Afroside, a 15β-Hydroxycardenolide

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Afroside (2) has the same carbohydrate as gomphoside (1), namely a 4,6-dideoxyhexosulose doubly linked to the aglycone at the 2α and 3β positions. It differs from gomphoside in having a 15β-hydroxy-group, the location of which is shown by n.m.r. (^{1}H and ^{13}C) and mass spectra, and is established by the formation of a 14,15-cyclic carbonate derivative (2e). The 14β,15β-diol group shows unusual inertness to glycol cleavage and to *OO*-isopropylidene derivative formation, in contrast with the behaviour of the 2′,3′-diol in the carbohydrate, and the 2α,3β-diol in the genin, afrogenin (4). Degradation of the carbohydrate in afroside gave afrogenin (4), and 'anhydroafrogenin' (9) which is a 15-ketone with 14α-H. The conformation of the highly crowded ring D is studied using ^{1}H and ^{13}C n.m.r.

THE cardiac glycosides afroside (2) and gomphoside (1) are constituents of 'milk-weed' or 'cotton bush' (*Asclepias fruticosa*, L., previously *Gomphocarpus fruticosus*, R.Br.; Asclepiadaceae) growing wild in Australia.[1,2] The structure of gomphoside was first proposed by Coombe and Watson from degradative studies.[2] Subsequently Reichstein and his co-workers [3] partially synthesised gomphogenin, the corresponding aglycone, from uzarigenin (5) thereby confirming its structure as 2α,3β,14β-trihydroxy-5α-card-20(22)-enolide (3). The structure proposed for the glycoside, gomphoside, contained the then unique carbohydrate, a 4,6-dideoxyhexosulose which was doubly linked to the aglycone through 2α- and 3β-hydroxy-groups. This carbohydrate, and modified forms of it, was identified subsequently as the sugar of a number of *Calotropis* and *Asclepias* glycosides.[4-6] Our recent studies using ^{1}H and ^{13}C n.m.r. measurements have defined the stereochemistry of the carbohydrate of gomphoside, and that of most of the *Calotropis* and *Asclepias* cardiac glycosides.[4,5] This communication gives evidence for the structure of afroside and of the aglycone afrogenin (4).

RESULTS AND DISCUSSION

Afroside.—The carbohydrate of afroside (2) is identical in structure and stereochemistry with that of gomphoside (1). This is shown by the isolation of the same compounds from the oxidative degradation of the sugar (see Experimental section), by the comparison of ^{1}H and ^{13}C n.m.r. spectra,[4] and by the formation of the same fragment ions under the same conditions in electron impact and chemical ionisation mass spectrometry (see below).

Afroside, $C_{29}H_{42}O_9$, differs from gomphoside, $C_{29}H_{42}O_8$, by having an extra, readily acetylated secondary hydroxy-group in the aglycone. The work described below establishes the position and configuration of that hydroxy-group to be 15β, so that afroside is 15β-hydroxygomphoside (2).

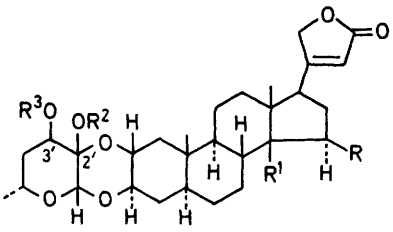
Gomphoside (1) and afroside (2), each with a *cis*-2′,3′-diol system on the sugar, formed the respective mono-*OO*-isopropylidene derivatives (1c) and (2c) when refluxed with acetone and acetone dimethyl acetal in the presence of toluene-*p*-sulphonic acid.[4] On acetylation gomphoside (1) yielded a 3′-monoacetate (1b),[4] and a 2′,3′-diacetate (1a).[2] Under similar conditions afroside forms a diacetate and a triacetate, while 2′,3′-*OO*-isopropylideneafroside gives a monoacetate. Comparing the ^{1}H n.m.r. spectra of corresponding pairs of compounds from the gomphoside and afroside series (Table 1), the extra hydroxy-group in afroside is characterised by a multiplet signal (width *ca.* 16 Hz) due to a carbinol proton (>C*H*–OH) at δ 4.5. Even at 270 MHz, this signal (and the corresponding one at δ 5.45 for the acetate derivatives) has a characteristic line-shape typical of virtual coupling to a proton on the β carbon. Since analogous ^{1}H signals are observed for the aglycone afrogenin and its triacetate (see below), this hydroxy is on the steroid nucleus. Evidence is given below that it is at position 15. For derivatives of both gomphoside and afroside, 17-H (which is allylic) resonates near δ 2.7. In the case of the afroside series, the signal near δ 2.7 consists of a two-proton multiplet (even at 270 MHz) due to two tightly coupled protons (17- and 16-H). Decoupling experiments † show that the carbinol proton (>C*H*–O–) is coupled to these two protons at δ 2.7, *and* to another 16-H resonating to higher field. The 'extra' hydroxy of afroside is thus determined to be at C-15, a conclusion which is supported by mass spectral data, discussed further below.

It is of interest that of the two 14,15-diols (as the 15-acetates) obtained by Reichstein and his co-workers [7] upon osmium tetraoxide oxidation of the Δ^{14}-cardenolide (7) the major one, assigned the 14β,15β-configuration [see (6)], showed in the published 100-MHz n.m.r. spectrum multiplet signals (for 15-H, and for 17- plus 16-H) which are similar in line-shape and chemical shift (see Table 1) to those of the afroside derivatives as discussed above.

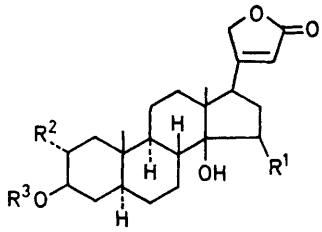
A β-configuration for the 15-hydroxy-group is indicated by the n.m.r. data of Reichstein and his co-workers [7] as cited above. For a definitive conclusion we turn to chemical evidence. The 14,15-diol group in afroside is stable to conditions under which the 2′,3′-diol forms

† Carried out at 100 MHz on diacetylafroside (2b) and on 2′,3′-*OO*-isopropylideneafroside (2c).

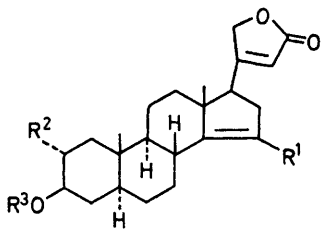
an isopropylidene derivative (see above). However, a 14,15-cyclic carbonate derivative (2e) was readily formed in good yield when 2′,3′-*OO*-isopropylidene-afroside (2c) was treated with phosgene in pyridine. The carbonate carbonyl resonates at δ 159 p.p.m. (Table 2) and absorbs at 1 785 cm^{-1} in the i.r. That the hydroxy-group at C-14 has the β-configuration is shown by measurements of lanthanide-induced n.m.r. shifts and signal-broadening described later. The formation of a cyclic carbonate is evidence that 15-hydroxy is also β. Afroside is thus 15β-hydroxy-gomphoside (2).



