Cardenolide Glycosides with 5,6-Unsaturation from *Asclepias vestita*

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Asclepias vestita from Commatti Canyon in California has yielded nine new 19-oxo cardenolide glycosides and one genin, all with the 5(6) double-bond, *viz* 5,6-dehydrocalactin, 5,6-dehydrocalotropin, 5,6-dehydroasclepin, 5,6-dehydrouscharidin, 5,6-dehydrocalatoxin, 16α-hydroxy-5,6-dehydrocalotropin, 16α-acetoxy-5,6-dehydrocalotropin, 16α-hydroxy-5,6-dehydroasclepin, 16α-acetoxy-5,6-dehydroasclepin, and the genin, 5,6-dehydrocalotropagenin. Most of these Δ^5-cardenolide glycosides were accompanied by the known 5α(H) analogues. Also found were two 5α-cardenolides with a 19-hydroxy group, *viz.* the glycoside 19-dihydrocalotropin and the known genin coroglaucigenin. The position of the unsaturation in the Δ^5-cardenolides was located by comparison of the 400 MHz n.m.r. data with those of the 5α-analogues, with consideration of the anisotropy effect of the Δ^5 double-bond. A pair of model Δ^5-19-oxo and 5α(H)-19-oxo steroids have also been studied for comparison.

Asclepias vestita Hook and Arn. of the Asclepiadacea family (milkweeds) is one of the foodplants of the monarch butterfly (*Danaus plexippus* L.).[1,2] Defence of the insect against vertebrate predators is provided by cardioactive cardenolide glycosides sequestered by the larvae from milkweeds.[3] *A. vestita* is notable in that specimens from three distinct geographical regions within California showed remarkable differences in total cardenolide glycoside content.[2] In continuation of our studies comparing the cardenolide glycosides from *Asclepias* plants with those from the insect which feed on them,[4–7] we recently described several new 5α(H)-cardenolide glycosides from *A. vestita* from Kimbler Ranch in California.[1] In this paper we report the structure of 11 new cardenolides from the same species but collected at Commatti Canyon in California. Many of these are unusual in possessing a Δ^5 function which is not found to a significant extent in cardenolides present in samples from the other two regions.

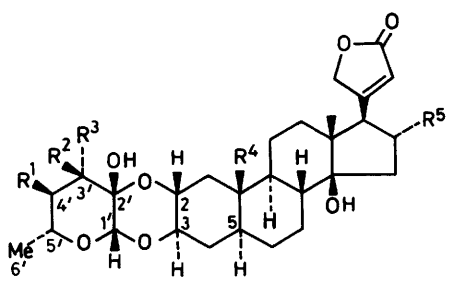
Most *A. vestita* cardenolide glycosides are related to gomphoside (**1**) which is one of the most potent inotropic glycosides known, causing the same increase in contractility of guinea pig heart at a fraction of the dose of the heart drug digoxin from *Digitalis spp.*[8] Unlike the *Digitalis* glycosides, gomphoside and its congeners are characterised by double-linkage of a 5α-steroid aglycone at 3β and 2α to a 6-deoxyhexosulose sugar *via* acetal and hemiacetal groups.[9]

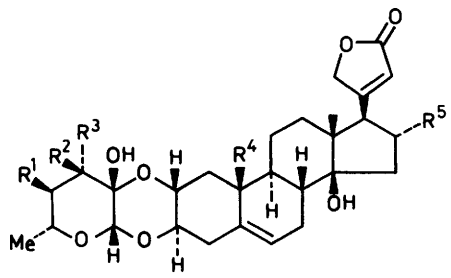
Among glycosides in *A. vestita* from all three regions are the known 19-oxo glycosides calactin (**2**) and calotropin (**3**), which are epimeric at position 3′,[10] and asclepin (calotropin 3′-acetate) (**5**).[11] The crude cardenolides of *A. vestita* from Commatti Canyon were separated by preparative thin-layer chromatography (t.l.c.) and high-pressure liquid-chromatography (h.pl.c.). The major fractions were calactin (**2**) and 5,6-dehydrocalactin (**2a**) in a ratio of *ca.* 1:3, and calotropin (**3**) and 5,6-dehydrocalotropin (**3a**) in a similar ratio. Unambiguous evidence is given below for the location of the Δ^5 double-bond in these two dehydrocaredenolide glycosides. Later in the paper, analogous 5,6-unsaturated structures are deduced for other new compounds (**5a**)—(**7a**), (**9a**)—(**12a**), and (**14a**). Since most compounds were obtained in sub-milligram quantities, structural elucidation has been based on careful comparison of the high-flux ^{1}H n.m.r. and the chemical ionisation (c.i.) mass spectral data with those of a series of known cardenlides.

