Pergamon

Tetrahedron, Vol. 53, No. 50, pp. 16959-16968, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)10174-0

Toxicarioside B and Toxicarioside C. New Cardenolides Isolated from *Antiaris toxicaria* Latex-Derived Dart Poison.

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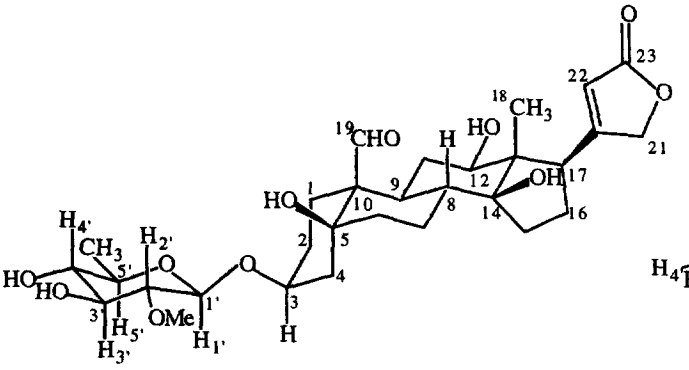
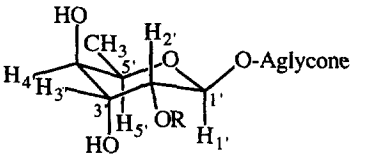
Abstract: Bioassay-guided fractionation of the chloroform/methanol extract of a dart poison from Indonesian Borneo (Kalimantan), derived from *Antiaris toxicaria* latex, has led to the isolation of two new cardenolides, toxicarioside B [**2**] and toxicarioside C [**3**]. Structures for **2** and **3** were deduced by analysis of spectroscopic data; these materials are isomeric with toxicarioside A [**1**] previously isolated in our earlier work with the same poison. The bioassay employed to isolate cardenolides **2** and **3** involves inhibition of Na^+/K^+-ATPase and mimics the suspected mode of action of these "cardiac-glycoside" toxins. © 1997 Elsevier Science Ltd.

INTRODUCTION

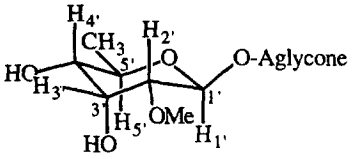
Hunting with poisoned darts is common among the indigenous peoples of Southeast Asia and typically employs material derived from the latex of *Antiaris toxicaria* (Pers.) Lesch (Moraceae). The geographical distribution and preparation of these dart poisons have been the subjects of extensive reports by Bisset,[1] while the chemical makeup of these poisons has been examined by both Bisset[2] and Reichstein[3] and, more recently, by Kopp,[4] among others. Early toxicological studies traced the toxicity of these poisons and their constituents to a family of steroid glycosides which cause severely irregular cardiac function and eventually death when injected into mammals.[3f] Hence, the common name of "cardiac" glycoside for these materials.[5] Although *A. toxicaria*-derived dart poisons prompted some early physiological studies,[6] these were not extensive and they examined only complex mixtures without making an attempt to pinpoint or quantitate the locus of dart poison toxicity. In fact, our recent work detailing the structure of toxicarioside A [**1**], was the first report of a rational, bioassay-driven fractionation of an *A. toxicaria*-derived dart poison.[7] The goal of this work was to systematically survey an *A. toxicaria*-derived dart poison, in an effort to isolate those constituents primarily responsible for toxicity, while employing a bioassay based upon the mode of toxic action generally accepted for cardiac glycosides: inhibition of Na^+/K^+-ATPase.[8] Herein, we report the structures of two new, isomeric cardiac glycosides, **2** and **3**, that have been isolated from the same *A. toxicaria* -derived dart poison that led to isolation of toxicarioside A [**1**].[7] Since **2** and **3** are isomeric with toxicarioside A, we have named them toxicarioside B and toxicarioside C, respectively. These new cardenolides were isolated from a partially purified fraction from the dart poison that displayed activity in our bioassay based upon inhibition of Na^+/K^+-ATPase, as had toxicarioside A. Cardenolides **1**, **2** and **3** are all stereoisomers of the known cardenolide antiarojavoside [**4**] originally isolated from the same source, *Antiaris toxicaria*.[9] Structure determination of **2** and **3**, which contain a rare C6′-deoxy-C2′-O-methylhexose unit, relied in part on comparisons with spectral data for the known cardiac glycoside α-antiarin [**5**], and with those for **1**, detailed in our studies predating these.[7]