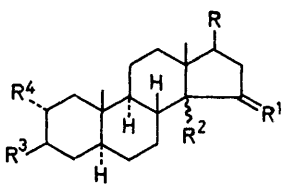
	R^3	R^2	R^1	R
(1)	H	H	OH	H
(1a)	Ac	Ac	OH	H
(1b)	Ac	H	OH	H
(1c)	—C(Me)$_2$—		OH	H
(2)	H	H	OH	OH
(2a)	Ac	Ac	OH	OAc
(2b)	Ac	H	OH	OAc
(2c)	—C(Me)$_2$—		OH	OH
(2d)	—C(Me)$_2$—		OH	OAc
(2e)	—C(Me)$_2$—		—O—C(=O)—O—	



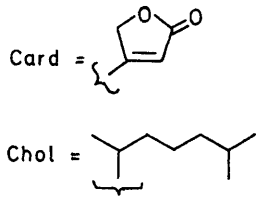
	R^3	R^2	R^1
(3)	H	OH	H
(4)	H	OH	OH
(4a)	Ac	OAc	OAc
(5)	H	H	H
(6)	Ac	H	OAc



	R^3	R^2	R^1
(7)	Ac	H	H
(8)	H	OH	H
(8a)	Ac	OAc	H
(9′)	H	OH	OH



	R^4	R^3	R^2	R^1	R
(9)	OH	OH	α-H	O	Card
(9a)	OAc	OAc	α-H	O	Card
(10)	OH	OH	β-OH	O	Card
(11)	H	H	α-H	O	H
(12)	H	H	α-H	O	Chol
(13)	OH	OAc	α-H	H_2	Card



The reluctance of the 14β,15β-diol to form an isopropylidene derivative is paralleled by its inertness to periodate. The quantitative oxidation of afroside (over 40 h at room temperature) required 1.14 mol of sodium metaperiodate per mol of the glycoside, the consumption of oxidant being mainly for cleavage of the 2′,3′-diol group in the carbohydrate moiety. Under similar conditions (but over 72 h) gomphoside took up 1.07 mol equiv. of periodate.[2]

The relative inertness of the 14β,15β-diol system may be ascribed to the high degree of steric crowding on the β-face of ring D. Shoppee and his co-workers in their work on digacetigenin observed that a mixture of diols resulting from the sodium borohydride reduction of the 14β-hydroxy-15-ketone system was oxidised very slowly if at all by periodate in methanol at 20°.[8]

Afrogenin and ' Anhydroafrogenin '.—A characteristic property of the *Calotropis* and *Asclepias* glycosides is their resistance to acid hydrolysis. Forcing conditions lead to extensive decomposition resulting in extremely complex mixtures of products and great loss of material.[2] The carbohydrate of afroside was therefore removed by periodate oxidation to the aldehyde (18) followed by acid hydrolysis, as described for the degradation of gomphoside.[2] From the hydrolysis, afrogenin, $C_{23}H_{34}O_6$, and ' anhydroafrogenin ', $C_{23}H_{32}O_5$, were isolated. Afrogenin forms a triacetate on acylation under mild conditions, and ' anhydroafrogenin ' a diacetate when treated similarly. From the mode of formation, and by comparison of the ^{13}C and ^1H n.m.r. spectra (Tables 1 and 2) the structure of afrogenin, *viz.* 2α,3β,14β,15β-tetrahydroxy-5α-card-20(22)-enolide (4), follows from that established [2,3] for gomphogenin (3). With gomphoside, dehydration at C-14 during the degradation gave rise to β-anhydrogomphogenin (8) which has Δ^{14}-unsaturation.[2] In the case of afroside, dehydration leads to a 15-ketone (9) *via* the 14-enol (9′). Thus the ^{13}C n.m.r. data of ' anhydroafrogenin ' diacetate (9a)

reveal the presence of a keto-group resonating at δ 211 p.p.m., and are in agreement with the formulation of ' anhydroafrogenin ' as 2α,3β-dihydroxy-15-oxo-5α,14α-card-20(22)-enolide (9). Analysis of the ^{13}C chemical shifts, particularly in relation to the assigned 14α-configuration, is presented in the next section The presence of one vicinal diol in ' anhydroafrogenin ' was confirmed by oxidation with periodate. The specific rate constant for lead tetra-acetate oxidation was found to be 1.40×10^{-3} l mol^{-1} s^{-1}, a value which compares very closely with that of known 2α,3β-diols of the 5α-steroids.[10]

From a similar degradation of afroside, but with a Jones oxidation between the periodate cleavage and the acid hydrolysis (see Experimental section), the 15-ketone analogue of afrogenin, *viz.* 15-oxogomphogenin (10), was obtained.

^{13}C *N.M.R.*—The ^{13}C chemical shifts of genins of the afroside and gomphoside series, as well as for the 14,15-carbonate (2e) are listed in Table 2. The assignments shown are based on multiplicities, chemical shift theory. internal consistency, and comparison with our assignments of the corresponding glycoside derivatives, the derivation of which had been fully discussed.[4]

Comparing derivatives of the afroside (and afrogenin) series with the gomphoside (and gomphogenin) analogues (Table 2; and ref. 4. Table 3), shift differences (≥0.3 p.p.m.) due to the additional oxygen function in the former compounds are observed at only certain carbons (see Table 3). As signal assignments are unambiguous for the quaternary carbons C-13 and -14, the pronounced perturbations observed at C-14 and -16 constitute evidence for a 15-hydroxy in afroside. As expected, C-16 becomes shielded by 3 p.p.m. upon acetylation at C-15 (Table 3), while the formation of a 14,15-carbonate is accompanied by strong deshielding of C-14 (by 16.5 p.p.m.) and C-15 (by 6.1 p.p.m.) (Table 2).

As discussed earlier, evidence that ' anhydroafrogenin ' (9) is a 15-ketone comes from the chemical shift of C-15 (δ 211 p.p.m.). Furthermore, ^{13}C shieldings provide information on the C–D junction. The substituent effect of a 15-oxo group (particularly for carbons in rings B and C) is expected to be sensitive to the configuration at C-14. In Table 4 this substituent effect, as derived from 5α,14α-androstan-15-one (11) [11–13] and 5α,14α-cholestan-15-one(12),[11,12] is compared with that estimated from ' anhydroafrogenin ' diacetate (9a). The agreement between these two sets of 15-oxo-substituent effects is compatible with a 14α-configuration for ' anhydroafrogenin '. In another approach, the substituent effect of a 17β-butenolide ring on rings C and D carbons, as derived from appropriate 14α-steroids, is compared with the shift difference between ' anhydroafrogenin ' diacetate (9a) and another 15-ketone, *viz.* androstan-15-one (11). The agreement shown in Table 4 serves to confirm that ' anhydroafrogenin ' is C–D-*trans*.