The Δ^5 double-bond is a structural feature which is unique among Asclepiadaceae cardenolide glycosides with doubly linked sugars. Such glycosides had hitherto been shown by *X*-ray crystallographic analyses to possess double-bonds at positions 9(11),[12] 7,[13] and 4.[14] Nevertheless the Δ^5-19-oxo functionality does occur in the cardenolide genin pachygenin (**16a**),[15] and is common among pregnane glycosides.[16]

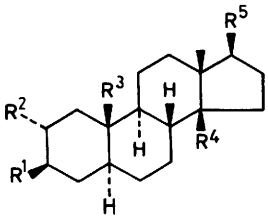
5,6-*Dehydrocalactin and* 5,6-*Dehydrocalotropin.*—The methane c.i. mass spectrum of each of the above-titled major dehydrocardenolide glycosides (**2a**) and (**3a**) (Table 1) shows protonated ions for the cardenolide glycoside molecule (*m/z* 531) as well as for the corresponding genin (*m/z* 403). These ions are 2 a.m.u. lower than the corresponding ions given by calactin (**2**) or calotropin (**3**) (Table 1). Identical sets of ions at *m/z* 129 and 111 originating from the doubly linked carbohydrate[6] were given by all four glycosides, showing gross structural identity of the carbohydrate moieties.

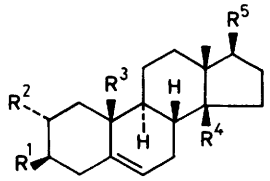
The ^{1}H n.m.r. spectra of the two dehydrocardenolide glycosides are generally similar to those of calactin (**2**) and calotropin (**3**), but show one extra vinyl proton signal at δ 6.10 (half height width 9.5 Hz). However, at 400 MHz, pronounced chemical shift changes compared with calactin/calotropin are observed for the 2β and 19 protons, which we show are due to the anisotropy effect of a Δ^5 double-bond. To draw structural information from the shift changes, we comment first on the 400 MHz spectra of calactin and calotropin (Table 2). In the region δ 3.5—4.8 are signals of protons on carbon bearing oxygen, *viz.* protons 2β and 3α on the steroid aglycone, and protons 1′, 3′, and 5′ on the carbohydrate. The axial 3′β-hydroxy group in calactin (**2**) causes 1,3-diaxial deshielding of the protons at 1′ and 5′, the signals of which are respectively 0.2 and 0.5 p.p.m. downfield of those in the 3′-epimer calotropin (**3**). Contrary to our earlier comments,[9] structural change at C-3′ can have a subtle effect on the chemical shift of the "remote" aglycone proton at 2β. Thus the signal of this proton in calactin (**2**) is 0.15 p.p.m. upfield of that in calotropin (**3**). This may be related to the conformation of the 2′-hydroxy group (which is 1,3-diaxial





	R^1	R^2	R^3	R^4	R^5	
(1)	H	OH	H	Me	H	
(2)	H	OH	H	CHO	H	(2a)
(3)	H	H	OH	CHO	H	(3a)
(4)	H	H	OAc	Me	H	
(5)	H	H	OAc	CHO	H	(5a)
(6)	H	—O—		CHO	H	(6a)
(7)	OH	OH	H	CHO	H	(7a)
(8)	OAc	OAc	H	CHO	H	(8a)
	H	H	OH	CHO	OH	(9a)
(10)	H	H	OH	CHO	OAc	(10a)
(11)	H	H	OAc	CHO	OH	(11a)
(12)	H	H	OAc	CHO	OAc	(12a)
(13)	H	H	OH	CH_2OH	H	





	R^1	R^2	R^3	R^4	R^5	
(14)	OH	OH	CHO	β-OH		(14a)
(15)	OH	H	CH_2OH	β-OH		
	OH	H	CHO	β-OH		(16a)
(17)	OAc	H	CHO	α-H		(17a)

to the 2β proton) as influenced by the 3′-hydroxy group in the two 3′-epimers (*cf.* Figure, but 5,6-saturated). Operation of such factors in influencing the relative ease of hemiacetal cleavage at 1′ in related cardenolide glycosides with 3′α and 3′β-hydroxy groups had been proposed.[17]

For the four cardenolide glycosides (**2**), (**3**), (**2a**) and (**3a**), ^1H–^1H decoupling was used to assign the signals of the protons at 1α, 1β, 2β and 3α (Table 2). For all four, the β proton at C-1 resonates near δ 2.45, some 0.6 p.p.m. downfield compared to the same proton in gomphoside (**1**) which is the 19-methyl analogue of calactin (**2**). This proton is in the deshielding region of a preferentially oriented C-19 formyl carbonyl (*cf.* Figure), a conclusion well supported by ^{13}C n.m.r.[9] As collated in Table 4. a striking 0.27 p.p.m. difference in the chemical shift of the aldehyde proton (19-H) is observed when this signal in calactin and calotropin (δ 10.01) is compared with that in 5,6-

Table 1. Methane chemical ionisation mass spectral ions[a]

	MH^+	$-H_2O$	−HOAc	GH^+	$-H_2O$	$-2H_2O$	−HOAc	SH^+	$-H_2O$	$-COCH_2$
(14a)	(see GH^+)			403	385	367				
(2)/(3)	533[b]	515		405[b]	387	369		129[b]	111	
(2a)	531[b]	513		403[b]	385	367		129[b]	111	
(3a)	531[b]	513[c]		403[b]	385	367		129[b]	111	
(6a)	529[b]	511		403	385	367		127[b]		
(5a)	573	555	513	403[b]	385	367		171[b]		129
(7a)				403[b]	385	367		145[b]	127	
(8a)	631[b]	613[d]	571[d]	403	385	367		229		
(9a)				419[b]	401	383		129[b]	111	
(11a)	589			419	401	383		171[b]		129
(10a)				461	443[e]		401[e]	129[b]	111	
(12a)	631[b]	613	571	461[b]	443			171[b]		129
(13)	535			407[b]	389	371		129[b]	111	
(15)	(see GH^+)			391[b]	373	355				

[a] MH^+, GH^+ and SH^+ refer to protonated molecular, genin, and sugar ions, respectively. [b] Accompanied by the corresponding (weaker) $C_2H_5^+$ and $C_3H_5^+$ adducts ions. [c] Also found were ions at *m/z* 495 and 477 by further loss of H_2O. [d] Further loss of HOAc led to ions at *m/z* 553 and 511. [e] Loss of H_2O or HOAc from these ions gave rise to an ion at *m/z* 383.

