

IRIDOID GLYCOSIDES AND A PYRIDINE MONOTERPENE ALKALOID
FROM *ORTHOCARPUS*. NEW ARTIFACTUAL IRIDOID DIENALS¹

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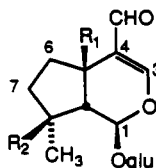
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ABSTRACT.—Euphroside [1] was found to be the major iridoid of *Orthocarpus purpureo-albus*, *Orthocarpus luteus*, *Orthocarpus tolmei*, and *Orthocarpus tenuifolius*, all species of the Section *Orthocarpus* in the genus *Orthocarpus* (Scrophulariaceae). Small amounts of plantarenaloside, adoxoside, ixoroside, geniposidic acid, and mussaenosidic acid were encountered in some of the species. Two closely related new iridoid glucoside dienals 2 and 3, which appeared to be the MeOH adducts from a bisdehydration process on euphroside [1], were isolated from extracts of all species. Attempts to prepare the dienals by dehydration of euphroside [1] were unsuccessful and yielded a monodehydrated MeOH adduct 6 rather than the isolated dienals. Euphrosine [16], a new pyridine monoterpene alkaloid, was isolated from *O. luteus*. It was synthesized from 1 through the intermediacy of 6.

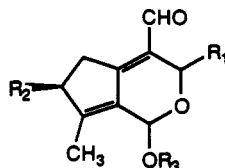
Orthocarpus is a western North American hemiparasitic genus of about 25 species (1) belonging to the subtribe Castillejinae of the Scrophulariaceae. The species were grouped (1) into two subgenera, *Orthocarpus* and *Triphysaria*, with the subgenus *Orthocarpus* being further divided into sections *Orthocarpus*, *Castillejoides*, and *Cordylanthoides*. An extensive study of chromosome numbers (2) and seed surface features (3) revealed the genus to be heterogenous, probably not monophyletic, and in need of generic realignment. Some species appeared to be close to *Cordylanthus* and others close to *Castilleja* (2,3). Because iridoid glycoside variation among *Castilleja* species may be providing evidence for groupings in that genus (4,5), we initiated a study of iridoids in *Orthocarpus*. We present here the results on four species of the Section *Orthocarpus*: *Orthocarpus luteus* Nutt., *Orthocarpus purpureo-albus* A. Gray ex S. Wats., *Orthocarpus tolmei* Hook & Arn., and *Orthocarpus tenuifolius* (Pursh.) Benth. The only previous report on chemistry of *Orthocarpus* was that of Kooiman (6), who suggested the presence of aucubin in *O. luteus* on the basis of paper chromatography.

RESULTS

The iridoid content of all four species was remarkably similar, with euphroside [1] being the major isolate from all species. *O. purpureo-albus* contained small amounts of plantarenaloside, adoxoside, mussaenosidic acid, and geniposidic acid; *O. luteus* small amounts of plantarenaloside and ixoroside; *O. tenuifolius* adoxoside and ixoroside; and *O. tolmei* plantarenaloside and adoxoside. Tlc evidence suggested that traces of mus-



- 1 $R_1 = R_2 = \text{OH}$
15 $R_1 = \text{OH}, R_2 = \text{H}$



- 2 $R_1 = \beta\text{-OMe}, R_2 = \text{H}, R_3 = \beta\text{-glucosyl}$
3 $R_1 = \alpha\text{-OMe}, R_2 = \text{H}, R_3 = \beta\text{-glucosyl}$
4 $R_1 = R_3 = \text{H}, R_2 = \text{OH}$
8 $R_1 = \text{OH}, R_2 = \text{H}, R_3 = \beta\text{-glucosyl}$

¹Chemistry of the Scrophulariaceae, 14. For Part 13 see K.M. L'Empereur and F.R. Stermitz, *J. Chem. Ecol.*, **15**, 187 (1990).

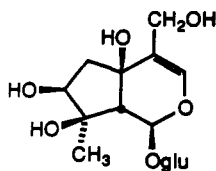
saenosidic acid and geniposidic acid were probably present in all species. Contrary to the previous report (6), *O. luteus* was devoid of aucubin, which was absent from the other *Orthocarpus* species as well.

