

## Cardenolide Glycosides of the Asclepiadaceae. New Glycosides from *Asclepias fruticosa* and the Stereochemistry of Uscharin, Voruscharin and Calotoxin

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Eight new cardenolide glycosides, 19-deoxyuscharin (1b), 4' $\beta$ -hydroxygomphoside (1k), 3'-didehydrogomphoside (1d), 3'-epigomphoside (1e), 3'-epiafroside (2e), 3'-epigomphoside 3'-acetate (1f), 3'-didehydroafroside (2d), and 3'-epiafroside 3'-acetate (2f), have been isolated from *Asclepias fruticosa* R. Br. The structures of all new compounds were established by spectral comparisons with known compounds. The chirality at C-3' of uscharin (3b), voruscharin, and 19-deoxyuscharin (1b) is proposed to be *S*. By comparison with 4' $\beta$ -hydroxygomphoside (1k), the  $\beta$  configuration of the 4'-hydroxy group of calotoxin (3k) (from *Calotropis procera*) has been established.

The structures of gomphoside (1a) and its 15 $\beta$ -hydroxy-analogue afroside (2a), two cardenolide glycosides isolated from *Asclepias fruticosa* R. Br. have been reported previously.<sup>1,2</sup> Various genera of the milkweed family (Asclepiadaceae), particularly *Asclepias* and *Calotropis*, produce glycosides that are unusually stable to acid hydrolysis of the carbohydrate group due to the double attachment of the sugar to the 3 $\beta$  and 2 $\alpha$  positions of the cardenolide aglycone through acetal and hemiacetal linkages respectively.<sup>1</sup> *Calotropis procera* produces glycosides in most of which C-19 of the steroid aglycone is present as an aldehyde. These include calactin (3a), calotropin (3e), uscharidin (3d), uscharin (3b), and calotoxin (3k).<sup>3</sup> *Asclepias* species also contain cardenolide glycosides with C-19 as an aldehyde, including asclepin (3f),<sup>4</sup> the 3'-monoacetate of calotropin, and humistratin, the  $\Delta^7$ -analogue of calactin (3a).<sup>5</sup> Other *Asclepias* species produce the more highly oxidised glycosides desglucosyrioxide (5a), labriformidin (5d), and labriformin (5b).<sup>6</sup>

*A. fruticosa* from near Sydney has recently been examined in order to obtain gomphoside (1a) for biological testing and chemical modification. By contrast with the original material collected from northern New South Wales,<sup>2</sup> the mixtures of glycosides from both leaves and stems of this new collection were particularly complex. This paper reports the isolation of 15 cardenolide glycosides including 8 new ones, and the elucidation of the stereochemistry of the *Calotropis* glycosides uscharin, voruscharin, and calotoxin.

The new compounds are all structural variants of known Asclepiadaceae cardenolide glycosides. Variations of the steroid moiety are represented by the three genins gomphogenin (I), afroginin (II), and calotropagenin (III). For all but one of the new glycosides variations of the carbohydrate portion are limited to C-3', while one contains extra oxygenation at C-4'.

The most abundant glycosides isolated from the leaves were uscharin (3b), 3'-epigomphoside 3'-acetate (1f), and 3'-epiafroside 3'-acetate (2f). Also isolated in substantial quantities were 3'-didehydrogomphoside (1d), uscharidin (3d), asclepin (3f), gomphoside (1a), and 19-deoxyuscharin (1b), whilst 3'-didehydroafroside (2d), calactin (3a), 3'-epigomphoside (1e), afroside (2a), and uzarigenin (IV), were minor constituents. Compounds (1b), (1d), (1e), (1f), (2d), and (2f) are new cardenolide glycosides. The major glycoside of the stem extract was afroside (2a). 3'-Epi-afroside (2e), uscharin (3b), 3'-epigomphoside 3'-acetate (1f), gomphoside (1a), 3'-epigomphoside (1e), and 4' $\beta$ -hydroxygomphoside (1k) were also present. The last two from the stems are previously unreported glycosides and were not found in the leaf extract.

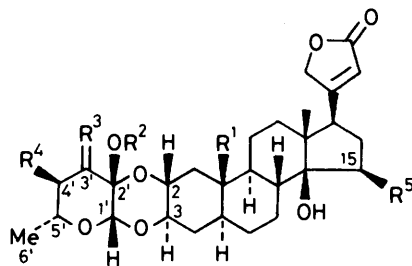
Methane chemical ionisation (c.i.) mass spectrometry



allows the assignment of the oxygenation pattern of the genin and carbohydrate portions of the molecule. Although in solution all glycosides in this series appear to exist exclusively in the 2'-hemiacetal form [cf. (A)], their methane c.i. mass spectra indicate that fragmentation occurs in the protonated opened keto alcohol form (B) shown in the Scheme. Thus ion *a* due to the 'pseudogenin', and ions *b* and *c* due to the carbohydrate portion can be observed.<sup>2</sup> Further, ions *d* (and *g*), *e*, and *f* appear to be characteristic of respectively keto, thiazoline (dihydrothiazole), and acetate functions at 3'. In those glycosides related to gomphogenin (I), a 'pseudogenin' ion occurs at *m/z* 391, and ions at *m/z* 373, 355, and 337 from the sequential loss of H<sub>2</sub>O are also dominant. Similarly, ions due to the 'pseudogenin' of calotropagenin (III) and afroginin (II) appear at *m/z* 405 or 407 respectively in the mass spectra of their related glycosides. It can thus be shown that all acetylated glycosides we isolated have the acetyl group on a carbohydrate hydroxy group. Furthermore, the c.i. mass spectrum of 4' $\beta$ -hydroxygomphoside (1k), which showed a 'pseudogenin' ion at *m/z* 391, and carbohydrate ions *b* and *c* at *m/z* 145 and 127, establishes that the additional hydroxy group occurs in the carbohydrate portion of the molecule (see later).

The mass spectral data, together with <sup>13</sup>C and <sup>1</sup>H n.m.r. comparisons, led to structural assignments for the 8 new compounds (1b), (1d), (1e), (1f), (1k), (2d), (2e), and (2f).

<sup>13</sup>C N.m.r. Results.—Table 1 shows <sup>13</sup>C n.m.r. data of all the new compounds and those of a representative series of known compounds. For glycosides related to gomphogenin (I) and calotropagenin (III), the C-14 resonance occurs near  $\delta_c$  84.5 p.p.m., C-16 resonates near  $\delta_c$  27 p.p.m., and C-17 resonates near  $\delta_c$  51 p.p.m. For those derived from afroginin (II), the  $\beta$ -OH group at C-15 causes an upfield shift of ca. 3.5 p.p.m. for C-14, a downfield shift of ca. 10.5 p.p.m. for the  $\beta$  carbon C-16, and an upfield shift of ca. 2 p.p.m. for C-17. In glycosides derived from calotropagenin (III), the aldehyde group attached to C-10 causes this carbon to resonate 15 p.p.m. downfield when compared with the 10-methyl analogues derived from gomphogenin (I) and afroginin (II). Shielding effects of the 19-carbonyl oxygen atom on C-1 (ca. 6 p.p.m.,  $\gamma$ -*gauche* effect)<sup>7</sup> and on carbons 5 and 9 (1–2 p.p.m.) indicate that the aldehyde group tends to adopt a conformation in which the carbonyl oxygen atom is *gauche* to C-1. Orientation of the carbonyl group is shown also by the pronounced deshielding of the  $\beta$  proton at C-1 in glycosides derived from calotropagenin (III);<sup>1</sup> e.g. 1 $\beta$ -H resonates at 2.48 p.p.m. in uscharin (3b) (Table 2), but at 1.91 p.p.m. in gomphoside 2',3'-diacetate (1i).

