Toxic Constituents of the Asclepiadaceae. Structure Elucidation of Sarcovimiside A-C, Pregnane Glycosides of *Sarcostemma viminale*

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The structure elucidation of three related pregnane glycosides, sarcovimiside A, B, and C from *Sarcostemma viminale*, is based on a detailed study of their highfield ^1H and ^{13}C NMR spectra. The results show that all the sugars are (1→4)-linked and both β-D- and α-L-cymarose residues are present in each glycoside chain. The aglycone of sarcovimiside A was identified as 12β-benzoyloxy-3β,8β,14,17-tetrahydroxy-14β,17α-pregn-5-en-20-one whereas in the case of both sarcovimiside B and C, oxidative cleavage of the C(8)–C(14) bond occurred and the aglycone is the 12,20-*O*,*O*-dibenzoyl derivative of (20*S*)-3β,5,12β,17,20-pentahydroxy-8,14-seco-5β,17α-pregn-6-ene-8,14-dione. Sarcovimiside C is the β-(1→4)-glucopyranosyl derivative of sarcovimiside B.

Sarcostemma viminale (L.) R.Br. (Asclepiadaceae) is a sturdy, succulent, leafless creeper which grows in and over trees and shrubs throughout Southern Africa. Poisoning of cattle and goats occurs in times when grazing is scarce, and specifically when the trees and shrubs which support the plant are chopped down. The plant affects the nervous system and nervousness, hypersensitivity, muscular tremors and convulsions occur, and affected animals may die of asphyxiation. Chronically intoxicated animals may become paralysed and die after 7 days.[1,2]

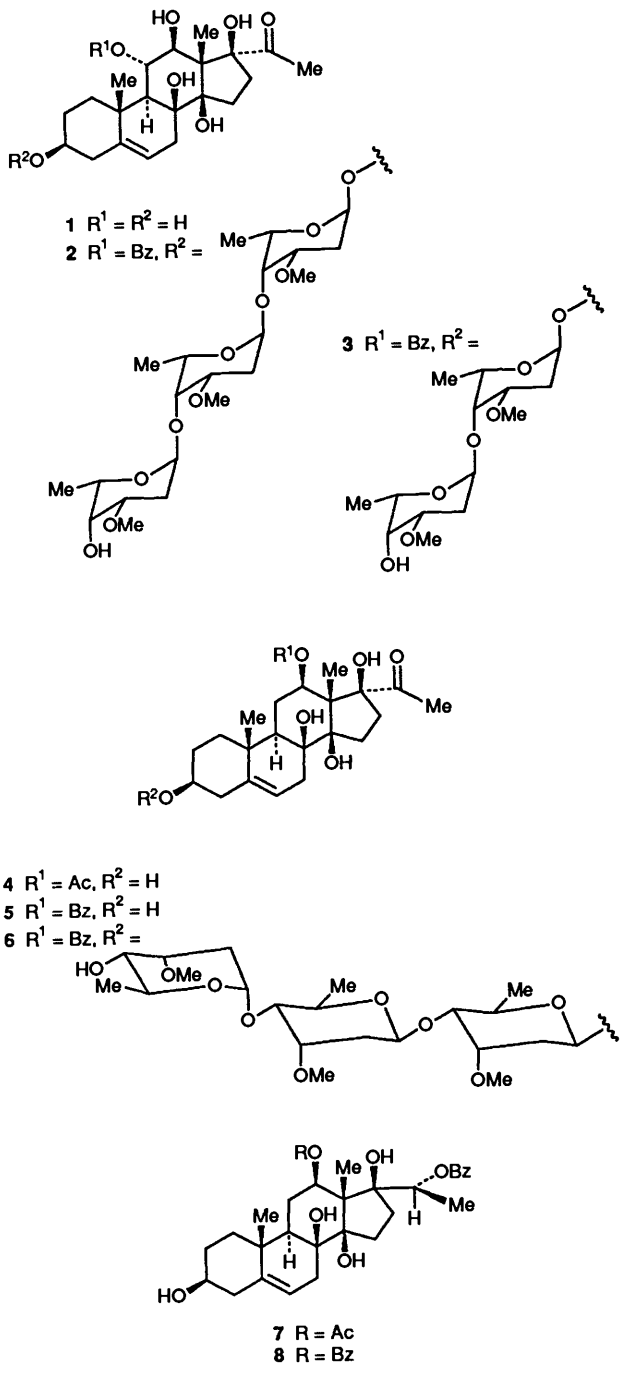
Investigation of *Sarcostemma* species by other workers led to the isolation from *S. brevistigma* of sarcogenin **1** (3β,8β,-11α,12β,14,17-hexahydroxy-14β,17α-pregn-5-en-20-one),[3] as well as brevine **2** and brevinine **3**, the 3-*O*-tri- and -diglycoside derivatives of 11-*O*-benzoylsarcogenin, respectively, involving in each case (1→4)-linked α-L-diginose residues.[4] In earlier work by Reichstein and co-workers[5,6] the isolation of metaplexigenin **4** and cynanforidine **5** (*i.e.* 12β-benzoyloxydeacetylmetaplexigenin) as well as (20*S*)-12β-acetoxy-20-benzoyloxysarcostin **7** and (20*S*)-12,20-dibenzoyloxysarcostin **8**, from a mixture obtained by acid-catalysed hydrolysis of a crude extract of *S. viminale*, is described. The structures of the corresponding glycosides, however, were not reported and remained unknown.

