Pergamon

Tetrahedron, Vol. 53, No. 40, pp. 13557-13566, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00895-8

Toxicarioside A. A New Cardenolide Isolated from *Antiaris toxicaria* Latex-Derived Dart Poison. Assignment of the ^{1}H- and ^{13}C-NMR Shifts for an Antiarigenin Aglycone.

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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 a new cardenolide, toxicarioside A [1]. The structure of **1** was deduced by analysis of spectroscopic data and has led to the first assignment of the ^{1}H- and ^{13}C-NMR shifts for an antiarigenin aglycone. The bioassay employed to isolate cardenolide **1** involves inhibition of Na^{+}/K^{+}-ATPase and mimics the suspected mode of action of these "cardiac-glycoside" toxins. © 1997 Elsevier Science Ltd.

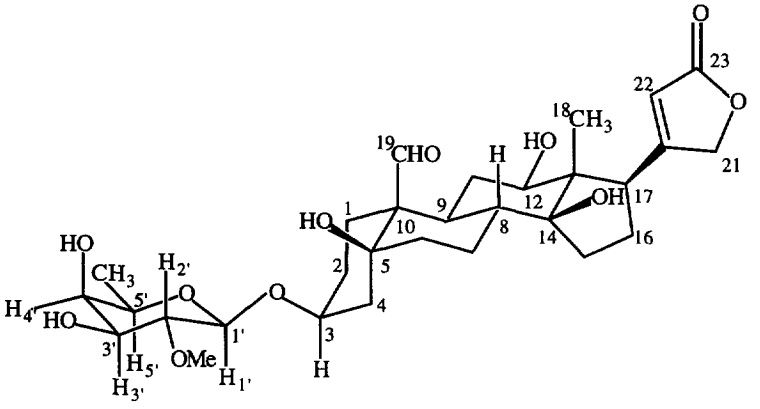
INTRODUCTION

It has been known for centuries that poisoned darts have been used widely by the indigenous peoples of Southeast Asia for hunting game. Although formulation practices vary from region to region, most poisons in this area are prepared by concentration of the latex harvested from *Antiaris toxicaria* (Pers.) Lesch (Moraceae) followed by application to darts or arrows.[1] Often, such poisons are modified through the addition of other toxic materials. Common additives have included plant extracts from *Strychnos*, *Amorphophallus*, *Psychotria*, and *Uvaria* species, but early reports that centipede and scorpion toxins have also been used as additives[2] have not been substantiated by more recent work.[3] Field observations among the Kayan of Borneo have shown that these poisons are prepared with a sophisticated knowledge of dose-response relationships depending upon the nature of the game being pursued.[4] Thus, a "level one" dart is used to kill birds and small game, while a "level seven" dart would be used to hunt prey as large as a tiger; a person will succumb to a "level three" dart. Indeed, killing humans with poisoned darts was practiced in Indonesia in the late 16th century.[5] Bisset reported that animals shot with poisoned darts "died with tetanic convulsions," but that the game was safe to eat, indicating that *A.toxicaria*-derived poisons function through the bloodstream and not the digestive tract.[1]

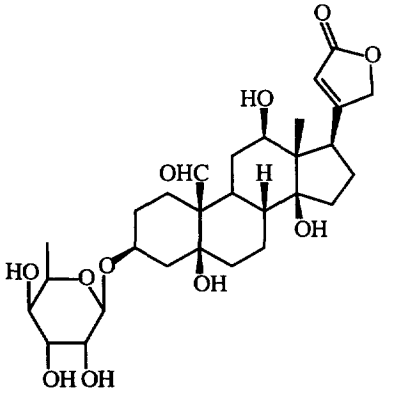
The notoriety of these materials long ago prompted investigations of their constituent natural products. Early work with *A. toxicaria* latex by Mulder[6] led to the isolation of several crystalline glycosides, one of which Kiliani much later determined[7] to be α-antiarin [2] a member of the cardenolide subfamily of steroid glycosides. Subsequent, extensive work by Reichstein and colleagues,[8] and recently extended by Kopp and coworkers,[9] has detailed the isolation/structure determination of several steroid glycosides from *A. toxicaria* latex, as well as from related plants originating in Africa[10] and South America;[11] some of these have included antiarigenin as aglycone.