Comparison of <sup>13</sup>C resonances of the carbohydrate ring



		R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>
Gomphoside	(1a)	Me	H	β-OH, α-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
Afroside	(2a)	Me	H			OH
Calactin	(3a)	CHO	H			H
<i>19-Deoxyuscharin</i>	(1b)	Me	H		$\left\{ \begin{array}{l} \text{H} \\ \text{H} \end{array} \right.$	H
Uscharin	(3b)	CHO	H			H
Voruscharin	(3c)	CHO	H		H	H
<i>3'-Didehydrogomphoside</i>	(1d)	Me	H	O	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
<i>3'-Dideohydroafroside</i>	(2d)	Me	H			OH
Uscharidin	(3d)	CHO	H			H
<i>3'-Epigomphoside</i>	(1e)	Me	H	α-OH, β-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
<i>3'-Epiafroside</i>	(2e)	Me	H			OH
Calotropin	(3e)	CHO	H			H
<i>3'-Epigomphoside 3'-acetate</i>	(1f)	Me	H	α-OAc, β-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
<i>3'-Epiafroside 3'-acetate</i>	(2f)	Me	H			OH
Asclepin	(3f)	CHO	H			H
	(1g)	Me	Ac	α-OAc, β-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
	(2g)	Me	Ac			OAc
	(3g)	CHO	Ac			H
	(1h)	Me	H	β-OAc, α-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \\ \text{H} \end{array} \right.$	H
	(2h)	Me	H			OAc
	(1i)	Me	Ac			H
	(2i)	Me	Ac	β-OAc, α-H	$\left\{ \begin{array}{l} \text{H} \\ \text{H} \end{array} \right.$	H
	(1j)	Me	Ac			OAc
<i>4'β-Hydroxygomphoside</i>	(1k)	Me	H	β-OH, α-H	$\left\{ \begin{array}{l} \text{OH} \\ \text{OH} \end{array} \right.$	H
Calotoxin	(3k)	CHO	H			H

Italicised names refer to new substances isolated in this work

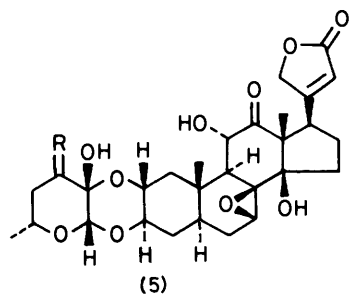
carbons for the 3'-ketones uscharidin (3d),<sup>6</sup> 3'-didehydrogomphoside (1d), and 3'-dideohydroafroside (2d) shows that an identical carbohydrate moiety is present in all three glycosides. The similarity of thiazoline and carbohydrate carbon shifts in the spectra of uscharin (3b) and its gomphoside analogue (1b) points not only to the same carbohydrate structure, but also to the same stereochemistry of the thiazoline ring (see below). Mercury(II) ion-catalysed hydrolysis of the thiazolines (3b) and (1b) gave the ketones (3d) and (1d) respectively.

The <sup>13</sup>C n.m.r. chemical shifts of the new compounds 3'-epigomphoside 3'-acetate (1f) and 3'-epiafroside 3'-acetate (2f) parallel those of gomphoside (1a) and afroside (2a) for steroidal carbons. Comparing the data of acetates (1f) and (2f) (Table 1) with those reported<sup>1</sup> for gomphoside 3'-acetate (1h) and afroside 3',15-diacetate (2h), significant differences in the resonances of the carbohydrate carbons are observed, carbons 1', 3', and 5' being ca. 2 p.p.m. to lower field in the acetates (1f) and (2f). These shifts to lower field are consistent with the proposed α-configuration for the acetoxy group at C-3' in these new compounds. Thus the shift differences at C-1' and C-5', both γ to the 3'-acetoxy group, reflect the differences between the periplanar heteroatom effect<sup>8,9</sup> of an equatorial 3'-acetoxy group present in the acetates (1f) and (2f), and the γ-gauche effect<sup>7</sup> of the corresponding axial acetoxy group in the 3'-epimeric analogues (1h) and (2h).<sup>1</sup>

The shift difference at C-3' is typical<sup>7</sup> of that between carbons attached respectively to axial and equatorial groups.

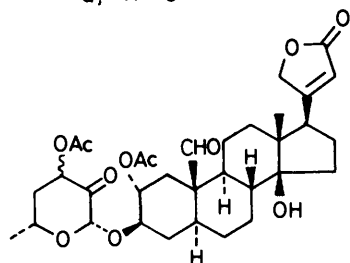
Hydrolysis of both acetates (1f) and (2f) with methanolic potassium hydrogen carbonate gave the parent alcohols epigomphoside (1e) and epiafroside (2e). Examination of the three 3'-epimeric pairs gomphoside (1a) and epigomphoside (1e), afroside (2a) and epiafroside (2e), and calactin (3a) and calotropin (3e), shows downfield shifts of ca. 2 p.p.m. at carbons 1', 3', and 5' for the 3'-equatorial epimers. A lesser shift to lower field (ca. 1 p.p.m.) is also observed for carbons 2' and 4'. As in the case of the acetates (above), these shift differences reflect the different configuration at C-3'.

<sup>1</sup>H N.m.r. Results.—<sup>1</sup>H N.m.r. data are shown in Table 2. In uscharidin (3d) and the related 3'-ketones (1d) and (2d), the low-field positions of the 5'-Me (6'-H<sub>3</sub>) and the 4'-H proton signals are particularly characteristic. The <sup>1</sup>H chemical shifts for the C-18 and C-19 methyl singlets in the 3'-ketones (1d) and (2d) are very similar to those of gomphoside (1a) and afroside (2a). Furthermore, the spectra of the 15β-hydroxy compounds (2a) and (2d) show a typical complex pattern due to 17-H and one 16-H as previously reported.<sup>2</sup> Spectra of the thiazolines uscharin (3b) and its 19-deoxy-analogue (1b) are almost superimposable in two sets of solvents, the sole difference being the replacement of a methyl singlet in the

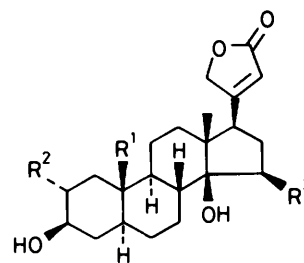
a; R =  $\beta$ -OH,  $\alpha$ -H

b; R =

d; R = O



(6)



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
Gomphogenin (I)	Me	OH	H
Afrogenin (II)	Me	OH	OH
Calotropagenin (III)	CHO	OH	H
Uzariogenin (IV)	Me	H	H

shielded by acetoxy groups on both C-2' and C-4', and resonates as a doublet ( $J$  3 Hz) at  $\delta$  6.05. The 4'-H signal appears as a doublet of doublets at  $\delta$  4.9.

The  $^{13}\text{C}$  n.m.r. data (Table 1) of 4 $\beta$ -hydroxygomphoside are in full accord with the structure (1k). Thus the addition of a hydroxy group on position 4' of gomphoside (1a) results in a  $\gamma$ -gauche shielding effect<sup>7</sup> on C-6' (3 p.p.m.) and  $\beta$  deshielding of carbons 3' and 5' (3 and 5 p.p.m.). The C-4' signal of 4 $\beta$ -hydroxygomphoside (1k) appears at  $\delta_{\text{c}}$  69 p.p.m.

spectrum of compound (1d) by an aldehyde singlet in that of uscharin (3b).

Formulation of the acetates (1f) and (2f) as respectively the 3'-acetate of 3'-epigomphoside (1e) and of 3'-epiafroside (2e), as based on  $^{13}\text{C}$  n.m.r. data, has been discussed earlier. The same assignments also follow from examining the  $^1\text{H}$  n.m.r. signal of 3'-H, which at 90 MHz appears as an X of an ABX spectrum ( $J'$  5 and 11 Hz) at  $\delta$  4.7 for each of compounds (1f) and (2f). These  $\delta$  values contrast with those reported<sup>1</sup> for the 3'-H signal for gomphoside 3'-acetate (1h) and afroside 3',15-diacetate (2h), *viz.*  $\delta$  4.95. Although the signals for the latter pair were masked by other signals, the 3'-H resonances were observed<sup>1</sup> as apparent triplets ( $J'$  3 Hz) in the 100 MHz spectra of gomphoside 2',3'-diacetate (1i) and afroside 2',3',15-triacetate (2i), indicating that in gomphoside (1a) and afroside (2a) the 3' proton is equatorial.<sup>1</sup> In contrast, the apparent vicinal coupling constants ( $J'$  5 and 11 Hz) observed for 3'-H in the acetates (1f) and (2f) indicate that 3'-H is axial in each of these compounds. For the parent alcohols 3'-epigomphoside (1e) and 3'-epiafroside (2e), the 3'-H signal appears at higher field ( $\delta$  ca. 3.65) forming a complex multiplet with the 5'-H signal.