In our continuing research on plants involved in the poisoning of livestock, we investigated the toxic principles of *S. viminale*, and we now report on the structure elucidation of three pregnane glycosides, the sarcovimisides. Fresh plant material of *S. viminale* was collected in September 1987 in the district of Christiana, Western Transvaal following a case of suspected poisoning. Extraction of the dried, milled plant material with methanol, followed by solvent–solvent partitioning and extensive column and preparative layer chromatography on both silica gel and reversed-phase silica gel, led to the isolation of three toxic compounds, named sarcovimiside A–C in order of decreasing R_f values on silica gel TLC plates.

The structural assignments of the sarcovimisides are based on a detailed study of the highfield ^1H and ^{13}C NMR spectral data of the compounds. The first stage in the characterization of the glycosides was the identification of the ^1H NMR signals belonging to isolated spin systems, *i.e.* to the protons of individual sugar residues or the aglycone, and was achieved by means of two-dimensional (2D) (^1H,^1H) correlation spectroscopy,[7,8] using the COSY-45 sequence. The multiplicities of the different resonances in the ^{13}C NMR spectra were deduced from the coupled ^{13}C NMR spectra as well as from the proton-decoupled CH, CH_2 and CH_3 subspectra obtained using the DEPT pulse sequence.[7,9] The ^{13}C resonances were partly assigned by correlation of the proton-bearing carbon atoms with specific proton resonances in 2D (^{13}C,^1H) chemical-shift experiments.[10,11] The signals of the quaternary carbon atoms were assigned by the observation of specific two- and three-bond (^{13}C,^1H) connectivities in heteronuclear $^{13}C\{^1H\}$ selective population inversion (SPI) experiments.[12] The relative stereochemistry of individual sugar residues followed from the magnitude of the (^1H,^1H) coupling constants. Sequence information and the site of the glycosidic bonds were deduced from interglycosidic long-range (^1H,^1H) couplings.[13] The ^1H and ^{13}C NMR data of sarcovimisides A–C are collated in Tables 1 and 2, respectively.

Sarcovimiside A **6** was obtained as an amorphous powder from aqueous methanol and analysed for $C_{49}H_{72}O_{16}\cdot 2.5H_2O$. The ion at m/z 939 $(M + Na)^+$ in the FAB mass spectrum of sarcovimiside A is consistent with the molecular formula of $C_{49}H_{72}O_{16}$. Acid hydrolysis of **6** led to the isolation of the aglycone, identified as cynanforidine **5** and a single sugar, cymarose, identified by comparison with an authentic sample.

The ^1H and ^{13}C NMR data for sarcovimiside A **6** pointed to the presence in the molecule of three *O*-methylated 2,6-dideoxy sugars which were identified from the ^1H NMR data as three cymarose moieties. The (^1H,^1H) coupling between 4-OH and 4-H of the terminal cymarose residue was the starting point in the assignment of the ^1H NMR resonances, and thus the corresponding ^{13}C resonances, to specific cymarose moieties. The magnitude of the (^1H,^1H) coupling constants indicated the presence of one equatorial (J 4.8 and 1.7 Hz) and two axial (J 9.6 and 2.0 Hz) anomeric protons. The COSY experiment established that the equatorial anomeric proton (δ_H 4.770) forms part of the connectivity network of the sugar moiety containing the 4-hydroxy substituent, the terminal cymarose, which on the basis of the (^1H,^1H) coupling constants of the anomeric proton (J 4.8 and 1.7 Hz) is involved in an α-glycosidic link. On the same basis the two cymarose residues with the axial anomeric protons are involved in β-glycosidic linkages.