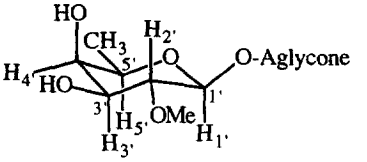
2, toxicarioside B
Aglycone = antiarigenin
sugar = 6-deoxy-2-O-methylglucose



3, toxicarioside C
R = CH_3, Aglycone = antiarigenin
sugar = 6-deoxy-2-O-methylgulose
5, α-antiarin
R = H, Aglycone = antiarigenin
sugar = β-6-deoxygulose



4, antiarojavoside
Aglycone = antiarigenin
sugar = 6-deoxy-2-O-methyl-D-allose



1, toxicarioside A
Aglycone = antiarigenin
sugar = 6-deoxy-2-O-methylgalactose

RESULTS AND DISCUSSION

Isolation of **2** and **3** commenced with the chloroform/methanol extract of a solid sample of Dayak dart poison which was submitted directly to bioassay. The bioassay consisted of measuring the rate of ATP hydrolysis catalyzed by commercially available porcine cerebral cortex Na^+/K^+-ATPase[10] both in the absence and presence of putative toxins. Significant depression of the rate of ATP hydrolysis in this system was interpreted as evidence of the presence of active toxin(s) in assayed fractions; results with ouabain,[11] a well known inhibitor of Na^+/K^+-ATPase, were used as a benchmark for the activity of a strong inhibitor. Inhibition of the bioassay system with the crude chloroform/methanol extract as a 10 mM aqueous solution was only slightly less pronounced than that observed for a 10 mM aqeuous sample of ouabain: K_m(mM): crude extract, 1.1; ouabain, 1.5; V_{max} (nmol P_i liberated/min/unit protein): crude extract, 11.6; ouabain, 7.4. Bioassay-guided chromatographic fractionation of the crude extract, first on flash silica gel followed by HPLC on a preparative scale reverse phase C-18 column, localized significant inhibitory activity in several peaks. Ultimately, further refinement of HPLC separation conditions produced a fraction that proved to be a mixture of **2**, **3** and a third

steroid glycoside whose structure is as yet undetermined. Separation of these three components led to isolation of **2** and **3** as a powdery solids upon lyophilization. As reported in our previous work with **1**,[7] no evidence for the presence of strychnine, the most common alkaloid additive in *A. toxicaria*-derived dart posions, was found in the crude extract by TLC or by treatment with Mayer's reagent.[12]

Due to their isomeric nature, significant portions of the spectroscopic data for **2** and **3** are similar, and these also correspond closely to the spectroscopic data we have reported for their stereoisomer, toxicarioside A **[1]**, which served as an invaluable reference compound during this work.[7] As a result, and in order to provide an economical presentation, data for **2** and **3** will be discussed concomitantly when possible. For example, mass spectral analyses for both **2** and **3** (*via* HRCIMS) showed a molecular ion at *m/z* 581 ($[M+1]^+$, CI, ammonia, 140 eV), from which molecular formulae of $C_{30}H_{44}O_{11}$ could be deduced, confirming that the correct carbon atom count for both **2** and **3** was 30. Further, ^{13}C-NMR, HMQC and DEPT spectra for **2** and **3** demonstrated that the aglycone portion of each molecule was antiarigenin. As shown in Table 1, this was established for **2** by comparison of the appropriate C1-C23 shifts with those measured for **1** under identical spectroscopic conditions. As can be seen, a majority of the carbon shifts for **1** and **2** are essentially identical (C1-C23: $\Delta\delta \leq 1.2$ ppm, typically ≈ 0.2 ppm); shift differences for the carbon signals in the sugars were significantly larger than the typical value. Establishing the identity of the aglycone in **3** required the measurement of ^{13}C-NMR (as well as HMQC) spectra for this material in d_5-pyridine owing to its limited solubility in d_6-acetone, the previous solvent of choice for analysis of **1** and **2**. In this case, a comparison of ^{13}C-NMR data for **3** was made with those for a sample of commercially available α-antiarin[13] **[5]** (also measured in d_5-pyridine). As before, the C1-C23 carbon shifts for these materials are essentially identical ($\Delta\delta$ typically $\leq$ 0.1 ppm). (It should be noted that, due to signal overlap, the ^{13}C-NMR spectra for both **2** and **3** each displayed only 29 peaks.) As reported in our previous work with **1**, carbon chemical shift assignments for the aglycone of **5** are based upon ^{13}C-NMR spectroscopic work by Kopp[14] with a disaccharide deriviative of antiarigenin, while assignments for the sugar in this known natural product are based upon our analysis of HMQC and ^{1}H, ^{1}H-COSY spectra.[7]