Tlc (Si gel) of the crude iridoid extract of each species also showed an iridoid-reacting spot which separated into two components on C₁₈ Si gel tlc. These components were isolated pure by vacuum liquid chromatography (vlc) and assigned structures **2** and **3** as follows. Presence of the highly conjugated dienal system was evident from the strong uv maximum at 303 nm (7), five sp² carbons in the ¹³C-nmr spectrum, and a conjugated aldehydic proton resonance in the ¹H-nmr spectrum (Table 1). A ¹H-¹³C HETCOR nmr spectrum of **2** showed that none of the sp² carbons, except the aldehydic one, bore protons. A three-proton methyl singlet occurred at 2.09 ppm in **2** and at 2.13 in **3**, suggesting a methyl on an sp² carbon. Two-proton broad triplets occurred in the ¹H-nmr spectrum of **2** at 2.75 and 3.11 ppm. The protons of these two groups were coupled to each other, but to no others, indicating an isolated -CH₂CH₂- grouping. The chemical shifts suggested that each CH₂ was bonded to an sp² carbon. Compound **3** showed a similar -CH₂CH₂- arrangement; thus both compounds contained a C=C(R)-CH₂CH₂-C(R)=C sequence. Coupling this information with the probable presence of conjugated aldehyde and MeC= groups led to the cyclopentene/dienal part of the structures. These part structures were analogous to a portion of **4**, obtained (7) by

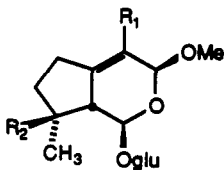
TABLE 1. ¹H-nmr Spectra Data of **2**, **3**, **2**-Ac, and **3**-Ac (300 MHz, ppm, *J* in Hz).^a

Proton	Compound			
	2	3	2 -Ac	3 -Ac
H-1	6.22 br s (1H)	6.09 br s (1H)	6.04 br s (1H)	5.94 br s (1H)
H-3	5.41 br s (1H)	5.50 br s (1H)	5.43 br s (1H)	5.43 br s (1H)
H-6	3.11 br t (2H)	3.04 dd (2H) (8.3, 3.6)	3.07 ddd (1H) (18.5, 6.2, 3.0) 2.97 ddd (1H) (18.5, 6.2, 3.0)	3.00 dd (1H) (2.7, 1.1) 2.98 dd (1H) (2.7, 1.1)
H-7	2.75 br t (2H)	2.69 br t (2H)	2.72 br m (1H) 2.62 br m (1H)	2.65 coalesced 2.64 (2H)
H-10	2.09 br s (3H)	2.13 br s (3H)	2.05 s (3H)	2.05 br s (3H)
H-11	9.71 s (1H)	9.66 s (1H)	9.72 s (1H)	9.72 s (1H)
OMe	3.55 s (3H)	3.50 s (3H)	3.53 s (3H)	3.52 s (3H)
H-1'	4.85 d (1H) (8)	4.80 d (1H) (7.8)	5.11 d (1H) (8.1)	5.04 s (1H)
H-2'	3.20 dd (1H) (9.2, 8)	3.26 dd (1H) (9.1, 7.8)	5.02 dd (1H) (9.3, 8.1)	5.06 d (1H) (4.9)
H-3'	3.43 t (1H) (9.2, 9)	3.42 m (1H)	5.23 t (1H) (9.4, 9.3)	5.27 dd (1H) (9.2, 4.9)
H-4'	3.27 t (1H) (9, 9)	3.32 m (1H)	5.10 t (1H) (10.0, 9.4)	5.09 dd (1H) (10.0, 9.2)
H-5'	3.39 ddd (1H) (9, 6.3, 2.1)	3.4 m (1H)	3.77 ddd (1H) (10.0, 4.6, 3.1)	3.76 ddd (1H) (10.0, 5.1, 2.8)
H-6'	3.93 dd (1H) (11.8, 2.1) 3.67 dd (1H) (11.8, 6.3)	3.91 dd (1H) (11.9, 1.8) 3.68 dd (1H) (11.9, 5.6)	4.25 dd (1H) (12.3, 3.1) 4.19 dd (1H) (12.3, 4.6)	4.23 dd (1H) (12.3, 5.1) 4.16 dd (1H) (12.3, 2.8)
OAc			2.02 s (3H) 1.98 br s (9H)	2.05 s (3H) 2.02 s (3H) 2.01 s (3H) 1.99 s (3H)

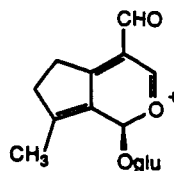
^a**2**, **3** in CD₃OD (3.30 ppm); **2**-Ac, **3**-Ac in CDCl₃ (7.24 ppm).