Early physiological study of the mode of action of these materials can be traced to Robinson and Ling who observed cardiac irregularities and death when extracts of *A. toxicaria*-derived dart poisons were injected

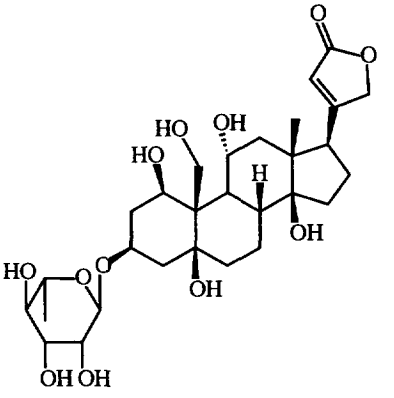
into cats.[12] These effects mirrored those produced by the known heart poison or "cardiac" glycoside, ouabain **[3]** isolated from the East African waba yo tree (*Strophanthus gratus*).[13] In 1983, Fujimoto *et al.* obtained



1, toxicarioside A
sugar = 2-O-methylfucose



2, α-antiarin
sugar = β-6-deoxygulose



3, ouabain
sugar = rhamnose

similar results in a study comparing the activity of *A. toxicaria* latex extract and ouabain.[14] This group also demonstrated that the latex extract inhibited Na^+/K^+-ATPase partially purified from guinea pig heart muscle. It is well-known that cardiac glycosides, in particular **3**, can inhibit or modulate the activity of Na^+/K^+-ATPase, and such effects at the level of cardiac muscle are generally regarded to be the mode of action of these toxins.[15] However, at the inception of our research, no systematic study of *A. toxicaria* latex or derived poisons had ever been performed using a Na^+/K^+-ATPase inhibition bioassay as a means of specifically isolating toxic constituents. Herein, we report the structure of a new cardiac glycoside **1**, that we have named toxicarioside A, that has been isolated from an *A. toxicaria*-derived dart poison using this biorational screening method.

Structure determination of **1**, which contains a rare C6′-deoxy-C2′-O-methyl hexose, relied in part on comparisons with α-antiarin [**2**]. Since only scant NMR spectroscopic data for the parent aglycone, antiarigenin, common to **1** and **2**, are available in the literature, we also herein report a full assignment for the ^{1}H- and ^{13}C-NMR resonances for this important steroid system.

RESULTS AND DISCUSSION

Isolation of **1** commenced with the chloroform/methanol (1/1) extract of a solid sample of Dayak dart poison, derived from *A. toxicaria* latex of known provenance, 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[16] 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 [**3**] 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; **3**, 1.5; V_{max} (nmol P_i liberated/min/unit protein): crude extract, 11.6; **3**, 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 distinct peaks, one of which provided **1** as a powdery solid upon isolation and lyophilization. Aqueous solutions (1 mM) of **1** and **3** showed essentially equivalent levels of activity in the bioassay: K_m(mM): **1**, 0.7; **3**, 0.9;V_{max} (nmol P_i liberated/min/unit protein): **1**, 11.9; **3**, 16.8. 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.[17]

The ^{1}H-NMR spectrum of **1** included two low field signals that immediately suggested the presence of the butenolide that forms a characteristic part of cardenolide systems: a one proton singlet at δ 5.82 (H22) and a two proton AB quartet at δ 4.81, 4.91 (H21a, H21b; J_{AB} = 18.1 Hz). In addition, an extremely low field one proton singlet indicated an aldehyde group that is characteristic of the antiarigenin aglycone system: δ 10.08 (H19). Other prominent signals included a high field methyl singlet at δ 0.80 (H18), also suggestive of a cardenolide nucleus, and a methyl doublet at δ 1.23 (J = 6.7 Hz, H6′) indicating that **1** was a glycoside incorporating a C6′-deoxy hexose unit. These features suggested that **1** was at least similar in structure to the known cardenolide α-antiarin [**2**] but examining the extent of similarity proved difficult since ^{1}H-NMR data for **2** are not available in the literature. However, comparison of our ^{1}H-NMR data for **1** with those we measured for a sample of commercially available α-antiarin,[18] under identical spectroscopic conditions, confirmed that these materials were similar but not identical. Particularly distinctive in the ^{1}H-NMR spectrum of **1** was a three proton singlet at δ 3.50 indicating the presence of a methyl ether.

