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CARDENOLIDES IN THE LATEX AND LEAVES OF SEVEN *ASCLEPIAS* SPECIES AND *CALOTROPIS PROCERA*

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Key Word Index—*Calotropis procera*; *Asclepias* spp; Asclepiadaceae; cardenolides; calotropagenin; calactin; calotropin; calotoxin; uscharidin; uscharin; voruscharin; desglucosyrioside; labriformidin; labriformin.

Abstract—The cardenolide content of the latex and leaves of seven *Asclepias* species and *Calotropis procera* was examined quantitatively by spectroassay and qualitatively by TLC. Species relatively rich in cardenolide, such as *C. procera*, *A. curassavica*, *A. eriocarpa*, *A. vestita*, and *A. cordifolia*, had latex–leaf cardenolide concentration ratios ranging from 79 (*C. procera*) to 1.5 (*A. cordifolia*). Two species, *A. californica* and *A. speciosa*, had measurable leaf cardenolide but no measurable amounts in the latex. *A. fascicularis* had no measurable cardenolide in either leaves or latex. *C. procera*, *A. curassavica*, *A. vestita* and *A. cordifolia* had primarily calotropagenin-derived cardenolides, while *A. eriocarpa* and *A. speciosa* had primarily desglucosyrioside and its derivatives. The latex of cardenolide-enriched species had greater proportions of lower polarity cardenolides, particularly those with a spiro NS ring or keto at 3′ of the sugar (uscharidin, uscharin, voroscharin. labriformin, labriformidin), than was present in the leaves. Uscharidin, uscharin and voruscharin were isolated from *A curassavica* latex, and labriformin from *A. eriocarpa* latex, in higher yields than reported from whole plants.

INTRODUCTION

Several genera of the milkweed (Asclepiadaceae) family contain biologically active chemicals [1–4]. Among these are species of *Asclepias* which contain cardenolides toxic to vertebrates [5, 6]. Certain insects, notably Monarch butterflies (*Danaus plexippus*) [7, 8], and milkweed bugs (*Oncopeltus fasciatus*) [9], sequester these chemicals from *Asclepias* host plants and, apparently, utilize them for defense against vertebrate predators [10]. Previous studies have shown that most *Asclepias* species examined contain cardenolide mixtures with concentrations which vary between species [11] and between plant parts within a given species [12]. Furthermore, there are at least two major structural groups of 2,3-dihydroxycardenolide glycosides in *Asclepias*, that is, derivatives of either calotrapagenin (**1**) or desglucosyrioside (**8**) [11, 13, 14]. Substitution in the sugar moiety and/or genin differentiates cardenolides in each group (Fig. 1).

We recently reported that the latex of *A. eriocarpa* contained much higher concentrations of total cardenolide than the leaves, stems, or roots, and was enriched in a relatively non-polar cardenolide derivative of desglucosyrioside, labriformin (**10**), having a spiro thiazoline ring at 3′ [12, 14, 15]. Earlier studies with the milkweed *Calotropis procera*, from which the calotropagenin-derived glycosides were first isolated [1, 16], showed that the latex was similarly a rich source of the 3′-thiazoline cardenolide, uscharin (**6**) [16]. We postulated that the latex may serve to isolate cardenolides from vascular tissue in milkweed species of high cardenolide content, that N,S-containing rings at 3′ may stabilize cardenolides in the latex suspension, and that a cardenolide-enriched latex may aid in deterring attack upon these plants by herbivores [12].

We report here a comparative quantitative and qualitative analysis of leaf and latex samples of several *Asclepias* species and *Calotropis procera*. This was carried out to test the generality of some postulates made from the examination of *A. eriocarpa*, and to identify sources for isolation of the relatively rare 2,3-dihydroxycardenolide glycosides of Asclepiadaceae for toxicological testing.