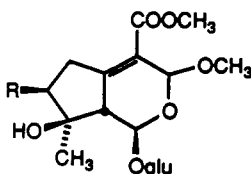
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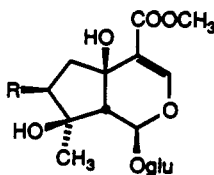
- 6 R₁=CH(OMe)₂, R₂=OH
 7 R₁=CHO, R₂=OH
 14 R₁=CHO, R₂=H



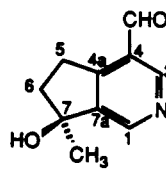
9



- 10 R=OH
 11 R=H



- 12 R=OH
 13 R=H



16

treatment of **5** with 0.2 N aqueous HCl. The spectra of both **2** and **3** showed ¹H and ¹³C resonances (Table 2) generally typical of the sugar portion of an iridoid glucoside. An H-1 resonance at 6.22 ppm in the spectrum of **2** and at 6.09 ppm in the spectrum of

TABLE 2. ¹³C-nmr Spectral Data of **2**, **3**, **2**-Ac, and **3**-Ac (300 MHz, ppm).^a

Carbon	Compound			
	2	3	2 -Ac	3 -Ac
C-1	91.5 d	91.0 d	89.3 d	90.0 d
C-3	96.1 d	96.1 d	95.0 d ^b	95.1 d
C-4	132.6 s	132.9 s	131.1 s	130.9 s
C-5	166.0 s	167.6 s	160.6 s	160.2 s
C-6	26.8 t	27.5 t	25.8 t	26.2 t
C-7	38.4 t	39.1 t	37.5 t	38.3 t
C-8	163.6 s	162.2 s	162.7 s	164.9 s
C-9	123.1 s	123.9 s	122.1 s	122.5 s
C-10	15.7 q	16.3 q	15.3 q	16.0 q
C-11	189.6 d	190.0 d	187.4 d	187.5 d
OMe	57.5 q	55.9 q	56.9 q	55.6 q
C-1'	99.4 d	100.3 d	95.1 d ^b	96.7 d
C-2'	74.9 d	74.9 d	70.9 d	71.0 d
C-3'	78.6 d	78.6 d	73.1 d	72.8 d
C-4'	71.7 d	71.7 d	68.7 d	68.6 d
C-5'	78.1 d	78.1 d	72.0 d	72.2 d
C-6'	63.1 t	62.9 t	61.9 t	62.0 t
-OC(O)Me			170.5 s	170.5 s
			170.3 s	170.1 s
			169.5 s	169.5 s
			169.4 s	169.4 s
-OC(O)CH ₃			20.7 q ^c	20.8 q
			20.6 q ^c	20.7 q
				20.6 q ^c

^a**2**, **3** in CD₃OD (49.0 ppm); **2**-Ac, **3**-Ac in CDCl₃ (77.0 ppm). Multiplicities assigned by DEPT.

^bSignals may be interchanged.

^cDouble intensities.

3 was also typical for an iridoid glucoside. The resonance was a singlet in both cases, and this suggested that there was no proton at H-9. Also absent was the vinylic H-3 resonance at about 7 ppm typical of most iridoids. All resonances were thus accounted for except for a three-proton singlet (3.55 ppm in the spectrum of **2** and 3.50 ppm in that of **3**) and a one-proton singlet at 5.41 (for **2**) and 5.50 ppm (for **3**). These could be assigned to the methyl ether group and the H-3 proton, respectively.

The nmr data (Tables 1 and 2) suggested $C_{17}H_{24}O_9$ molecular formulas for **2** and **3**, but the expected molecular ions were not observable in their mass spectra, even with fabms. Acetylation yielded tetraacetates **2-Ac** and **3-Ac**, which were characterized by nmr (Tables 1 and 2). **2-Ac** gave a fabms which established a $C_{25}H_{32}O_{13}$ molecular formula, and this confirmed the formula for **2** (as well as those for **3** and **3-Ac** by analogy). Assignment of the configurations at C-3 was accomplished by nOe experiments on **2-Ac** and **3-Ac**. Irradiation of H-1 in **2-Ac** showed a 3.5% enhancement of H-3 and no enhancement of the OMe methyl proton resonance, while irradiation of H-1 in **3-Ac** showed a 6% enhancement of the OMe methyl resonance.

A conformational difference in the pyran ring and in the sugar portion was also evident between the two epimeric acetates. From nmr studies, we were able to establish the conformational structures shown in Figure 1. Thus, the irradiation at H-1 in **2-Ac** also caused an 8% enhancement in the C-10 methyl signal, while that enhancement was 2% or less in **3-Ac**. In **2-Ac**, with both the OMe and the *O*-glucosyl groups pseudoaxial, the H-1 proton is in close proximity to the protons of the C-10 methyl. In the case of **3-Ac**, either the OMe or the *O*-glucosyl group would have to adopt a pseudoequatorial position, and the nOe results indicate that it is the OMe which must be pseudoaxial. With the *O*-glucosyl group in the pseudoequatorial position, one favorable anomeric-effect interaction with the pyran ring oxygen is lost. This can be recovered if the glucosyl ring adopts a twist boat conformation, which places the glucosyl ring oxygen above the plane of the ring and an oxygen nonbonded electron pair in an axial position. That this occurs is evident from analysis of the sugar portion of the 1H -nmr spectrum of **3-Ac** in comparison with that of **2-Ac**. The main indication is the anomalous occurrence of H-1' as a singlet in **3-Ac**, while it is a typical doublet ($J = 8.1$ Hz) in **2-Ac** as in other iridoid glucoside spectra which have been reported. A singlet would result if the pyranose ring adopted a twist-boat conformation since there would then be a