Sequence information on the sugars of the glycoside was deduced from a long-range (^{1}H,^{1}H) COSY-45 experiment. Although four-bound couplings are small[15] [^4J(HCOCH) < 0.2 Hz], they can be detected in a COSY experiment in which the fixed delay in the pulse sequence is adjusted (0.4 s) to enhance correlations through long-range couplings. In this manner a correlation was observed between the anomeric proton of the terminal cymarose moiety (δ_H 4.770) and the 4-H (δ_H 3.205) of the neighbouring cymarose sugar which enabled us to assign the ^{1}H and ^{13}C resonances to the different cymarose residues. The corresponding correlations for the protons involved in β-linkages were not observed.

A survey of closely related glycosides from the Asclepiadaceae family[14,16-20] reveals that all the β-linked 2,6-dideoxy sugars have the D configuration, whereas the α-linked sugars are mostly L-sugars. Additional evidence for the C-1 configuration of the 2-deoxy sugars present in the sarcovimisides was

Table 1 ^{1}H NMR data (J/Hz) for sarcovimiside A **6** and B **9**

Proton	**6**	**9**
Aglycone		
1	1.89 m	1.69 m
	1.14 m	1.24 m
2	1.88 m	1.42 m
	1.60 m	0.85 m
3	3.52 m	3.46 m
4	2.380 ddd, J 12.8, 4.9, 1.8	1.84 m
	2.25 m	1.44 m
6	5.354 m	6.403 d, J 10.3
7	2.206 m	5.792 d, J 10.3
9	1.547 d, J 9.7	2.439 dd, J 10.9, < 1.0
11	1.96 m	1.792 ddd, J 14.5, 10.9, < 1.0
		1.485 ddd, J 14.5, 9.4, < 1.0
12	4.823 d, J 9.9	5.810 dd, J 9.4, < 1.0
15†	2.02 m	2.971 ddd, J 19.0, 11.0, 2.2
		2.503 ddd, J 19.0, 9.5, 9.5
16†	2.839 ddd, J 14.8, 11.8, 5.6	2.355 ddd, J 13.7, 11.1, 9.1
	1.86 m	2.064 ddd, J 13.6, 9.6, 2.1
18	1.518 s	1.402 s
19	1.105 s	0.672 s
20	—	5.099 q, J 6.2
21	2.041 s	1.343 d, J 6.2
OCO*Ph*	7.912 m (2 H)	7.722 m (2 H)
	7.530 m (1 H)	7.527 m (2 H)
	7.408 m (2 H)	7.478 m (1 H)
		7.439 m (1 H)
		7.284 m (2 H)
		7.174 m (2 H)
	D-Cymarose	D-Digitoxose
1	4.827 dd, J 9.6, 2.0	4.629 dd, J 9.7, 2.1
2_{eq}	2.060 ddd, J, 13.9, 3.7, 2.0	1.959 ddd, J 13.9, 3.5, 2.1
2_{ax}	1.558 ddd, J 13.9, 9.5, 2.6	1.560 ddd, J 13.9, 9.7, 3.1
3	3.777 ddd, J 3.7, 3.0, 2.6	4.126 ddd, J 3.5, 3.1, 3.0
4	3.193 dd, J 9.6, 3.0	3.085 dd, J 9.3, 3.0
5	3.824 dd,[a] J 9.6, 6.3	3.609 dd, J 9.3, 6.3
6	1.183 d,[b] J 6.3	1.084 d, J 6.2
OMe	3.462 s[d]	—
	D-Cymarose	D-Cymarose
1	4.742 dd, J 9.6, 2.0	4.747 dd,[c] J 9.7, 2.1
2_{eq}	2.117 ddd, J 13.8, 3.7, 2.0	2.057 ddd, J 13.8, 3.8, 2.1
2_{ax}	1.629 ddd, J 13.8, 9.6, 2.6	1.571 ddd, J 13.9, 9.8, 2.4
3	3.673 ddd, J 3.7, 2.8, 2.6	3.635 ddd, J 3.8, 2.7, 2.4
4	3.205 dd, J 9.6, 2.8	3.173 dd, J 9.6, 2.7
5	3.850 dd,[a] J 9.6, 6.3	3.870 dd, J 9.6, 6.2
6	1.191 d,[b] J 6.3	1.152 d, J 6.2
OMe	3.426 s[d]	3.459 s
	L-Cymarose	L-Cymarose
1	4.770 dd, J 4.8, 1.7	4.746 dd,[c] J 4.7, 1.8
2_{eq}	2.255 ddd, J 14.9, 3.9, 1.7	2.236 ddd, J 15.0, 3.9, 1.8
2_{ax}	1.712 ddd, J 14.9, 4.8, 3.4	1.701 ddd, J 15.0, 4.8, 3.4
3	3.562 ddd, J 3.9, 3.6, 3.4	3.552 dd, J 3.9, 3.6, 3.4
4	3.231 dd, J 9.2, 3.6[e]	3.227 dd, J 9.1, 3.6[e]
5	4.018 dd, J 9.2, 6.4	3.996 dd, J. 9.2, 6.3
6	1.240 d, J 6.4	1.230 d, J 6.3
OMe	3.364s	3.344 s