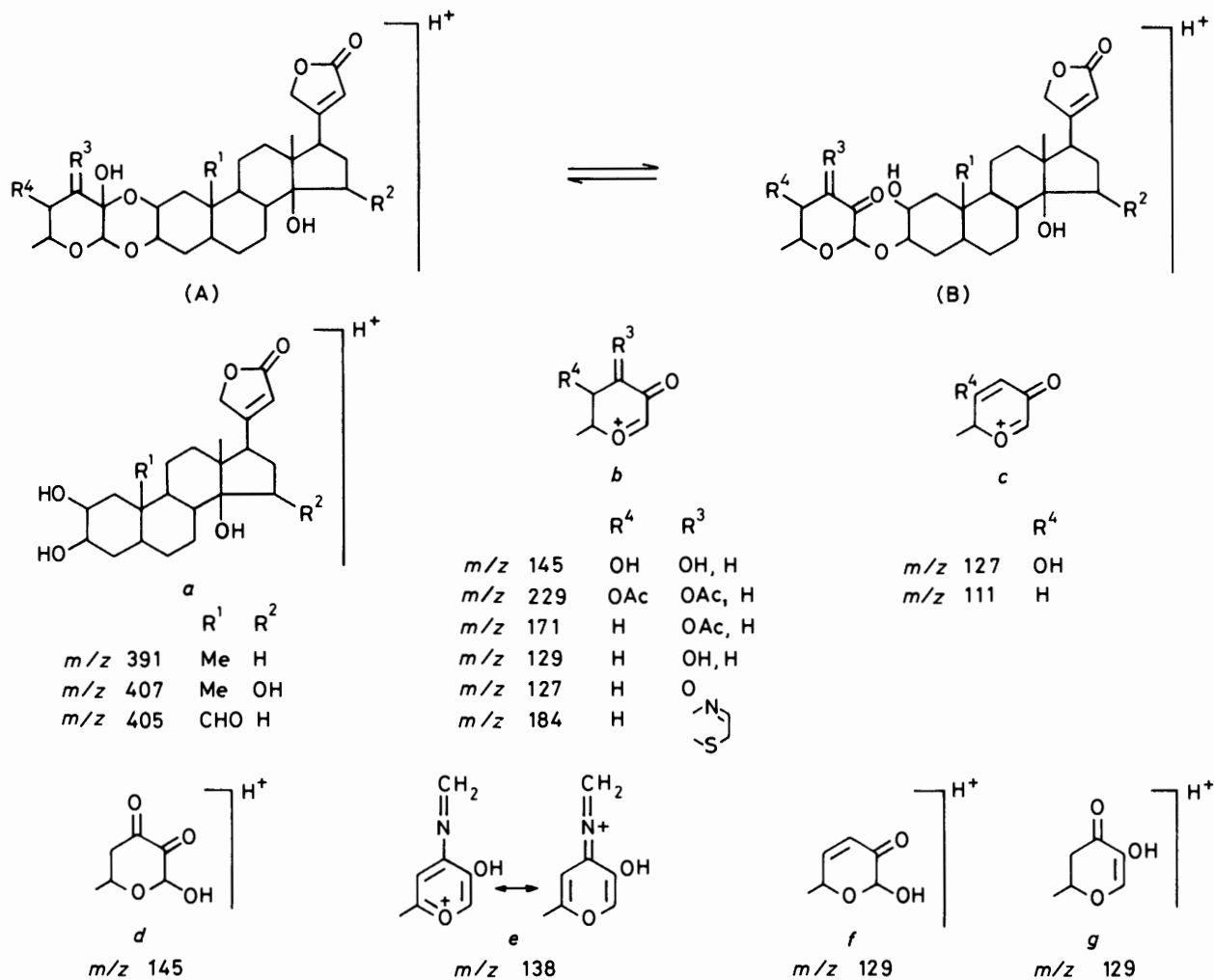
**Structure of 4 $\beta$ -Hydroxygomphoside (1k).**—The position and stereochemistry of the hydroxy groups of the carbohydrate of 4 $\beta$ -hydroxygomphoside (1k) were determined by analysis of the  $^1\text{H}$  n.m.r. spectra of the alcohol (1k) and its triacetate (1j). The signal for 5'-H occurred at  $\delta$  3.85 in the spectrum of (1k), and on saturation of the 5'-Me doublet, this complex signal collapsed to a doublet ( $J$  10 Hz). Since 5'-H is axial,<sup>1</sup> the result establishes that C-4' is hydroxylated, and that 4'-H is *trans*-diaxial to 5'-H. 3'-H Resonates as a doublet of  $J$  3 Hz at  $\delta$  3.6, suggesting an axial-equatorial coupling between it and 4'-H. It is thus equatorial, and hence  $\alpha$ .

The  $^1\text{H}$  n.m.r. spectrum of the 2',3',4'-triacetate (1j) of 4 $\beta$ -hydroxygomphoside supports the stereochemistry assigned. Thus the complex signal of 5'-H collapsed to a doublet ( $J$  10 Hz) when 5'-Me was irradiated. 3'-H is de-

**Stereochemistry of Calotoxin.**—Before this work, calotoxin (3k)<sup>3</sup> was the only example of an Asclepiadaceae-type cardenolide glycoside with a 4'-hydroxy group. It was proposed<sup>1</sup> to have the same 1',2'-*cis*-fused carbohydrate rings as in related glycosides, but the configurations at 3' and 4' remained unresolved. A sample of calotoxin which we isolated<sup>10</sup> from *Calotropis procera* has  $^1\text{H}$  n.m.r. (Table 2) and  $^{13}\text{C}$  n.m.r. signals<sup>10</sup> for the carbohydrate moiety which are identical with those of 4 $\beta$ -hydroxygomphoside (1k). It is clear that calotoxin (3k) has  $\beta$ -hydroxy groups at 3' and 4', and is the 19-oxo-analogue of 4 $\beta$ -hydroxygomphoside (1k).

**Structure of Calotropin Diacetate.**—Calotropin 3'-acetate (3f) (asclepin)<sup>4</sup> yielded calotropin diacetate on acetylation with acetic anhydride-pyridine catalysed by 4-dimethylaminopyridine (see Experimental section). Similar acetylation of 3'-epigomphoside 3'-acetate (1f) and 3'-epiafroside 3'-acetate (2f) gave the acetates (1g) and (2g).

In the  $^1\text{H}$  n.m.r. spectrum of calotropin diacetate, both 1'-H and 3'-H are deshielded by acetoxy groups and resonate at  $\delta$  5.55 and 5.8 respectively. Similar chemical shift values are also observed for 1'-H and 3'-H in 3'-epigomphoside 2',3'-diacetate (1g) and 3'-epiafroside 2',3',15-triacetate (2g) (Table 2). Singh and Rastogi<sup>4</sup> noted a marked downfield shift of 1'-H (1 p.p.m.) upon acetylation of calotropin 3'-acetate (3f). These authors were led to suggest that calotropin diacetate should be formulated as the diacetate (6) with an open keto-alcohol structure. An alternative explanation has since been offered.<sup>1</sup> We now provide  $^{13}\text{C}$  n.m.r. evidence to disprove the suggestion of Singh and Rastogi. Our sample of calotropin diacetate has  $^1\text{H}$  n.m.r. spectral data identical with those previously reported.<sup>4</sup> However, in the  $^{13}\text{C}$  n.m.r. spectrum of this compound (Table 1) the methine carbon C-1' resonates at 99.2 p.p.m. There is no resonance near 200 p.p.m. which would be expected for the carbonyl carbon present in the suggested alternative structure (6). Thus calotropin 2',3'-diacetate is correctly formulated as structure (3g).



Scheme. Methane chemical ionisation mass spectra

**Stereochemistry of the Thiazoline Ring in Uscharin (3b) and 19-Deoxyuscharin (1b).**—The configurations at carbons 1', 2', 3', and 5' of the hexosulose in a large number of *Asclepias* and *Calotropis* cardenolides were determined by us earlier.<sup>1</sup> However, in the case of uscharin (3b) the stereochemistry at C-3', the point of attachment of the thiazoline ring to the hexosulose, remained unassigned. Below we present evidence which indicates that the configuration at C-3' in uscharin is *S*.

Uscharin (3b) and 19-deoxyuscharin (1b) show <sup>1</sup>H and <sup>13</sup>C n.m.r. signals for carbohydrate and thiazoline nuclei which are identical. These two compounds must therefore have the same chirality at C-3'. The chemical shifts of C-1' and C-5' in the <sup>13</sup>C n.m.r. spectra of the 3'-thiazoline compounds (3b) and (1b) differ little from those of the same carbons in the 3'-α-hydroxycardenolides (1e), (2e), and (3e), there being an upfield shift for C-1' of 0.7 p.p.m. and none for C-5' (Table 1). This shows that the combined *γ-gauche* and *γ-anti* effects of the heteroatoms <sup>7-9</sup> N and S in the thiazoline compounds are about the same as the *γ-anti* effect of O in the 3'-α-alcohols. However, the relative orientation of N *vs.* S is not obvious from the <sup>13</sup>C n.m.r. data.

By the use of <sup>1</sup>H n.m.r. decoupling experiments, including some at high magnetic fields (see Table 2), the <sup>1</sup>H chemical shifts of the multiplet due to 5'-H in uscharin (3b), 3'-didehydrogomphoside (1d), 3'-epigomphoside (1e), 3'-epiafroside

(2e), and afroside (2a) were determined. In Table 3, these shift values, as well as those of the 1'-H singlet and of other selected signals, are compared. As expected, structural changes at C-3' have no effect on the chemical shift values of the remote aglycone protons at 2β and 3α, but do cause small changes in the chemical shifts of the 5'-methyl group (6'-H<sub>3</sub>). When compared with the 3'-α-alcohols (1e) and (2e), the 3'-thiazoline ring in uscharin (3b) causes a pronounced deshielding of 1'-H (0.46 p.p.m.) and 5'-H (0.62 p.p.m.). Inspection of a model shows that if N and S were β and α respectively in uscharin (3b), protons 1' and 5' would be in the deshielding region of the CH=N bond on the β-face of the hexosulose ring,\* and be approximately symmetrically placed with respect to it. This anisotropic effect will be absent when the CH=N bond is reduced. In voruscharin (3c) (from *A. curassavica*)<sup>11</sup> which has a saturated (thiazolidine) ring, 1'- and 5'-H resonate some 0.2 p.p.m. upfield of 1'- and 5'-H in uscharin (3b) (Table 3). If the C-3' configuration were reversed, the deshielding effect of a 3'β sulphur atom on 1'-H and 5'-H, which would each be 1,3-diaxial to it, would not change significantly when the CH=N bond is saturated.