The corresponding ^{1}H-NMR spectra of **2** and **3** exhibited signals typical of the antiarigenin cardenolide nucleus (for example, one proton singlets for H22 in the γ-lactone (**2**: δ 5.83; **3**: δ 5.82) and for the C19 aldehyde (**2**: δ 10.05; **3**: δ 10.07)), supporting the ^{13}C-NMR assignments. These spectra also displayed a methyl doublet near δ 1.2 for H6′ (**2**: δ 1.21; **3**: δ 1.18), and they included an especially distinctive three proton singlet near δ 3.5 (**2**: δ 3.50; **3**: δ 3.33) corresponding to the O-methyl ether unit. Analysis of these data, successively, allowed us to conclude that both **2** and **3** were: a) cardenolides incorporating an antiarigenin aglycone; b) glycosides carrying a 6′-deoxy-hexose different from that in α-antiarin (6′-deoxygulose) and that in toxicarioside A (6'-deoxy-2'-O-methylgalactose); and c) O-methyl ethers at one of the available hydroxyl groups in each molecule, presumably in the sugars since the aglycone carbon resonances correspond so closely.

Characterization of the hexose components in **2** and **3** commenced with assignment of the glycoside protons in the corresponding ^{1}H-NMR spectra (Table 2). These assignments were supported by analysis of the corresponding ^{13}C-NMR and HMQC data and through comparison with the appropriate signals in spectral data for **1**.[7] Identification of the H1′ anomeric proton in ^{1}H-NMR spectra of **2** and **3** was straightforward due to the unique chemical shift (^{1}H-NMR: **2**: δ 4.40, **3**: δ 4.73; ^{13}C-NMR: **2**: δ 101.1, **3**: δ 98.5) and doublet multiplicity (**2**: J = 7.9 Hz; **3**: J = 8.1 Hz) of this position; the same was the case for the H6′ methyl group protons

Table 1. ^{13}C-NMR Data for **1**, **2**, **3** and **5**.

	Shifts in d_6-acetone		Shifts in d_5-pyridine	
Carbon Number	**δ, 2**	**δ, 1**	**δ, 3**	**δ, 5**
Aglycone				
1	18.8	18.9	19.2	19.2
2	25.7	25.8	26.2	26.3
3	74.5	74.7	73.7	74.1
4	37.3	37.7	38.1	38.2
5	72.8	74.0	74.1	74.1
6	35.6	35.7	35.3	35.2
7	25.1	25.2	25.5	25.5
8	41.9	42.1	42.1	42.0
9	36.9	36.9	37.2	37.1
10	56.5	56.5	56.9	56.9
11	31.4	31.7	32.1	32.0
12	74.9	74.7	74.7	74.6
13	55.4	55.5	55.6	55.5
14	85.6	85.7	85.4	85.3
15	32.5	32.8	32.9	32.9
16	27.9	28.0	28.2	28.1
17	46.4	46.5	46.7	46.6
18	9.6	9.7	10.5	10.4
19	208.9	208.6	209.1	209.1
20	177.2	176.5	176.7	176.8
21	74.5	74.1	74.4	74.3
22	117.6	117.6	117.9	117.9
23	175.6	174.5	175.1	175.1
Glycoside				
1′	101.1	101.7	98.5	99.9
2′	84.8	82.4	79.5	69.6
3′	77.7	75.0	69.6	70.3
4′	76.7	72.9	73.6	73.3
5′	74.2	71.4	70.3	74.0
6′	18.1	16.9	17.2	17.3
O-Methyl Ether				
	61.0	61.0	56.9	----

(^{1}H-NMR: **2:** δ 1.21, d, J = 6.0 Hz; **3:** δ 1.18, d, J = 6.6 Hz; ^{13}C-NMR: **2:** δ 18.1; **3:** δ 17.2). Moreover, although signals for H3′ and H5′ in ^{1}H-NMR spectra of **2** were patially overlapped, the associated signals forH2′ and H4′ were well resolved, and the corresponding ^{1}H, ^{1}H-COSY spectrum allowed unambiguous assignment of the entire H1′-H6′ spin system. Analysis of vicinal coupling constants for the H2′ (^{1}H-NMR: δ 2.85, dd, J = 7.9, 9.0 Hz; ^{13}C-NMR: δ 84.8) and H4′ (^{1}H-NMR: δ 2.98, dd, J = 9.3, 9.3 Hz; ^{13}C-NMR: δ