† Assignments may be interchanged for **6**. [a-d] May be interchanged. [e] After D_2O exchange.

provided by ^{13}C NMR data. An analysis of the ^{13}C chemical-shift values for the anomeric carbon atom of the 2-deoxy sugars (cymarose, oleandrose and digitoxose) of a large number of steroid glycosides reveals that C-1 of the β-D-sugars resonates at δ_C 35–38[14,16-19] and that of α-D-sugars at δ_C 35–36[20] whereas the corresponding carbon atom of the α-L-sugar appears in the δ_C 30–32 region. The chemical shift values for C-2 of the sugar moieties in sarcovimiside A **6** show that the terminal α-linked cymarose (δ_C 30.91) has the L-configuration and the two β-linked cymaroses (δ_C 35.89 and 35.72) the D-

Table 2 ^{13}C NMR data for sarcovimiside A **6**, B **9** and C **10**

Carbon	6	9	10
Aglycone			
1	38.79 T	25.23 T	25.23 T
2	28.91 T	25.83 T	25.82 T
3	77.80 D	74.31 D	74.29 D
4	38.79 T	35.86 T	35.42 T
5	140.61 S	73.97 S	74.02 S
6	117.59 D	153.05 D	152.99 D
7	34.24 T	127.08 D	127.10 D
8	74.27 S	201.50 S	201.52 S
9	43.67D	46.97 D	46.97 D
10	37.14 S	45.53 S	45.53 S
11	24.16 T	27.43 T	27.43 T
12	73.19 D	71.79 D	71.79 D
13	58.33 S	62.17 S	62. 19 S
14	88.00 S	217.02 S	217.18 S
15	33.25 T[a]	33.63 T	33.66 T
16	31.98 T[a]	28.89 T	28.90 T
17	91.43 S	83.02 S	83.03 S
18	9.49 Q	11.00 Q	11.03 Q
19	18.55 Q	17.42 Q	17.43 Q
20	209.28 S	74.24 D	74.23 D
21	27.33 Q	14.23 Q	14.25 Q
O-Benzoate			
OCOPh	165.12 S	166.61 S	166.61 S
		164. 21 S	164.23 S
OCOPh	133.07 D	132.94 D	132.96 D
		132.87 D	132.89 D
	129.98 S	129.90 S	129.87 S
		129.78 S	129.76 S
	129.50 D (2×)	129.58 D (2×)	129.59 D (2×)
		129.40 D (2×)	129.41 D (2×)
	128.39 D (2×)	128.16 D (4×)	128.18 D (4×)
	D-Cymarose	D-Digitoxose	D-Digitoxose
1	96.06 D	97.01 D	96.93 D
2	35.72 T	36.77 T	36.78 T
3	77.20 D[b]	66.16 D	66.19 D
4	81.55 D	82.03 D	82.09 D
5	68.69 D	68.15 D	68.13 D
6	18.28 Q[c]	18.00 Q[d]	17.98 Q
OMe	58.10 Q	—	—
	D-Cymarose	D-Cymarose	D-Cymarose
1	99.62 S	98.30 D	98.24 D
2	35.89 T	36.01 T	35.80 T
3	77.12 D[b]	77.08 D	77.08 D
4	82.43 D	81.40 D	81.77 D
5	68.54 D	69.02 D	68.86 D
6	18.15 Q[c]	18.18 Q	18.08 Q
OMe	58.10 Q	58.24 Q	58.00 Q
	L-Cymarose	L-Cymarose	L-Cymarose
1	98.19 D	98.30 D	98.57 D
2	30.91 T	30.91 T	30.63 T
3	74.71 D	74.72 D	72.87 D
4	72.12 D	72.05 D	78.91 D
5	65.66 D	65.74 D	65.23 D
6	18.02 Q	17.97 Q[d]	18.22 Q
OMe	56.19 Q	56.21 Q	56.06 Q

[a–d] May be interchanged.

configuration. In the light of the foregoing evidence, the structure of sarcovimiside A **6** was established as 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosylcynanforidine.