Table 2a. Chemical shifts (with half-height widths in Hz) of cardenolides with oxygen substituent at 2α[a] (*continued over*)

Proton	Gomphoside (1)	Calactin (2)	5,6-*Dehydrocalactin* (2a)	Calotropin (3)	5,6-*Dehydrocalotropin* (3a)	3′-*epi*-Gomophoside 3′-acetate (4)	Asclepin (5)	5,6-*Dehydroasclepin* (5a)	Uscharidin (6)	5,6-*Dehydrouscharidin* (6a)	Calotoxin[i] (7)
1-H_α	1.12t	1.13t	1.10dt[b]	1.11t	1.09dt	1.12t	1.14t	1.11dt	1.12t		1.09t
1-H_β	1.83dd	2.45dd	2.42dd	2.47dd	2.45dd		2.50dd	2.49dd	2.45dd	2.42dd[b]	2.42dd
2-H	4.01ddd	3.76ddd	4.11m[c]	3.91ddd	4.26ddd	4.09ddd	3.90ddd	*ca.* 4.25[b]	3.96ddd	*ca.* 4.3[b]	3.88ddd
3-H	3.94ddd	4.02ddd	4.00ddd	3.97ddd[e]	3.95ddd	3.96ddd	3.98ddd	*ca.* 3.96[b]	4.11dt	*b*	3.94
6-H			6.10m (9.5)		6.10m (9.5)[h]			6.11m (9)		6.10m (10)	
16-H				*ca.* 2.2	*ca.* 2.04, 2.25						2.78dd
17-H	2.78dd	2.76dd	2.83m (17)[d]	2.76dd	2.83m (16)[d]	2.78dd	2.76dd	2.82m (16)[d]	*ca.* 2.75[b]	*ca.* 2.8[b]	0.81
18-H	0.87	0.82	0.79	0.82	0.79	0.87	0.82	0.78	0.82	0.78	
19-H	0.87	10.01 (2.5)	9.75d (3)	10.02 (2.5)	9.75d (3)[f]	0.88	10.01 (2.5)	9.75d (3)	10.01 (2.5)	9.74d (3)	10.01 (2.5)
21-H	4.80	4.79	4.81	4.79	4.81	4.81	4.79	4.82	4.79	4.81	4.84
(2 dd)	4.97	4.94	4.93	4.95	4.93	4.99	4.95	4.93	4.94	4.93	5.00
22-H	5.87	5.88	5.94	5.88	5.94	5.87	5.87	5.94	5.88	5.93	5.88
1′-H	4.80	4.78	4.80	4.58	4.60	4.60	4.57	4.60	4.63	4.66	4.72
3′-H	3.73dd	3.73t	3.75t	3.63dd[g]	*ca.* 3.65[g]	4.78dd	4.76dd	4.78dd			3.65d
4′-H				*ca.* 1.85m	*ca.* 1.85m				2.77dd(β)	2.77dd(β)	3.31dd
5′-H	4.09m	4.12m	4.11[c]	*ca.* 3.65[g]	*ca.* 3.65[g]	3.70m	3.70m	*ca.* 3.75m[b]	2.48dd(α)	2.47dd(α)	3.80dq
6′-H	1.26d	1.24d	1.24d	1.28d	1.28d	1.30d	1.30d	1.30d	3.75m	3.73m	1.32d
3′-OAc						2.16	2.17	2.18	1.42d	1.41d	

Table 2a (*concluded*)