76.7) signals was pivotal in the characterization of the hexose unit in **2**, providing a determination of the relative configuration for the entire set of glycoside carbons through consideration of the well-known dependence of J on substitution pattern in pyranoses.[15] Specifically, a diaxial relationship for the H1′ and H2′ positions (and a β-pyranose form for the glyscoside) was indicated by the large, 7.9 Hz H1′/H2′ coupling constant, while the second large coupling observed in H2′ (9.0 Hz) implied that H3′ also must have been axial. Likewise, the two large and identical couplings observed in the H4′ signal (9.3 Hz) indicated diaxial relationships for the H3′/H4′ and H4′/H5′ systems. Hence, all five of the ring positions in the pyranose unit in **2** carry axial protons and equitorial substituents, as shown in the structure above.

Table 2. ^{1}H-NMR Data in d_6-acetone for **2** and **3**.

Proton	δ, (multiplicity, J)	
	2	**3**
Aglycone		
3	4.16 (br. s)	4.16 (br. s)
12	3.35 (m)[a]	3.38 (m)
17	3.35 (m)[a]	3.40 (m)
18	0.75 (s)	0.80 (s)
19	10.05 (s)	10.07 (s)
21a	4.82 (dd, J = 1.7, 18.3 Hz)	4.81 (dd, J = 1.7, 17.9 Hz)
21b	4.92 (dd, J = 2.0, 18.3 Hz)	4.92 (dd, J = 1.3, 17.9 Hz)
22	5.83 (s)	5.82 (s)
Glycoside		
1′	4.40 (d, J = 7.9 Hz)	4.73 (d, J = 8.1 Hz)
2′	2.85 (dd, J = 7.9, 9.0 Hz)	3.26 (dd, J = 8.1, 3.1 Hz)
3′	3.35 (m)[a]	4.19 (br. m)
4′	2.98 (dd, J = 9.3, 9.3 Hz)	3.52 (br. m)
5′	3.24 (m)[a]	3.99 (dq, J = 6.6, 1.3 Hz)
6′	1.21 (d, J = 6.0 Hz)	1.18 (d, J = 6.6 Hz)
C2′-O-Methyl Ether	3.50 (s)	3.33 (s)

[a] Signals overlapped.

Determining the relative configuration of the C1′-C5′ centers in the hexose unit of **3** was somewhat more complicated. Analysis of ^{1}H, ^{1}H-COSY spectra of **3** allowed assignment of the C1′-C6′ spin system and, as was the case with **2**, the relative configuration of the C1′, C2′ and C3′ centers could be deduced by analysis of vicinal coupling constants. Thus, a H1′/H2′ diaxial relationship was indicated by the 8.1 Hz coupling observed at H1′ while the small, second coupling constant observed in the well resolved double doublet signal for H2′ (δ 3.26, J = 8.1, 3.1 Hz) demonstrated that H3′ was equatorial. However, the signals for H3′ (δ 4.19) and H4′ (δ 3.52) appeared as broadened multiplets from which neither accurate coupling constants, nor unambiguous conclusions about relative configuration, could be extracted. The signal for H5′, on the other hand, appeared as a well resolved double quartet (δ 3.99, J = 6.6, 1.3 Hz), with the smaller J value, coresponding to the H4′/H5′ interaction, ruling out only a diaxial relationship for these two protons.