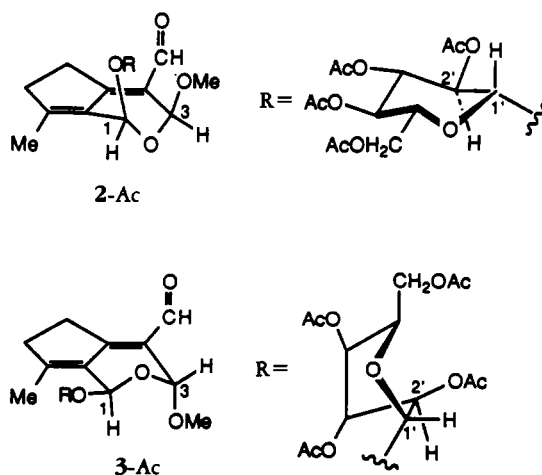


FIGURE 1. Conformations of **2-Ac** and **3-Ac** from 1H nmr analysis.

dihedral angle of about 85° (Dreiding models) between H-1' and H-2'. In this conformation, the dihedral angle between H-2' and H-3' (about 135°) would be small relative to that in a chair conformation (about 180°), and this accounts for the small 5 Hz coupling constant as compared to that expected (9 Hz or greater). As far as we are aware, the twist-boat conformation has not previously been reported for an iridoid glucoside derivative, although such a conformation has indeed been reported in the solid state for some substituted sugars (8).

The structures of **2** and **3** suggested that they might be artifacts resulting from dehydration of **1**. Compound **1** was treated with MeOH at room temperature for 24 h, but no **2** or **3** was formed. A trace of each was observable after heating **1** in MeOH for 5 days at 50° . Compound **1** was also treated with MeOH dilute acids (citric, H_2SO_4 , HCl, *p*-TsOH) under a variety of conditions, and each treatment gave an identical product, but no **2** or **3**. The product formed was assigned structure **6** based upon the 1H - and ^{13}C -nmr spectra (see Experimental) and DEPT, HETCOR, and nOe experiments. In the nOe work, irradiation of the H-1 resonance (5.19 ppm) gave enhancements of 4% in the H-10 and 10% in the H-1' resonances, but no other significant enhancements. This indicated no basic change in the stereochemistry at C-1 or at C-8 and suggested (because there was no enhancement of a methoxy methyl group) that the 3-OMe was oriented β . As confirmation, it was necessary to identify and irradiate the 3-OMe. Irradiation of a 4.95 ppm resonance resulted in a 10% enhancement of one methoxy signal only, that at 3.45 ppm, while irradiation of a 4.90 resonance gave enhancements (7 and 4%) of the other two methoxy signals (3.32 and 3.26 ppm). Thus, the 4.95 one-proton singlet could be assigned to H-3 and the 3.45 ppm three-proton singlet to the 3-OMe. Irradiation of the 3.45 ppm resonance resulted in enhancement only of the H-3 resonance (6%). If the 3-OMe were oriented α , an enhancement of H-1 should have been seen. Only **6** was produced in any of the above reactions, and none of the C-3 epimer could be detected. More vigorous acid treatment of **6** yielded only materials that appeared to have lost the glucosyl moiety. Upon storage, **6** was gradually converted to the free aldehyde **7**, which was separated pure and characterized spectroscopically.

Portions of *O. purpureo-albus* plant material were extracted with the use of absolute EtOH instead of MeOH. Workup yielded euphoside as before, as well as a mixture of compounds similar to **2** and **3**, but the nmr spectrum of the mixture suggested that the compounds contained OEt instead of OMe groups at C-3. Some plant material was ground into a fine paste with H_2O and $CaCO_3$ in order to neutralize any plant acids and then extracted with MeOH. Compounds **2** and **3** were again found after workup. If plant material was extracted with H_2O and worked up without MeOH in the procedure, euphoside was found, but not **2** or **3**.

Two pyridine monoterpene alkaloids (PMT) were isolated from *O. luteus*: the known PMT derivative of plantarenaloid, boschniakine, and the new PMT derivative of euphoside for which we propose the name euphosine [**16**].

