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THREE NEW CARDENOLIDES FROM THE MILKWEEDS *ASCLEPIAS ERIOCARPA* AND *A. LABRIFORMIS*

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Key Word Index—*Asclepias eriocarpa*; *A. labriformis*; Asclepiadaceae; milkweed; new cardenolides; isolation; partial structures; labriformin; labriformidin; eriocarpin.

Abstract—*Asclepias eriocarpa* and *A. labriformis* contain three new cardenolides, the structures of which have been partially assigned by their spectral properties and comparison with the known cardenolides of *A. curassavica*. They include labriformin ($C_{31}H_{39}O_{10}NS$), labriformidin ($C_{29}H_{36}O_{11}$), and eriocarpin.

INTRODUCTION

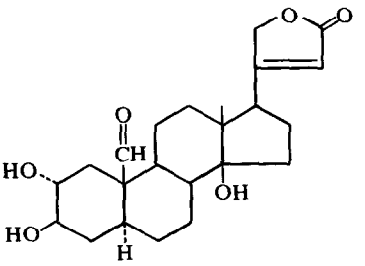
Some members of the milkweed plant family (Asclepiadaceae) contain biologically active components [1–3]. Included among them are species of the North American genus *Asclepias* which have caused fatal poisoning of livestock [4] and may serve as a source of cardenolides sequestered by insect herbivores for defense against predation [5–7]. Analysis of several *Asclepias* species showed that the cardenolide concentration varies greatly among species, from less than 0.01 to over 3.0% dry weight expressed as digitoxin equivalents [8]. Furthermore, the cardenolides in these species fall into at least two distinct groups, differentiated by profiles obtained upon TLC analysis [8].

Milkweed cardenolides which have been fully characterized include those first isolated from *Calotropis procera* [1]. These compounds, since isolated from *A. curassavica* [9, 10], are for the most part glycosides of calotropagenin (**1**). Uscharin (**2**), uscharidin (**3**) and the isomeric pair calactin and calotropin (**4**) which differ in stereochemistry at C-2′ and C-3′, are examples; their structures have recently been revised to those shown here [11, 12]. Reported cardenolide isolations from other *Asclepias* species have been summarized elsewhere [8, 13].

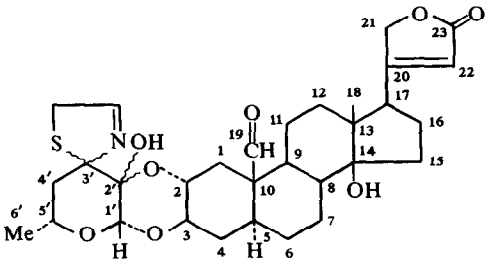
A. eriocarpa and *A. labriformis*, two species of particularly high toxicity to livestock [4], have a relatively high cardenolide content, 0.1–0.5% dry weight, and a cardenolide TLC pattern which differs from those of *A. curassavica* and several other *Asclepias* species [8]. We report here the isolation and partial characterization of three major cardenolides from *A. eriocarpa* and *A. labriformis*, and provide evidence that they represent a new structural group of cardenolides.

RESULTS AND DISCUSSION

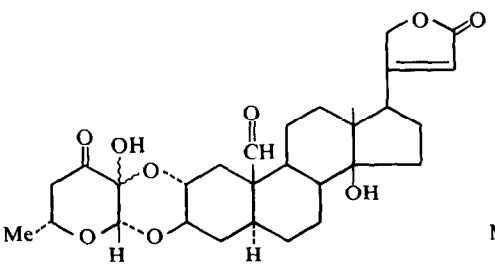
Fractionation of plant extracts, patterned after the



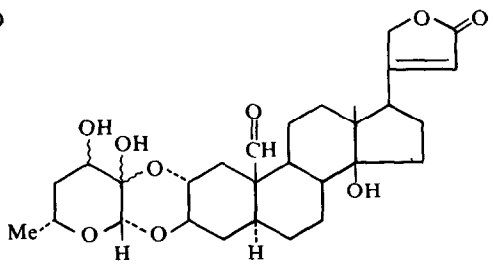
Calotropagenin (1)



Uscharin (2)



Uscharidin (3)