Establishing the correct C4′/C5′ relative configuration in **3**, as well as the site of the pyranose/aglycone junction, depended upon NOE studies. In the ^{1}H,^{1}H-NOESY spectrum of **3**, an NOE was observed from H1′ to H5′, indicating that H5′ was axial. Moreover, the lack of an NOE from H1′ to H3′ corroborated our previous equatorial assigment for the H3′ position. Also observed in this spectrum was an NOE from H1′ to H3, allowing us to conclude that the pyranose was joined to the antiarigenin aglycone at C3. In our previous work with toxicarioside A [**1**], ^{1}H,^{1}H-NOESY spectra established C2′ as the site for attachment of the O-methyl ether by displaying an NOE correlation from H2′ to the O-methyl ether position. Unfortunately, because the O-methyl ether singlet (δ 3.33) and the H2′ signal in ^{1}H-NMR spectra of **3** were separted by less than 30 Hz, it was impossible to determine unambiguously if an NOE could be oberserved between these positions. However, just as had been the case in our work with **1**, the conclusion that **3** was a C2′-O-methyl ether was supported both by the relatively high shift observed for C2′ in the ^{13}C-NMR spectrum of **3** (δ 79.5) and by the narrow line width observed for the H2′ signal in the corresponding ^{1}H-NMR spectrum (indicating the absence of coupling to an attached hydroxyl group).

In like fashion, the structure for **2** was completed by analysis of ^{1}H,^{1}H-NOESY data for this material. In these spectra, definitive NOEs could be observed from H1′ to H3′ and to H5′, corroborating our previous axial assignments for all three positions. Also observed in this spectrum were NOEs from H1′ to H3 and to the methyl group in the O-methyl ether. These NOEs allowed us to conclude that the pyranose was joined to the antiarigenin aglycone at C3 and that the O-methyl ether attachement point was at C2′ of the pyranose, just as in **3**. As above, support for the latter conclusion was provided by the high shift observed for C2′ (δ 84.81) and by the narrow line width observed for H2′ in the corresponding ^{13}C- and ^{1}H-NMR spectra, respectively, for **2**. Not surprisingly, since **1**, **2** and **3** all include antiarigenin as aglycone, the ^{1}H-NMR spectra of these materials are very similar. In fact, the aglycone regions of these spectra (δ 1.25-2.20) are essentially identical. As a result, data presented in Table 2 are only for unique signals in ^{1}H-NMR spectra of **2** and **3**. A complete assignment of the protons in the antiarigenin aglycone may be found in our report detailing the structure of **1**.[7]

It should also be noted that the carbon and proton assignments and their corresponding connectivities discussed above were supported by HMBC experiments for both **2** and **3**, as shown by the data presented in Table 3. Thus, for example, in both cases, correlations from H2′ to the carbon atom in the OCH_3 group support the attachment point for the O-methyl ether. Correlations from H6′ to C5′ and to C4′ in both **2** and **3** help support the assigned connectivities for these three positions. Likewise, correlations from H3′ to C2′ and to C5′ in 2, and from H3′ to C1′, C2′ and to C5′ in **3**, help support the assigned connectivities amongst positions 1′-3′ in the corresponding sugars.

Table 3. Diagnostic Heteronuclear Couplings for **2** and **3**.

2- and 3-Bond ^{1}H, ^{13}C-Couplings Observed[a]	
2	**3**
H1′: C5′	H1′: C3′, C5′
H2′: C1′, OCH_3	H2′: C1′, OCH_3
H3′: C2′, C5′	H3′: C1′, C2′, C5′
H4′: C3′, C6′	H4′: C2′, C3′
H5′: --	H5′: C1′, C3′, C6′
H6′: C4′, C5	H6′: C4′, C5′
[a]From HMBC experiments optimized for J = 4.5 and 7.0 Hz.	

Although a small number of other antiarigenin-based cardiac glycosides are known (including α-antiarin [**5**], and its C6′ isomer, β-antiarin (antiarigenin-rhamnoside)),[3e, 3f] cardenolides **2** and **3**, which we have named toxicariosides B and C, respectively, have not been reported previously in the literature. Especially noteworthy in the structures of **2** and **3** are the unusual pyranoses they contain. In **2**, the pyranose is the 2-O-methyl derivative of 6-deoxyglucose, known as quinovose (or chinovose) in its L-form, and as either isorhamnose or isorhodeose in its D-form. Pyranose **3** is also a 2-O-methyl derivative but of 6-deoxygulose, known as antiarose in its D-form. As its name implies, antiarose was originally obtained *via* hyrolysis of α-antiarin after isolation from *A. toxicaria*;[3a] the same sugar has also been isolated from *Erysimum helveticum*,[16] *Convallaria keiskei*,[17] and *Ipomea parasitica*.[18] However, the 2-O-methyl derivative of this sugar has never appeared previously in the literature. Likewise, natural sources of quinivose are known[19] and isorhamnose has been isolated from cardiac glycoside-bearing plants,[18, 20] but only the 3-O-methyl derivative (also known as thevetose or cerberose) has been found as a component of cardiac glycosides;[21] and the corresponding 2-O-methyl derivative has not been reported previously in the literature.