Proton	5,6-*Dehydro-calotoxin*[j] (**7a**)	(**8**) (with 2′-acetylated)	Calotoxin 3′,4′-diacetate (**8**)	5,6-Dehydro-calotoxin 3′,4′-diacetate (**8a**)	16α-*Hydroxy*-5,6-*dehydro-calotropin*[j] (**9a**)	16α-*Acetoxy*-5,6-*dehydro-calotropin* (**10a**)	16α-*Hydroxy*-5,6-*dehydro-asclepin* (**11a**)	16α-*Acetoxy*-5,6-*dehydro-asclepin* (**12a**)	Calotropagenin (**14**)[20]	5,6-*Dehydro-calotropagenin* (**14a**)	19-*Dihydro-calotropin* (**13**)
1-H$_\alpha$	1.07dt	1.16t	1.15t	1.13dt	1.10dt			1.15[b]	0.98t	0.96dt	
1-H$_\beta$	2.39dd	2.50dd	2.45dd	2.44dd	2.45dd	2.46dd	2.49dd	2.50dd	2.59dd	2.55dd	2.38dd
2-H	*ca.* 4.2m[b]	3.71ddd	3.92dt[b]	4.28m[b]	4.24m	4.28m	4.25m[b]	4.25ddd[b]	3.46 (25)	3.83m	4.12m[b]
3-H	3.95m	4.03dt[b]	*ca.* 4.0m[b]	3.97m[b]	3.95m	3.94m	3.97m	3.97m		3.42ddd	4.02ddd
6-H	6.10m (10)			6.10m (9)	6.09m (9)	6.11m (9.5)	6.10m (10)	6.12m (9)		6.11m (9)	
16-H					4.61dt[b]	5.34dt	4.69dt	5.33dt			
17-H	2.83m[d]	2.77dd	2.77dd	2.83m[d]	2.65d	2.69d	2.66d	2.69m	2.77dd	2.83m[d]	2.79dd
18-H	0.78	0.84	0.82	0.79	0.75	0.77	0.76	0.78	0.83	0.79	0.92
19-H	9.74d (3)	9.97 (2.5)	9.99 (2.5)	9.73 (3)	9.73 (3)	9.73d (3)	9.73d (3)	9.74d (3)	10.04	9.73d (3)	3.68d, 3.92[m]
21-H (2 dd)	4.83 4.96	4.79 4.94	4.79 4.94	4.81 4.94	4.80 4.90	4.86 4.91	4.78 4.87	4.86 4.92	4.79 4.94	4.82 4.94	4.81 4.97
22-H	5.94	5.89	5.88	5.94	6.00	6.00	6.01	5.99	5.88	5.94	5.89
1′-H	4.74	4.84	4.76	4.77	4.58	4.60	4.60	4.60			4.62
3′-H	*ca.* 3.7[b]	6.06[d]	5.15d	5.17d	*ca.* 3.65m[b]	*ca.* 3.65m[b]	4.78[b]	4.80dd			*ca.* 3.65[b]
4′-H	*b*	4.88dd	4.89dd	4.90dd[b]		1.86m	1.87m	1.87m			*ca.* 1.85m
5′-H	3.79dq	3.99dq[b]	4.01dq[b]	4.03m[b]	*ca.* 3.65m[b]	*ca.* 3.65m[b]	*b*	*ca.* 3.65m[b]			*ca.* 3.65[b]
6′-H	1.33d	1.48d	1.48d	1.48d	1.27d	*b*	*b*	1.30d			1.29d
3′-OAc		2.00, 2.10[k]	2.03	2.05			2.18	2.18			
4′-OAc		2.07	2.07	2.07							
16-OAc						2.04		2.05			
15-H[l] (2 dd)					2.01, 2.74	2.19, 2.34	2.05, 2.99	2.18, *ca.* 2.35[b]			

[a] Chemical shifts in p.p.m. from $SiMe_4$ measured at 400 MHz in $CDCl_3$ solvent, except where otherwise stated. Symbols d, t, q, and m refer to doublet, *apparent* triplet, quartet, and multiplet respectively. Signals inter-related by decouplings are linked by lines. Compounds with italicised names refer to new cardenolices of *A. vestita* from Commatti Canyon. For the other compounds, n.m.r. samples were derived from other sources (see 'Acknowledgements', also from *A. fruticosa*[6]). Data of 5α(H)-analogues of Δ^5-cardenolides (**10a**)—(**12a**) isolated from the same species from Kimbler Ranch in California were given in ref. 1. A sample of glycoside (**12a**) isolated from *A. californica* (C. J. Nelson, unpublished) and identical to that from *A. vestita* was used for n.m.r. [b] Overlaps with OH or other signal. [c] Signals of 2-H$_\beta$ and 5′-H overlap to give a combined peak with $W_{h/2}$ 22 Hz. [d] Multiplet due to virtual coupling. [e] Mutual decouplings involving this signal located the signal of 4-H$_\alpha$ (ddd) and 4-H$_\beta$ (part of apparent q observed) at δ 1.77 and *ca.* 1.4 respectively. [f] Upon irradiation of 1-H$_\alpha$, the 1.5 Hz splitting was removed, and the signal was narrowed by 1.5 Hz. [g] Signals of 3′-H and 5′-H overlap to give a combined peak with $W_{h/2}$ 20 Hz. [h] Signal narrowed by 3 Hz upon saturation of H-7ξ at *ca.* δ 2.25. [i] In CD_3OC–$CDCl_3$ (1:4, v/v). [j] In CD_3OD–$CDCl_3$ (1:20, v/v). [k] Also 2′-OAc. [l] Assigned by decoupling. [m] $J_{19,19}$ 11.5 Hz.

Table 2b. Some coupling constants in Hz of cardenolides with oxygen substituent at 2α

	1-H_α	1-H_β	3-H_α	21-H_x	22-H	5′-H
1-H_α		12.5[a]				
2-H_β	12[b]	4.5[a,b]	*ca.* 10[b]			
4-H_α			4[c]			
4-H_β			*ca.* 11.5[c]			
21-H_γ				18	1.5	
22-H				1.5		
6′-H						6

[a] Not observed for compounds (**4**) and (**6a**). [b] Data derived from compounds (**2**), (**3a**), (**4**), (**6**), and (**8**; with 2′-acetylated) only, since for the others either $\Delta\nu/J < 4$ for the 2β and 3α protons, or the signal of 2-H cannot be readily analysed. [c] Data derived from compounds (**2**), (**2a**), (**3a**), (**4**), and (**6**) only, since for the others either $\Delta\nu/J < 4$ for the 2β and 3α protons, or the signal of 3-H cannot be readily analysed.