RESULTS AND DISCUSSION

Quantitative analysis of total cardenolide content was done by visible spectrophotometry (spectroassay) of the 2, 2′, 4, 4′-tetranitrodiphenyl (TNDP) cardenolide complex, referenced to that of a digitoxin standard [18, 19]. Recoveries through the lead acetate precipitation clean-up [12] were obtained by comparing spectroassay results for crude ethanol extracts with those from cleaned extracts; recoveries exceeded *ca* 65% for all cardenolide-positive leaf and latex samples (Table 1). Average latex–leaf cardenolides ratios after clean-up exceeded 1 for *C. procera* (79), *A. curassavica* (51 and 55 for two different sample sets), *A. vestita* subsp. *parishii* (28),

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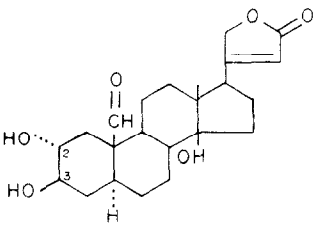
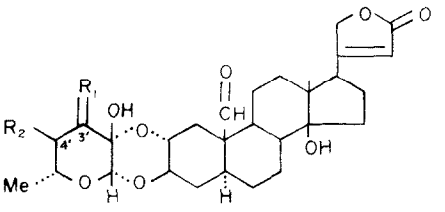
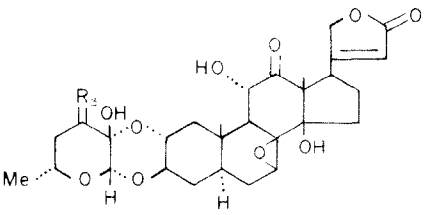


Calotropagenin I





Compound	Trivial name	R_1	R_2
2	Calactin	α-H, β-OH	H
3	Calotropin	β-H, α-OH	H
4	Calotoxin	ξ-H, ξ-OH	ξ-H, ξ-OH
5	Uscharidin	O	H
6	Uscharin		H
7	Voruscharin		H

Compound	Trivial name	R_2
8	Desglucosyrioside	α-H, β-OH
9	Labriformidin	O
10	Labriformin	

Fig. 1. Some cardenolides in *Asclepias* species and *Calotropis procera*. Complete stereochemistry is described elsewhere [13].

A. eriocarpa (8.7), *A. vestita* subsp. *vestita* (3.9 and 10.0 for two different sample sets) and *A. cordifolia* (1.5). Ratios were less than 1 for *A. speciosa* and *A. californica*, both of which had measurable cardenolide in the leaf but not in the latex. For *A. fascicularis*, neither leaf nor latex had cardenolide above the detection limit of the spectroassay method.

These quantitative results for *A. curassavica* leaves are somewhat higher than we reported previously for a different sample where 3.8 mg/g was obtained prior to clean-up [11]. The present results for *A. eriocarpa* compare favorably with those obtained previously for uncleaned samples from the same month of collection (July), but from a different location (leaves 4.9, and latex 67.5 mg/g [12]). Reported mean leaf cardenolide content for 172 uncleaned *A. eriocarpa* samples from six locations in California was 4.21 mg/g with s.d. of 1.70 [20]. The latter study showed that location had a relatively minor effect on leaf cardenolide content for *A. eriocarpa*. Apparent variations in some results for other *Asclepias* species reported here and in our previous study [11] may be due to the fact that only leaves were analysed here, while a mixture of leaves and stems constituted the earlier samples; also, different clean-up methods were employed in the two studies. The lead acetate method [12] provides higher recoveries overall, and a much higher recovery of more polar cardenolides than a solvent partition method used previously [11]. None of these variations will negate principal findings of the present study, that *Asclepias* species vary distinctly in cardenolide content and latex–leaf cardenolide ratios, and that species with relatively high leaf cardenolide content tended toward higher latex–leaf ratios than cardenolide-poor species.

Cleaned extracts were analysed by TLC using the cardenolide-selective TNDP spray reagent, and the relative amount of individual components subjectively scored for all TNDP-positive spots (Table 2). These measurements were taken from plates developed in solvent system I with a second plate developed in solvent system II providing confirmation of assignments and the relative proportions for components which overlapped in solvent system I. Assignments were based on coincidence of TLC R_f with standards run on the same plates.