The ^{13}C-NMR spectrum of **1** showed 29 signals but analysis of the corresponding DEPT and HMQC spectra established that two methine carbons (C3 and C12) were overlapped at δ 74.7 in these spectra, bringing the total number of carbon atoms in **1** to 30. This conclusion was confirmed by mass spectral data for **1** which showed a molecular ion at *m/z* 581 ($[M+1]^{+}$, CI, ammonia, 140 eV) from which a molecular formula of

$C_{30}H_{44}O_{11}$ could be deduced *via* HRMS analysis. Our ^{13}C-NMR data for **1** and **2**, measured under identical spectroscopic conditions, are presented in Table 1. These data show that a majority of the carbon shifts for the

Table 1. ^{13}C-NMR Data in d_6-acetone for **1** and **2**.

Carbon Number	δ, 1	δ, 2, α-antiarin	Δδ
Aglycone			
1	18.9	18.3	0.6
2	25.8	25.3	0.5
3	74.7	73.5	1.2
4	37.7	37.2[a]	0.5
5	74.0	73.4	0.6
6	35.7	34.9[a]	0.8
7	25.2	24.7	0.5
8	42.1	41.5	0.6
9	36.9	36.2	0.7
10	56.5	55.9	0.6
11	31.7	31.0	0.7
12	74.7	74.1	0.6
13	55.5	54.8	0.7
14	85.7	85.1	0.6
15	32.8	32.2	0.6
16	28.0	27.4	0.6
17	46.5	45.9	0.6
18	9.7	9.1	0.6
19	208.6	208.1	0.5
20	176.5	175.9	0.6
21	74.1	73.5	0.6
22	117.6	117.0	0.6
23	174.5	174.0	0.5
Glycoside			
1′	101.7	99.3	2.4
2′	82.4	68.7	13.7
3′	75.0	69.2	5.8
4′	72.9	72.6	0.3
5′	71.4	72.8	-1.4
6′	16.9	15.9	1.0
O-Methyl Ether			
	61.0	----	----

[a] Shifts reversed in original literature assignment.[19]

aglycones of **1** and **2** are essentially identical (C1-C23: $\Delta\delta \leq 1.2$ ppm, typically ≈ 0.6 ppm). Shift differences for the sugar carbon signals (C1′-C5′) were significantly larger, and one signal for **1** (corresponding to the methyl ether carbon at δ 61.0) had no corresponding signal in spectra of **2**. Carbon chemical shift assignments for the aglycone of **2** are based upon ^{13}C-NMR spectroscopic work by Kopp[19] with a disaccharide deriviative of antiarigenin; assignments for the sugar are based upon analysis of HMQC, HMBC and ^{1}H, ^{1}H-COSY spectra, as discussed below. The above data taken together allowed us to conclude that **1** was: a) a cardenolide incorporating an antiarigenin aglycone; b) a glycoside carrying a 6′-deoxyhexose different from that in α-antiarin (β-6′-deoxygulose); and c) an O-methyl ether at one of its available hydroxyl groups, presumably in the sugar given the close correspondence of aglycone carbon resonances.