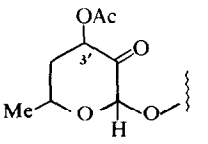
Calactin and Calotropin (4)

scheme of Kupchan *et al.* [3] for *A. curassavica*, employed partitioning between aqueous MeOH and organic solvents of increasing polarity. Labriformin was occasionally obtained by crystallization of the C_6H_6 partition phase; as this compound partially decomposed on column chromatography, failure to crystallize at the appropriate point in the scheme precluded its isolation from some batches. Labriformidin and eriocarpin, purified by column chromatography of the CH_2Cl_2 partition phase, were obtained as amorphous powders whose mps varied with different preparations. Shortcomings in the isolation scheme led to recoveries of only 2–10% of the amount of each cardenolide estimated as present in the plants. Labriformin was first isolated from *A. labriformis* but more consistent preparations of the three cardenolides were obtained from *A. eriocarpa*. TLC mobilities (Table 1) and response to tetranitrodiphenyl spray reagent, and IR absorptions for the butenolide ring at *ca* 1780, 1745 (C=O) and 1630 cm^{-1} (C=C) were used to assess the purity of isolated cardenolides.

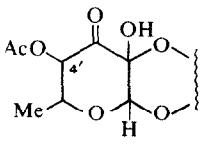
Labriformin, 1 N atom from combustion analysis, contains a thiazoline ring at C-3′ from the following evidence: the IR spectrum had absorption at 1650 cm^{-1} (C=N) as in the spectrum of uscharin (**2**) [14]. Acid hydrolysis led to loss of N and generation of labriformidin; **2** formed uschardin (**3**) under the same reaction conditions [15]. The PMR spectrum of labriformin had peaks at δ 7.65 (—CH=N—) and 3.91 (—S—CH_2—), similar to the positions of corresponding groups in **2** [11]. Aside from the usual peaks due to butenolide ring protons, labriformin and **2** also had in common PMR peaks at *ca* δ 5.1 (1H, *s*, C-1′) and 1.2 (3H, *d*, C-6′). These indicate similar sugars in the two compounds, namely a 6-deoxyhexosone attached to C-2 and C-3 of the genin through hemiketal (at C-2′) and acetal bonds (at C-1′) as in the calotropagenin glycosides **2**–**4** [11] and gomphoside [16].

Labriformin differed from uscharin (**2**) in lacking aldehyde functionality at C-19; C-19 is a Me in labriformin, as shown by the PMR singlet at δ 1.21 which was not present in the spectrum of **2**. Also, C-18 protons of labriformin were shifted downfield, to δ 1.09, relative to their position at δ 0.8 in **2**. This indicates substitution in rings C and/or D of the steroid nucleus of labriformin. The di-*O*-acetyl derivative of panogenin, a cardenolide with an 11-β OH, had C-18 H absorption at about the same position as for labriformin [17].

Labriformin also had C-19 as a methyl group, PMR δ 1.19, and C-18 H shifted relative to its position in uscharin (**2**) and uscharidin (**3**) to δ 1.05. But similarities in other features of the PMR spectra showed that labriformidin and **3** [11] conform to the same 7-ring geometry. The PMR spectrum of labriformidin monoacetate had acetyl Me at δ 2.20 and an apparent doublet (1H, J = 13 Hz) at 5.68 which was not visible in the spectrum of labriformidin. This is assigned to the C-4′ proton which, in labriformidin, underlies PMR peaks at δ 4.77 (2H, *s*, C-21) and 4.58 (1H, *s*, C-1′). A *ca* 1 ppm downfield shift occurred at the C-3′ proton upon acetylation of asclepin to acetylasclepin (**5**) [18]. In **5** the C-3′ proton is adjacent to C=O just as we suppose to be the case for the C-4′ proton in labriformidin monoacetate.



Acetylasclepin (**5**)



Labriformidin monoacetate

High resolution MS of labriformidin had the first visible fragment at *m/e* 542, $C_{29}H_{34}O_{10}$, apparently from dehydration of M^+ (Scheme 1). The base peak, at *m/e* 459 ($C_{25}H_{31}O_8$), resulted from loss of the butenolide ring from $C_{29}H_{34}O_{10}$, the sugar group in the latter having been stabilized by C=C—C=O conjugation during initial dehydration. Major fragments also occurred at *m/e* 417 ($C_{23}H_{29}O_7$) and 399 ($C_{23}H_{27}O_6$). These are apparently the genin minus 1 H and its dehydration product, respectively. MS of uscharidin (**3**), calactin/calotropin (**4**), and related compounds showed the genin, but it was frequently quite weak [11]. Subsequent fragmentation of $C_{23}H_{27}O_6$ was by loss of H_2O and CO, as reported for the calotropagenin glycosides [11]. For labriformidin monoacetate M^+ (*m/e* 602) was visible but quite weak. Loss of MeCOOH from M^+ gave the same *m/e* 542 peak as observed for labriformidin, and subsequent fragmentation was similar to that of labriformidin.