The 1H -nmr spectrum of **16** (Table 3) was typical of a pyridine alkaloid, exhibiting the characteristic singlets for H-1 and H-3 at 8.76 and 8.89 ppm, respectively. A singlet (1-H) at 10.2 ppm indicated the presence of an aromatic aldehyde, while a three-proton singlet at 1.65 ppm was consistent with a methyl group β to both a hydroxyl functionality and an aromatic system. Two methylene protons between 3.1 and 3.5 ppm indicated an aromatic-bond CH_2 ; these protons were coupled to only one methylene group (2.25–2.35 ppm), confirming the presence of a $-C-CH_2CH_2-Ar$ moiety. The ^{13}C -nmr spectrum exhibited ten resonances and confirmed the presence of a Me-C-OH unit by the singlet resonance at 79.8 ppm. NOe experiments were performed in order to make unambiguous proton assignments. Irradiation of the methyl

TABLE 3. ^1H - and ^{13}C -nmr Spectral Data (300/75 MHz, CDCl_3) for Euphrosine [16].

Atom	$^1\text{H}^a$	$^{13}\text{C}^b$
1	8.76 s	149.0 d
3	8.89 s	152.7 d
4	—	128.1 s
4a	—	153.4 s
5	3.42 ddd (18.7, 8.1, 5.6) 3.19 ddd (18.7, 8.0, 6.3)	29.1 t
6	2.30 dd (8.0, 5.6) 2.28 dd (8.1, 6.3)	42.2 t
7	—	79.8 s
7a	—	145.7 s
8	1.65 s	27.7 q
9	10.20 s	191.3 d

^aValues in parentheses are *J* in Hz.

^bMultiplicities assigned by DEPT.

resonance (1.65 ppm) produced 1.6% enhancements in both the signals at 8.76 and at 2.35–2.25 ppm. Irradiation of the aldehydic resonance (10.2 ppm) produced an 8% enhancement in the signal at 8.89 ppm. Thus, the pyridine protons could be definitively assigned, and a HETCOR experiment defined the carbons as well (Table 3).

Many reported pyridine monoterpene alkaloids are iridoid artifacts arising from isolations involving basification with NH_3 (17, 18). Others occur naturally and can be isolated even when NH_3 is not used. In the present case, both **16** and boschniakine could be isolated with basification by $\text{NaOH}/\text{Na}_2\text{CO}_3$ rather than aqueous NH_3 , although the yield was markedly lower: 0.003% (with $\text{NaOH}/\text{NaHCO}_3$) vs. 0.03% (with aqueous NH_3).

The conversion of euphroside [**1**] to euphrosine [**16**] was attempted in order to provide additional evidence for the identity of the isolated alkaloid. We were unable to do this directly from euphroside via either enzymatic or acid hydrolysis, followed by reaction with NH_3 . Compound **16** was, however, prepared by first converting **1** to the acetal **6** in acidic MeOH and then treating **6** with dilute aqueous HCl, followed by basification with gaseous NH_3 .

DISCUSSION

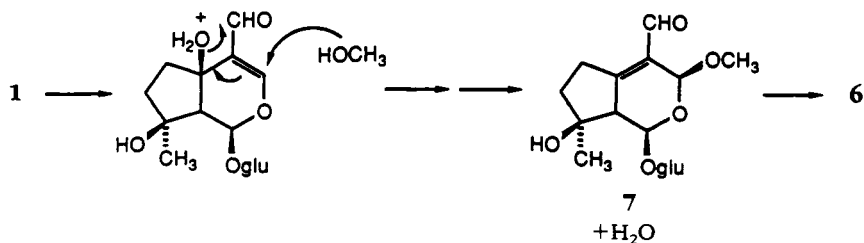
SYSTEMATIC RELATIONSHIPS.—All four species were very similar in iridoid content, and the content was quite different from that of *Castilleja* species. Euphroside [**1**], by far the major iridoid in the *Orthocarpus*, has not yet been found in any of the twelve *Castilleja* species we have so far studied. The *Castilleja* appear to be dominated by aucubin and catalpol, iridoids which lack C-4 substituents, with some occurrence of iridoids with C-4 carboxyl groups but not carboxaldehydes. Preliminary results (C. Boros) on *Orthocarpus densiflorus*, *Orthocarpus purpurascens*, and *Orthocarpus attenuatus*, all species of Section Castillejoideis, indicate that mussaenoside and verbascoside are the main components of each, with euphroside completely lacking. Thus, initial results tend to indicate chemical uniformity within the established botanical sections, but diversity among them.

The previous (incorrect) report (6) of aucubin in *O. luteus* was based on paper chromatography comparisons, and it is likely that this error was due to similar tlc behavior between aucubin and euphroside. Care should be taken when using the Kooiman paper (6) for any taxonomic conclusions or hypotheses until the iridoid identifications reported have been confirmed.