Four species, *C. procera*, *A. curassavica*, *A. vestita*, and *A. cordifolia*, had several calotropagenin-derived cardenolides in common. *C. procera* leaves contained principally calotropagenin (**1**), calactin (**2**), calotropin (**3**), and calotoxin (**4**). All four compounds were present in the latex and, along with uscharin (**6**) and voruscharin (**7**), constituted over half of the latex cardenolide. The content of *A. curassavica* was similar to that of *C. procera*, except that **5** and **7** were present in greater proportions in both leaves and latex of the former. **7** was not present in the leaves of this species. **1–6** have been reported previously from *C. procera* ([1, 16] references in [17]) and *A. curassavica* [6, 21, 22]. *A. vestita* leaves and latex had no **7** in

Table 1. Moisture content, cardenolide content (as mg equiv. to digitoxin/g dry wt) and per cent recovery through clean-up procedure for leaf and latex samples of eight milkweed species

Species Sample type (n)	Moisture ($\bar{x}$% ± s.d.)	Cardenolide $\bar{x}$(mg/g ± s.d.) Uncleaned	 Cleaned	Recovery ($\bar{x}$% ± s.d.)
A. curassavica				
Sample A:				
leaf (5)	79 ± 2	6.58 ± 0.76	6.07 ± 0.67	92 ± 3
latex (5)	87 ± 1	422.0 ± 34.0	312.0 ± 27.0	75 ± 6
Sample B:				
leaf (5)	80 ± 1	5.77 ± 1.84	5.55 ± 1.85	96 ± 4
latex (5)	90 ± 1	392.0 ± 57.0	303.0 ± 40.0	78 ± 8
C. procera				
leaf (2)	81 ± 1	3.11 ± 0.84	2.04 ± 0.67	65 ± 4
latex (2)	81 ± 4	213.0 ± 25.0	162.0 ± 19.0	76 ± 0
A. vestita subsp. *vestita*				
Kimbler Ranch:				
leaf (5)	71 ± 2	25.1 ± 5.8	21.8 ± 4.6	87 ± 3
latex (5)	76 ± 2	118.0 ± 31.0	85.8 ± 19.2	76 ± 18
Commatti Canyon:				
leaf (5)	81 ± 2	6.50 ± 2.43	6.18 ± 2.13	97 ± 15
latex (5)	81 ± 8	68.1 ± 17.9	61.9 ± 12.9	92 ± 8
A. vestita subsp. *parishii*				
leaf (5)	83 ± 1	1.87 ± 0.81	1.52 ± 0.71	78 ± 2
latex (5)	84 ± 2	50.3 ± 5.3	42.7 ± 4.7	85 ± 7
A. eriocarpa				
leaf (5)	77 ± 1	4.97 ± 0.49	4.25 ± 0.52	86 ± 10
latex (5)	67 ± 14	54.0 ± 22.8	37.2 ± 14.1	70 ± 7
A. cordifolia				
leaf (5)	74 ± 1	2.63 ± 1.20	2.52 ± 0.73	105 ± 26
latex (5)	73 ± 2	4.68 ± 1.60	3.89 ± 1.24	87 ± 22
A. speciosa				
Sample A:				
leaf (5)	81 ± 2	3.09 ± 0.43	2.04 ± 0.36	66 ± 8
latex (5)	71 ± 3	< 0.57	< 0.14	—
Sample B:				
leaf (5)	78 ± 0	1.27 ± 0.09	0.98 ± 0.16	77 ± 8
latex (5)	65 ± 4	< 0.57	< 0.29	—
A. californica				
leaf (5)	69 ± 2	1.15 ± 0.35	0.96 ± 0.46	84 ± 39
latex (5)	77 ± 4	< 0.57	< 0.29	—
A. fascicularis				
leaf (5)	73 ± 1	< 0.57	< 0.14	—
latex (5)	51 ± 15	< 0.57	< 0.29	—

either of two subspecies and only small amounts of **6** in subspecies *vestita*, but a notably high concentration of **2** in both types of samples from two subspecies. **1–5** constituted over half of the *A. vestita* latex cardenolide. *A. cordifolia* leaves and latex also lacked **7**. For this species the latex cardenolide was principally a mixture of **2–6**. There were several unidentified cardenolide TLC spots in all of these species, with a trend to larger proportions with low or zero R_f in leaves than occurred in latex.