\* In contrast, 1'-H and 5'-H are well away from the deshielding region of the carbonyl group of the 3'-ketones (1d), (2d), and (3d). In these ketones 1'-H and 5'-H are not deshielded in comparison with the *epi*-alcohols (1e) and (2e) (Table 3).

Table 1. <sup>13</sup>C Chemical shifts δ (in p.p.m.)

Carbon number	(1a) <sup>c</sup>	(1e) <sup>b</sup>	(1f) <sup>b</sup>	(1d) <sup>c</sup>	(1b) <sup>a</sup>	(1k) <sup>d</sup>	(1k) <sup>g</sup>	(2a) <sup>b</sup>	(2e) <sup>d</sup>	(2f) <sup>b</sup>	(2d) <sup>c</sup>	(3a) <sup>f</sup>	(3e) <sup>f</sup>	(3e) <sup>e</sup>	(3g) <sup>a</sup>	(3b) <sup>b</sup>
1'	94.2	95.8	96.0	97.3	95.3	94.0	95.1	94.4	96.0	96.3	95.3	94.1	95.9	95.8	99.2	95.1
2'	90.3	91.1	90.6	91.3	99.8 <sup>h</sup>	92.1	93.3	90.5	91.3	90.9	91.0	90.5	91.5	91.4	92.3	98.8 <sup>h</sup>
3'	70.7	72.9	73.8	202.0	91.7	74.0	75.0	70.8	72.8	75.5	202.4	70.2	72.4	72.9 <sup>i</sup>	70.3	91.5
4'	36.6	38.2	35.3	45.0	42.7 <sup>j</sup>	69.1	69.8 <sup>*</sup>	36.7	38.5	35.6	45.0	37.2	38.6	38.2	36.3	42.7 <sup>j</sup>
5'	66.2	68.0	67.8	67.8	68.2	71.3	72.5	66.4	68.2	67.9	67.8	65.6	67.6	68.1	67.8	68.2
6'	20.5	20.8	20.8 <sup>*</sup>	21.3	20.9	17.3	18.4	20.6	20.6	20.7	21.3	21.0	21.3	21.0	20.9	20.7
1	42.2	42.0	42.0	42.0	42.3	42.2	42.5	42.3	42.2	42.3	41.8	35.8	35.8 <sup>j</sup>	36.1 <sup>j</sup>	35.8	36.0 <sup>j</sup>
2	69.3	69.0	68.8	69.6	70.0	69.1	69.1 <sup>*</sup>	69.4	68.9	69.1	69.4	68.7	68.4	68.8	68.6	69.7
3	72.5	72.7	72.6	72.8	72.3	72.6	72.7	72.6	72.7 <sup>**</sup>	72.7 <sup>**</sup>	72.4 <sup>**</sup>	71.3	71.5	71.8	71.0	71.4
4	32.0	31.9	32.0	32.1	32.2	32.1	32.6	32.1	32.1	32.2	31.8	31.7	31.8	32.1	32.4	31.9
5	44.8	44.8	44.8	44.8	45.1	44.9	44.7	44.9	44.9	45.1	44.0	43.0 <sup>**</sup>	42.9 <sup>**</sup>	43.4 <sup>**</sup>	43.5 <sup>**</sup>	43.4 <sup>**</sup>
6	27.8	27.7	27.7	27.9	27.8	27.8	27.9	27.9	27.9	27.9	27.5	27.6 <sup>*</sup>	27.6 <sup>*</sup>	27.7 <sup>*</sup>	27.6 <sup>*</sup>	27.6 <sup>*</sup>
7	27.3	27.2	27.2	27.3	27.4	27.2	27.5	26.3	26.3	26.5	27.5	27.3 <sup>**</sup>	27.5 <sup>**</sup>	27.5 <sup>**</sup>	27.5 <sup>**</sup>	27.3 <sup>**</sup>
8	40.5	40.7	40.7	40.6	41.2	40.5	40.7	40.5	40.5	40.7	40.6	41.9 <sup>**</sup>	42.0 <sup>**</sup>	42.2 <sup>**</sup>	42.5 <sup>**</sup>	42.1 <sup>**</sup>
9	49.7	49.6	49.7	49.8	50.0	49.6	49.6	49.3 <sup>*</sup>	49.2 <sup>*</sup>	49.4 <sup>*</sup>	49.6 <sup>*</sup>	48.1	48.0	48.6	48.7	48.6
10	37.8	37.8	37.9	38.1	38.1	37.8	37.6	38.0	38.0	38.1	38.2	52.6	52.6 <sup>h</sup>	52.7 <sup>h</sup>	52.8	52.8 <sup>h</sup>
11	21.3	21.3	21.3	21.3	21.5	21.3	21.3	21.1	21.1	21.2	20.7	21.6	21.7	21.8	22.0	21.9
12	39.6	39.6	39.6	39.7	39.9	39.6	39.3	38.5	38.5	38.6	38.9	38.9	38.9	39.2	39.4	39.2
13	49.7	49.6	49.7	49.8	49.6	49.8	49.1	48.8	48.8	48.8	49.4	49.4	49.4	49.5	49.4	49.4
14	84.8	84.9	84.8	84.9	85.4	84.6	84.1	81.6	81.6	81.6	81.2	83.5	83.6	84.2	84.9	84.4
15	32.4	32.6	32.4	32.5	33.1	32.4	32.7	72.8 <sup>**</sup>	72.8 <sup>**</sup>	72.9 <sup>**</sup>	72.5 <sup>**</sup>	33.3	33.4	33.3	33.1	33.0
16	26.8	26.8	26.8	27.0	27.0	26.8	27.0	37.1	37.1	37.3	37.5	26.6	26.6	26.7	26.8	26.7
17	50.9	50.7	50.8	50.9	51.0	50.9	51.0	49.1 <sup>*</sup>	49.0 <sup>*</sup>	49.1 <sup>*</sup>	48.5 <sup>*</sup>	50.4	50.4	50.7	50.7	50.6
18	15.5	15.6	15.6	15.7	15.8	15.5	15.8	16.5	16.4	16.5	16.5	15.7	15.7	15.7	15.7	15.4
19	13.4	13.6	13.5	13.6	13.8	13.4	13.4	13.7	13.7	13.8	13.9	207.7	208.4	207.3	206.9	207.7
20	176.4	175.5	176.2	176.1	174.4	176.6	175.5	174.9	175.4	174.2	174.9	175.6	175.9	174.7	174.3	175.3
21	73.9	73.7	73.9	74.0	73.4	73.7	73.3	73.9	73.9	73.6	73.2	73.2	73.3	73.4	73.5	73.7
22	117.0	117.3	117.0	117.2	117.7	116.9	117.3	117.6	117.5	117.9	117.4	116.9	116.9	117.5	117.9	117.4
23	175.8	175.2 <sup>h</sup>	175.6 <sup>h</sup>	175.8	174.4	176.6	175.5	175.3 <sup>h</sup>	175.6	174.6	174.9	174.9	174.0	174.4	174.6	175.3
CH <sub>3</sub> CO	—	—	20.6 <sup>*</sup>	—	—	—	—	—	—	20.7	—	—	—	—	20.9	—
CH <sub>3</sub> CO	—	—	171.8	—	—	—	—	—	—	172.0	—	—	—	—	22.0	—
CH=N	—	—	—	—	159.9	—	—	—	—	—	—	—	—	—	170.2	—
CH <sub>2</sub> S	—	—	—	—	47.4	—	—	—	—	—	—	—	—	—	—	160.6
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	47.1

