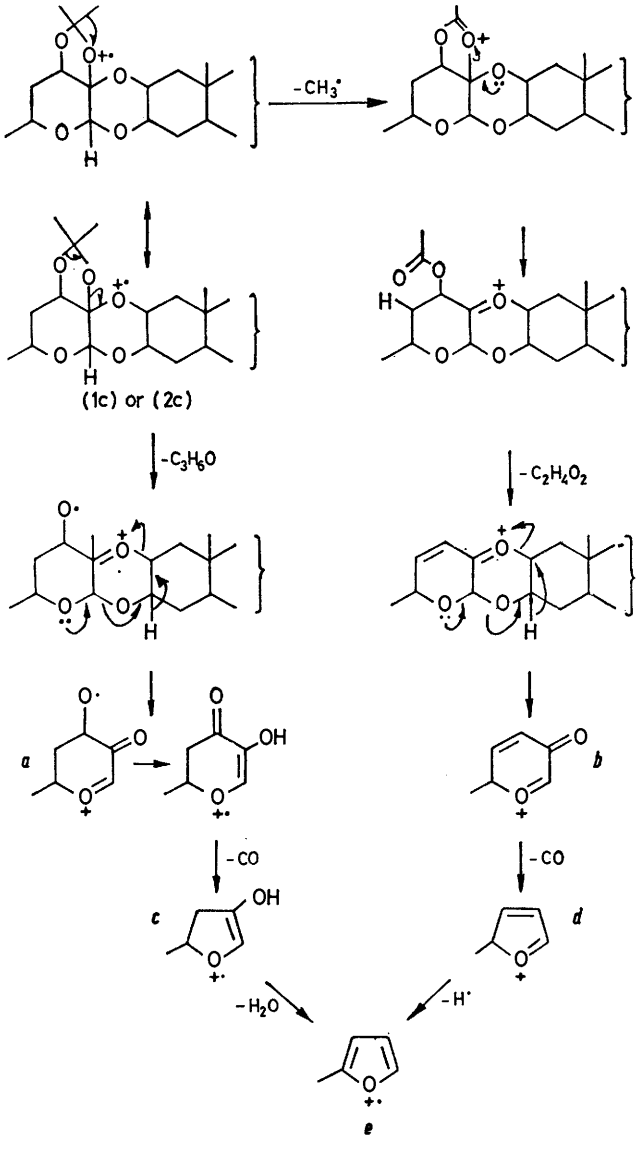
[a] Ref. 21. [b] Ref. 19.

* The effect of a preferentially oriented carbonyl is further seen when 2′,3′-diacetylcalactin (3a) is compared with 2′,3′-diacetylgomphoside (1a). In the former compound C-1, which is *γ-gauche* to the C-19 carbonyl oxygen, is shielded by 6 p.p.m., while H-1 (at δ 2.47 with $J_{1\beta,1\alpha}$ 12.7 Hz and $J_{1\beta,2\beta}$ 4.6 Hz) is deshielded by at least 0.4 p.p.m. We thanks Professor T. Reichstein for a copy of the 270-MHz spectrum of (3a).[7]

† Compared with $CDCl_3$ solutions, chemical shifts (relative to $SiMe_4$) are *ca.* 1 p.p.m. upfield for CD_3SOCD_3 solutions [see (1) and (2) in Table 3]; and about 0.5 p.p.m. downfield for 3 : 2 $CDCl_3$–CD_3OD and for pentadeuteriopyridine solutions, which were used by Tori *et al.*[20] and Yamauchi *et al.*[21] in their studies on 5β- and 5α-cardenolides respectively.

Resonances of these glycosides near 69.3 and 72.3 p.p.m. are thus assigned to carbons 2 and 3 respectively.

Each of the glycosides listed in Table 3 shows four upfield methine signals (in the range 40—51 p.p.m.) of which that due to C-17 is recognized from 'Birdsall plots' [18] since this carbon carries a relatively deshielded proton. The observed effects of a 15β-hydroxy- or acetoxy-group (*viz.* none at C-5, *ca.* —0.5 p.p.m. at C-8, *ca.* —1 p.p.m. at C-9, and *ca.* — 2 p.p.m. at C-17) are in agreement with the assignments.



SCHEME

Of the methylene signals in the range 26—28 p.p.m. that which is most deshielded and which remains within 0.1 p.p.m. of 27.8 p.p.m. for all glycosides in Table 3 is assigned to C-6. That occurring at 26.9 p.p.m. for gomphoside derivatives is assigned to C-16 since an identical resonance is found for both 3-acetyluzarigenin (13) and its 5-epimer 3-acetyldigitoxigenin (14) (Table 5). The remaining signal, which is due to C-7, is more shielded in afroside derivatives having a 15β-acetoxy-function [26.0—26.1 p.p.m. for (2a), (2b), and (2d)] than in the gomphoside analogues [27.2—27.3 p.p.m. for (1a), (1b), and (1c)]. The corresponding δ shielding effect of the 15β-hydroxy-group on C-7 is 0.7 p.p.m. [(2c) *vs.* (1c)], and is similar in magnitude to the effect of a 15α-hydroxy-group (0.9 p.p.m.). Models show that these epimeric hydroxy-groups are both subjected to non-bonded interaction with C-7. The latter substitutent effect is derived from the data of Yamauchi *et al.*[21] as re-assessed by us (see Table 5).

Mass Spectra of the 2′,3′-OO-Isopropylidene Derivatives of Gomphoside and Afroside.—The electron-impact mass spectra of (1c) and (2c) are characterized by the high relative intensity of a number of low-mass ions which originate from the carbohydrate (see Experimental section).

A proposal for the origin of ions *a*—*e* is given in the Scheme.