Characterization of the hexose commenced with assignment of the glycoside protons in the ^{1}H-NMR spectrum of **1** (Table 2) which was supported by analysis of the corresponding ^{13}C-NMR and HMQC data. Its unique chemical shift (^{1}H-NMR: δ 4.36; ^{13}C-NMR: δ 101.7) and doublet multiplicity (J = 7.8 Hz) made identification of the the H1′ anomeric proton straightforward; the same was the case for the H6′ methyl group protons (^{1}H-NMR: δ 1.23, d, J = 6.7 Hz; ^{13}C-NMR: δ 16.9). Although the signal for H2′ was well resolved (^{1}H-NMR: δ 3.13, dd, J = 7.8, 9.1 Hz; ^{13}C-NMR: δ 82.4), signals for the 3′, 4′, and 5′ positions were closely spaced in the proton and carbon spectra, making unambiguous assignments for the protons and carbons at these three positions difficult. However, the ^{1}H, ^{1}H-COSY spectrum clearly revealed the H1′-H3′ and H6′-H5′ spin systems, making assignment of H3′ (^{1}H-NMR: δ 3.55, ddd, J = 3.5, 6.5, 9.1 Hz; ^{13}C-NMR: δ 75.0) and H5′ (^{1}H-NMR: δ 3.63, br. dq, J = < 1, 6.7 Hz; ^{13}C-NMR: δ 71.4) straightforward, and making an assignment of H4′ (^{1}H-NMR: δ 3.59, br. dd, J = <1, 3.5, 4.7 Hz; ^{13}C-NMR: δ 72.9) possible by default. Analysis of the HMBC spectrum of **1** (Table 3) corroborated the connectivities established above. For example, H6′ showed 2-bond coupling to C5′ which, in turn, showed 3-bond couplings to C3′ and C1′, establishing the positions of these four carbons. Likewise, H4′ showed 3- and 2-bond couplings, respectively, to C2′ and C3′.

Analysis of vicinal coupling constants for the sugar proton signals allowed determination of the relative configuration for almost the entire set of glycoside carbons through consideration of the well-known dependence of J on substitution pattern in pyranoses.[20] Thus, the large, 7.8 Hz H1′/H2′ coupling constant indicated a diaxial relationship for H1′ and H2′ (and a β-pyranose form for the glycoside), while the second large coupling observed in H2′ (9.1 Hz) indicated that H3′ also was axial. Likewise, the small coupling constant observed at H3′ (3.5 Hz) indicated that H4′ must be equatorial. (Decoupling studies demonstrated that coupling with the C3′ OH proton at δ 4.25 accounted for the 6.5 Hz coupling observed at H3′.) Unfortunately, the small J value observed at H5′ for the H4′/H5′ vicinal coupling (< 1 Hz) was consistent with either an axial or equatorial orientation for H5′.

Determining the correct configuration at C5′, as well as the point of attachment for the O-methyl ether and the pyranose/aglycone junction, depended upon NOE studies (Table 3). In the ^{1}H,^{1}H-NOESY spectrum of **1**, NOEs were observed from H1′ to H3′ and H5′, corroborating our previous diaxial assigments for H1′ and H3′ and indicating that H5′ also was axial. 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 attachment point was at C2′ of the pyranose. The latter conclusion was supported both by the relatively low field shift observed for C2′ in the ^{13}C-NMR spectrum of **1** (δ 82.4) and by the narrow line width observed for the H2′ double doublet signal in the corresponding ^{1}H-NMR

spectrum (indicating the absence of coupling to an attached hydroxyl group). Support for the former conclusion was found in 1D NOE difference (DNOE) experiments that showed a significant NOE (2%) at H1′ upon irradiation of H3. Finally, in the HMBC spectrum of **1**, H2′ showed 3-bond coupling to the carbon atom in the O-methyl ether, firmly establishing that this moiety was attached to the sugar at C2′.

Due to extensive overlap of most of the signals arising from protons in the aglycone (δ 1.0-2.6), complete assignment of these shifts in 1D ^{1}H-NMR spectra of cardiac glycosides is extremely difficult, even at very high (>10T) field strengths. For example, complete assignments of the ^{1}H-NMR spectra for **3** and the closely related cardiac glycoside digoxin (digoxigenin-trisdigitoxoside) have only recently been reported,[21] and such an assignment has never been reported for a cardiac glycoside bearing an antiarigenin aglycone.