Conformation of Ring D.—As the 14,15-glycol group of

TABLE 1

^{1}H Chemical shifts of genins or of aglycone portions of glycosides [a]

Compounds	Functional groups at positions: 2α	3β	14	15	15α-H	16α- and 17α-H	18-H (13-Me)	19-H (10-Me)	21-H	22-H	$CH_3CO{\cdot}O-$
(8) [9]	OH	OH	Δ[14]		5.23m	2.80 (17-H)	0.82	0.88	4.78 4.78	5.88	
(8a) [2]	OAc	OAc	Δ[14]		5.24 [c]		0.82	0.94	4.76 4.76	5.89	2.02 2.02
(9a)	OAc	OAc	α-H	C=O			0.70	0.94	4.80 4.80	5.88	2.01 2.01
(10) [d]	OH	OH	β-OH	C=O			0.72	0.88	4.96 4.96	6.02	
(4) [e]	OH	OH	β-OH	β-OH	4.50m [h]	*ca.* 2.65m [i]	0.94	0.85	4.89 5.10 [j]	5.88	
(4a)	OAc	OAc	β-OH	β-OAc	5.44m [h]	*ca.* 2.7m [i]	0.93	0.94	*ca.* 4.8 *ca.* 5.1	5.90	2.02 2.02 2.12
(6)[7]	H	OAc	β-OH	β-OAc	5.4m [h]	*ca.* 2.7m	0.93	0.82	*ca.* 4.9	5.83	1.96 2.05
(2) [b,f,g]	Sugar		β-OH	β-OH	4.49m [h]	*ca.* 2.6m [i]	0.91	0.87	4.83 5.09 [j]	5.84	
(2b) [b,g]	Sugar		β-OH	β-OAc	5.47 [h]	*ca.* 2.7m [i]	0.93	0.86	4.80 5.08 [j]	5.89	2.11 2.13
(2a) [b]	Sugar		β-OH	β-OAc	5.46 [h]	*ca.* 2.7m [i]	0.93	0.83	4.81 5.04 [j]	5.89	2.06 2.07 2.10
(2c) [b]	Sugar		β-OH	β-OH	4.52m [h]	2.6m [i]	0.93	0.88	4.87 5.01 [j]	5.86	
(2d) [b]	Sugar		β-OH	β-OAc	5.44m [h]	2.7m [i]	0.93	0.87	4.79 5.05 [j]	5.88	2.11
(2e)	Sugar		–O–CO–O–		5.01d [k]	*ca.* 2.8m	1.16	0.89	4.79 4.79	5.98	

[a] Measured relative to $SiMe_4$ in $CDCl_3$ at 100 MHz unless otherwise stated. [b] For carbohydrate protons of glycosides (2)—(2d) see ref. 4. [c] $J_{15,16\alpha} + J_{15,16\beta}$ *ca.* 6 Hz. [d] In CD_3SOCD_3. [e] In CD_3OD–$CDCl_3$ (1 : 3) mixture. [f] 10% CD_3SOCD_3 added. [g] 270 MHz. [h] For appearance of this multiplet signal, see text. [i] Multiplet due to tightly coupled protons, see text. [j] AB quartet (J_{AB} 19.5 Hz) further split (*ca.* 2 Hz) by 21-H. [k] $J_{15\alpha,16\alpha}$ 6.5, $J_{15\alpha,16\beta} < 1$ Hz.

afroside shows unusual inertness, n.m.r. coupling, shift, and relaxation parameters were used to establish the β configuration of 14-hydroxy and to compare the conformation of ring D in afroside derivatives with that in gomphoside. Derivatives of afroside with the hydroxy-groups at 2′,3′, and 15 protected would complex with lanthanide chelates predominately at 14-hydroxy, yielding stereochemical information on ring D from the paramagnetic shift induced by a shift reagent, and from the relaxation rate changes caused by a relaxation reagent. The latter results will be discussed here, and the former in the next section. Relaxation rate changes induced by $Gd(fod)_3$ (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionatogadolinium), as measured by broadening of ^1H signals, are expected to have an inverse sixth-power dependence upon the Gd–H distance.[15] Thus another chelating hydroxy-group at the other end of the molecule, *viz.* at C-2′, will not affect the relative signal broadening for ring D protons. Pronounced broadening of the 22-H signal was observed for 3′-acetylgomphoside (1b) (Table 5). This indicates that in solution the orientation of the lactone ring is similar to that in the solid state as determined by *X*-ray analysis on digitoxigenin [(5), but 5β],[19] *viz.* with 22-H approximately above C-15. The vicinal coupling constants between 17-H and the protons at C-16, *viz.* 5 and 8.5 Hz [as measured on 2′,3′-diacetylgomphoside (1a) at 270 MHz] are also compatible with the *X*-ray results,[16] which showed that ring D is an α-envelope with C-14 out of the plane formed by C-13, -17, -16, and -15.

On addition of a 15β-acetoxy onto the crowded β face of ring D, a conformational change is discerned. Thus the vicinal couplings between 17-H and the C-16 protons

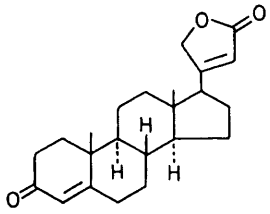
TABLE 2

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

Carbon	(3) [b]	(8a) [a]	(4a) [a]	(9a) [a]	(2e) [a]
C-1	45.3	42.2	42.4	42.3	42.1
C-2	72.7	71.6	71.6	71.7	71.8
C-3	76.1	74.3	74.4	74.3	72.4
C-4	35.6	32.6	32.5	32.7	31.9
C-5	44.6	43.5	43.5	44.2	44.1
C-6	28.1 *	27.1	27.5	27.1	26.6 *
C-7	27.5 *	29.5	25.9	31.8	25.6 *
C-8	40.9	34.4	40.3	30.1	37.9
C-9	50.0	53.2 *	48.6	53.8	48.9
C-10	37.7	37.1	37.4	37.2	37.9
C-11	21.5	21.7	*ca.* 21	20.8	20.5
C-12	40.0	40.9	38.3	38.1	37.9
C-13	50.0	48.4	48.1	43.2	49.6
C-14	85.2	153.3	81.9	65.0	98.4
C-15	32.8	117.1 [d]	75.6	211.4	81.7
C-16	27.2	33.5	34.1	39.1	35.5
C-17	51.2	52.5 *	48.6	46.2	50.0
C-18	15.9	18.2	16.0	14.3	17.3
C-19	13.3	12.5	13.1	12.9	13.7
C-20	176.5	170.4	172.7	167.6 *	169.6 †
C-21	74.2	73.4 [d]	73.3	73.4	73.4
C-22	117.4	116.2 [d]	118.6	117.6	118.2
C-23	176.1 [c]	173.8 [c]	*e*	173.1 *	173.0 †
–OCOCH$_3$		21.0	21.2	21.1	
		21.0	21.2	21.1	
			21.2		
–OCOCH$_3$		170.4	170.5	170.6	
		170.4	170.5	170.6	
			169.7		
C-1′					97.9
C-2′					96.4
C-3′					77.4
C-4′					33.8
C-5′					68.0
C-6′					20.9
–O–CO–O–					158.9
CH$_3$ \ C / O–					109.4
CH$_3$ / O–					
CH$_3$ \ C / O–					26.2, 27.9
CH$_3$ / –					

*,† Signals within a vertical column may be reversed.