<sup>a</sup> In CDCl<sub>3</sub> with δ<sub>c</sub> 77.1 p.p.m. <sup>b</sup> In 10 : 1 v/v CDCl<sub>3</sub>-CD<sub>3</sub>OD with δ<sub>c</sub> (CDCl<sub>3</sub>) 77.1 p.p.m. <sup>c</sup> In 3 : 1 v/v CDCl<sub>3</sub>-CD<sub>3</sub>OD with δ<sub>c</sub> (CDCl<sub>3</sub>) 77.3 p.p.m. <sup>d</sup> In 1 : 1 v/v CDCl<sub>3</sub>-CD<sub>3</sub>OD with δ<sub>c</sub> (CDCl<sub>3</sub>) 77.4 p.p.m. <sup>e</sup> In 10 : 1 v/v CDCl<sub>3</sub>-CD<sub>3</sub>SOCD<sub>3</sub> with δ<sub>c</sub> (CDCl<sub>3</sub>) 77.3 p.p.m. <sup>f</sup> In 1 : 2 v/v CDCl<sub>3</sub>-CD<sub>3</sub>SOCD<sub>3</sub> with δ<sub>c</sub> (CDCl<sub>3</sub>) 78.8 p.p.m. <sup>g</sup> In pentadeuteriopyridine with δ<sub>c</sub> of C-4 of C<sub>5</sub>D<sub>5</sub>N set at 135.2 p.p.m. <sup>h</sup> This signal is observed as a doublet in the single-frequency off-resonance decoupled (s.f.o.r.d.) spectrum due to J<sub>CC</sub>H. The distinction of the C-2' from the C-3' signal [for (1b) and (3b)] and the distinction of the C-23 from the C-20 signal are based on this observation. The latter distinction is based also on the expectation that C-23 gives a weaker signal under the conditions employed. <sup>i</sup> Sharp doublet in s.f.o.r.d. spectrum showing that the attached proton is loosely coupled to vicinal proton(s). <sup>j</sup> d of d in s.f.o.r.d. spectrum showing that the attached protons are well separated in chemical shifts (see Table 2).

\*\*\* Signals within a vertical column may be reversed.

Table 2. <sup>1</sup>H Chemical shifts (*J* in Hz)<sup>a</sup>

Compd.	1'-H	3'-H	4'β-H	4'α-H	6'-H <sub>3</sub> <sup>f</sup>	5'-H	2β-H	3α-H
(1d) <sup>k</sup>	4.65	—	2.45 ( <i>J</i> 2, 14)	2.8 ( <i>J</i> 11.5, 14)	1.41	3.7m <sup>m</sup>	← 4.0—4.2m <sup>n</sup> →	
(2d) <sup>k</sup>	4.65	—	2.45 ( <i>J</i> 2, 14.5)	2.8 ( <i>J</i> 11.5, 14)	1.39	3.7m <sup>m</sup>	← 4.0—4.2m →	
(3d) <sup>k</sup>	4.65	—	2.45 ( <i>J</i> 2, 14)	2.75 ( <i>J</i> 11.5, 14)	1.39	ca. 3.75m <sup>l</sup>	← 3.8—4.15m →	
(1b) <sup>o</sup>	5.1	—	<sup>e</sup>	2.25 ( <i>J</i> 10, 13)	1.21		← 4.0—4.4m →	
(3b) <sup>b,p</sup>	5.07	—	1.72dd <sup>b</sup>	2.23dd <sup>b</sup>	1.23	4.27m <sup>b</sup>	3.96ddd <sup>b</sup>	4.09ddd <sup>b</sup>
(3c) <sup>b</sup>	4.83	—	1.82dd <sup>b</sup>	1.99dd <sup>b</sup>	1.21	4.12m <sup>b</sup>	3.96ddd <sup>b</sup>	4.07ddd <sup>b</sup>
(1f)	4.6	4.7 ( <i>J'</i> 5, 11.5)			1.28	3.75m	← 3.9—4.2m →	
(2f)	4.6	4.7 ( <i>J'</i> 5, 11)			1.29	<sup>e</sup>	← 3.9—4.2m →	
(3f) <sup>c</sup>	4.55	4.75 ( <i>J'</i> 6, 11)			1.29		← 3.5—4.1m →	
(1g)	5.55	5.85 ( <i>J'</i> 6, 11)			1.25		← 3.6—4.1m →	
(2g)	5.55	5.85 ( <i>J'</i> 6.5, 10.5)			1.25		← 3.7—4.2m →	
(3g)	5.55	5.8 ( <i>J'</i> 6.5, 10)			1.24		← 3.6—4.2m →	
(1e)	4.6	ca. 3.65m <sup>e</sup>			1.27	ca. 3.65m <sup>e</sup>	← 3.9—4.2m →	
(2e)	4.6	ca. 3.65m <sup>e</sup>			1.30	ca. 3.65m <sup>e</sup>	← 3.9—4.2m →	
(3e) <sup>c</sup>	4.55	3.65 <sup>e</sup> ( <i>J'</i> 5.5, 11.5)			1.28	ca. 3.65m <sup>e</sup>	← 3.8—4.1m →	
(2a) <sup>d</sup>	4.78	3.69t ( <i>J'</i> 3)			1.24	ca. 4.1	3.94	4.07
(3k) <sup>c</sup>	4.75	3.65d ( <i>J</i> 3)	—	3.45dd <sup>e</sup> ( <i>J</i> 3, 9.5)	1.28	3.85dq <sup>e,l</sup> ( <i>J</i> 10, 6)	← 3.8—4.1m →	
(1k) <sup>c</sup>	4.75 <sup>e</sup>	3.6d ( <i>J</i> 3)	—	3.45dd <sup>e</sup> ( <i>J</i> 3, 9.5)	1.27	3.85dq <sup>e,l,m</sup> ( <i>J</i> 10, 6)	← 3.8—4.1m →	
Compd.	1β-H	15α-H	17α-H	18-H <sub>3</sub>	19-H	21-H <sub>2</sub> <sup>g</sup>	22-H <sup>h</sup>	CH <sub>3</sub> CO
(1d) <sup>k</sup>			2.7m	0.87	0.87	5.0, 4.8	5.85	—
(2d) <sup>k</sup>		4.55m <sup>l</sup>	2.65m <sup>j</sup>	0.89	0.86	5.0, 4.8	5.85	—
(3d) <sup>k</sup>	<sup>e</sup>		ca. 2.7m <sup>e</sup>	0.81	10.0	4.95, 4.75	5.85	—
(1b) <sup>o</sup>			2.75m	0.86	0.86	5.0, 4.85	5.9	—
(3b) <sup>b,p</sup>	2.48dd <sup>b</sup>		2.76 ( <i>J'</i> 5.5, 9.5)	0.82	10.0	4.97, 4.80	5.88	—
(3c) <sup>b</sup>	2.48dd <sup>b</sup>		2.76 ( <i>J'</i> 5.5, 10)	0.83	10.0	4.94, 4.79	5.90	—
(1f)			2.75m	0.86	0.86	5.0, 4.8	5.85	2.15
(2f)		4.45m <sup>l</sup>	2.65m <sup>j</sup>	0.90	0.86	5.0, 4.8	5.85	2.15
(3f) <sup>c</sup>	ca. 2.5 <sup>e</sup>		ca. 2.7m <sup>e</sup>	0.80	9.95	5.0, 4.85	5.85	2.14
(1g)			2.7m	0.86	0.86	4.95, 4.8	5.85	2.05, 2.09
(2g)		5.45m <sup>l</sup>	2.75m <sup>j</sup>	0.90	0.86	5.0, 4.8	5.9	2.04, 2.08, 2.08
(3g)	2.55 <sup>e</sup> ( <i>J</i> 3.5, 11.5)		ca. 2.7m <sup>e</sup>	0.76	10.0	4.9, 4.75	5.85	2.0, 2.05
(1e)			2.75m	0.87	0.87	5.0, 4.8	5.9	—
(2e) <sup>c</sup>		4.5m <sup>c</sup>	2.7m <sup>j</sup>	0.91	0.86	5.0, 4.8	5.9	—
(3e)	2.5 ( <i>J</i> 2.5, 13)		ca. 2.75m <sup>e</sup>	0.81	9.8	5.0, 4.85	5.85	—
(2a) <sup>d</sup>		4.49m	ca. 2.6m <sup>j</sup>	0.91	0.87	5.09, 4.83	5.84	—
(3k) <sup>c</sup>	ca. 2.45 <sup>e</sup>		ca. 2.75m	0.81	10.1	5.05, 4.85	5.9	—
(1k) <sup>c</sup>			2.75m	0.86	0.86	5.05, 4.9	5.9	—