Table 2 represents a complete assignment of all proton shifts in **1**, including all resolvable $^3J_{H,H}$ coupling

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

Proton	δ, J (Hz)	Proton	δ, J (Hz)
Aglycone		15α[a]	1.98 (br. d, J15α,15β=12.9)
1α	1.67 (br. dd, J1α,1β=14.8; 1α,2α=2; 1α,2β=2)	15β[a]	1.71 (br. d)
1β	2.17 (ddd, J1β,2α=14.8; 1β,2β=3.5)	16α	2.08 (br. m, J16α,16β=15.3; 16α,17=5.8; 16β,17=9.5)
2α	1.71 (br. dd, J2α,2β=13.4; 2α,3=2)	16β	2.00 (br. m)
2β	1.88 (br. d, J2β,3=2)	17	3.39 (dd)
3	4.19 (br. dd, J3,4α=2; 3,4β=4)	18	0.80 (s)
4α	1.65 (br. dd, J4α,4β=15.4)	19	10.08 (s)
4β	2.19 (br. dd)	21a	4.81 (dd, J =1.7; J_{AB} = 18.1)
5 OH	4.34 (s)	21b	4.91 (dd, J = 1.7; J_{AB} = 18.1)
6α	1.61 (ddd, J6α,6β=13.8; 6α,7α=4.3; 6α,7β=2)	22	5.82 (s)
6β	2.08 (ddd, J6β,7α=13.8; 6β,7β=4.9)	**Glycoside**	
7α	1.31 (dddd, J7α,7β=13.4; 7α,8=12.6)	1′	4.36 (d, J = 7.8)
7β	2.16 (br. d, J7β,8=3.6)	2′	3.13 (dd, J = 7.8, 9.1)
8	1.96 (ddd, J8,9=12.6)	3′	3.55 (ddd, J = 3.5, 6.5, 9.1)
9	1.77 (ddd, J9,11β=12.6; 9,11α=3.6)	3′ OH	4.25 (br. d, J = 6.5)
11α	1.71 (br. dd, J11α,11β=12.6; 11α,12=4.7)	4′	3.59 (br. dd,[b] J = <1, 3.5, 4.7)
11β	1.26 (br. ddd, J11β,12=12.6)	4′ OH	3.69 (br. d, J = 4.7)
12	3.38 (ddd, J12,12OH=5.8)	5′	3.63 (br. dq, J = < 1, 6.7)
12 OH	3.80 (br. d)	6′	1.23 (d, J = 6.7)
14 OH	3.44 (br. s)	**C2′-O-Methyl**	3.50 (s)

[a]assignments may be interchanged; [b]complete multiplicity deduced through decoupling studies.

constants. Assignments for signals in the δ 1.0-2.6 region were obtained using a variety of techniques, including double quantum filtered ^1H, ^1H-COSY spectroscopy, HOM2DJ spectroscopy, DNOE spectroscopy, HMBC spectroscopy, and through analysis of cross sections taken through the proton dimension in an HMQC spectrum of **1**, an approach that has been used successfully with other steroids.[22] Analysis of HMQC spectra also served to confirm and refine the ^{13}C-NMR chemical shift assignments presented in Table 1. For example, these data led us to reverse Kopp's[19] original assignments for the ^{13}C shifts of carbons 4 and 6. This revised assignment was also consistent with our HMBC spectra for **1** in which H4 showed 2-bond coupling to C3 (Table 3). On the other hand, our HMQC, $^3J_{H,H}$ (Table 2), and HMBC data (Table 3) were consistent with Kopp's original assignments[19] for the ^{13}C shifts of C1 and C7, a point which bears noting since these positions can be difficult to assign. Distinguishing alpha and beta positions in diastereotopic methylene groups in the steriod skeleton, and refining coupling constant measurements in general, was aided by DNOE experiments, the results for which are summarized in Table 3. For example, saturation at the H18 methyl group produced a significant NOE (2%) at the 11β proton, allowing us to distinguish this signal from that for the 11α proton. Only the C15 and C16 protons proved problematic in this series of analyses. Although an NOE from H21 allowed us to identify the signal for H16β, the signals for H15α and H15β could not be assigned using this approach. Their assignments, with H15α downfield from H15β, are in analogy with those reported by McIntyre for the same positions in **3**[21] which has a D ring substitution pattern indentical to that in **1**. The complexity of the H15 signals, and the non-first order nature of the H16 signals, also made it impossible to measure anything other than geminal coupling constants for these protons. Finally, it should be noted that saturation at H1′ produced strong NOEs at H3′ and H5′, supporting our assignment of axial positions for these protons.