[a] In $CDCl_3$ solvent with δ 77.1 p.p.m. [b] In CD_3OD–$CDCl_3$ with δ 77.6. [c] Doublet in single-frequency off-resonance spectrum due to J_{CCH}. [d] Assignment of ^{13}C and ^1H inter-related by Birdsall-type plot.[14] [e] Not observed.



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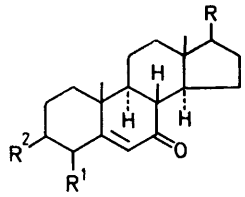
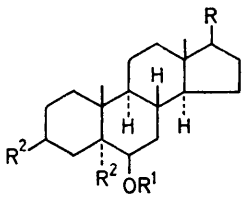
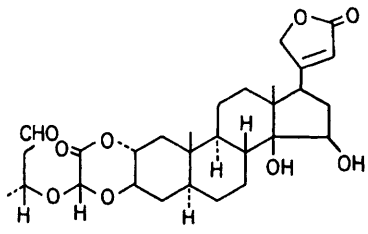


	R^2	R^1	R
(15a)	β-OAc	H	Card
(15b)	π-bond		H



	R^2	R^1	R
(16a)	OH	H	Card
(16b)	OH	Ac	Card
(17a)	H	H	H
(17b)	H	Ac	H



(18)

TABLE 3

Effect of 15-substituents on ^{13}C shieldings (in p.p.m., $CDCl_3$ solvent) for cardenolides with 14β-hydroxy-groups

	C-7	C-8	C-9	C-12	C-13	C-14	C-16	C-17
Δδ (15β-hydroxy) [(2c) *versus* (1c)] [a]	−0.7	−0.5	−0.7	−1.3	−0.8	−3.5	+10.2	−1.9
Δδ (15β-acetoxy) [(2a) *versus* (1a); (2b) *versus* (1b)] [a]	−1.2	−0.5	−0.9	−1.2	−1.4	−3.4	+7.1	−2.0

change to 10 Hz (to the deshielded 16-H at δ 2.7) and <1 Hz. These extreme values rule out the possibility that ring D exists as interconverting forms, and show that the protons on C-16 and C-17 are no longer eclipsing.* The change in $Gd(fod)_3$-induced signal broadening (see Table 5) shows that although the configuration of the 14-hydroxy remains β, there is a small increase in the Gd–22-H distance (see Table 6) relative to the Gd–13-Me one. However, it does not necessarily follow that the 14-OH–22-H distance is increased, since an altered LIS for the carbohydrate protons. The LIS values for ring D protons confirm the β-configuration of the 14-hydroxy. However as these are little affected by the presence of a 15-acetoxy (see item 4 *versus* item 5; item 1 *versus* item 3), little conclusion may be drawn on the influence of this group on ring D conformation.

An identical ring D is present in substrates (2d and b), and parallel sets of LIS for protons of the ring D region are obtained (see items 3 and 4). The ratio between LIS of individual protons from the two sets, *viz.* 0.75,

TABLE 4

Effects of 15-oxo and 17β-butenolide groups on ^{13}C shieldings (in p.p.m.) for 14α-steroids [a]

	C-7 [c]	C-8 [c]	C-9	C-11	C-12	C-13	C-14	C-16	C-17	C-18
(a) Δδ (15-oxo)										
5α,14α-Androstan-15-one (11) [12,13] *versus* 5α,14α-androstane	−1.9	−3.5	−0.1	−0.5	+0.4	−1.6	+8.8	+14.6	−5.2	+0.7
5α,14α-Cholestan-15-one (12) [12] *versus* 5α,14-cholestane	−1.4	−3.5	−0.4	−0.4	−0.1	−0.2	+9.4	+12.7	−4.7	+1.0
'Anhydroafrogenin' diacetate (9a) *versus* steroid (13) [b]	+0.1	−4.9	−0.5	−0.6	+0.2	−1.1	+9.2	+13.2	−4.5	+1.0
(b) Δδ[17β-(2,5-dihydro-5-oxo-3-furyl)]										
Steroid (15a) [18] *versus* steroid (15b) [19]				+0.5	−1.5	+3.2	−0.1	+5.2	+11.7	−4.2
Steroid (16a) [18] *versus* steroid (17a); [19] (16b) [18] *versus* (17b) [20]				+0.5	−0.5	+4.2	+1.7	+5.8	+10.8	−4.0
'Anhydroafrogenin' diacetate (9a) *versus* 5α,14α-androstan-15-one (11) [12,13]				+0.4	−1.3	+4.0	+1.5	+4.0	+10.9	−4.0

[a] Data refer to $CDCl_3$ solvent except for compounds (16a and b) which were run in CD_3OD–$CDCl_3$ (3 : 7).[15] [b] Shieldings shown for steroid (13) are estimated by modifying those of steroid (14) with the appropriate effects [11] of structural change at ring A. [c] The slight variation in the extent of the γ- and δ-effects of the 15-oxygen on C-7 and -8 probably reflects the perturbation of C-17 and ring A substituents on these carbons, known to be as much as 1—2 p.p.m. (see p. 463 of ref. 11).

location of the lanthanide atom (*e.g.*, upon bidentate co-ordination) cannot be excluded.

The C-17—C-20 torsion angle appears to be little affected by an extra hydroxy-function at 15β. Thus for both gomphoside and afroside [and for (2c)] the observed coupling between 17α-H and C-22 (*ca.* 14 Hz) implies that these two nuclei are approximately *anti*, in agreement with the *X*-ray results for digitoxigenin.[16] A Karplus relationship is generally accepted for the angle-dependence of J_{COCH}.[21]

Lanthanide-induced Shifts (*LIS*).—Substrates (1b) and (2b) have free hydroxy-groups at both C-2′ and -14; the LIS for the carbohydrate protons (see identical values in the lower part of Table 7) are caused mainly by complexation at the 2′-hydroxy, while the LIS for protons in the ring D region (upper part of Table 7) are caused by 14-hydroxy. On the other hand, the isopropylidene derivatives (2d) and (1c) have only one free hydroxy-group (at C-14), and yield small but identical corresponds to the fraction of the lanthanide reagent which complexes with the 14β-hydroxy-group.

Electron Impact Mass Spectra of Afrogenin and Derivatives.†—The base peak in the spectrum of afrogenin (4) at *m/e* 296 is due to the loss of 110 a.m.u. from the molecular ion. Although cardenolides do not commonly yield an $(M - 110)^+$ ion, most do form the $(M - 110 - H_2O)^+$ ion (usually <20% abundance). Based on labelling experiments, Flaskamp and Budzikiewicz [22] proposed structures for these ions the generation of which involves rupture of the 13,17- and 15,16-bonds. The hydroxy-group at C-15 in afrogenin (4) would facilitate this fragmentation, and resonance-stabilise the resulting $(M - 110)^+$ ion (Scheme 1). Retention of the 15-functional group in the $(M - 110)^+$ ion derived from 15-hydroxy- and 15-acetoxy-cardenolides was observed by Fayez and Negm.[23] A high abundance of an *m/e* 111 ion, formed by a McLafferty rearrangement and first formulated as *a* by Shannon,[24] is also observed for afrogenin. Further support to the presence of a 15-hydroxy-group in afrogenin comes from the observation of ions of *m/e* 140 and 266 $(M - 140)^+$ due to cleavages at 14,15 as is proposed in Scheme 1.