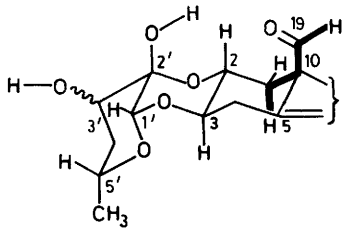


Figure.

dehydrocalactin (**2a**) and 5,6-dehydrocalotropin (**3a**) (δ 9.75). A model shows that with the aldehyde carbonyl oriented as discussed above, 19-H will be located above the plane of the Δ^5 double-bond, and hence shielded. For the dehydrocardenolides (**2a**) and (**3a**), a 1.5 Hz splitting of the signal of 19-H (half-height width 3 Hz) is observed, due to w-type coupling[18] to the proton at 1α (see thickened path in Figure). This data defines the preferred conformation of the formyl group as one in which the carbonyl approximately eclipses the C(10)–C(1) bond. For the 5α(H)-cardenolides (**2**) and (**3**), the conformation adopted appears to be somewhat less favourable for w-coupling, since the half-height width of the unsplit 19-H signal is less (2.5 Hz). A further long-range coupling to 5-H_α could have masked any splitting of the 19-H signal.*

To check the validity of the above-mentioned effects, we examined as models the corresponding data (Table 3) for methyl 3β-acetoxy-19-oxochol-5-en-24-oate (**17a**)[19] and methyl 3β-acetoxy-19-oxo-5α-cholan-24-oate (**17**) (obtained on hydrogenation over Pd on charcoal). As shown in Tables 3 and 4, 5,6-unsaturation is once again associated with a pronounced shielding of 19-H (0.36 p.p.m.) and a 1.5 Hz splitting of its signal which was removed upon irradiation of the 1α proton. As in the cases of the 5α-cardenolides (**2**) and (**3**), the 5α-steroid (**17**) has a smaller long-range coupling between the 19 and 1α protons, the singlet signal of the former proton narrowing by only 0.4 Hz, upon irradiation of the latter.

Another anisotropy effect of the Δ^5 double-bond is observed for the 2β signal. This signal, found at δ 3.76 and 3.91 for calactin (**2**) and calotropin (**3**) respectively, is 0.35 p.p.m. downfield in the spectra of the corresponding dehydro analogues (Table 4). For the model steroids (**17**) and (**17a**), the shift difference is 0.23 p.p.m. (Table 4). A model shows that the 2β proton is approximately co-planar with the 5,6 double-bond, thus accounting for the observed deshielding effect.

Among cardenolide glycosides with doubly linked carbohydrates, unsaturation at 9(11),[12] 7,[13] and 4[14] are known. The above-discussed anisotropy effects are not compatible with a double-bond at 9(11) which would cause severe deshielding at position 1. Neither is the unsaturation at 7, since humistratin from *A humistrata*, shown by *X*-ray crystallography to be 7,8-dehydrocalactin, has a more shielded and broader vinyl proton signal (δ 5.73, half-height width 14 Hz).[13] Unsaturation at 4 would give rise to coupling between the vinyl and 3α protons. Instead, the vinyl proton is coupled to a proton in the allylic region (7-H, *ca.* δ 2.25) by *ca.* 3 Hz (Table 2a, footnote h).

Interestingly, the conformational transmission caused by a Δ^5 double-bond is manifested in a 0.06—0.07 p.p.m. deshielding of 17-H and 22-H (Tables 2, 4), and degeneration of the 17-H signal from a doublet of doublets to a multiplet (Table 2) due to virtual coupling even at 400 MHz. Both effects are observed also for 5,6-dehydrocalotropagenin (**14a**) (described below) with no attached sugar (Tables 2, 4).

5,6-Dehydro Analogues of Asclepin, Uscharidin, Calotoxin, and of 16α-Hydroxy- and 16α-Acetoxycardenolide Glycosides.—Apart from calactin and calotropin, a number of other 5α-cardenolide glycosides occur in *A. vestita* from Commatti Canyon. These are the known glycosides[10,11,17] asclepin (**5**) (calotropin 3′-acetate), uscharidin (**6**) (with 3′-keto group), calotoxin (**7**) (4′β-hydroxycalactin), and 3 glycosides newly isolated from *A. vestita* collected at Kimbler Ranch,[1] *viz.* 16α-acetoxycalotropin (**10**), 16α-hydroxyasclepin (**11**), and 16α-acetoxyasclepin (**12**). They were acompanied by generally bigger amounts of their respective 5,6-dehydro analogues, which tended to be found in the same or adjacement h.p.l.c. fractions. The Δ^5-cardenolide glycosides (**5a**)—(**7a**) and (**10a**)—(**12a**) have protonated ions in the methane c.i. mass spectra for the molecule and for the genin which are 2 a.m.u. lower than those from the corresponding 5α-cardenolides; the protonated sugar ions are identical (see Table 1 and ref. 1). The 400 MHz n.m.r. spectra of the Δ^5-cardenolide glycosides (Table 2) parallel those of the corresponding 5α-cardenolides (Table 2, and ref. 1), except for the characteristic differences introduced by the 5,6 double-bond as discussed above (see Table 4), and the presence of a multiplet signal (half-height width *ca.* 9 Hz) near δ 6.1 for a vinyl proton at position 6. Also a doublet signal (*J* 1.5 Hz) for 19-H, diagnostic of the Δ^5-19-oxo function as discussed above was observed. In the case of 5,6-dehydrocalotoxine (**7a**), the above comparison was repeated after it and calotoxin (**7**) were converted to the respective 3′,4′-diacates (**8a**) and (**8**) respectively (Table 2,4).†