Table 3. Diagnostic Heteronuclear Couplings and NOEs for **1**.

2- and 3-Bond $^1H,^{13}C$-Couplings[a]	NOEs[b]
H1′: C3′, C5′	H3: H1′ (2%), H2′ (<1%), 2α (2%), 2β (2%), 4α (2%), 4β (3%)
H2′: C1′, C4′, OCH_3	H18: H8 (2%), H11β (2%), H17 (1%), H21 (2%), H22 (1%)
H3′: C2′	H19: C5OH (1%), H6β (4%), H8 (4%), H11β (<1%)
H4′: C2′, C3′	H21: H16β, (2%), H17 (1%), H18 (2%)
H5′: C1′, C3′, C6′	H1′: H3 (5%), H2′ (3%), H3′ (7%), H5′ (8%)
H6′: C5′	H6′: H5′ (2%), H4′ (1%)
H1: C2, C3, C10	-
H4: C3, C10	-
H7: C6, C9	-
[a]From HMBC experiments optimized for J = 4.5 and 7.0 Hz.	[b]From DNOE experiments; saturation resonance listed before colon.

Although a small number of other antiarigenin-based cardiac glycosides are known (including α-antiarin, **2**, and its C6′ isomer, β-antiarin (antiarigenin-rhamnoside)), has not been reported previously in the literature. Especially noteworthy in the structure of **1** is the unusual pyranose, 2-O-methylfucose (6-deoxy-2-O-methylgalactose). Both antipodes of this sugar are known in nature but both are exceedingly rare.[23] Indeed, O-methylpyranose 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, all isomeric with 2-O-methylfucose, include: acofriose (3-O-methyl-L-rhamnose),[24] digitalose (3-O-methyl-D-fucose),[25] sordarose (4-O-methyl-6-deoxy-D-altrose),[26] vallarose (3-O-methyl-6-deoxy-L-altrose),[27] and acovenose (3-O-methyl-6-deoxy-D-allose).[28] Cardiac glycosides bearing 2-O-methylpyranoses include kanaloside (a 2,3-di-O-methyl-D-fucose derivative)[29] and antiarojavoside,[30] an antiarigenin-class cardenolide isomeric with **1** and bearing the rare sugar javose (2-O-methyl-6-deoxy-D-allose, isomeric with 2-O-methyl fucose) at the 3 and 4 positions. To the best of our knowledge, ours is the first report of 2-O-methylfucose in a cardiac glycoside. Studies to assign the absolute configuration of the sugar are in progress. Ongoing work in our lab has recently isolated two additional cardiac glycosides which are isomeric with **1**, but which are also not antiarojavoside, and therefore appear to be new. Structural studies with these materials will be reported in due course, but these developments have prompted us to name cardenolide **1** as toxicarioside A. Efforts to determine the structures of other dart poison constituents that displayed significant activity in our bioassay also are in progress.

EXPERIMENTAL

General. NMR spectrometers: ^{1}H-NMR spectra were recorded in d_6-acetone (0.5 mL) with temperature regulation (25 °C) using 6 mg of **1** on a Bruker DPX300 (70.46 kG, ^{1}H 300 MHz, ^{13}C 75 MHz) and a Varian UNITY PLUS 400 (93.94 kG, ^{1}H 400 MHz, ^{13}C 100 MHz); ^{13}C-NMR spectra were measured under the same conditions using either the above Bruker system or a JEOL GSX-270 (63.41 kG, 270 MHz, ^{13}C 67.5 MHz). Spectra were referenced against the appropriate solvent lines for d_6-acetone: ^{1}H-NMR: center line of the residual CD_3COCD_2H pentet (δ 2.05); ^{13}C-NMR: center line of the $^{13}CD_3COCD_3$ septet (δ 29.92). Pulse sequences employed standard Brüker (XWIN-NMR 1.3), Varian (VNMR 5.3) or JEOL (Plexus 3.1) software. The HMQC spectra were optimized for an average $^1J_{C,H}$ of 140 Hz and employed a null delay of 0.2 sec; HMBC spectra were optimized for $J_{C,H}$ of 4.5 and 7.0 Hz. Difference NOE (DNOE) experiments were measured with 1152 accumulated transients each of the on-resonance (saturation) and off-resonance (negative) spectra acquired in repetitive blocks of 4 transients each. The irradiation delay was set to 6.6 sec; samples were not degassed prior to recording DNOE spectra. Infrared spectra were recorded on a Perkin-Elmer 1800 FTIR or a Perkin-Elmer 710 B spectrometer. Mass spectra were measured on a Finnigan MAT 90 in either CI (ammonia, 140 eV) or EI (70 eV) mode, as indicated. Optical rotations were measured on a Rudolph Research Autopol III polarimeter (concentration: g/dL).