Indeed, O-methyl pyranose derivatives in general are only rarely isolated from natural sources. Several examples are known from cardiac glycoside-bearing plants, but 3-O- or 4-O-methyl derivatives are most common. Examples of such unusual sugars include: acofriose (3-O-methyl-L-rhamnose),[22] digitalose (3-O-methyl-D-fucose),[23] sordarose (6-deoxy-4-O-methyl-D-altrose),[24] vallarose (6-deoxy-3-O-methyl-L-altrose),[25] and acovenose (6-deoxy-3-O-methyl-D-allose).[26] Cardiac glycosides bearing 2-O-methylpyranoses include kanaloside (a 2,3-di-O-methyl-D-fucose derivative)[27] and antiarojavoside [**4**],[9] an antiarigenin-class cardenolide, originally isolated from *Antiaris toxicaria*, which is isomeric with **1**, **2** and **3** and which bears the rare sugar javose (6-deoxy-2-O-methyl-D-allose) at the 3 position. We have yet to observe **4** in any of our *A. toxicaria*-derived dart poisons, although work with other components that remain unidentified is ongoing. Direct spectroscopic comparisons between antiarojavoside and **1**, **2** and **3**, which would be both interesting and very useful in supporting our structural assignments, have not been possible since we have been unable to locate a source for authentic **4**. To the best of our knowledge, ours is the first report of 6-deoxy-2-O-methylglucose and of 6-deoxy-2-O-methylgulose in cardiac glycosides. Studies to assign the absolute configuration of these sugars are in progress as are efforts to determine the structures of other dart poison constituents that displayed significant activity in our bioassay. Studies detailing the activity levels for pure **2** and **3** in the bioassay system will be reported in due course.

EXPERIMENTAL

General. Spectroscopic and chromatographic equipment and materials used were the same as previously reported.[7] ^{13}C-NMR spectra obtained in d_5-pyridine were referenced against the center line of the residual triplet at δ 135.91. Additional isocratic preparative scale HPLC employed a Rainin Rabbit HPLC equipped with a Gilson UV absorbance detector (set at 220 nm) and a Phenomenex preparative HPLC column (Microsorb C18, 250 mm x 10 mm). Flow rates were 2 mL/min and 5 mL/min for analytical and preparative scale HPLC respectively. Injection volumes for analytical and preparative scale HPLC, were 5-20 μL and 25-300 μL, respectively. Gradient HPLC was performed with any of the following: Gradient System 1, Gradient System

2, or Gradient System 3. Gradient Systems 1 and 2 are based upon the work of Tittel, et al.[28] Specific composition details for these systems are summarized in Tables 4-6.

Table 4. Gradient System 1.

Step Time (min)	% Solvent A	% Solvent B	% Pure CH_3CN	Gradient Shape[29]
2	100	--	--	--
40	83	17	--	-9.0
10	60	40	--	4.0
5	--	100	--	1.0 (linear)
5	--	--	100	1.0 (linear)
Solvent A: 10:90 (v:v) $CH_3CN:H_2O$; Solvent B: 50:50 (v:v) $CH_3CN:H_2O$				

Table 5. Gradient System 2.

Step Time (min)	% Solvent A	% Solvent B	% Pure CH_3CN	Gradient Shape[29]
2	100	--	--	--
40	91.2	8.8	--	-8.5
10	60	40	--	4.0
5	--	100	--	1.0 (linear)
5	--	--	100	1.0 (linear)
Solvent A: 10:90 (v:v) $CH_3CN:H_2O$; Solvent B: 50:50 (v:v) $CH_3CN:H_2O$				

Table 6. Gradient System 3.