MS and PMR data lead to partial formulation of labriformidin as **7**. It follows that labriformidin monoacetate is **8** and labriformin is **6**. The partial structures and major MS fragments of **7** and **8** are in Scheme 1.

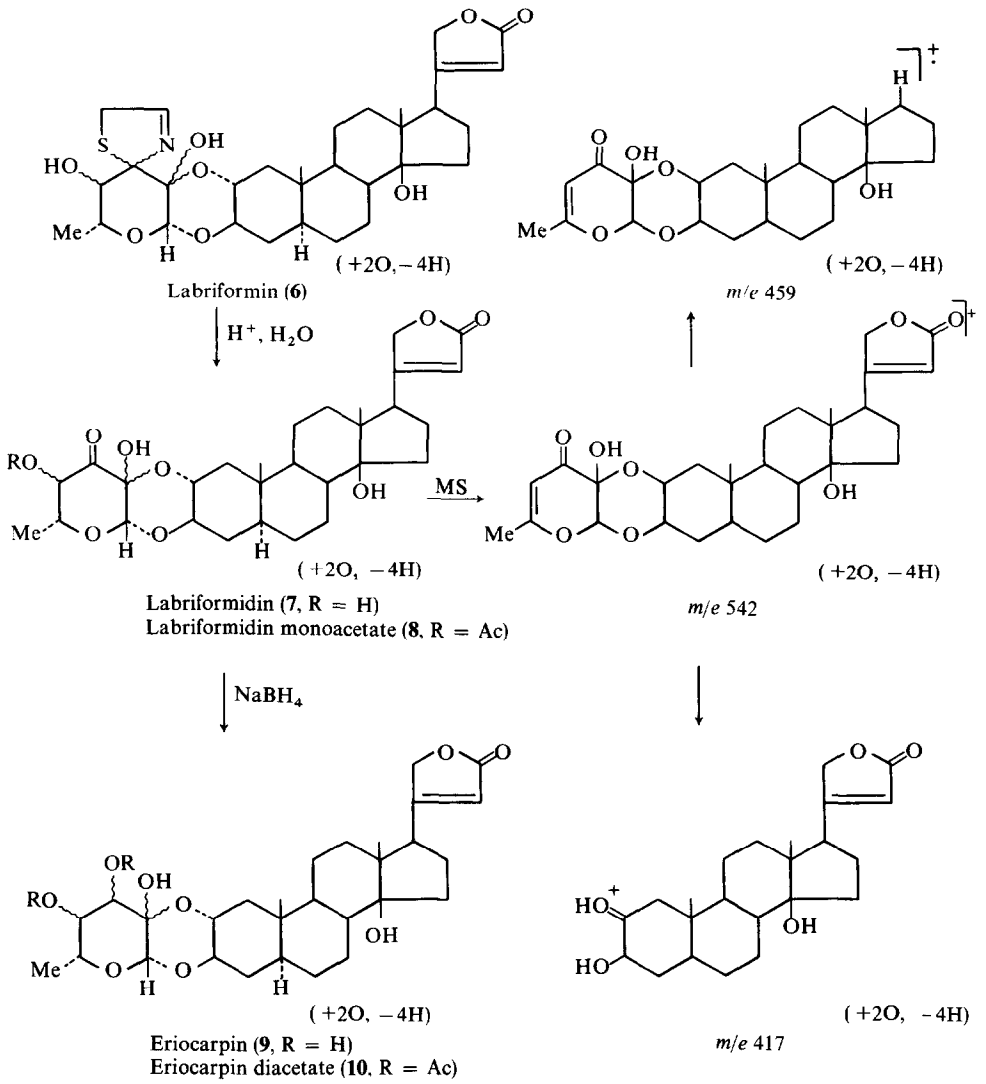
The PMR spectra of eriocarpin and labriformidin were quite similar. Additional OH in the former was indicated by its lower TLC R_f and by the fact that acetylation under the same conditions as for conversion of labriformidin (**7**) to its monoacetate (**8**) gave rise to a diacetate (PMR singlets at δ 2.10 and 2.24). Partial reduction of labriformidin with $NaBH_4$ gave a small amount of eriocarpin along with other unidentified products, one of which was predominant. Under similar reduction conditions uscharidin (**3**) formed calotropin (**4**) along with products from further reduction [19]. This evidence suggests that eriocarpin is represented by **9** (Scheme 1) and bears the same relationship to labriformidin as does calactin/calotropin (**4**) to uscharidin (**3**).