<sup>a</sup> Determined at 90 MHz in CDCl<sub>3</sub> unless otherwise stated; δ values relative to SiMe<sub>4</sub> (δ 0) or CHCl<sub>3</sub> (δ 7.27) are quoted to nearest 0.05 p.p.m. except for Me signals (to 0.01 p.p.m.); d, t, m, and bs refer to doublet, triplet, multiplet, and broad singlet respectively. *J'* refers to apparent coupling. <sup>b</sup> Determined at 400 MHz in CDCl<sub>3</sub>, δ relative to SiMe<sub>4</sub> being given to nearest 0.01 p.p.m. Coupling constants in Hz measured directly or from decoupled spectra are: 4'α,4'β 13.2; 4'α,5'β 11.1; 4'β,5'β 1.7; 5'β,6' 6.2; 1α,1β 12.6; 1α,2β 11.6; 1β,2β 4.2; 2β,3α 10.0; 3α,4α 4.2; and 3α,4β 10.0 (the last two being apparent *J*). <sup>c</sup> Determined in CDCl<sub>3</sub>-CD<sub>3</sub>OD (5:1) mixture with δ (SiMe<sub>4</sub>) 0. <sup>d</sup> Determined at 270 MHz in CDCl<sub>3</sub>-CD<sub>3</sub>SOCD<sub>3</sub> (10:1) mixture, δ relative to SiMe<sub>4</sub> being quoted to nearest 0.01 p.p.m. Data of ref. 1,2. <sup>e</sup> Masked by other signals. <sup>f</sup> Doublet (*J* 6 Hz). <sup>g</sup> Doublet (*J* 1.5 Hz) of AB quartet (*J*<sub>AB</sub> 18.5 Hz). <sup>h</sup> Triplet (*J* 1.5 Hz). <sup>i</sup> For characteristic shape of this signal see ref. 2. <sup>j</sup> For shape of this signal, which contains also one 16-H, see ref. 2. <sup>k</sup> In dilute CDCl<sub>3</sub> solution the signal for 2'-OH, which is strongly H-bonded to C=O, appeared as a peak at δ 4.0 which could be removed by saturation transfer. <sup>l</sup> On irradiation of 6'-H, this signal in compound (3d) formed a d of d (*J* 2.5, 11 Hz), while the signal in compounds (3k) and (1k) formed a doublet (*J* 10 Hz). <sup>m</sup> Coupling to 6'-H and 4'-H removed upon saturation of this signal. <sup>n</sup> Appearances of 6'-H and 4'-H signals not affected by saturation of this signal. <sup>o</sup> δ 3.85 bs (CH<sub>2</sub>S), 7.55 bs (N=CH). <sup>p</sup> δ 3.88, 3.89 (*J* 17, 1.5 Hz) (CH<sub>2</sub>S); 7.53 (*J* 1.5 Hz) (N=CH).

**Table 3.** Effects of structural changes at 3'-H on chemical shifts of carbohydrate protons<sup>a</sup>

Compd.	6'-H <sub>3</sub>	1'-H	5'-H	2β-H	3α-H
(2a) <sup>b</sup>	1.24	4.78	ca. 4.1	3.94	4.07
(1e)	1.27	4.61	3.65	3.9—4.2	
(2e)	1.30	4.60	3.65	3.9—4.2	
(3b)	1.23	5.07	4.27	3.96	4.09
(3c)	1.21	4.83	4.12	3.96 4.07	
(1d)	1.41	4.64	3.7	4.0—4.2	
(2d)	1.39	4.63	3.7	4.0—4.2	
(3d)	1.39	4.63	3.75	3.8—4.15	

<sup>a</sup> In p.p.m. from SiMe<sub>4</sub> in CDCl<sub>3</sub> at 270 MHz (2a), 90 MHz [(1e), (2e), (1d), (2d), and (3d)], and 400 MHz [(3b) and (3c)]. <sup>b</sup> 10% CD<sub>3</sub>SOCD<sub>3</sub> added; data from ref. 1.

Voruscharin (3c), shown by this study to have a 3'β-NH, in fact gives rise to 1'- and 5'-H signals which are substantially the same as those in afroside (2a) which has a 3'β-OH (Table 3).

While the <sup>1</sup>H chemical shift data discussed above favour a 3'β-configuration for the nitrogen in uscharin (3b), supporting evidence comes from long-range <sup>1</sup>H-<sup>1</sup>H coupling measurements. The signal for the axial 4'α-H in both uscharin (3b) and 19-deoxyuscharin (1b) appears as a doublet of doublets near δ 2.25, the assignment of which is based on the observed large coupling constants (Table 2), and by double-resonance experiments. The 4'β-H signal of uscharin could also be observed at 400 MHz as a doublet of doublets at δ 1.72. On irradiation of the lowfield broad singlet signal of -CH=N- (δ 7.53), the outer lines of the 4'α-H signal narrowed from 1.8 Hz to 1.6 Hz width, whilst the valley between the overlapping inner lines deepened significantly. No concurrent narrowing of the two pairs of lines of the 4'β-H was observed. The long-range coupling over 5 bonds thus observed shows that there is an approximately planar and extended W-path<sup>12</sup> between the -CH=N- and 4'α protons. This coupling is possible in uscharin (3b) if the nitrogen is β but not if it is α. Thus uscharin (3b), and hence 19-deoxyuscharin (1b) and voruscharin (3c), has *S* chirality at C-3'.

## Experimental

High-pressure liquid chromatography (h.p.l.c.) separations were performed on a Whatman Magnum 9 10/50 Partisil semi-preparative column. Separations were monitored by a Waters R403 refractive index detector. T.l.c. separations were performed on Merck silica gel plates. All solvents were redistilled. Light petroleum refers to the b.p. 40–70 °C fraction. Most <sup>13</sup>C and <sup>1</sup>H n.m.r. data were collected on a JEOL FX 90Q Fourier-transform spectrometer operating at 89.6 (<sup>1</sup>H) or 22.5 MHz (<sup>13</sup>C). 400 MHz <sup>1</sup>H n.m.r. spectra were run on a Bruker WM 400 spectrometer. Mass spectra were obtained using a Finnigan 3200E GC mass spectrometer and associated Finigan 6110 data system. All mass spectral data refer to chemical ionisation (c.i.) with methane as the reagent gas.

*Separation of Cardenolides.*—*Asclepias fruticosa* collected near Wiseman's Ferry, New South Wales in March 1982 was dried at 75 °C. (a) Powdered leaf material (4 kg) was percolated successively (three times each) with light petroleum and chloroform at room temperature. The combined chloroform extracts were concentrated to 3 l and filtered under reduced pressure through powdered charcoal (100 g) dry-packed into a Buckner funnel.<sup>13</sup> The charcoal was washed with chloroform (2 l), and the combined filtrates were concentrated under

reduced pressure to give a dark orange gum (40 g, 1%). This gum was dissolved in chloroform (1 l) and chromatographed on Merck Kieselgel H (300 g), dry-packed under vacuum into a Buckner funnel to form a bed 13 × 13 cm.<sup>13</sup> A flow rate of ca. 15 ml min<sup>-1</sup> was obtained by applying a water-pump vacuum and the column was eluted successively with chloroform (fraction No. 1), ethyl acetate–light petroleum mixtures [1 : 1 (No. 2), 2 : 1 (No. 3), 3 : 1 (No. 4), 4 : 1 (No. 5), 5 : 1 (No. 6)], ethyl acetate (No. 7), and ethyl acetate–methanol mixtures [98 : 2 (No. 8), 97 : 3 (No. 9), 95 : 5 (No. 10) and 90 : 10 (No. 11)], with 500 ml of each elution solvent. Cardenolides were monitored by t.l.c. [ethyl acetate and chloroform–acetone (7 : 3)] by spraying with 0.4% 2,2',4,4'-tetranitrophenyl in toluene followed by 10% potassium hydroxide in 1 : 1 methanol–water. Overspraying with 2% vanillin in concentrated sulphuric acid followed by heating to 120 °C revealed non-cardenolide products.

Fractions 4 and 5 contained compounds (1f) and (1d) together with ca. 80% non-cardenolides, and were combined to give a dark yellow gum (6 g). Fractions 6 and 7 (11 g) contained compounds (3d), (3b), (2f), (1f), (3f), (2d), (1b), and (1d), with a trace of polar cardenolides and ca. 20% non-cardenolide substances. Fractions 8–10 (6 g) contained compounds (1a), (3b), (2a), (IV), (1e), and (3a) with ca. 10% non-cardenolides.

Fractions 4 and 5 were rechromatographed on Kieselgel H (100 g) by the vacuum method detailed above using increasing concentrations of methanol (1–10%) in chloroform as eluant. The cardenolides (1f) and (1d), essentially free of other cardenolides, were eluted with 4% methanol in chloroform. Separation was achieved by preparative h.p.l.c. (ethyl acetate) to yield 3'-didehydrogomphoside (1d) (120 mg) and 3'-epi-gomphoside 3'-acetate (1f) (550 mg).

Crystalline material (900 mg; from ethyl acetate) which separated from fractions 6 and 7 was recrystallised from chloroform–methanol–ethyl acetate to give uscharin (3b) (620 mg). The combined mother liquors were chromatographed on Kieselgel H (150 g) by the vacuum procedure above. Elution with chloroform–3% methanol (fraction A) (2.6 g) gave a mixture of compounds (1d), (1f), (3d), and (2d); chloroform–4% methanol (fraction B) (2.3 g) gave a mixture of cardenolides (2f), (1b), and (3f), whilst chloroform–5% methanol gave a fraction rich in uscharin (3b) which was separated by crystallisation from ethyl acetate (400 mg). The mother liquors were returned to fraction B.