The most abundant fragment ion, *m/e* 266, from compound (10), the 15-oxo-analogue of afrogenin, arises by the loss of $C_7H_6O_3$ (138 a.m.u.) from the molecular ion. Another major ion, *m/e* 248, is the dehydrated

* Values of $J_{16,17}$ were derived by saturation of 15α-H, and analysis of the AB of ABX signals due to 17- and 16-H in a $Eu(fod)_3$-shifted spectrum of 2′,3′-*OO*-isopropylidene-15-acetylafroside (2d). From 3′,15-diacetylafroside (2b) values of $J_{15,16}$ were estimated to be *ca.* 6.5 Hz (derived from the 15-H doublet upon saturation of the deshielded 16-H and of 17-H) and *ca.* 9 Hz (from collapse of the signal due to the deshielded 16-H on saturation of 15-H). An α-configuration is assigned to the deshielded 16-H (at δ 2.7) based on these coupling constants. It is possible that 16α-H is deshielded by van der Waals repulsion resulting from increased folding of rings C and D;[17] the complementary shielding at C-9 and -12 may be discerned (ref. 4, Table 3).

† For full spectral data see Experimental section.

TABLE 5

Broadening of half-height width of ^1H signals by $Gd(fod)_3$ [a]

	Ring D region						[Carbohydrate region]			
Substrate	22-H	21-H (low-field)	21-H (high-field)	18-H	17α-H	15β-OAc	1′-H	3′-H	19-H (10-Me)	3′-OAc
3′-Acetylgomphoside (1b) [b] observed	2.15	0.35	0.36	*d*	*d*		0.24	1.18	0.04	0.77
normalised	2.87 [e]	0.47 [e]	0.48 [e]	*d*	*d*		0.96 [f]	4.72 [f]	0.16 [f]	3.08 [f]
2′,3′-*OO*-Isopropylidenegomphoside (1c) [c]	2.92	0.50	0.45	0.16	0.11					
2′,3′-*OO*-Isopropylidene-15-acetylafroside (2d) [c]	1.97	*ca.* 0.52 [d]	0.14	0.30	*d*	0.44				

[a] Expressed as Hz per 10^{-4} mol (with respect to substrate) of added $Gd(fod)_3$; and measured by sequential addition of μl quantities of a *ca.* 3×10^{-3}M $CDCl_3$ solution of $Gd(fod)_3$ to a $CDCl_3$ solution containing the substrate and $Eu(fod)_3$ and determination of the slope of the resulting linear plot of signal broadening *versus* $Gd(fod)_3$ concentration. Correction is made for intermolecular paramagnetic relaxation [as indicated by signal broadening of $CHCl_3$, and in the case of substrates (1c) and (2d), also of 1′-H] which is less than 0.1 Hz per 10^{-4} mol. $[^2H_{27}]Eu(fod)_3$ was used with substrate (1c). [b] 0.11M-Substrate (1b), 0.05M-$Eu(fod)_3$. [c] 0.08M-Substrate, 0.04M-$Eu(fod)_3$. [d] Data subject to error due to signals overlapping. [e] Normalised by 1/0.75 to account for the decreased *effective* concentration of $Gd(fod)_3$ with respect to the 14β-OH, part of the reagent being taken up by the 2′-OH of this substrate. From lanthanide-induced shift experiments, 75% of the reagent is co-ordinated with 14β-OH. [f] Normalised by 1/0.25; see note *e*.

TABLE 6

Estimated *relative* Gd–H distances (in arbitrary units) [a]

	Ring D region						Carbohydrate region			
Substrate	22-H	21-H (low-field)	21-H (high-field)	18-H	17α-H	15β-OAc	1′-H	3′-H	19-H (10-Me)	3′-OA
(1c), (1b)	4.2	5.6	5.7	6.8	7.2		5.0	3.9	6.8	4.2
(2d)	4.5	5.6	6.9	6.1		5.7				

[a] Calculated from the signal-broadening data (in Hz per 10^{-4} mol) of Table 5, and expressed as K/(signal broadening)$^{1/6}$, K being taken arbitrarily as 5.0.

TABLE 7

Lanthanide-induced shifts of ^1H in p.p.m. per mol equiv. of shift reagent [a]

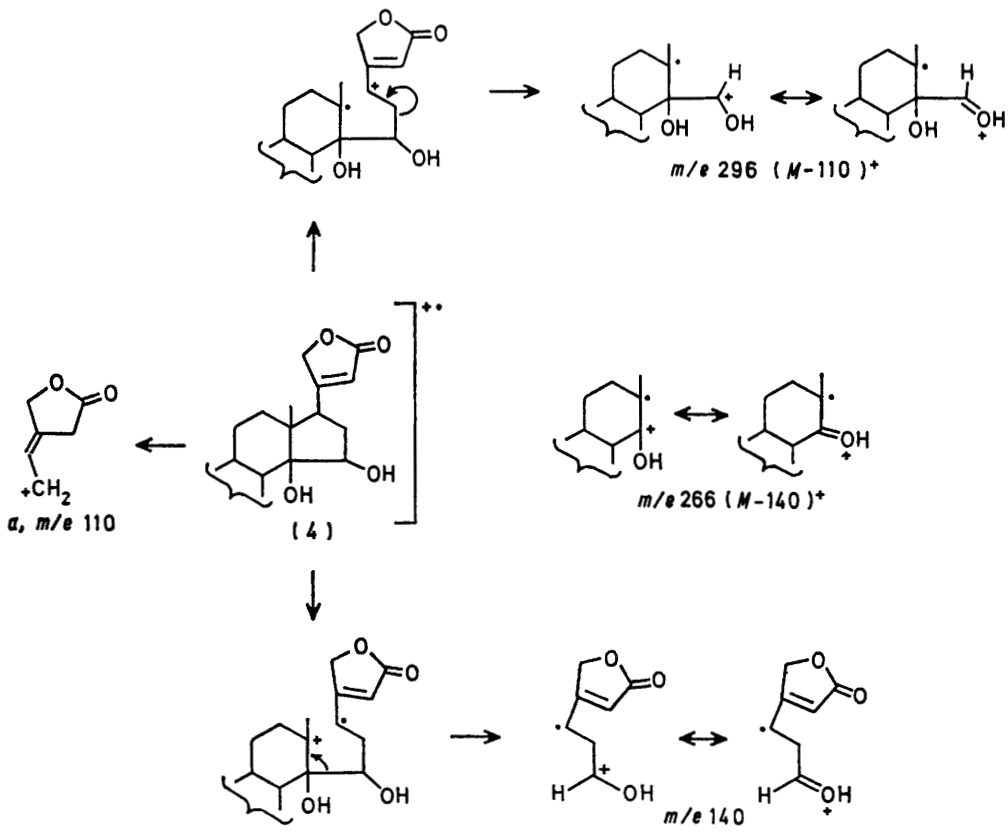
			Ring D region					
Item	Substrate	Shift reagent	22-H	21-H (low-field)	21-H (high-field)	18-H (13-Me)	16α- and 17α-H	15β-OAc
1	3′-Acetylgomphoside (1b)	$Eu(fod)_3$	3.3	2.2	2.1	*ca.* 0.65	*ca.* 0.7	
2	3′-Acetylgomphoside (1b)	$[^2H_{27}]Pr(fod)_3$	−3.45	−1.9	−1.9	−0.7	*ca.* −0.7	
3	3′,15-Diacetylafroside (2b)	$Eu(fod)_3$	3.0	2.2	2.2	0.75	*ca.* 0.7	1.65
4	2′,3′-*OO*-Isopropylidene-15-acetylafroside (2d)	$Eu(fod)_3$	3.85	3.0	2.9	1.0	*ca.* 1.15	2.2
5	2′,3′-*OO*-Isopropylidene-gomphoside (1c)	$[^2H_{27}]Eu(fod)_3$	3.9	2.7	2.7	1.0	*ca.* 1.0	
	Ratio $\frac{\text{item 3}}{\text{item 4}}$		0.78	0.72	0.76	0.75		0.74