The partial structures proposed for labriformin (**6**), labriformidin (**7**) and eriocarpin (**9**) must be regarded as hypotheses for the present. Comparison with their empirical formulae, derived from combustion data and high resolution MS, shows that mass 28 has yet to be accounted for. Placement of groups in the steroid ring satisfying +2 oxygen, −4 hydrogen could account for

Table 1. Amounts (estimated by TLC) and properties of cardenolides isolated from *A. eriocarpa* and their derivatives

Compound	Present in plant (% dry wt)	mp	TLC $R_{digitoxin}$*	
			I	II
Labriformin (**6**)	0.1	213–215°d	2.1	1.3
Labriformidin (**7**)	0.02	amorphous	2.1	1.6
Labriformidin monoacetate (**8**)	—	216–219° (sinter)	2.3	1.6
Eriocarpin (**9**)	0.1	amorphous	1.2	1.3
Eriocarpin diacetate (**10**)	—	—†	2.4	1.6

* Si gel, developed 4 × in system I, $CHCl_3$–MeOH–formamide (90:6:1), and 2 × in system II, EtOAc–MeOH (97:3). † Impure waxy solid, insufficient amount for recrystallization.



Scheme 1. Partial structures of cardenolides present in *Asclepias eriocarpa* and *A. labriformis* and their derivatives, and major MS fragments of labriformidin (7) and labriformidin monoacetate (8).

this deficiency, and the anomolous PMR shift of the C-18 protons in **6**, **7** and **9**. It is clear, however, that the structures of the three new cardenolides described herein, while bearing some analogy with those of the calotropagenin glycosides **2**–**4**, depart in a major way from them.

Labriformin (**6**), labriformidin (**7**) and eriocarpin (**9**) are all highly toxic [20]. The minimum lethal oral dose for a mixture of the three in sheep was about 6 mg/kg, on the same order as for digitoxin. Their LD_{50}s by i.p. administration to mice were 9.2 (**6**), 3.1 (**7**) and 6.5 (**9**), respectively, on the same order as for ouabain (6.8 mg/kg). In addition, the ability of the three new cardenolides to inhibit sheep cardiac ATPase was similar to that of ouabain, digitoxin, uscharidin (**3**) and calotropin (**4**). TLC analysis has shown that at least one other milkweed, *A. erosa*, contains cardenolides **6**, **7** and **9**.

EXPERIMENTAL

Mps are uncorr. IR were taken in KBr. PMR spectra were recorded at 100 MHz with solns in $CDCl_3$ using TMS as int. stand. 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 at least twice in each solvent. Visualization was by spraying with a satd soln of 2,4,2',4'-tetranitrodiphenyl in C_6H_6, then a 10% soln of KOH in 50% aq. MeOH. Biosil A silicic acid, 100–200 mesh, was used with a slight head pressure for CC.

Extraction and crude fractionation of A. eriocarpa. Above ground parts of *A. eriocarpa* Benth., collected on 15 June 1976, at the flowering stage near Road 19, Woodland (Yolo County), California, were air dried and milled to pass a 20 mesh screen. Identification was made by the staff of the Botany Department Herbarium, University of California, Davis (voucher specimen 71895). A 0.65 kg batch was extracted by alternatively blending for 90 sec and heating at 60° for 30 min with 6 l. of 95% EtOH. Combined filtrates from 2.6 kg of plant material were evapd and the residue dissolved in CH_2Cl_2 (3 l.). The soln was washed with H_2O (2 × 1 l.) and satd $NaHCO_3$ soln (2 × 1 l.), partially decolorized with Nuchar charcoal (10 g), dried and evapd to give the crude residue (95.5 g). This was dissolved in MeOH (600 ml) and added to warm (35°) H_2O (2.6 l.). The suspension was extracted with petrol (3 × 1 l.), C_6H_6 (4 × 1 l.), Et_2O (3 × 1 l.) after adding 20 g NaCl and CH_2Cl_2 (3 × 800 ml).