The 5α-analogue of one Δ^5-cardenolide glycoside from Commatti Canyon, *viz.* 16α-hydroxy-5,6-dehydrocalotropin (**9a**), was not previously isolated from the Kimbler Ranch sample.[1] In its absence, the structure of 16α-hydroxy-5,6-dehydrocalotropin (**9a**) is based on the observation of ^{1}H n.m.r. signals (Table 2) for protons at 6 and 19 diagnostic of the Δ^5-19-oxo function (see above): and for protons at 16 (doublet of triplet at δ 4.61, *J* 4.5, 8 Hz) and 3′ (multiplet near δ 3.65) characteristic of α hydroxy groups at these positions [*cf.* the corresponding signals of the Δ^5-cardenolides (**11a**); and (**10a**)

* The 2β proton in gomphoside (**1**) is 0.25 p.p.m. less shielded than in calactin (**2**), possibly reflecting the shielding of the formyl carbonyl in the latter and the steric compression of the C-10 methyl in the former. A similar shift difference is observed between 3′-*epi*-gomphoside 3′-acetate (**4**) and asclepin (**5**) (Table 2).

† From calotoxin (**7**) further acetylation gives the 2′,3′,4′-triacetate (**8**; with 2′-acetylated). For this compound, the carbonyl of the tertiary 2′-acetate is directed towards the 3′ proton, thus strongly deshielding it [δ 6.06 *vs.* δ 5.15 in the 3′,4′-diacetate (**8**)], and causing shielding at the 2β proton (δ 3.71 *vs.* δ 3.92 in the diacetate). Analogous comments on the preferred conformation of the same acetate group in gomphoside 2′,3′-diacetate was given earlier.[9]

Table 2c. Other coupling constants in Hz of cardenolides with oxygen substituent at 2α

	1α,19	15α,16β 15β,16β	16α,17α	16β,17α	3′β,4′α	3′β,4′β	3′α,4′α	3′α,4′β	4′α,5′β
(1), **(2)**			9.5	5.5			*ca.* 3	*ca.* 3	
(2a)	1.5		*a*	*a*			*ca.* 3	*ca.* 3	
(3)[e]			9.5	5.5	*ca.* 12[b]	*ca.* 4,5[b]			
(3a)	1.5		*a*	*a*	*b*	*b*			
(4), **(5)**			9.5	5.5	12	5			
(5a)	1.5		*a*	*a*	12	5			
(6)[c], **(6a)**	1.5 (**6a**) only		*f*	*f*					12
(7)[d], **(8)**, (8′, with 2′ acetylated)			9.5	5.5			3		10
(8a)	1.5		*a*	*a*			3		10
(9a)[d], **(10a)**	1.5	8		4	*b*	*b*			
(11a), **(12a)**	1.5	8		4	12	5			
(13), **(14)**			9.5	5.5					
(14a)	1.5		*a*	*a*					

[a] Not observed due to virtual coupling between 17-H_α and a proton at 15. [b] Signals of 3′-H_β and 5′-H_α overlap. [c] $J_{4'\alpha,4'\beta}$ 14 Hz, $J_{4'\beta,5'\beta}$ 2 Hz. [d] Coupling constants data in this Table and in Tables 2b, 3b refer to $CDCl_3$ solvent, except for compounds (**7**) (CD_3OD–$CDCl_3$; 1:4, v/v) and (**9a**) (1:20). [e] $J_{4\alpha,5\alpha}$ 3 Hz, $J_{4\alpha,4\beta}$ 13 Hz, $J_{4\beta,5\alpha}$ *ca.* 12 Hz. [f] Not observed due to masking of 17-H signal.

Table 3a. Chemical shifts (with half-height widths in Hz) of steroids with no oxygen substituent at 2α[a]

Proton	Methyl 3β-acetoxy-19-oxo-5α-cholan-24-oate (**17**)[b]	Methy l3β-acetoxy-19-oxochol-5-en-24-oate (**17a**)[b]	Coroglaucigenin (**15**)[d]
1-H_α	0.95dt[c]	1.13m[c]	0.78dt
1-H_β	2.41dt	2.56dt	2.49dt
2-H_α	1.30m	1.94m[c]	1.86m[c]
2-H_β	1.38m	1.61m[c]	*ca.* 1.37m[c]
3-H	4.71m	4.60m	3.64m
4-H_α		2.49ddd[e]	*ca.* 1.68m[c]
4-H_β		1.83dt	0.96br dt
5-H			
6-H		5.88br d (9)[f]	
16-H			1.86m, 2.14m
17-H			2.78dd
18-H			0.91
19-H	10.02 (2.5)	9.66d (3)	3.73d, 3.86d
21-H			4.83dd, 5.00dd
22-H			5.88

[a] See footnote *a* of Table 2a. [b] Only signals of protons in the ring A region listed. [c] Overlaps with OH or other signal. [d] In CD_3OD–$CDCl_3$ (1:20, v/v). [e] Showing W-type coupling to 2-H_α. [f] Signal sharpened to a sharp doublet (*J* 5 Hz) and narrowed by 0.2 Hz upon irradiation of 4-H_β.

and (**3a**) respectively in Table 2]. The methane c.i. mass spectrum (Table 1) is in agreement with the proposed structure.