			Carbohydrate region						
Item	Substrate	Shift reagent	1′-H	3′-H	5′-H	6′-H (5′-Me)	19-H (10-Me)	3′-OAc	$(CH_3)_2C<^{O-}_{O-}$
1	3′-Acetylgomphoside (1b)	$Eu(fod)_3$	1.7	3.8	b	0.4	0.25	2.3	
2	3′-Acetylgomphoside (1b)	$[^2H_{27}]Pr(fod)_3$	−1.75	−3.8	*ca.* −1.05	−0.35	−0.25	−2.25	
3	3′,15-Diacetylafroside (2b)	$Eu(fod)_3$	1.8	3.8	*ca.* 1.05	0.3	0.25	2.35	
4	2′,3′-*OO*-Isopropylidene-15-acetylafroside (2d)	$Eu(fod)_3$	0.55	*ca.* 0.15	b	*ca.* 0.15	*ca.* 0.15		*ca.* 0.05
5	2′,3′-*OO*-Isopropylidene-gomphoside (1c)	$[^2H_{27}]Eu(fod)_3$	0.55	*ca.* 0.15	b	*ca.* 0 05	*ca.* 0.15	*ca.* 0.15	*ca.* 0.05

[a] Quoted to nearest 0.05 p.p.m.; + and − refer to downfield and upfield shifts respectively. [b] Subject to error due to overlapping of signals.

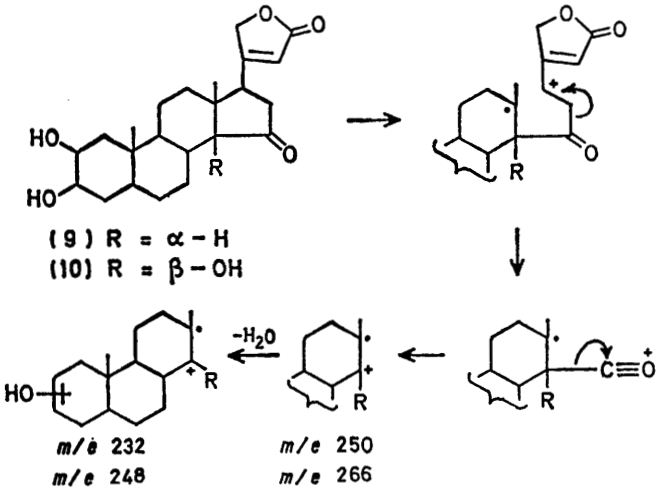
analogue. For the 14H-15-ketone ' anhydroafrogenin ' (9) * the loss of 138 a.m.u. is also a highly favoured process. This yields an ion at *m/e* 250, and by loss of water from ring A, at *m/e* 232 (100%). As proposed in afrogenin (4). Likewise *a*, *m/e* 111, is formed as for afrogenin.

Hydrogen Chemical Ionisation Mass Spectra.—Chemical ionisation with hydrogen as reactant gas



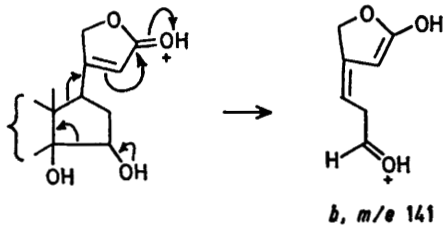
SCHEME 1 Fragmentation mechanisms suggested for afrogenin (4). Bond cleavage at 13,17 could occur later than shown

Scheme 2, formation of these ions from the 15-ketones (10) and (9), involving cleavages at 13,17 and 14,15 is similar to the production of the $(M - 140)^+$ ion from



SCHEME 2 Fragmentation mechanisms suggested for 15-oxo-cardenolides. Bond cleavage at 13,17 could occur later than shown

(H_2CI) gives more useful information on cardenolide glycosides than by electron impact. Hydrogen with higher energy produces more fragment ions than the commonly used methane, and the quasimolecular ion $(MH)^+$ is readily detected [as are those due to the elimination of H_2O or CH_3COOH or $(CH_3)_2CO$].



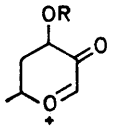
SCHEME 3

Using afrogenin (4) as a model, the major ions are found to result from the successive loss of 4 H_2O from $(MH)^+$ (*m/e* 407). Ions corresponding to the loss from $(MH)^+$ of 140 a.m.u., together with 1—3 molecules of

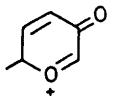
* Mechanisms offered to rationalise the spectrum of ' anhydroafrogenin ' as given in ref. 24 were based on earlier erroneous hypothesis for the structure.

water, are important. A prominant ion of *m/e* 141 is probably formed as shown in Scheme 3.

The H_2CI spectrum of afroside (2) shows a prominent ion at *m/e* 407 which corresponds to the quasimolecular ion of the genin [referred to hereafter as $(G + H)^+$], and ions due to successive loss from it of 4 H_2O. 3′,15-Diacetylafroside (2b) likewise forms an ion of *m/e* 449 due to the quasimolecular ion of the 15-acetylated genin [referred to as $(G' + H)^+$], and ions resulting from the



c, R = Ac, *m/e* 171
d, R = H, *m/e* 129



e, *m/e* 111

subsequent and successive loss of acetic acid and water. A peak at *m/e* 171 in the spectrum of 3′,15-diacetylafroside (2b) is attributed to the ion *c* derived from the carbohydrate; it is found also in 2′,3′-diacetylgomphoside (1a).[6] For afroside (2), the analogous ion *d* gives rise to the base peak, *m/e* 129. The dehydrated form of ion *d*, *viz.* ion *e* (*m/e* 111) is also observed, but this may arise partly as the isomeric ion *a* from ring D cleavage.