The present results for *A. eriocarpa* agreed with those reported [12], that is, that labriformidin (**9**) and labriformin (**10**) were the principal latex cardenolides and, with desglucosyrioside (**8**), constituted the identified cardenolides. *A. eriocarpa* leaves also contained **8–10** but in lower proportions than in the latex. *A. speciosa* leaves had **8** and several unidentified cardenolides coinciding with ones in *A. eriocarpa*, but were lacking **9** and **10**. *A. speciosa* latex had only faint cardenolide spots (one sample) at the level spotted on TLC. Both species had an abundance of unidentified cardenolides of low R_f in leaves, with *A. speciosa* leaves particularly enriched in a component of R_f 0.5 relative to digitoxin in solvent system I.

A. californica had very faint cardenolide TLC spots, and only in the leaves; among the species examined it most closely resembled *A. curassavica*. Our previous results had suggested the presence of calotropagenin-derived cardenolides in *A. californica* [11]. *A. fascicularis* had no cardenolide TLC spots observed in either leaves or latex.

Previous results indicated that uzarigenin was present in several of the species examined [11]; our results for *A. curassavica* [23] and *A. eriocarpa* [12]

Table 2. Estimated relative cardenolide abundances in cleaned leaf and latex samples of eight milkweed species, as determined from TLC*

		Calotropagenin and derivatives							Unidentified cardenolides			Desgluco-syrioside and derivatives		
	R_d values	2.1	1.9	1.7	1.08	1.03	0.84	0.62	≥ 1.00	0.2–0.98	0	3.03	3.03	1.30
Species Sample type	Compound Name/No.	**7** Voruscharin	**5** Uscharidin	**6** Uscharin	**3** Calotropin	**2** Calactin	**1** Calotropagenin	**4** Calotoxin	Unidentified	Unidentified	Unidentified	**10** Labriformin	**9** Labriformidin	**8** Desglucosyrioside
A. curassavica														
Sample A:										(2)				
leaf		—	+++	++	++	++	++	++	—	++	++	—	—	—
									(1)	(2)				
latex		+++	+++	++	—	++	++	++	+	++	++	—	—	—
A. curassavica														
Sample B:									(2–3)	(2)				
leaf		—	++	+	+	++	++	++	+	+++	+++	—	—	—
									(2)	(2)				
latex		+++	+++	++	—	++	++	++	+	+++	++	—	—	—
C. procera									(1)	(3)				
leaf		—	+	+	++	++	+++	++	+	+++	+++	—	—	—
									(1)	(2)				
latex		+	+	+++	+	++	+++	++	+	++	+	—	—	—
A. vestita subsp. *vestita*														
Kimbler Ranch										(1–3)				
leaf		—	++	+	++	+++	++	++	—	++	+++	—	—	—
										(1)				
latex		—	++	+	+++	+++	++	++	—	+	+	—	—	—
Commatti Canyon									(1–2)	(4–6)				
leaf		—	++	—	+	+++	++	++	++	+++	++	—	—	—
									(2)	(3–4)				
latex		—	+	—	+	+++	++	+	++	++	++	—	—	—
A. vestita subsp. *parishii*									(1)	(1–4)				
leaf		—	+	—	+	++++	++	++	+	++	++	—	—	—
									(1)	(1–2)				
latex		—	+++	—	++	+++	++	++	+	++	++	—	—	—
A. eriocarpa									(4–5)	(4–6)				
leaf		—	—	—	—	—	—	—	++	+++	+++	+	+	+
									(4–5)	(4)				
latex		—	—	—	—	—	—	—	+++	+++	++	+	+	+
A. cordifolia									(2–3)	(1–4)				
leaf		—	++	++	+	+	++	++	++	++	+	—	—	—
										(3–4)				
latex		—	+++	++	++	++	+	++	—	+++	+++	—	—	—
A. speciosa														
Sample A:									(3–7)	(4–5)				
leaf		—	—	—	—	—	—	—	+++	++++	++	—	—	—
latex		—	—	—	—	—	—	—	—	—	—	—	—	—
A. speciosa														
Sample B:									(1)	(3–4)				
leaf		—	—	—	—	—	—	—	+	++++	—	—	—	+
										(2)				
latex		—	—	—	—	—	—	—	—	+++	—	—	—	—
A. californica									(3)	(5–7)				
leaf		—	—	—	—	—	—	—	+	++++	+++	—	—	—
latex		—	—	—	—	—	—	—	—	—	—	—	—	—
A. fascicularis														
leaf		—	—	—	—	—	—	—	—	—	—	—	—	—
latex		—	—	—	—	—	—	—	—	—	—	—	—	—

*Estimates are means of at least two samples (Table 1). R_f values relative to digitoxin (R_d) in solvent system I appear next to cardenolide name and structural number. Parenthetical values refer to approximate number of unidentified cardenolide TLC spots in solvent system I. Code is: ++++ > 35% of total; +++ 16–35%; ++ 6–15%; + trace–5%; —none observed.

suggest that this genin is restricted to stem tissue and, thus, would not have been included in the present analyses.