IRIDOID DIENALS.—Dienals such as **2** and **3** have not previously been reported, although the aglucone **4** was obtained (7) by acidic treatment of **5**. In spite of retaining the sugar portion, **2** and **3** are apparently artifacts because they could not be isolated from H₂O extracts of the plant and because EtOH extraction yielded the related ethoxy derivatives. Our failure to produce them from euphroside [**1**] under conditions similar to those attained during actual isolation (although they do begin to form after extended heating with MeOH) suggests that the precursor might be some other component, such as the hemiacetal **8**. On the other hand, it is not possible to exactly reproduce extraction methods when plant material is involved. We attempted to produce acidic conditions that could occur with plants by use of the citric acid procedure, but this also led to formation of **6**. Alternatively, the extraction in the presence of CaCO₃ might have been expected to avoid acid conditions, but again, **2** and **3** were produced. The occurrence of both the epimers **2** and **3** suggests that they arise from the oxonium ion **9**.

The monodehydration products **6** and **7** are comparable to **10** and **11**, obtained (9) by acidic resin treatment of lamiide [**12**] and ipolamiide [**13**]. Further dehydration to products related to **2** and **3** was not reported. Compound **7** is very similar to pinifolin [**14**], an isolate reported (10) from *Penstemon pinifolius*. Although not presented as such (10), it is likely that **14** is an artifact since the major iridoid of *P. pinifolius* is plantarenalioside [**15**] and the extraction procedure involved boiling MeOH.

Although the stereochemistry at C-3 was not determined for **10** and **11**, it was reported (9) that only a single epimer was formed. Similarly, only the epimer **14** was obtained from *P. pinifolius*. These findings, along with the present results, suggest that these compounds are formed by the reaction path of Scheme 1, rather than by a free allylic cation as previously proposed (9). Syn addition of the nucleophile is generally favored in S_N2' reactions (11), and this would account for formation of only the single epimer.



SCHEME 1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Instruments used were as follows: ¹H- and ¹³C-nmr spectra (chemical shifts reported in ppm from TMS), Bruker ACE-300, Bruker IBM WP-270, or Nicolet NT-360 spectrometers; uv, Varian DMS 80; ir, Beckman 4240; optical rotations, Rudolph Research Autopol III. Tlc used 0.25 mm Si Gel 60 F-254 (Merck) plates, CH₂Cl₂/MeOH elution, and *p*-anisaldehyde visualization.

PLANT COLLECTIONS.—*O. purpureo-albus* was collected 2.5 miles (4 km) north of Dolores, Montezuma County, Colorado, on August 4, 1986 (voucher FRS 307). *O. solmei* was collected on August 21, 1985, at the south end of Fish Lake, Sevier County, Utah (voucher FRS 274). *O. luteus* was collected on August 14, 1985, at Michigan Hill, near U.S. Highway 285, southwest of Jefferson, Park County, Colorado (voucher GH 166). *O. tenuifolius* was collected on a hill east of Rattlesnake Creek and above Elk Ridge Road northeast of Missoula, Missoula County, Montana, on July 24, 1988 (voucher FRS 365). The vouchers were deposited in the Colorado State University Herbarium, and the identities of all specimens were confirmed by D.M. Wilken, Department of Biology, Colorado State University.

IRIDOID ISOLATION.—Whole, air-dried, and crushed *O. luteus* (148 g) was soaked in MeOH for 48

h; the mixture was filtered and concentrated in vacuo. This crude extract was then partitioned between H₂O and Et₂O, and the aqueous phase was concentrated to a residue which was then triturated several times with absolute MeOH. The MeOH was evaporated to yield 4.4 g of crude iridoids.

Part of this crude iridoid mixture (1 g) was adsorbed onto Si gel 60 (Merck), applied to the top of a vlc column (30 ml fritted glass funnel, 3.0 × 3.2 cm), and eluted with a CH₂Cl₂/MeOH gradient. Forty 30-ml fractions were collected. Fractions 9–12 (11% MeOH) contained a mixture of **2** and **3** (32 mg); fractions 13–15 (15% MeOH) contained adoxoside (35 mg); fractions 17–19 (20% MeOH) contained ixoroside (29 mg); fractions 23–25 (28% MeOH) contained plantarenaloid (52 mg); fractions 27–30 (32 to 35% MeOH) contained euphroside (265 mg); fractions 35–38 (45 to 50% MeOH) contained geniposidic acid, mussaenosidic acid, and sugars. Fractions containing geniposidic and mussaenosidic acids had such small amounts that the individual acids could not be separated. These fractions were combined from several extractions and the mixture acetylated. Sugar acetates were separated from the iridoid acid acetates by differential pH extraction, and pure geniposidic acid pentaacetate and mussaenosidic acid tetraacetate were isolated by mini-*vlc* (disposable Pasteur pipettes).