Elemental analysis established the molecular formula of sarcovimiside B **9** as $C_{55}H_{74}O_{18}\cdot 2H_2O$ in agreement with the ion at *m/z* 1045 $(M + Na)^+$ in the FAB mass spectrum. The ^1H and ^{13}C NMR data of **9** once again indicated the presence of three 2,6-dideoxy sugars but in this case involving a digitoxose and two cymarose moieties. The location of a hydroxy group at C-4 and C-3 in two separate sugars was deduced from the ^2H-induced β-isotope shifts for the δ_C 72.04 ($\Delta\delta$ −0.114 ppm, C-4) and 66.16 ($\Delta\delta$ −0.120 ppm, C-3) resonances in the proton-decoupled ^{13}C NMR spectrum when the exchangeable protons were partially exchanged with ^2H upon addition of D_2O–H_2O (1:1 v/v).[21] The relative stereochemistry of the sugar moieties, derived from the (^1H,^1H) coupling constants of the sugar protons, and the methoxy resonances at δ_H 3.459 and 3.344, identify two of the sugars as cymarose residues. The three-bond (^{13}C,^1H) connectivities between the methoxy protons (δ_H 3.344 and 3.459) and the C-3 carbon atoms which resonate at δ_C 74.72 and 77.08, respectively, were established in $^{13}C\{^1H\}$ SPI experiments.[10] The δ_C 74.72 resonance furthermore, shows a one-bond (^{13}C,^1H) correlation with the resonance at δ_H 3.552, which was assigned to 3-H of the connectivity network of the sugar containing the 4-hydroxy group. The terminal sugar moiety is, therefore, a cymarose which on the basis of the (^1H,^1H) coupling constants of the anomeric proton (*J* 4.7 and 1.8 Hz) is involved in an α-linkage. The (^1H,^1H) coupling constants indicate that the remaining two sugars, one of which is a cymarose, are β-linked (*J* 9.7 and 2.1 Hz).

Table 3 Two- and three-bond (^{13}C,^1H) connectivity pattern for sarcovimiside B **9**

	δ_H	Correlation signals/δ_C
1	0.672 (19-H)	73.97 S (C-5), 46.97 D (C-9), 45.53 S (C-10), 25.23 T (C-1)
2	1.343 (21-H)	83.02 S (C-17), 74.24 D (C-20)
3	1.406 (18-H)	217.02 S (C-14), 83.02 S (C-17), 71.79 D (C-12), 62.17 S (C-13)
4	3.344 (OCH_3)	74.72 D (C-3: L-Cymarose)
5	3.459 (OCH_3)	77.08 D (C-3: D-Cymarose)
6	5.099 (20-H)	83.02 S (C-17), 62.17 S (C-13), 28.89 T (C-16), 14.23 Q (C-21)
7	5.792 (7-H)	73.97 S (C-5)
8	6.403 (6-H)	201.50 S (C-14)

The sequence of the different sugars in sarcovimiside B **9** was established in a long-range (^1H,^1H) COSY-45 experiment which showed a correlation between the anomeric proton of the terminal cymarose moiety (δ_H 4.746) and the 4-H (δ_H 3.173) of the β-linked neighbouring sugar, cymarose, It therefore follows that the digitoxose sugar is involved in the β-linkage with the aglycone.

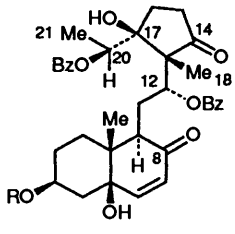
The C-2 ^{13}C chemical-shift values for the sugar residues in sarcovimiside B **9** indicate that the terminal α-linked cymarose (δ_C 30.91) has the L-configuration and the β-linked cymarose (δ_C 36.01) and digitoxose (δ_C 36.77) the D-configuration.

With the structure of the trisaccharide to hand, attention was now directed towards identification of the aglycone of sarcovimiside B. Several structural features were evident from the ^1H NMR spectrum of **9**. The three-proton singlets at δ_H 1.406 and 0.672 were assigned to C-18 and C-19, respectively. The presence of two benzoate groups was deduced from the multiplets in the δ_H 7–8 region. First-order analysis of a number of multiplets which exhibited fine structure, identified four isolated spin systems. The first system is characterised by a three-proton doublet resonance at δ_H 1.343 (*J* 6.2 Hz) which is coupled to the resonance at δ_H 5.099 (q, *J* 6.2 Hz) and is assigned to 21-H and 20-H, respectively. The chemical shift for the 20-H resonance, which correlates with the resonance at δ_C 74.24, indicates that the oxygen present at the methine carbon atom forms part of an ester moiety, a benzoate group. The presence of two hydroxy groups at C-5 and C-17 was inferred from the β-isotope shifts observed for the δ_C 73.97 ($\Delta\delta$ −0.113 ppm) and 83.02 ($\Delta\delta$ −0.124 ppm) resonances upon