For all of the species examined which had cardenolides in the latex, the trend was to cardenolides of lower polarity (R_f > digitoxin) in this fluid than occurred in leaves. The latex was a particularly good source of N,S-containing cardenolides, **6** in *C. procera*, **6** and **7** in *A. curassavica*, **6** in *A. cordifolia*, and **10** in *A. eriocarpa*. In addition to the N,S-containing cardenolides, the latex of cardenolide-positive species also contained glycosides with 3'-keto (**5**, **9**) and 3'-hydroxy groups (**2**, **3**, **8**). The 4'-hydroxy derivative, **4**, and the genin **1** were also measurably present in the latex of four of the species tested.

The latex of *A. curassavica* was examined as a source for cardenolide isolation. From 54 ml of latex (6.45 g of solid matter) purified cardenolide yields were 0.23, 0.22, and 0.30 g for **5**, **6**, and **7**, respectively. Considerably larger quantities of these three compounds were present in unresolved mixtures from the isolation scheme. By contrast, 1.95 kg of dried aerial plant parts (leaves, stems, and residual latex) had previously yielded only 0.034 g of **5** along with 0.138 g of uzarigenin and 0.339 g of **3** [23]. Similarly, 85 ml of *A. eriocarpa* latex yielded 0.045 g of pure **10** in the present study, where previously 2.6 kg of dried whole plant yielded 0.21 g of impure **10** [15]. The latex of *A. curassavica* and *A. eriocarpa* is thus a better source for isolation of some cardenolides than whole plant. Although not tested here, it may be surmised that *A. vestita* latex might be a preferred source for isolation of **2** and **3**, while *C. procera* latex could furnish **1**, **4**, and **6**. *A. eriocarpa* latex could furnish **9** either directly or after acid hydrolysis of **10** [15]. Previous results indicate that *A. erosa* and *A. labriformis* may substitute for *A. eriocarpa* for isolation of **10** and, perhaps, other derivatives of **8** [15].

In summary, the present results support the hypothesis that *Asclepias* species relatively enriched in cardenolides favor higher content in the latex than occurs in leaves, and that cardenolides containing the spiro N,S ring at 3' are principally associated with the latex. Furthermore, the latex of some milkweed species may provide a more convenient source for isolation of relatively rare 2,3-dihydroxycardenolide derivatives of the calotropagenin and desglucosyrioside series than whole plants.

EXPERIMENTAL

Mps are uncorr. IR were taken in KBr. ^{1}H NMR spectra were recorded at 360 MHz with solns in $CDCl_3$ using 1% TMS as int. standard. TLC was carried out on Si gel G with solvent systems I, $CHCl_3$–MeOH–formamide (90:6:1) and II, EtOAc–MeOH (97:3). Plates were developed 4× in I and 2× in II, then sprayed with a 4% soln of 2, 2', 4, 4'-tetranitrodiphenyl in toluene, followed by a 10% soln of KOH in 50% aq. MeOH. Color xeroxes of photographs of freshly sprayed plates were used to score cardenolide proportions. Sample clean-up for quantitative analysis was by lead acetate precipitation as reported in [12]. CC for isolation used Biosil A silicic acid, 100–200 mesh, with a slight head pressure. Spectroassay was done as reported [18, 19].