IRIDOID IDENTIFICATIONS.—High field ¹H- and ¹³C-nmr spectra in comparison with literature values were used to identify euphroside (12), plantarenaloid (13), ixoroside (12, 14), and geniposidic acid pentaacetate (15). Adoxoside was similarly compared with a previous isolate (5). The high field ¹H-nmr spectrum of mussaenosidic acid tetraacetate was compared to the literature (16), and the ¹³C-nmr spectrum (not previously reported) was consistent with the structure: (75 MHz, CDCl₃, ppm) 170.6, 170.2, 169.4, 169.2 (OCOMe), 152.0 (C-3), 112.0 (C-4), 95.9 (C-1'), 94.0 (C-1), 79.5 (C-8), 72.5 (C-5' or C-3'), 72.1 (C-3' or C-5'), 70.7 (C-2'), 68.2 (C-4'), 61.6 (C-6'), 51.0 (C-9), 40.7 (C-7), 30.3 (C-5), 29.2 (C-6), 24.3 (C-10), 20.7, 20.6 (2), 20.3 (OCOMe).

(1*S*,3*S*)-1-(β-D-Glucopyranosyloxy)-1,3,5,6-tetrahydro-3-methoxy-7-methylcyclopenta[*c*]pyran-4-carboxaldehyde [**2**].—¹H nmr see Table 1; ¹³C nmr see Table 2; uv λ max (MeOH) 303 nm; ir (CHCl₃) 3400, 2910, 1715, 1655, 1628, 1595, 1400, 1230, 1056 cm⁻¹. Converted to **2**-Ac: Hrfabms [M + Li]⁺ 547.2027, calcd for C₂₅H₃₂O₁₃Li, 547.2003; [α]_D²⁶ -29.0° (c = 0.5, CHCl₃); ¹H nmr see Table 1; ¹³C nmr see Table 2; uv λ max (CHCl₃) 304 nm (log ε 4.2).

(1*S*,3*R*)-1-(β-D-Glucopyranosyloxy)-1,3,5,6-tetrahydro-3-methoxy-7-methylcyclopenta[*c*]pyran-4-carboxaldehyde [**3**].—¹H nmr see Table 1; ¹³C nmr see Table 2; uv λ max (MeOH) 300 nm. Converted to **3**-Ac: ¹H nmr see Table 1; ¹³C nmr see Table 2; uv λ max (CHCl₃) 300 nm (log ε 3.8); [α]_D²⁶ -13.5° (c = 0.62, CHCl₃).

CONVERSION OF EUPHROSIDE [**1**] TO **6** AND **7**.—Euphroside [**1**] (339 mg) was dissolved in 35 ml of MeOH, to which was added 3 drops of 1 N HCl. Na₂CO₃ was added after 24 h to neutralize, and the mixture was filtered and evaporated to yield 343 mg of brown foam. This residue was chromatographed (*vlc*, Si gel, 30 ml funnel, 30 ml fractions, CH₂Cl₂/MeOH gradient) to yield 110 mg of pure **6** as a gum (fractions 16–22, 10 to 11.5% MeOH). Hrfabms [M + Na]⁺ 459.1846, calcd for C₁₉H₃₂O₁₁Na, 459.1842; ¹H nmr (CD₃OD, 300 MHz, ppm, *J* in Hz) 5.19 (d, 8.6, H-1), 4.95 (br s, H-3), 4.90 (br s, H-11), 4.69 (d, 7.7, H-1'), 3.83 (dd, 11.9, 2.4, H-6'), 3.75 (dd, 11.9, 4.2, H-6'), 3.45 (s, 3H, 3-OMe), 3.43 (m, H-4'), 3.41 (t, 6.3, H-3'), 3.32 (s, 3H, 11-OMe), 3.31 (m, H-2'), 3.28 (m, H-5'), 3.26 (s, 3H, 11-OMe), 2.57 (d, 8.6, H-9), 2.55 (m, H-6), 2.43 (dd, 19.3, 10.5, H-6), 1.81 (q, 11.0, H-7), 1.70 (ddd, 12.3, 8.7, 2.3, H-7), 1.16 (s, 3H, H-10); ¹³C nmr (CD₃OD, 75 MHz, ppm) 144.2 (s, C-4), 127.1 (s, C-5), 103.4 (d, C-11), 100.5 (d, C-1'), 99.3 (d, C-3), 93.7 (d, C-1), 79.0 (s, C-8), 78.2 (d, C-5'), 77.8 (d, C-3'), 74.5 (d, C-2'), 71.0 (d, C-4'), 62.1 (t, C-6'), 56.2 (q, 3-OMe), 55.1 (d, C-9), 54.6 (q, 11-OMe), 52.9 (q, 11-OMe), 40.3 (t, C-7), 26.6 (t, C-6), 21.7 (q, C-10).