EXPERIMENTAL

General laboratory procedures are as in ref. 4. Specific rotations refer to 0.5—1.5% solutions. For details of n.m.r. experiments using lanthanide reagents, see footnotes to the relevant Tables. For ^1H and ^{13}C n.m.r. data see Tables 1 and 2. Details of the hydrogen chemical ionisation (H_2CI) mass spectra are given at the end of this section, but *m/e* listed under individual compounds refer to electron impact.

Separation of Cardenolides.—The crude glycosides (15 g) of *Asclepias fruticosa* were subjected to partition chromatography as described earlier [2] yielding, in order of elution: uzarigenin (5) (1 g); gomphoside (1) (2 g); afroside (2) (10 g), m.p. 259—264 °C, $[\alpha]_D$ −20° (pyridine) (Found: C, 64.8; H, 7.0. Calc. for $C_{29}H_{42}O_9$: C, 65.1, H, 7.0%).

Di- and Tri-acetates of Afroside.—Afroside (1 g) in pyridine (10 ml) and acetic anhydride (10 ml) was allowed to stand at room temperature for two days. It was poured into an ice–water mixture and the precipitate collected was crystallised repeatedly from ethanol to give 2′,3′,15-triacetylafroside (2a) [1] as plates, m.p. 204—206 °C $[\alpha]_D$ −20° (chloroform), and 3′,15-*diacetylafroside* (2b), m.p 228—230 °C (Found: C, 62.3; H, 7.6. $C_{33}H_{46}O_{11},H_2O$ requires C, 62.4; H, 7.4%. Found: *M*H by H_2CI, 619.315. $C_{33}H_{47}O_{11}$ requires *M*H, 619.312)

Reaction of Afroside with Sodium Metaperiodate.—(a) Afroside (22 mg, 0.413×10^{-4} mol) was dissolved in methanol (10 ml) and sodium metaperiodate (40.5 mg, 1.89×10^{-4} mol) in water (1 ml) was added. The solution was allowed to stand at room temperature for 48 h. Sodium arsenite (0.049M, 20 ml), excess of sodium hydrogencarbonate, and then 0.6M-potassium iodide (1 ml) solutions were added, and the mixture titrated after 20 min with standard 0.1M aqueous iodine. The amount of sodium metaperiodate consumed was 0.468×10^{-4} mol.

(b) To afroside (160 mg) in methanol (90 ml) and water (10 ml) was added sodium metaperiodate (0.50 g). After 16 h at room temperature the solution was filtered and the methanol removed under vacuum while water (30 ml) was added gradually. The chloroform extract of the resulting aqueous solution was dried and evaporated. The amorphous aldehyde product (18) (143 mg), $\nu_{max.}$(KCl) 3 500, 3 320, 1 745, 1 720, and 1 625 cm^{-1}, gave strong reducing tests with ammoniacal silver nitrate and Fehling's solution, and was converted into the mono-2,4-dinitrophenylhydrazone derivative (60 mg), m.p. 133—135 °C after chromatography on Bentonite–Celite (Found: C, 58.0; H, 6.3; N, 8.0. Calc. for $C_{35}H_{44}O_{12}N_4$: C, 59.0; H, 6.2; N, 7.9%).

(c) The amorphous aldehyde (18) isolated as in (b) was dissolved in a mixture of methanol (30 ml) and 2M-sulphuric acid (30 ml) and refluxed for 4 h. The mixture was steam-distilled into a trap containing 2,4-dinitrophenylhydrazine reagent, and the precipitate obtained was chromatographed on neutral alumina to give crotonaldehyde 2,4-dinitrophenylhydrazone (60 mg), m.p. 195 °C, $\lambda_{max.}$(ethanol) 374 nm ($\log_{10}$ ε 4.40), identical with (i.r., u.v., and mixed m.p.) an authentic sample (Found: C, 48.7; H, 4.0; N, 22.1. Calc. for $C_{10}H_{10}O_4N_4$: C, 48.0; H, 4.0; N, 22.4%).

(d) The aldehyde (18) isolated as in (b) was dissolved in ethanol (80 ml) containing 2,4-dinitrophenylhydrazine (96 mg). Hydrochloric acid (10M, 22 ml) was added and the solution refluxed for 2 h. The ethanol was removed by distillation under vacuum at room temperature, water (30 ml) was added gradually, and the solution extracted with chloroform. The combined chloroform extract was repeatedly washed with 0.1M-sodium hydrogencarbonate. The aqueous solution was extracted with chloroform after acidification, dried, and evaporated. The yellow residue obtained was chromatographed on Florisil to give glyoxylic acid 2,4-dinitrophenylhydrazone (16 mg), m.p. 188—190 °C, identical with an authentic sample (i.r., u.v., and mixed m.p.).

2′,3′-OO-*Isopropylideneafroside* 14,15-*Carbonate* (2e).—To an ice-cold solution of 2′,3′-*OO*-isopropylideneafroside (2c) [4] (254 mg) in dry pyridine (0.5 ml) was added a 20% solution of phosgene in toluene (0.5 ml) over a period of 10 min. The solution was allowed to warm to room temperature, and more phosgene solution (0.5 ml) was added over 30 min. The mixture was diluted with ice–water and extracted with chloroform (3 × 10 ml). The chloroform extract was washed with hydrochloric acid (4M), saturated aqueous sodium hydrogencarbonate, and water, then dried (Na_2SO_4) and evaporated to yield crude product (207 mg, 78%). Crystallisation from methanol–acetone gave 2′,3′-OO-*isopropylideneafroside* 14,15-*carbonate* (2e), m.p. 297 °C, $\nu_{max.}$(Nujol) 1 785 and 1 730 cm^{-1} (Found: C, 65.9; H, 7.3. $C_{33}H_{44}O_{10}$ requires C, 66.0; H, 7.4%).

Afrogenin (4), '*Anhydroafrogenin*' (9), *and their Acetates.*—The aldehyde (18) obtained as in (b) from afroside (470 mg) (but with reaction in aqueous ethanol) was dissolved in ethanol (30 ml), 2M-sulphuric acid (30 ml) was added, and the solution was refluxed for 2 h. The volatile fragments (see above) were removed by steam distillation, and on cooling a crop of crystals (148 mg) was collected. After several days a second crop (82 mg) was obtained.