Plant samples. Except where noted, latex and matching leaves were gathered from three plants. Five samples were taken for each species at one location. Latex was collected from the stem after cutting at the tip, leaf-stem juncture, and/or pod-stem juncture. Samples were frozen immediately in dry ice. *Asclepias californica* Greene was collected on 25 July 1979, near Pine Flat Reservoir (Fresno County, CA). *A. cordifolia* (Benth.) Jeps. was collected on 19 July 1979, near Monticello Dam (Napa County, CA). *A. curassavica* L. was collected from flowering greenhouse specimens on 18 July 1979, and from a cultivated garden plot on 24 September 1979. *A. eriocarpa* Benth. was collected on 13 July 1979, in an open field off Hwy 49 near Auburn (Placer County, CA). These plants had been damaged from insect feeding. *A. fascicularis* Dcne. was collected on 19 July 1979, near road 95 west of Davis (Yolo County, CA). *A. speciosa* Torr. was collected on 19 July 1979, from a field south of Davis (Yolo County, CA) and on 2 August 1979, from a pasture off Allegany road near Hwy 49 (Nevada County, CA). *A. vestita* subsp. *vestita* was collected on 25 July 1979, at the Kimbler Ranch near Humphrey's station east of Fresno (Fresno County, CA); and on 29 May 1981, at Commatti Canyon (Fresno County, CA). *A. vestita* subsp. *parishii* was collected on 17 May 1981, at Cajon Pass (San Bernardino County, CA). *Calotropis procera* R. Br. was collected on 11 October 1979, from two cultivated greenhouse plants. Identifications were based on voucher specimens at the Botany Department Herbarium, University of California, Davis, except for two *A. vestita* samples, for which voucher specimens are on file with Dr. Steven Lynch, Louisiana State University, Shreveport, LA, under 5097 (Commatti Canyon) and 5095 (Cajon Pass).

Analysis. Frozen material was thawed, dried 16–24 hr at 60°, and allowed to come to room temp. in a desiccator. Leaf samples were ground to pass a 0.84-mm Wiley mill screen, and latex samples were crushed with a mortar and pestle. Samples (0.2 or 0.5 g) were extracted with 95% EtOH (10 or 25 ml) for 1 hr at 70–78°. Total cardenolide content was then determined by spectroassay, referencing to a standard curve generated from 1.5×10^{-5}–15×10^{-5} M solutions of digitoxin. One or two 6-ml aliquots of EtOH extract were evaporated to dryness, cleaned up using the lead acetate precipitation method, then reassayed to determine cardenolide recoveries through the procedure.

TLC plates were spotted with the standards digitoxin, digitoxigenin and uzarigenin. In addition, latex and leaf samples from *C. procera*, *A. curassavica*, *A. vestita*, *A. cordifolia* and *A. californica* were chromatographed with standards of **1**–**7**; samples from *A. speciosa* and *A. ericarpa* were similarly compared with standards of **8**–**10** and syriogenin.

Isolation of cardenolides from A. curassavica *latex.* Latex (54 ml) collected during July and August, 1980, from plants growing outdoors at the University of California, Davis, was dried (40–50°) for 3 days, yielding 6.4 g. The residue was extracted 3× by alternately warming (53°) and sonicating with 100 ml portions of MeOH. The solution was evaporated to dryness, the residue (5.4 g) dissolved in 1:1 EtOH–H_2O (1.1 l), and the resulting solution extracted 3× with $MeCl_2$ (500 ml). The combined $MeCl_2$ solution was extracted with satd $NaHCO_3$ (500 ml), 0.1 N HCl (500 ml), and H_2O (500 ml), then evaporated to dryness. The residue (2.8 g) was applied in $CHCl_3$ (20 ml) to a column containing 46 g Si gel. Elution was with $CHCl_3$ (200 ml), 25% increments of EtOAc in $CHCl_3$ (200 ml/increment), and MeOH (200 ml).

Uscharidin (**5**). The 25% EtOAc–75% $CHCl_3$ Si gel elution solvent was evaporated to dryness and the residue (0.53 g) was dissolved in a minimum vol. of $MeCl_2$. Et_2O was added

dropwise to the cloud-point, and the solution was cooled for 2 days yielding a white powder. Recrystallization in a similar way gave crystalline **5** (116 mg), mp 193–196° (lit. mp 201°/295–297°, MeOH–Et_2O and 195°, $CHCl_3$ [25]) and $[\alpha]_D^{22}$ + 25.6° in EtOH (lit. $[\alpha]_D$ + 34.6° in EtOH [17] and + 24° in EtOH [25]). This sample agreed in TLC, IR, and NMR with reported values for **5** [17, 23]; its mp and $[\alpha]_D$ suggested that it was the $MeCl_2$ solvate of **5** [25, 26]. Additional **5** (120 mg) was obtained by similar processing of the mother liquor from the 50% EtOAc–50% $CHCl_3$ Si gel elution after voruscharin was crystallized out (below).