Labriformin (**6**). Residue from the partially decolorized C_6H_6 extract (8.5 g) was dissolved in MeOH (80 ml), insoluble material filtered and discarded, and the soln poured in H_2O (400 ml). The suspension was extracted with petrol (3 × 150 ml) and C_6H_6 (4 × 150 ml). The C_6H_6 residue (0.65 g) contained one cardenolide by TLC. A soln in MeOH (10 ml) gave crystals of **6** (0.21 g), mp 200–220°d. Recrystallization from CH_2Cl_2–MeOH (1:10) gave colorless crystals, mp 213–215°d. (Found: C, 58.7; H, 6.8; N, 2.1. $C_{31}H_{39}O_{10}NS{\cdot}H_2O$ requires: C, 58.6; H, 6.5; N, 2.2%). IR ν_{max} cm^{-1}: 3500 (OH), 1780, 1745, 1630 (butenolide ring), 1650 (C=N), 1460, 1385, 1315, 1050 (broad, several), 970 890, 840, 780. PMR: δ 1.09 (3H, *s*, C-18), 1.21 (3H, *s*, C-19), 1.25 (3H, *d*, J = 6 Hz, C-6'), 3.91 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, —S—CH_2—), 3.6–4.5 (*m*, C-2, C-3, C-5'), 4.90 (2H, *s*, $W_{\frac{1}{2}}$ = 6 Hz, C-21), 5.15 (1H, *s*, C-1'), 6.18 (1H, *s*, C-22), 7.65 (1H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, —CH—N—). MS (probe, 235°) *m/e* (calc formula; rel. int.): 417 ($C_{23}H_{29}O_7$, 21), 416 ($C_{23}H_{28}O_7$, 80), 399 ($C_{19}H_{27}O_6$, 27), 398 ($C_{23}H_{26}O_6$, 57), 380 ($C_{23}H_{24}O_5$, 16), 370 ($C_{22}H_{26}O_5$, 31).

Labriformidin (**7**). Residue from the partially decolorized CH_2Cl_2 extract (6.3 g) was dissolved in MeOH (80 ml) and the soln poured in 1% NaCl soln (400 ml). The suspension was extracted with Et_2O (3 × 150 ml) and CH_2Cl_2 (3 × 150 ml). The CH_2Cl_2 residue (5.0 g) was applied in $CHCl_3$ (15 ml) to a column containing 50 g Si gel. Elution with $CHCl_3$ (200 ml) and 1% increments of MeOH in $CHCl_3$ (200 ml) gave impure **7** in the 1 and 2% fractions. This material (0.22 g) was chromatographed on 20 g Si gel eluting with 0.5% increments of MeOH in $CHCl_3$ (100 ml). Residue from the 0.5 and 1.0% fractions (84 mg) was pptd from cold Et_2O–CH_2Cl_2 (1:10) to give amorphous **7** (62 mg) as a white powder, mp 206–210° (best prepn). IR ν_{max} cm^{-1}: 3500 (OH), 1785, 1750, 1635 (butenolide ring), 1470, 1390, 1160, 1070 and 1045 (broad, several), 970, 895, 810. PMR: δ 1.05 (3H, *s*, C-18), 1.19 (3H, *s*, C-19), 1.36 (3H, *d*, J = 6 Hz, C-6'), 3.3–4.3 (*m*, C-2, C-3, C-5'), 4.58 (1H, *s*, C-1'), 4.6–4.8 (1H, C-4'), 4.77 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-21), 5.95 (1H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-22). MS (probe, 230°) *m/e* (calc formula; rel. int.): 542 ($C_{29}H_{34}O_{10}$, 0.8), 487 ($C_{26}H_{31}O_9$, 5), 460 ($C_{25}H_{32}O_8$, 27), 459 ($C_{25}H_{31}O_8$, 100), 441 ($C_{25}H_{29}O_7$, 14), 417 ($C_{23}H_{29}O_7$, 27), 399 ($C_{23}H_{27}O_6$, 33), 381 ($C_{23}H_{25}O_5$, 26), 363 ($C_{23}H_{23}O_4$, 12), 353 ($C_{22}H_{25}O_4$, 10).