Cardenolide Genins and 19-Dihydrocalotropin.—Found in the Commatti Canyon sample were calotropagenin (**14**) and 5,6-dehydrocalotropagenin (**14a**) which are the 'parent' genins to the above described 5α- and Δ^5-cardenolide glycosides. The former was identified by direct comparison. The structure of the latter was deduced by comparison of its ^1H n.m.r. (Table 2) and mass spectral data (Table 1) with those recorded for calotropagenin (**14**).[20] Another genin found is the 19-hydroxy compound coroglancigenin (**15**), identified by direct comparison.

A glycoside with protonated molecular and genin ions 2 a.m.u. higher than those of calactin/calotropin (Table 1) gives rise to a pair of doublets in the n.m.r. spectrum which is of similar chemical shift (δ 3.7, 3.9) and geminal coupling constant (11.5—12 Hz) as the signals of the 19-CH_2OH group in coroglaucigenin (**15**). The pattern of signals for protons 1′,3′-6′

Table 3b. Coupling constants in Hz of steroids (see Table 3a) with no oxygen substituent at 2α[a]

	1-H_α	1-H_β	3-H_α	4-H_β	H-5$_\alpha$
1-H_α		13.5			
2-H_α	4[b,c]	3.5		2[d]	
2-H_β	13[b,c]	3.5			
4-H_α			4[d]		⩽ 3[d]
4-H_β			*ca.* 11[d]	13.5[d]	12[d]

[a] For cardenolide (**15**), $J_{19.19}$ 12 Hz; $J_{16.17}$ 5.5 and 9.5 Hz. For steroid (**17a**), $J_{1\alpha.19}$ 1.5 Hz; $J_{4\beta.6}$ *ca.* 0.5 Hz; $J_{6.7}$ 5 and ⩽ 1 Hz. [b] Observed for compound (**15**). [c] Observed for compound (**17**). [d] Observed for compound (**17a**).

Table 4. Chemical shift differences in p.p.m. between 5α- and Δ[5]-steroids with 19-aldehyde groups[a]

	19-H	2-H_β	17-H	22-H
Methyl 3β-acetoxy-19-oxo-5α-cholan-24-oate (**17**) and methyl 3β-acetoxy-19-oxochol-5-en-24-oate (**17a**)	−0.36	+0.23		
Calatcin (**2**) and 5,6-dehydrocalactin (**2a**)	−0.26	+0.35	+0.07	+0.06
Calotropin (**3**) and 5,6-dehydrocalotropin (**3a**)	−0.27	+0.35	+0.07	+0.06
(**5**) and (**5a**)	−0.26	*ca.* +0.35	+0.06	+0.07
(**6**) and (**6a**)	−0.25	*ca.* +0.35	*ca.* +0.05	+0.06
(**7**) and (**7a**)	*ca.* −0.25[b]	*ca.* +0.35[b]	*ca.* +0.05[b]	*ca.* +0.05[b]
(**8**) and (**8a**)	−0.26	+0.36	+0.06	+0.06
(**10**) and (**10a**)	−0.28	*ca.* +0.35	+0.04[c]	+0.06
(**11**) and (**11a**)	−0.28	*ca.* +0.35	+0.04[c]	+0.06
(**12**) and (**12a**)	−0.28	+0.35	+0.04[c]	+0.05
(**13**) and (**13a**)	−0.30	+0.37	+0.06	+0.06

[a] In $CDCl_3$ unless otherwise stated. Where approximate (due to *e.g.* overlapping signals), shift differences are rounded to the nearest 0.05 p.p.m. [b] The two samples compared differed in the amount of CD_3OD in the $CDCl_3$ solvent. [c] Shift differences may be affected by the nature of the 16α substituent.

shows that the sugar is identical to that of calotropin (**3**) but not that of calactin. It is thus 19-dihydrocalotropin (**13**).

Experimental

New cardenolides obtained in sub-milligram quantities were characterised by methane c.i. mass spectral (Table 1) and 400 MHz ^1H n.m.r. data (Tables 2, 3). Known cardenolide glycosides obtained had h.p.l.c. and t.l.c. characteristics identical with those of authentic samples, which were used for the n.m.r. data in Tables 2 and 3. General conditions for obtaining n.m.r. spectra and for separation by preparative h.p.l.c. were as given in ref. 7 unless otherwise specified. Mass spectral data were collected using a Finnigan TSQ-46 MS/MS quadrupole spectrometer and associated Finnigan-Incos data system. Evaporation of solvents was carried out under reduced pressure.