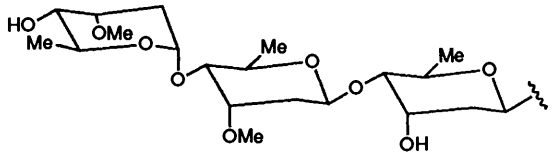
partial exchange of the protons of the hydroxy groups with deuterium.

The chemical shifts of the resonances at δ_H 6.403 and 5.792 and the coupling constant (J 10.3 Hz) of the two-proton second spin system point to the presence of a Z double bond. The corresponding carbon resonances at δ_C 153.05 and 127.08 in conjunction with the resonance at δ_C 201.50 suggest the presence of an α,β-unsaturated ketone. The one-proton double doublet at δ_H 5.810 (J 9.4 and < 1.0 Hz), assigned to C-12, serves as the terminus for the four-proton third spin system. The chemical-shift value indicates that this proton is situated on an oxygen-bearing carbon atom and that the oxygen function is part of an ester moiety, *i.e.* the second benzoate group. The magnitude of the vicinal coupling constants observed for the C-12 (J 9.4 and < 1.0 Hz) and C-9 (J 10.9 and < 1.0 Hz) protons with the 11-methylene protons at δ_H 1.729 (J 10.9 and < 1.0 Hz) and 1.485 (J 9.4 and < 1.0 Hz) points to the existence of a preferred conformation, as a result of restricted rotation around the C(9)–C(11) and the C(11)–C(12) bonds, with approximate dihedral angles of either 0 or 180°, and *ca.* 90° for these protons. The proton–proton connectivity pattern of the fourth spin system, containing four protons, was determined by 2D (^{1}H,^{1}H) correlation spectroscopy using the COSY-45 sequence. The chemical shift value of the C-15 protons (δ_H 2.971 and 2.503) and the geminal coupling constant (J 19.0 Hz) suggest that this carbon atom is bonded to an sp^2 hybridised carbon atom. The cross peaks in the COSY spectrum showed that the methylene protons correlate only with two other methylene protons (16-H) at δ_H 2.355 and 2.064.

The location of the different spin systems in the aglycone structure of sarcovimiside **B 9** followed from the two- and three-bond (^{13}C,^{1}H) connectivity pattern as determined in a series of ^{13}C{^{1}H} SPI experiments as well as a COLOC experiment in which the Δ_1 and Δ_2 delays were optimised for (^{13}C,^{1}H) couplings of 5 Hz. The results, summarised in Table 3, locate



9 R =



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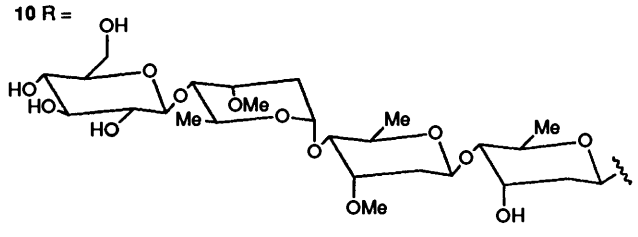

the carbonyl group of an α,β-unsaturated ketone (δ_C 201.50) at C-8 and a ketone carbonyl group (δ_C 217.02) at C-14 and implies that cleavage of the C(8)–C(14) bond must have occurred. The stereochemical assignment at C-9 was deduced from the proton–proton NOEs. Irradiation of the 19-H resonance in an NOE experiment, followed by measurement of the resultant NOE in the difference mode, resulted in an NOE enhancement of the 11-H resonance at δ_H 1.729. Both the 10-methyl group and the C-9 pendant carbon chain must, therefore, be on the β-side of the molecule. The aglycone of sarcovimiside **B** is thus (20*S*)-12β,20-dibenzoyloxy-3β,5,17-trihydroxy-8,14-seco-5β,17α-pregn-6-ene-8,14-dione.