Eriocarpin (**9**). The 3% MeOH in $CHCl_3$ fraction from the 1st CC of the CH_2Cl_2 residue contained impure **9**. This material (0.72 g) was chromatographed on 20 g Si gel eluting with 1% increments of MeOH in $CHCl_3$. Residue from the 3% fraction (132 mg) was kept in cold CH_2Cl_2–Et_2O (1:5) to give amorphous **9** (70 mg) as a white powder, mp over a wide range from 150–220°. IR ν_{max} cm^{-1}: 3500 (OH), 1780, 1755, 1735 (butenolide ring), 1460, 1390, 1165, 1070 and 1045 (broad, several), 970, 890, 800. PMR: δ 1.04 (3H, *s*, C-18), 1.17 (3H, *s*, C-19), 1.19 (3H, *d*, J = 6 Hz, C-6'), 3.3–4.3 (*m*, C-2, C-3, C-5'), 4.74 (1H, *s*, C-1'), 4.7–4.9 (1H, C-4'), 4.79 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-21), 5.97 (1H, *s*, $W_{\frac{1}{2}}$ = 6 Hz, C-22). MS (probe, 230°) *m/e* (calc formula, rel. int.): 416 ($C_{23}H_{28}O_7$, 7.2) 400 ($C_{23}H_{28}O_6$, 3.5), 398 ($C_{23}H_{26}O_6$, 3.3).

Isolation of labriformin (**6**) *from* A. labriformis. Dried arial parts (1 kg) of *A. labriformis* M. E. Jones supplied by R. F. Keeler, USDA Poisonous Plant Laboratory, Logan, Utah, were processed as described for *A. eriocarpa*. Crystalline **6** (29 mg) from one batch was identical to that from *A. eriocarpa*. No pure **7** or **9** were obtained, although they were present in some fractions as shown by TLC analysis.

Hydrolysis of labriformin (**6**). A soln of **6** (10 mg) in MeOH (5 ml) and 2 N H_2SO_4 (5 ml) was refluxed under N_2 for 2 hr, then extracted with $CHCl_3$ (3 × 20 ml). Workup gave 5 mg of residue containing **7** as the major cardenolide TLC spot in systems I and II.

Labriformidin monoacetate (**8**). Impure **7** (63 mg) was stirred at room temp. for 8 hr in Py (2 ml) and Ac_2O (2.5 ml). Workup gave a residue (66 mg) which in 5 ml MeOH gave crystals (17.4 mg). Recrystallization from MeOH–Et_2O (1:10) gave **8**, mp 216–219° (sinter) which did not melt completely at less than 300°. PMR: δ 1.04 (3H, *s*, C-18), 1.12 (3H, *s*, C-19), 1.39 (3H, *d*, J = 6 Hz, C-6'), 2.20 (3H, *s*, Ac), 3.1–4.2 (*m*, C-2, C-3, C-5'), 4.61 (1H, *s*, C-1'), 4.78 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-21), 5.68 (1H, *d*, J = 13 Hz, C-4'), 5.96 (2H, *s*, $W_{\frac{1}{2}}$ = 4 Hz, C-22). MS (probe, 230°) *m/e* (rel. int.). 602 (0.14), 542 (0.16), 459 (4.9), 441 (1.8), 417 (1.8), 416 (1.4), 399 (3.6), 398 (2.2), 381 (4.1), 363 (2.5), 353 (2.2).

Reduction of labriformidin (**7**). A soln of $NaBH_4$ (0.034 mg) in 80% EtOH (1 ml) was added dropwise during 1 hr to a soln of **7** (2.0 mg) in 80% EtOH (1 ml) kept at room temp. The resulting soln was acidified with dil. H_2SO_4 and extracted with $CHCl_3$. The residue upon evapn of $CHCl_3$ contained, by TLC analysis, unreacted **7**, eriocarpin (**9**) and an unidentified product $R_{digitoxin}$ 1.25 (solvent system I) and $R_{digitoxin}$ 1.15 (solvent system II). The amount of the unidentified product was *ca* 4 × that of **9**. When the reaction was repeated using 0.13 mg of $NaBH_4$, the unidentified product comprised *ca* 75% of the product mixture, and unreacted **7**, **9** and two more polar compounds accounted for the remaining *ca* 25%.

Eriocarpin diacetate (**10**). Impure **9** (20 mg), acetylated as described for **7**, gave 5 mg of **10** as an impure waxy solid. PMR: δ 1.02 (3H, *s*, C-18), 1.12 (3H, *s*, C-19) 1.23 (3H, *d*, J = 6 Hz, C-6'), 2.10 (3H, *s*, Ac), 2.24 (3H, *s*, Ac), 3.4–4.2 (*m*, C-2, C-3, C-5'), 4.70 (1H, *s*, C-1'), 4.76 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-21), 5.68 (1H, *d*, J = 12 Hz, C-4'), 5.98 (2H, *s*, $W_{\frac{1}{2}}$ = 5 Hz, C-22).

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