Step Time (min)	% Pure H_2O	% Pure CH_3CN	Gradient Shape[29]
1	100	--	--
30	84.8	15.2	-5.0
12	63	37	4.0
5	50	50	1.0 (linear)
5	--	100	1.0 (linear)

Isolation of 2 and 3. A sample of authentic dart poison, prepared from *A. toxicaria* latex, was obtained from the Puak Kenyah in East Kalimantan (Indonesian Borneo) on the Pujangan River. A solid, glassy sample of the poison (5.06 g) was finely ground and extracted successively (7 X) with 25 mL volumes of 1:1 $CHCl_3$:CH_3OH (50 °C). The extract was filtered, concentrated *in vacuo*. A 10 mM aqueous solution of the crude extract displayed only slightly weaker activity in the bioassay than a 10 mM aqeuous sample of ouabain: K_m(mM): crude extract, 1.1; ouabain, 1.5; V_{max} (nmol P_i liberated/min/unit protein): crude extract, 11.6; ouabain, 7.4. The crude extract was fractionated on a flash silica gel column eluted with $CHCl_3$:CH_3OH:H_2O (75:25:2). The resulting column fraction of intermediate polarity (containing spots with R_f 0.1-0.6) showed the highest activity in the bioassay and it was concentrated *in vacuo*. The resulting residue was dissolved in $CH_3OH:H_2O$ (2:1) and then subjected to preparative HPLC employing Gradient System 1, giving a series of fractions numbered 1-6 in order of decreasing polarity. Fractions 3 and 4 displayed the highest levels of inhibition in the bioassay. Fraction 3 was then resolved into a series of individual components α, β, γ, δ, and ε, in order of decreasing polarity, employing Gradient System 2. The resulting residue was dissolved in $CH_3OH:H_2O$ (2:1) and then subjected to sequential rounds of preparative HPLC fractionation using Gradient

System 3. The so-called ε fraction was homogeneous and ultimately provided toxicarioside A [**1**].[7] The so-called δ fraction (a broad peak collected in each preparative run with retention time ≈ 27-32 min) proved to be a partially resolved triad of peaks (δ_1, δ_2, and δ_3); the mixture was not subjected to the bioassay. Instead, pooled fractions containing these peaks were lyophilized to give 18 mg of a mixture of **2** (δ_3), **3** (δ_2) and an unidentified glycoside (δ_1) as a white powder. Preparative HPLC separation was performed on a ca. 6 mg sample of this mixture, under isocratic conditions (H_2O:CH_3OH:*i*-PrOH:CH_3CN, v/v/v/v, 7:1:1:1), providing pure **2** (1.8 mg) and **3** (1.3 mg) following concentration *in vacuo*.

Toxicarioside B [2]. $[a]_D^{25}$ +0.19° (c 0.18, MeOH), UV (MeOH) λ_{max} 220 nm; ^{1}H- and ^{13}C-NMR see Tables 1 and 2; IR (KBr) 3429, 2956, 1727, 1708, 1452, 1163 cm^{-1}; HRCIMS (ammonia, 140 eV) *m/z* ([M+1]) 581.2974 calc'd for $C_{30}H_{45}O_{11}$ 581.2949.

Toxicarioside C [3]. $[a]_D^{25}$ -0.10° (c 0.38, MeOH), UV (MeOH) λ_{max} 220 nm; ^{1}H- and ^{13}C-NMR see Tables 1 and 2; IR (KBr) 3439, 2939, 1736, 1449, 1071 cm^{-1}; HRCIMS (ammonia, 140 eV) *m/z* ([M+1]) 581.3001 calc'd for $C_{30}H_{45}O_{11}$ 581.2949.

ACKNOWLEDGMENTS

We thank Professor Nancy Roseman of Williams College for assistance with developing and conducting bioassays and Mr. Michael Creech of Boston University for measuring mass spectra. We thank Dr. John Burley and Dr. Andrew McDonald of the Harvard Arboretum and Mr. Koko Iskandar of BIOTROP Laboratories (Bogor, Indonesia) for their kind assistance in procuring supplies of *A. toxicaria* latex as well as samples of dart poison derived from it. We also thank Professor Brigette Kopp and Dr. Martina Jelovina, Institute of Pharmacognosy, University of Vienna for supplying unpublished spectroscopic data on antiarigenin derivatives from their research. D.P.R thanks Professor John K. Snyder of Boston Univeristy for sponsoring a sabbatical year during which this manuscript was completed. Financial support from the National Science Foundation RUI Program (grant no. CHE-9208927) and from the Bronfman Science Center at Williams College is also gratefully acknowledged.

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29. "Gradient shape" refers to the rate of change of the solvent composition in a given program step. Thus, a linear gradient (i.e. 1.0) varies linearly with time; a negative gradient (i.e. -9.0) involves a solvent program that changes very quickly initially but then changes slowly towards the end of that program step; a positive gradient (i.e. > 1.0) is the opposite of a negative gradient.

(*Received in USA* 29 *August* 1997; *accepted* 9 *October* 1997)