Fraction A was separated by h.p.l.c. [ethyl acetate–light petroleum (4 : 1)] to give compound (1d) (30 mg), compound (1f) (320 mg), and a mixture of uscharin (3d) and 3'-didehydroafroside (2d) (1.2 g; ratio 4 : 1), a portion of which was separated into the two pure components by h.p.l.c. chloroform–acetone (3 : 1)] by small injections and peak shaving.

Fraction B was crystallised twice from chloroform to yield 3'-epiafroside 3'-acetate (2f) (500 mg). The mother liquors were separated by h.p.l.c. (ethyl acetate) to give 19-deoxyuscharin (1b) (130 mg), and a mixture of compound (2f) and calotropin 3'-acetate (asclepin) (3f) (1.3 g), a portion of which was separated by h.p.l.c. [chloroform–acetone (3 : 1)] by peak shaving.

Fractions 8–10 (3 g) were separated by h.p.l.c. [ethyl acetate followed by chloroform–acetone (3 : 1)] to give uscharin (3b) (100 mg), gomphoside (1a) (350 mg), 3'-epi-gomphoside (1e) (30 mg), calactin (3a) (30 mg), afroside (2a) (40 mg), and uzarigenin (IV) (40 mg).

(b) Powdered stems (6 kg) were percolated three times with methanol and the extracts were concentrated to 2 l under reduced pressure. The orange extract was decanted from an insoluble wax (ca. 100 g), diluted with water (5 l), and extracted with chloroform (2 × 2 l). The wax was triturated

with light petroleum (2 × 500 ml) and the residue (ca. 10 g) was added to the chloroform extract which was then filtered through charcoal as above and concentrated under reduced pressure to give an orange gum (40 g, 0.66%). The gum in chloroform (1 l) was chromatographed on Kieselgel H (300 g) by the vacuum procedure above. Elution with light petroleum-ethyl acetate gave a fraction (1 g) from which 3'-epigomphoside acetate (1f) (70 mg) was obtained by trituration with diethyl ether. Elution with ethyl acetate (1 l) gave a mixture of cardenolides (6 g) of which uscharin (3b), gomphoside (1a), and 3'-epiafroside 3'-acetate (2f) were the major components. Elution with ethyl acetate-methanol (98 : 2) (600 ml) followed by concentration gave afroside (2a) (700 mg). The mother liquors gave a 1 : 1 mixture of afroside (2a) and 3'-epiafroside (2e), part of which (700 mg) was separated by h.p.l.c. [ethyl acetate-methanol (98 : 2)]. Further elution with ethyl acetate-methanol (96 : 4) (400 ml) gave a mixture (600 mg) of afroside (2a), 3'-epiafroside (2e), and 4β-hydroxygomphoside. This fraction gave a crystalline mixture of 4β-hydroxygomphoside (1k) and afroside (2a) (123 mg; ratio ca. 9 : 2) which was recrystallised from methanol-ethyl acetate to give pure 4β-hydroxygomphoside (1k). The mother liquors were acetylated and separated as detailed later to give the acetate (1j).

3'-Didehydrogomphoside (1d) crystallised from ethyl acetate as needles, m.p. 302—304 °C; *m/z* 517 (25%, MH<sup>+</sup>), 499 (16, MH - H<sub>2</sub>O), 391 (2, a), 373 (28, a - H<sub>2</sub>O), 355 (40, a - 2H<sub>2</sub>O), 337 (16, a - 3H<sub>2</sub>O), 173 (14), 145 (100, d), 144 (17), 129 (7, g), 127 (5, b), 117 (10), 109 (4, b - H<sub>2</sub>O), and 101 (10, g - CO) (Found: C, 67.15; H, 7.75. C<sub>29</sub>H<sub>40</sub>O<sub>8</sub> requires C, 67.4; H, 7.8%).

3'-Epigomphoside 3'-acetate (1f) separated from ethyl acetate-methanol as needles, m.p. 247—248 °C; *m/z* 561 (2%, MH<sup>+</sup>), 543 (4, MH - H<sub>2</sub>O), 419 (5), 391 (13, a) 373 (8, a - H<sub>2</sub>O), 355 (15, a - 2H<sub>2</sub>O), 337 (9, a - 3H<sub>2</sub>O), 171 (15, b) 129 (100, f) 128 (20), 111 (6, c), and 101 (24, f - CO) (Found: C, 64.9; H, 7.8. C<sub>31</sub>H<sub>44</sub>O<sub>9</sub>·CH<sub>3</sub>OH requires C, 64.8; H, 8.2%).

3'-Epiafroside 3'-acetate (2f) separated from chloroform as needles, m.p. 199—201 °C; *m/z* 577 (1%, MH<sup>+</sup>), 559 (1, MH - H<sub>2</sub>O), 435 (1), 407 (2, a), 389 (1, a - H<sub>2</sub>O), 371 (1, a - 2H<sub>2</sub>O), 199 (7), 171 (30, b), 129 (100, f) 128 (28), 111 (4, c), and 101 (13, f - CO) (Found: C, 64.25; H, 7.55. C<sub>31</sub>H<sub>44</sub>O<sub>10</sub> requires C, 64.6; H, 7.7%).

3'-Epiafroside (2e) separated from ethyl acetate-methanol as prisms, m.p. 210—212 °C; *m/z* 535 (14%, MH<sup>+</sup>), 407 (22, a), 389 (6, a - H<sub>2</sub>O), 371 (13, a - 2H<sub>2</sub>O), 141 (16), and 129 (100, b) (Found: C, 63.2; H, 7.8. C<sub>29</sub>H<sub>42</sub>O<sub>9</sub>·H<sub>2</sub>O requires C, 63.0; H, 8.0%).

19-Deoxyuscharin (1b) separated from chloroform as very pale yellow crystals, m.p. 243.5—244.5 °C; *m/z* 574 (46%, MH<sup>+</sup>), 556 (46, MH - H<sub>2</sub>O), 538 (8, MH - 2H<sub>2</sub>O), 528 (85, MH - CH<sub>2</sub>S), 510 (8, 528 - H<sub>2</sub>O), 431 (10), 419 (29), 391 (94, a), 375 (18), 373 (42, a - H<sub>2</sub>O), 355 (48, a - 2H<sub>2</sub>O), 337 (18, a - 3H<sub>2</sub>O), 200 (17), 186 (27), 184 (70, b), 156 (52), 154 (50), 138 (100, e) 128 (41), and 126 (47) (Found: C, 53.55; H, 6.25; N, 1.7. C<sub>31</sub>H<sub>43</sub>O<sub>7</sub>NS·CHCl<sub>3</sub> requires C, 54.05; H, 6.5; N, 1.95%).

Uscharin (3b) separated from ethyl acetate as pale yellow prisms, m.p. 263—265 °C (lit.,<sup>3</sup> 270 °C); *m/z* 588 (82%, MH<sup>+</sup>), 570 (12, MH - H<sub>2</sub>O), 542 (18, MH - CH<sub>2</sub>S), 433 (10), 405 (26, a), 387 (23, a - H<sub>2</sub>O), 369 (10, a - 2H<sub>2</sub>O), 184 (78, b), 156 (12), 154 (13), 152 (34), 138 (100, e), 128 (44), and 126 (46).

3'-Dideohydroafroside (2d) separated from chloroform as needles, m.p. 172—173 °C; *m/z* 533 (27%, MH<sup>+</sup>), 407 (8, a), 389 (28, a - H<sub>2</sub>O), 371 (20, a - 2H<sub>2</sub>O), 173 (27), 145 (67, d), 129 (100, g), 127 (54, b), 111 (10), 101 (88, g - CO), 100 (24), 99 (33, b - CO), 87 (48), and 83 (29) (Found: C, 63.45; H, 7.5. C<sub>29</sub>H<sub>40</sub>O<sub>9</sub>·H<sub>2</sub>O requires C, 63.25; H, 7.7%).

3'-Epigomphoside (1e) separated from ethyl acetate-methanol as a microcrystalline solid, m.p. 250—251 °C; *m/z* 519 (0.5%, MH<sup>+</sup>), 501 (0.5, MH - H<sub>2</sub>O), 483 (0.5, MH - 2H<sub>2</sub>O), 419 (5), 401 (2), 391 (15, a), 373 (11, a - H<sub>2</sub>O), 355 (18, a - 2H<sub>2</sub>O), 333 (10, a - 3H<sub>2</sub>O), 157 (8), 129 (100, b/f), 111 (10, c), and 101 (2, 129 - CO) (Found: C, 66.25; H, 7.95. C<sub>29</sub>H<sub>42</sub>O<sub>8</sub>·½H<sub>2</sub>O requires C, 66.0; H, 8.2%).