Flash chromatography was conducted on silica gel (50 μ average particle size) from J.T. Baker (Phillipsburg, PA). Constituent spots were localized by thin layer chromatography using precoated silica gel plates with fluorescent indicator (254 nm) from Aldrich Chemical Company (Milwaukee, WI); visualization of spots employed a *p*-anisaldehyde-based spray reagent following the procedure of Touchstone and Dobbins.[31] HPLC analysis and preparative isolation were performed using a Perkin-Elmer Series 410 Pump and a Perkin-Elmer LC95 UV/Visible detector equipped with a PE Nelson 1022 LC Plus System data station. Alltech reverse phase HPLC columns (Deerfield, IL) were used; analytical HPLC employed an Absorbosphere C18 5μ column (250 mm x 4.6 mm) while preparative HPLC employed an Absorbosphere C18 5μ column 250 mm x 10 mm). All HPLC grade solvents were obtained from Fisher Scientific (Pittsburgh, PA) and were filtered through Alltech 0.2m Nylon 66 membrane filters prior to use. Fractions were concentrated using a Büchi 011

Rotovapor; lyophilization employed a Model 77510 Lyph-Lock 4.5 Liter Freeze Dry System from Labconco (Kansas City, MO).

The bioassay used to guide chromatographic purification measured the rate of hydrolysis of ATP (Boehringer Mannheim GmbH, Germany) catalyzed by porcine cerebral cortex Na^+/K^+-ATPase[16] (Sigma Chemical Company, St. Louis, MO) in the absence and presence of putative toxins. Fractions exhibiting the most significant decreases in ATP hydrolysis rate (which was interpreted to indicate the presence of active toxin(s)), in each stage of the isolation were carried forward into the next round of purification. Inhibition activity displayed by the common Na^+/K^+-ATPase inhibitor ouabain [**3**] (Sigma Chemical Company, St. Louis, MO) was used as a positive control.

Isolation of ***1***. 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 and then 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; **3**, 1.5; V_{max} (nmol P_i liberated/min/unit protein): crude extract, 11.6; **3**, 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 a 100% H_2O to 100% CH_3CN gradient 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 a new set of 100% H_2O to 100% CH_3CN gradient conditions. In sequential rounds of preparative HPLC fractionation employing these conditions, homogeneous peak ε (retention time ≈ 34 min) was collected in each preparative run. Pooled fractions containing this peak were lyophilized to give 13.6 mg of **1** as a white powder. Aqueous solutions (1 mM) of **1** and **3** showed essentially equivalent levels of activity in the bioassay: : K_m(mM): **1**, 0.7; **3**, 0.9;V_{max} (nmol P_i liberated/min/unit protein): **1**, 11.9; **3**, 16.8.

Toxicarioside A [1]. $[\alpha]_D^{25}$ +0.24 (c 0.23, MeOH), UV (MeOH) λ_{max} 216, 296 nm; ^{1}H- and ^{13}C-NMR see Tables 1 and 2; IR (KBr) 3401, 2943, 2890, 1734, 1638, 1066 cm^{-1}; HRMS (CI, ammonia, 140 eV) *m/z* ([M+1]) 581.2953 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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(*Received in USA* 3 *June* 1997; *accepted* 4 *August* 1997)