Voruscharin (**7**). The 50% EtOAc–50% $CHCl_3$ Si gel elution solvent was evaporated to dryness and the residue (0.67 g) dissolved in a minimum vol. of hot MeOH. Cooling the solution produced crystalline **7** which, after a further recrystallization from MeOH, gave 230 mg, mp 190–192° and $[\alpha]_D^{22}$ + 42.6°. Recrystallization from acetone gave **7** as a powder, mp 168–171° and $[\alpha]_D^{22}$ + 60.7° in EtOH (lit. mp 165–166° from acetone and $[\alpha]_D$ − 60.8° in EtOH [27]). IR ν_{max} cm^{-1}: 3500 (OH), 2860 (CHO), 1780, 1750, 1630 (butenolide ring), 1450, 1370, 1160, 1070, 860, 720 (broad, several). ^1H NMR δ 0.82 (3H, *s*, C-18), 1.20 (3H, *d*, J = 6 Hz, C-6'), 2.96 (2H, *m*, CH_2–N), 4.0–4.1 (*m*, C-2, C-5'), 4.82 (1H, *s*, C-1'), 4.94 and 4.70 (2H, *d* × *d*, J = 18, 2 Hz, C-21), 5.87 (1H, *t*, J = 2 Hz, C-22), 10.01 (1H, *s*, C-19). Our spectral data was consistent with the structure of **7**, and our mp agreed with that reported for **7**. The reason for the discrepancy in sign of rotation between our sample and that reported for **7** is not known.

Additional **7** was obtained as follows: the 75% EtOAc–25% $CHCl_3$ Si gel elution solvent was evaporated to dryness and the residue (440 mg) was applied to 11 g of Si gel in a second chromatography column. Elution was with 1, 1.25, 1.50, 1.75 and 2.0% MeOH in $CHCl_3$. The 1.25% elution phase contained an equal mixture of **6** and **7** by TLC; the residue was dissolved in 7:3 H_2O–MeOH (100 ml), made 0.1 N in HCl and extracted with $MeCl_2$ (3 × 100 ml). The H_2O–MeOH phase was neutralized with $NaHCO_3$ to pH 6.5 and extracted with $MeCl_2$ (3 × 50 ml); evaporation of $MeCl_2$ to dryness gave a residue. This residue was dissolved in a minimum vol. of boiling MeOH, 2 drops of H_2O added, and the solution kept at − 10° for 3 days. Recrystallization of the resulting crystals by the same method gave 73 mg **7**, mp 190–191°.

Uscharin (**6**). The 1.5% MeOH in $CHCl_3$ elution phase from the second chromatography column was evaporated to dryness and the residue dissolved in a minimum vol. of warm MeOH with sonication to aid in dissolution. The solution was cooled to room temp, a few drops of Et_2O added, and the resulting solution kept at − 10° for 3 days. Recrystallization by the same method gave crystalline **6** (22 mg), mp 250–253° (lit. mp 265° *d* [17]), and $[\alpha]_D^{22}$ + 37.8° in $CHCl_3$ (lit. $[\alpha]_D$ + 29.0° in $CHCl_3$ [17]) which agreed in its ^1H NMR spectrum with reported data [17].

Isolation of labriformin (**10**) *from* A. eriocarpa *latex.* Latex (85 ml) collected in June from the Woodland, Yolo Co., site was mixed with MeOH (90 ml) and H_2O (360 ml) and the suspension extracted with petrol (4 × 150 ml) and C_6H_6 (4 × 150 ml). Residue from the C_6H_6 extract (0.32 g) was dissolved in MeOH (25 ml) and mixed with H_2O (100 ml). This suspension was extracted with petrol (3 × 25 ml) and C_6H_6 (5 × 25). Residue from the C_6H_6 extract (0.13 g) crystallized on adding MeOH (5 ml). Recrystallization from MeOH gave 45 mg of crystalline **10**, mp 213–215° d (lit. mp 213–215° d [15]) with spectra identical to a previous sample [15].

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