EXPERIMENTAL

^{1}H N.m.r. spectra were determined either at 270 MHz on a Bruker WP-270 Fourier-transform (FT) spectrometer (at the Australian National N.M.R. Centre) or at 100 MHz on a Varian HA-100 continuous-wave instrument. ^{13}C N.m.r. data were collected on a Varian CFT-20 (20 MHz) FT spectrometer. N.O.e. measurements were carried out in the continuous-wave mode at 100 MHz, using *ca.* 0.2M-solutions in deuteriochloroform which were degassed. Mass spectra were obtained, using A.E.I. MS-902 and MS-30 spectrometers, from solid samples heated to 200 °C and ionized at 70 eV.

Formation of 2′,3′-OO-Isopropylidene Derivatives of Gomphoside (1) *and Afroside* (2).—A solution of gomphoside (1) (250 mg), 2,2-dimethoxypropane (2.0 ml), and toluene-*p*-sulphonic acid (2 mg), in dry acetone (150 ml) was refluxed until t.l.c. showed the complete disappearance of gomphoside. The chilled reaction mixture, after being treated with cold aqueous sodium hydrogencarbonate, was extracted with chloroform (3 × 50 ml). The washed (H_2O) and dried (Na_2SO_4) extract was evaporated to give 2′,3′-OO-*isopropylidenegomphoside* (1c) (265 mg), m.p. 265—267 °C (from aqueous methanol), *m/e* 543.295 (0.5%, M^+ — Me), 500.279 (0.5, $M^+ - C_3H_6O$, *m** 448), 483.277 (2, [543 — $C_2H_4O_2$]$^+$), 454.271 (10, [$C_{28}H_{38}O_5$]$^+$), 427.249 (8, [$C_{26}H_{35}O_5$]$^+$), 373.238 (10, [$C_{23}H_{33}O_4$]$^+$), 355.227 (13, [$C_{23}H_{31}O_3$]$^+$), 128.048 (8, [$C_6H_8O_3$]$^+$, *a*), 111.045 (18, [$C_6H_7O_2$]$^+$, *b*), 100.052 (53, [$C_5H_8O_2$]$^+$, *c*), 83.049 (62, [C_5H_7O]$^+$, *d*), and 82.042 (100, [C_5H_6O]$^+$, *e*) (Found: C, 68.9; H, 8.5. $C_{32}H_{46}O_8$ requires C, 68.8; H, 8.3%).

On similar treatment, afroside (2) yielded 2′,3′-OO-*isopropylideneafroside* (2c) (95%), m.p. 278—284 °C, *m/e* 499.268 (2%, M^+ — Me — $C_2H_4O_2$), 481.256 (4, [499 — H_2O]$^+$), 480.249 (2, [$C_{29}H_{36}O_6$]$^+$), 470.267 (10, [$C_{28}H_{38}O_6$]$^+$), 452.256 (6), 433 (7), 128.049 (10, [$C_6H_8O_3$]$^+$, *a*), 111.044 (19, [$C_6H_7O_2$]$^+$, *b*), 100.052 (52, [$C_5H_8O_2$]$^+$, *c*), 83.049 (55, [C_5H_7O]$^+$, *d*), 82.042 (100, [C_5H_6O]$^+$, *e*) (Found: C, 67.1; H, 8.3. $C_{32}H_{46}O_9$ requires C, 66.9; H, 8.1%).

On treatment with acetic anhydride (0.5 ml) in pyridine at room temperature for 16 h, compound (2c) (222 mg) yielded 2′,3′-OO-*isopropylidiene*-15-*acetylafroside* (2d) (180 mg), m.p. 234—236 °C (Found: C, 66.3; H, 7.6. $C_{34}H_{48}O_{10}$ requires C, 66.2; H, 7.8%). ^{1}H N.m.r. data of the

above three isopropylidene derivatives are listed in Tables 2 and 6.

Removal of the OO-*Isopropylidene Group*.—2′,3′-*OO*-isopropylidenegomphoside (1c) (23 mg) in anhydrous methanol (50 ml) containing toluene-*p*-sulphonic acid (5 mg) was stored at 15—20 °C for 10 d. The mixture was concentrated under reduced pressure to 10 ml, diluted with water, and extracted with chloroform. On evaporation of the washed (aqueous sodium hydrogencarbonate, then H_2O) and dried chloroform solution, gomphoside (1) (18 mg) was obtained which had ^1H n.m.r. and infrared spectra identical with those of an authentic sample.

TABLE 6

Chemical shifts of protons in the ring-D region

Compound	H-15	H-18	H-21 [a]	H-22 [a]	Ac
(1c)		0.87	4.76, 5.03	5.84	
(2c)	4.55	0.93	4.81, 5.05	5.86	
(2d)	5.44	0.93	4.79, 5.05	5.88	2.11

[a] $J_{21.21}$ 18; $J_{21.22}$ 1.5 Hz.

3′-*Acetylgomphoside* (1b).—This was obtained as one of the products of the acetylation of gomphoside with acetic anhydride in pyridine,[22] m.p. 204—205 °C (from aqueous methanol) (Found: C, 63.9; H, 8.0. $C_{31}H_{44}O_9 \cdot H_2O$ requires C, 64.3; H, 8.0%).

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Note added in Proof.—After submission of this paper, the *X*-ray analysis of the 5β-cardenolide affinoside B (from the Apocynaceae) was reported (T. Yamauchi, K. Miyahara, F. Abe, and T. Kawasaki, *Chem. Pharm. Bull.*, 1979, **27**, 2463) showing that the 3-*O*-methyl-4,6-dideoxy-2-hexosulose group therein has the same stereochemistry as shown here for the sugar in gomphoside.

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