The first crop was recrystallised from methanol–water to give '*anhydroafrogenin*' [2α,3β-*dihydroxy*-15-*oxo*-5α,14α-*card*-20(22)-*enolide*] (9), m.p. 289—301 °C, $[\alpha]_D$ −28° (pyridine); $\nu_{max.}$(KCl) 3 400, 1 780, 1 750, 1 710, 1 640, and 1 060 cm^{-1}; *m/e* 388 (15%, $M^{+\cdot}$), 370 (35, $M - H_2O^+$), 352 (13, $M - 2H_2O^+$), 250 (26, $M - 138^+$), 233 (18), 232 (67,

250 — H_2O^+), 231 (9), 137 (100), and 111 (70) (Found: C, 70.6; H, 8.3. $C_{23}H_{32}O_5$ requires C, 71.1; H, 8.3%). The second crop was recrystallised from ethanol to give *afrogenin* [2α,3β,14β-*trihydroxy*-5α-*card*-20(22)-*enolide*] (4), m.p. 250—254 °C, $[\alpha]_D$ +25° (methanol); *m/e* 406 (8%, $M^{+\cdot}$), 388 (9, *M* — H_2O^+), 297 (19), 296 (100, *M* — 110$^+$), 278 (12, 296 — H_2O^+), 266 (17, *M* — 140$^+$), 196 (20), 178 (12), 140 (9), 111 (40), and 101 (47) (Found: C, 68.3; H, 8.6. $C_{23}H_{34}O_6$ requires C, 68.0; H, 8.4%).

Afrogenin (4) (120 mg) was allowed to stand in dry pyridine (2 ml) and acetic anhydride (2 ml) for 16 h. The crude acetate (137 mg) was chromatographed on neutral alumina (10 g) and crystallised from methanol–water to give *2,3,15-triacetylafrogenin* [14β-*hydroxy*-2,3,15-*triacetoxy*-5α-*card*-20(22)-*enolide*] (4a), m.p. 238—240 °C, $[\alpha]_D$ −71° (chloroform) (Found: C, 64.9; H, 7.5; Ac, 24.0. $C_{29}H_{40}O_9$ requires C, 65.4; H, 7.6; 3Ac, 24.2%). 'Anhydroafrogenin' (9) (60 mg) was similarly acetylated to give, on crystallisation from chloroform–light petroleum, *2α,3β-diacetoxy*-15-*oxo*-5α,14α-*card*-20(22)-*enolide* ('*anhydroafrogenin' diacetate*) (9a) (61 mg), m.p. 258—260°, $[\alpha]_D$ +15° (chloroform) (Found: C, 68.3; H, 7.6. $C_{29}H_{38}O_8$ requires C, 67.7; H, 7.4%).

Reactions of 'Anhydroafrogenin' with Sodium Metaperiodate and with Lead Tetra-acetate.—'Anhydroafrogenin' (9) (22 mg, 0.56 × 10^{-4} mol) was oxidised by sodium metaperiodate as described above for afroside. The amount of periodate consumed after 20 h was 0.70 × 10^{-4} mol.

Oxidation of 'anhydroafrogenin' by lead tetra-acetate at 25 °C was carried out under conditions identical with those for the oxidation of β-anhydrogomphogenin (8), and the specific rate constant was found to be 1.40 × 10^{-3} l mol^{-1} s^{-1}.

15-Oxogomphogenin.—The crude aldehyde (18) (5 g) from periodate oxidation of afroside (see above) was dissolved in acetone (300 ml) and was titrated with Jones reagent until a permanent brown colour was obtained. After addition of methanol (0.5 ml) to discharge the excess of chromic acid, the mixture was diluted with water (2 l), extracted with chloroform (6 × 100 ml) and the chloroform solution worked up to give a crude product (2 g). Nitrogen was bubbled through a solution of this product (2 g) in ethanol (80 ml) containing 10M-hydrochloric acid (10 ml). After 16 days at room temperature the mixture was diluted with water (1 l) and extracted with chloroform (6 × 100 ml). The chloroform solution was repeatedly washed with 0.6M-aqueous sodium hydrogencarbonate and worked up to yield a gum (1.2 g). On chromatography on acid-washed alumina, *15-oxo-2α,3β,14β-trihydroxy*-5α-*card*-20(22)-*enolide* (*15-oxogomphogenin*) (10) was obtained as plates from ethanol, m.p. 262—268 °C; $\nu_{max.}$ 1 735 and 1 710 cm^{-1}; *m/e* 404.219 0 (12%, $M^{+\cdot}$) ($C_{23}H_{32}O_6$ requires *M*, 404.219 9), 386 (4, *M* — H_2O^+), 376 (3, *M* — CO^+), 267 (18), 266.188 (100, *M* — $C_7H_6O_3^+$), 248.177 (9, $C_{16}H_{24}O_2^+$), 163 (3), 138 (7), and 111 (8); o.r.d. (*c* 0.054 in dioxan), amplitude −5.05 × 10^6 °mol^{-1}, λ_0 321 nm.

Hydrogen Chemical Ionisation Mass Spectra.—Afrogenin (4), *m/e* 407 (76%, *M*H$^+$), 389 (62, *M*H — H_2O^+), 371 (100, *M*H — $2H_2O^+$), 353 (87, *M*H — $3H_2O^+$), 335 (25, *M*H — $4H_2O^+$), 249 (18, 389 — 140$^+$), 231 (35, 249 — H_2O^+), 213 (20, 231 — H_2O^+), 141 (65, *b*), and 111 (13, *a*); afroside (2), *m/e* 535 (3%, *M*H$^+$), 517 (1, *M*H — H_2O^+), 407 (82, *M*H — 128$^+$, *G* + H$^+$), 389 (60, *G* + H — H_2O^+), 371 (76, *G* + H — $2H_2O^+$), 353 (29, *G* + H — $3H_2O^+$), 337 (8), 335 (8, *G* + H — $4H_2O^+$), 249 (7), 231 (20), 213 (10), 141 (35, *b*), 129 (100, *d*), 111 (15, *a/e*), 101 (8), and 83 (15); 2′,3′-*OO*-isopropylideneafroside (2c), *m/e* 575 (38%, *M*H$^+$), 557 (24, *M*H — H_2O^+), 539 (9, *M*H — $2H_2O^+$), 517 [76, *M*H — $(CH_3)_2CO^+$], 499 [38, 557 — $(CH_3)_2CO^+$], 481 (9), 389 (57, 499 — 110$^+$), 371 (28), 129 (38, *d*), and 59 (100); 3′,15-*diacetylafroside* (2b), *m/e* 619 (38%, *M*H$^+$), 601 (30, *M*H — H_2O^+), 559 (17, *M*H — HOAc$^+$), 541 (29, 601 — HOAc$^+$), 523 (2, *M*H — $2H_2O$ — HOAc$^+$), 499 (3, *M*H — 2HOAc$^+$), 481 (8, 523 — 42$^+$); 449 (8, *G*′ + H$^+$), 431 (9), 413 (4), 389 (3), 371 (8), 353 (6), 171 (19, *c*), 129 (25, *d*), 111 (3, *a/e*), and 43 (100); 2′,3′-*OO*-isopropylideneafroside 14,15-carbonate (2e), *m/e* 601 (27%, *M*H$^+$), 585 (2), 557 (2, *M*H — 44$^+$), 543 [16, *M*H — $(CH_3)_2CO^+$], 539 (14, 557 — H_2O^+), 525 (2, 585 — 60$^+$), 499 (5), 481 (5), 415 (11, 543 — 128$^+$), 353 (9), 349 (9), 335 (5), 331 (9), 273 (61), 213 (14), 153 (11), 129 (14, *d*), 111 (18, *a/e*), 101 (5), 83 (11), 69 (30), and 59 (100).

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