Extraction of A. vestita *and Preparative T.l.c. Separation of Cardenolides.*—Dried ground leaf material (4.69 g) of *A. vestita* collected[2] at Commatti Canyon in California was extracted three times with 75% aqueous ethanol (100 ml) at 70—75 °C for 1 h. The residue obtained on evaporation of the ethanol extracts* was dissolved in methanol (10 ml). The solution was precipitated with 10% aqueous lead(II) acetate (20 ml), and after 20 min at 0 °C was centrifuged. The precipitate was washed with methanol-water (2:1, v/v; 5 ml), and the supernatant and washings were combined. Excess of lead in the combined solutions was precipitated by the addition of solid ammonium sulphate and, after centrifugation, the supernatant was extracted with dichloromethane (3 × 10 ml). The residue from evaporation of the dried (Na_2SO_4) organic extracts was separated on two 0.5 mm silica t.l.c. plates (20 × 20 cm) (pre-washed by development with 1% ammonia in diethyl ether), with development (× 3) with ethyl acetate. Ten major bands of cardenolides (visualised by spraying an edge of the plate with 0.4% 2,2′,4,4′-tetranitrobiphenyl in toluene followed by 10% potassium hydroxide in methanol-water (1:1, v/v) were scraped off and eluted with 30—100% methanol in ethyl acetate. The cardenolide fractions so obtained were individually subjected to further separation by preparation h.p.l.c.†

Preparative H.p.l.c. Separation of Cardenolides.—T.l.c. fraction 1 (see above), the least polar one, was fractionated by h.p.l.c. on a preparative silica gel column with a mobile phase of propan-2-ol–hexane (1:5, v/v) at a flow rate of 2 ml min^{-1}, giving 5,6-dehydrouscharidin (**6a**) (R_t 71 min), 5,6-dehydroasclepin (**5a**) (R_t 90 min), and two compounds (R_t 106 min) one of which was 16α-acetoxy-5,6-dehydroasclepin (**12a**). T.l.c. fraction 2 consisted mainly of 5,6-dehydrocalactin (**2a**).

Preparative h.p.l.c. of t.l.c. fraction 3 as for fraction 1, but with the use of propan-2-ol–hexane (1:3, v/v), yielded 5,6-dehydrocalotropin (**3a**) (R_t 83 min) as a major component, 16α-acetoxy-5,6-dehydrocalotropin (**10a**) (R_t 105 min), and 60α-hydroxy-5,6-dehydroasclepin (**11a**) (R_t 116 min). T.l.c. fraction 4 was subjected to preparative h.p.l.c. on an analytical silica gel column (10 μm, Brownlee lab.) using propan-2-ol–hexane (1:4, v/v) at a flow rate of 1.5 ml min^{-1} giving 5,6-dehydrocalotoxin (**7a**) (R_t 25 min). After determination of spectroscopic data (see below), 5,6-dehydrocalotoxin was treated with acetic anhydride in pyridine at 25 °C for 4 h to give the 3′,4′-diacetate (**8a**). Under the same h.p.l.c. conditions, t.l.c. fraction 5 yielded coroglaucigenin (**15**) (R_t 18 min) the h.p.l.c. and t.l.c. behaviour of which was identified with that of an authentic sample. Before comparison, this fraction was further purified by h.p.l.c. using reduced mobile phase polarity (2:9, v/v) and reduced flow rate (1 ml min^{-1}). T.l.c. fractions 6 and 7 were likewise subjected to preparative h.p.l.c. using propan-2-ol–hexane (3:10 v/v) at a flow rate of 1.5 ml min^{-1} yielding from fraction 6, 5,6-dehydrocalotropagenin (**14a**) (R_t 45 min) and 16α-hydroxy-5,6-

* Assayed[2] to contain 40 mg of total cardenolides.

† In two other extractions on somewhat smaller states, aqueous ethanol was used in the work-up, and alcohol was evaporated from the supernatant before extraction (with chloroform). The residue (*ca.* 40 mg each) from evaporation of the dried extract was separated by t.l.c. and h.p.l.c.

dehydrocalotropin (**9a**) (R_t 57 min), and from fraction 7,19-dihydrocalotropin (**13**) (R_t 44 min). In the above separations, h.p.l.c. fractions were combined and re-chromatographed where appropriate.

On a silica gel h.p.l.c. column as described above, each of the 5,6-dehydrocardenolides co-chromatographed with lesser amounts of the corresponding 5α(H)-analogue, as evident by n.m.r. and mass spectral data. However the presence of a 5α-analogue of 16α-hydroxy-5,6-dehydrocalotropin (**9a**) could only be tentatively inferred from the mass spectral data.

Separation of a 5,6-dehydrocardenolide from its 5α-analogue could be achieved using C18 reverse phase columns. For example, with the use of a semi-preparative C18 column and mobile phase of methanol-phosphate (1:1, v/v) buffer (pH 7.4) at a flow rate of 5 ml min^{-1}, minor amounts of calotropin (**3**) (R_t 11 min) was separated from the major component 5,6-dehydrocalotropin (**3a**) (R_t 8 min) of t.l.c. fraction 3. Details of further separations on reverse phase columns and the quantitative aspects of cardenolide content in relation to location of the plant will be given in a subsequent publication.

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