Calotropin 3'-acetate (asclepin) (3f) was isolated as crystals, m.p. 299—305 °C (lit.,<sup>4</sup> 308—309 °C), on evaporation of an h.p.l.c.-purified fraction under reduced pressure; *m/z* 585 (0.5%, MH<sup>+</sup>), 557 (0.5, MH - CO), 405 (7, a), 387 (8, a - H<sub>2</sub>O), 369 (5, a - 2H<sub>2</sub>O), 351 (2, a - 3H<sub>2</sub>O), 199 (10), 171 (40, b), 129 (100, f), 111 (20, c), and 101 (38, f - CO).

4β-Hydroxygomphoside (1k) separated from ethyl acetate-methanol as a microcrystalline solid, m.p. 224—226 °C; *m/z* 535 (1%, MH<sup>+</sup>), 517 (1, MH - H<sub>2</sub>O), 391 (7, a), 373 (4, a - H<sub>2</sub>O), 355 (5, a - 2H<sub>2</sub>O), 337 (1, a - 3H<sub>2</sub>O), 145 (100, b), and 127 (75, c) (Found: C, 61.7; H, 7.65. C<sub>29</sub>H<sub>42</sub>O<sub>9</sub>·½H<sub>2</sub>O requires C, 62.0; H, 8.05%). The 2',3',4'-triacetate (1j) obtained by acetylation as described below for the preparation of the acetates (3g) and (2g), followed by h.p.l.c. separation [chloroform-methanol (97 : 3)] had *m/z* 661 (15%, MH<sup>+</sup>), 601 (16, MH - HOAc), 391 (1, a), 373 (1, a - H<sub>2</sub>O) and 229 (30, b).

Other cardenolide glycosides. Calactin (3a), m.p. 250—260 °C (lit.,<sup>3</sup> 265—268 °C), gomphoside (1a), m.p. 235 °C (lit.,<sup>14</sup> 235—242 °C), afroside (2a), m.p. 255—260 °C (lit.,<sup>2</sup> 259—264 °C), and uzarigenin (IV), m.p. 237 °C were isolated by concentration of appropriate h.p.l.c. fractions under reduced pressure. Each had n.m.r. spectral data as shown in Tables 1 and 2. Evaporation of an h.p.l.c. fraction containing uscharidin (3d) and 3-dideohydroafroside (2d) in the ratio 9 : 1 gave crystals which were dissolved in chloroform. Storage at 0 °C overnight gave pure uscharidin (3d) as the chloroform solvate (80% recovery of uscharidin), m.p. 195—196 °C (lit.,<sup>15</sup> 194—196 °C).

*Formation of 3'-Epigomphoside (1e) and 3'-Epiafroside (2e) by Hydrolysis of the Acetates (1f) and (2f).*—To a solution of 3'-epigomphoside 3'-acetate (1f) (20 mg) in methanol (3 ml) was added a saturated solution of potassium hydrogen carbonate in methanol (3 ml), and the mixture was kept at room temperature overnight. The mixture was diluted with water (10 ml), kept at room temperature for 2 h, and the precipitate was collected, washed with water, and dried. Recrystallisation from chloroform-methanol gave 3'-epigomphoside (1e), m.p. 251—252 °C (14 mg) with spectral characteristics identical with those of the natural product (see above). A similar hydrolysis of 3'-epiafroside 3'-acetate (2f) gave 3'-epiafroside (2e), m.p. 210 °C.

*Conversion of the Thiazolines (1b) and (3b) into the 3'-Ketones (1d) and (3d).*—To a solution of the thiazoline (1b) (20 mg) in acetone (3 ml) and water (0.5 ml) was added a solution of mercury(II) chloride (50 mg) in acetone (2 ml) and the mixture was kept at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate (3 × 15 ml). Concentration of the dried (Na<sub>2</sub>SO<sub>4</sub>) combined extracts gave 3'-dideohydrogomphoside (1d) (11 mg) identical with the natural product. Similar treatment of uscharin (3b) gave uscharidin (3d).

*Separation of Calotropin 2',3'-Diacetate (3g) and 3'-Epiafroside 2',3',15-Triacetate (2g).*—To a mixture of asclepin (3f) and 3'-epiafroside 3'-acetate (2f) (100 mg; ratio 1 : 5) in acetic anhydride (5 ml) and pyridine (5 ml) was added 4-dimethylaminopyridine (5 mg), and the solution was kept overnight. The mixture, diluted with water (60 ml), was



extracted with ethyl acetate (2 × 20 ml) and the combined extracts were washed in turn with 1M HCl (2 × 10 ml), aqueous sodium hydrogencarbonate (2 × 10 ml), and water (20 ml). Concentration of the dried (Na<sub>2</sub>SO<sub>4</sub>) organic layer gave a gum which was separated by h.p.l.c. [ethyl acetate–light petroleum (3 : 1); 10 mg injections in CHCl<sub>3</sub>]. Concentration of appropriate fractions gave (i) calotropin 2',3'-diacetate (3g) as an amorphous solid<sup>4</sup> (28 mg), *m/z* 617 (0.1%, (MH<sup>+</sup>), 557 (0.4, MH – HOAc), 497 (0.2, MH – 2HOAc), 391 (0.2), 171 (2, *b*), 129 (10, *f*), 103 (5), 101 (6), 83 (8), and 61 (100); and (ii) 3'-epiafroside 2',3',15-triacetate (2g) (70 mg) which separated from ethyl acetate–hexane as prisms, m.p. 171–172 °C; *m/z* 661 (25%, MH<sup>+</sup>), 601 (33, MH – HOAc), 541 (11, MH – 2HOAc), 171 (16, *b*), 129 (20, *f*), 111 (39, *c*), 101 (10, *f* – CO), and 61 (100) (Found: C, 63.5; H, 7.35. C<sub>35</sub>H<sub>48</sub>O<sub>12</sub> requires C, 63.6; H, 7.35%).

3'-Epigomphoside 2',3'-diacetate (1g) was prepared from 3'-epigomphoside 3'-acetate (1f) (20 mg) by the acetylation procedure above and crystallised from ethyl acetate–hexane as prisms, m.p. 185.5–186.5 °C; *m/z* 603 (6%, MH<sup>+</sup>), 543 (5, MH – HOAc), 225 (2), 171 (3, *b*), 129 (4, *f*), 111 (6, *c*), 103 (4), 101 (9, *f* – CO), and 61 (100) (Found: C, 65.4; H, 7.55. C<sub>33</sub>H<sub>46</sub>O<sub>10</sub> requires C, 65.75; H, 7.7%).

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#### References

- 1 H. T. A. Cheung and T. R. Watson, *J. Chem. Soc., Perkin Trans. I*, 1980, 2162.
- 2 H. T. A. Cheung, R. G. Coombe, W. T. L. Sidwell, and T. R. Watson, *J. Chem. Soc., Perkin Trans. I*, 1981, 64.
- 3 F. Brüscheiler, W. Stöcklin, K. Stöckel, and T. Reichstein, *Helv. Chim. Acta*, 1969, **52**, 2086 and the work of G. Hesse *et al.* cited therein.
- 4 B. Singh and R. P. Rastogi, *Phytochemistry*, 1972, **11**, 757.
- 5 S. Nishio, M. S. Blum, J. V. Silverton, and R. J. Highet, *J. Org. Chem.*, 1982, **47**, 2154.
- 6 H. T. A. Cheung, T. R. Watson, J. N. Seiber, and C. Nelson, *J. Chem. Soc., Perkin Trans. I*, 1980, 2169 and references cited therein.
- 7 G. C. Levy, R. L. Lichter, and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance Spectroscopy,' Wiley-Interscience, New York, 1980, 2nd edition.
- 8 E. L. Eliel, F. W. Bailey, L. D. Kopp, R. L. Willer, D. M. Grant, R. Bertrand, K. A. Christensen, D. K. Dalling, M. W. Duch, E. Wenkert, F. M. Schell, and D. W. Cochran, *J. Am. Chem. Soc.*, 1975, **97**, 322.
- 9 E. L. Eliel and K. M. Pietrusiewicz in 'Topics in Carbon-13 N.M.R. Spectroscopy,' ed. G. C. Levy, Wiley-Interscience, New York, 1979, vol. III, pp. 203–205.
- 10 H. T. A. Cheung, F. C. K. Chiu, T. R. Watson, and R. J. Wells, unpublished results.
- 11 J. N. Seiber, C. J. Nelson, and S. M. Lee, *Phytochemistry*, 1982, **21**, 2345.
- 12 S. Sternhell, *Quart. Rev.*, 1969, **23**, 236.
- 13 N. M. Targett, J. P. Kilcoyne, and B. Green, *J. Org. Chem.*, 1979, **44**, 4962.
- 14 J. Cable, R. G. Coombe, and T. R. Watson, *Aust. J. Chem.*, 1964, **17**, 1423.
- 15 D. H. G. Crout, C. H. Hassall, and T. L. Jones, *J. Chem. Soc.*, 1964, 2187.

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