Sarcovimiside C **10** analysed for $C_{61}H_{84}O_{23}\cdot 4H_2O$ in agreement with the ion at m/z 1207 $(M + Na)^+$ in the FAB mass spectrum. The four signals in the anomeric region (δ_C 95–103) pointed to the presence of four sugar residues in the molecule. From the ^{1}H NMR data it was evident that three of the sugars are 2,6-dideoxy sugars and the fourth, glucose. A comparison of the ^{1}H and ^{13}C NMR data of sarcovimiside B and C indicated that the presence of a glucopyranosyl moiety is the only difference between the two compounds. This finding was confirmed by the enzymatic hydrolysis of sarcovimiside C **10** using β-glucosidase to yield a product which was identified as sarcovimiside **B 9**. The β-linkage of the terminal D-glucose moiety followed from the coupling constant (J 7.6 Hz) of the anomeric proton at δ_H 4.334 and the (1→4) sequence with the α-linked L-cymarose sugar was established by the glycosidation shifts observed for the C-3 (δ_C 72.87, $\Delta\delta$ −0.15 ppm), C-4 (δ_C 78.91, $\Delta\delta$ +6.86 ppm), and C-5 (δ_C 65.23, $\Delta\delta$ −0.51 ppm) resonances of the L-cymarose residue in the ^{13}C NMR spectrum of **10**.

A feature of the sarcovimisides A–C is that each include both D- and L-cymarose in the glycoside moiety. This finding has been reported previously only for two *Cynanchum* species [12,14–16] and is of chemotaxonomic interest for plants of the Asclepiadaceae family.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus. FAB mass spectra were taken on a Finnigan MAT-90 spectrometer. NMR spectra were measured for $CDCl_3$ solutions on a Bruker WM-500 (11.7 T) or AC-300 (7.0 T) spectrometer. All chemical shifts are reported as δ-values downfield from Me_4Si, and the J values are given in Hz. Merck silica gel 60 (particle size 0.063–0.200 mm) and Merck Lichroprep RP-8 (40–63 μm) were used for column chromatography. The toxicity of the plant extracts and fractions was monitored by dosing to weaned guinea-pigs. Fractions which did not cause the death of the animals within 48 h at dose levels of 50 mg kg^{-1} were considered non-toxic.

Isolation of the Toxic Principles.—*S. viminale* plants were collected in September 1987 in the district of Christiana, Western Transvaal. The plant material was air-dried, milled to a coarse powder (13.5 kg) and extracted with methanol (3 × 30 dm^3). The combined extracts were evaporated under reduced pressure and the resultant syrup was partitioned between water and ethyl acetate. The aqueous phase was non-toxic. Evaporation of the ethyl acetate fraction yielded a toxic syrup which was partitioned between light petroleum (b.p. 60–80 °C) and 95% aqueous methanol. The toxic residue (215 g) from the methanol fraction was fractionated by column chromatography on silica gel (1.2 kg) using ethyl acetate as eluent. Appropriate fractions of the eluate were combined to give fraction 1 (23.8 g) and fraction 2 (50.3 g) containing toxic material. Fraction 1 (23.8 g) was purified by column chromatography on silica gel (300 g) using ethyl acetate–methanol (9:1, v/v), followed by chromatography of the toxic material (7.0 g) obtained in this way, twice on silica gel (300 g) using chloroform–acetone–methanol (80:18:2, v/v/v). In this way two toxic fractions, *viz.* fraction 3 (195 mg) and fraction 4 (161 mg) were obtained which were purified by preparative layer chromatography on silica gel using chloroform–acetone–methanol (85:13:2, v/v/v).

Fraction 3 gave sarcovimiside A **6** (95 mg) which crystallised from methanol–water, m.p. 137–140 °C; *m/z* 939 $(M + Na)^+$ (Found: C, 61.3; H, 7.9. $C_{49}H_{72}O_{16}\cdot 2.5H_2O$ requires C, 61.17; H, 8.06%).

Fraction 4 gave sarcovimiside B **9** (71 mg), m.p. 129–132 °C (from methanol–water); *m/z* 1045 $(M + Na)^+$ (Found: C, 62.05; H, 7.45. $C_{55}H_{74}O_{18}\cdot 2H_2O$ requires C, 62.36; H, 7.42%).

Fraction 2 (50.3 g) was subjected to a series of column chromatographic separations on silica gel using chloroform–acetone–methanol–water (i) (60:28:10:2.5, v/v/v/v) and (ii) (60:40:10:2.5, v/v/v/v) as eluent to give toxic material (2.5 g). This toxic material was purified by reversed phase column chromatography on Lichroprep RP-8 (70 g) using acetone–water (7:3, v/v). Recrystallisation of the toxic material (700 mg) obtained from methanol–water gave sarcovimiside C **10** (100 mg), m.p. 158–160 °C; *m/z* 1207 $(M + Na)^+$; δ_H aglycone: 1-H: 1.69, 1.25; 2-H: 1.44, 0.88; 3-H: 3.48; 4-H: 1.84, 1.36; 6-H: 6.405 (1 H, d, *J* 10.3); 7-H: 5.797 (1 H, d, *J* 10.3); 9-H: 2.441 (1 H, dd, *J* 10.9 and 1.0); 11-H: 1.731 (1 H, ddd, *J* 14.5, 10.9 and < 1.0) and 1.468 (1 H, ddd, *J* 14.5, 9.4 and < 1.0); 12-H: 5.814 (1 H, dd, *J* 9.4 and < 1.0); 15-H: 2.996 (1 H, ddd, *J* 19.0, 11.0 and 2.2) and 2.508 (1 H, ddd, *J* 19.0, 9.4 and 9.4); 16-H: 2.36 (1 H) and 2.10 (1 H); 18-H: 1.409 (3 H, s); 19-H: 0.671 (3 H, s); 20-H: 5.097 (1 H, q, *J* 6.2); 21-H: 1.352 (3 H, d, *J* 6.2). *O*-Benzoate: 7.721 (2 H, m), 7.524 (2 H, m), 7.478 (1 H, m), 7.439 (1 H, m), 7.284 (2 H, m), 7.173 (2 H, m). D-Digitoxose: 1-H: 4.632 (1 H, dd, *J* 9.7 and 2.1); $2\text{-}H_{eq}$: 1.96 (1 H); $2\text{-}H_{ax}$: 1.56 (1 H); 3-H: 4.120 (1 H); 4-H: 3.083 (1 H, dd, *J* 9.2 and 3.0); 5-H: 3.611 (1 H, dd, *J* 9.3 and 6.2); 6-H: 1.085 (3 H, d, *J* 6.2). D-Cymarose: 1-H: 4.726 (1 H, dd, *J* 9.7 and 2.1); $2\text{-}H_{eq}$: 2.08 (1 H); $2\text{-}H_{ax}$: 1.56 (1 H); 3-H: 3.668 (1 H); 4-H: 3.147 (1 H, dd, *J* 9.6 and 2.7); 5-H: 3.848 (1 H, dd, *J* 9.6 and 6.3; 6-H: 1.139 (3 H, d, *J* 6.2). L-Cymarose: 1-H: 4.750 (1 H, dd, *J* 4.7 and 1.8); $2\text{-}H_{eq}$: 2.19 (1 H); $2\text{-}H_{ax}$: 1.76 (1 H); 3-H: 3.668 (1 H); 4-H: 3.49 (1 H); 5-H: 4.174 (1 H, dd, *J* 8.0 and 6.4; 6-H: 1.204 (3 H, d, *J* 6.4) (Found: C, 58.1; H, 7.1. $C_{61}H_{84}O_{23}\cdot 4H_2O$ requires C, 58.26; H, 7.37%).

Acid Hydrolysis of Sarcovimiside A **6**.—Sarcovimiside A **6** (30 mg) in methanol (5 cm^3) and 0.1 mol dm^{-3} sulfuric acid (5 cm^3) was heated at 60 °C for 2 h. Water (10 cm^3) was added and the mixture was concentrated to 5 cm^3. The solution was heated for a further 60 min. The solution was extracted with ether and the residue, obtained by evaporation of the ether, was purified by column chromatography (hexane–acetone, 1:1 v/v) on silica gel to yield cynanforidine (12β-benzoyloxy-3β,8β,14,17-tetrahydroxy-14β,17α-pregn-5-en-20-one) **5**,[5,14] m.p. 268–272 °C (lit.,[5] m.p. 268–274 °C).

The aqueous layer was neutralised with a saturated aqueous barium hydroxide. The precipitate was filtered off and the filtrate evaporated. Column chromatography ($CHCl_3$–MeOH–H_2O, 12:3:1) of the residue yielded cymarose, identical with an authentic sample.

Enzymatic Hydrolysis of Sarcovimiside C **10**.—A solution of sarcovimiside C **10** (30 mg) in ethanol (1 cm^3) was added to a solution of β-glucosidase (150 mg) in a 0.2 mol dm^{-3} NaOAc buffer (pH 5.0; 10 cm^3). The reaction mixture was incubated for 48 h at 37 °C. Extraction with chloroform (3 × 20 cm^3) and purification by PLC on silica gel plates using chloroform–acetone–methanol (85:13:2, v/v/v) yielded sarcovimiside B **9** (18 mg).

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