Euphroside [**1**] was similarly treated with 1 M H₂SO₄, citric acid, and *p*-TsOH. In all cases, the presence of **6** was evident by *tlc* and nmr, but neither **2** nor **3** could be detected.

Storage of **6** in the freezer for 6 months converted it essentially quantitatively to **7**: ¹H nmr (CD₃OD, 300 MHz, ppm, *J* in Hz) 9.73 (s, H-11), 5.30 (s, H-3), 5.28 (d, 8.5, H-1), 4.71 (d, 7.7, H-1'), 3.83 (dd, 11.9, 2.4, H-6'), 3.75 (dd, 11.9, 4.2, H-6'), 3.7–3.2 (m, H-2'–H-5'), 3.50 (s, 3H, OMe), 2.93 (m, H-6), 2.79 (bd, 8.5, H-9), 2.11 (dd, 11.6, 1.1, H-6), 1.96 (m, H-7), 1.86 (ddd, 12.6, 8.4, 2.4, H-7), 1.21 (s, 3H, H-10); ¹³C nmr (CD₃OD, 75 MHz, ppm) 189.9 (d, C-11), 165.1 (s, C-5), 131.2 (s, C-4), 100.4 (d, C-1'), 97.3 (d, C-3), 92.9 (d, C-1), 79.1 (s, C-8), 78.2 (d, C-5'), 77.8 (d, C-3'), 74.5 (d, C-2'), 70.9 (d, C-4'), 62.0 (t, C-6'), 56.5 (d, C-9), 56.4 (q, OMe), 40.1 (t, C-7), 26.3 (t, C-6), 22.0 (q, C-10).

ISOLATION OF BOSCHNIAKINE AND EUPHROSINE [**16**].—Dried *O. luteus* (12.25 g) was ground and soaked in 150 ml MeOH (room temperature, 20 h). This was filtered and concentrated, and the residue was dissolved in 35 ml 10% NaHCO₃. The basic solution was extracted with *n*-BuOH–toluene (1:1) (3 × equal vol). The organic layer was concentrated in vacuo to 30 ml and extracted with 1 M H₂SO₄ (3 × equal vol). The acidic solution was extracted with Et₂O (2 × 100 ml) and then divided into two equal portions.

One portion was basified to pH 9 with aqueous NH_3 , and the other with NaOH and NaHCO_3 . Each was then extracted with CHCl_3 (4×50 ml). Upon concentration of the organic layers, the NH_3 -basified portion yielded 3.3 mg of a crude alkaloid mixture (positive spray reaction with IPA) identified as a mixture of boschniakine and **16** by ^1H nmr and mass spectra of the mixture. The $\text{NaOH}/\text{NaHCO}_3$ -basified portion yielded 1.8 mg of a similar crude alkaloid mixture (positive spray reaction with IPA). NH_3 cims of each crude alkaloid mixture gave an m/z 178 peak $[\text{M} + \text{H}]^+$ for **16** and an m/z 162 peak $[\text{M} + \text{H}]^+$ for boschniakine.

Dried *O. luteus* (148 g) was similarly extracted using $\text{NaOH}/\text{NaHCO}_3$ to basify, and 5 mg (0.003% dry wt) of alkaloid mixture was obtained. Dried *O. luteus* (35 g) extracted in the same manner but basified with aqueous NH_3 yielded 9 mg (0.03% dry wt) of alkaloid mixture which was chromatographed by preparative tlc [Si gel, 20×20 cm \times 0.25 mm, MeCN-MeOH (1:1)] to furnish 1 mg of boschniakine and 2 mg of pure **16**.

CONVERSION OF EUPHROSIDE [**1**] TO EUPHROSINE [**16**].—Euphroside [**1**] (500 mg) was dissolved in 30 ml MeOH to which was added 1 ml 1 N HCl. After 70 min, this solution was concentrated in vacuo (50° , 5 min) to yield a bright purple residue which contained mainly **6**. This residue was dissolved in 20 ml H_2O , and 0.5 ml 1 N HCl was added to achieve pH 1. After 2 min the solution was basified to pH 10 by bubbling in NH_3 gas. The solution was then stirred under N_2 in 25 ml CH_2Cl_2 for 20 h. Concentration of the organic layer yielded 86 mg of crude alkaloid which was chromatographed by vlc (pipet column, $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to yield 60 mg of euphrosine [**16**] as a light yellow residue.

EUPHROSINE [**16**].—Light yellow residue: $[\alpha]^{23\text{D}} + 3.3^\circ$ ($c = 0.21$, CHCl_3); uv λ max (EtOH) (log ϵ) 238 (3.4), 266 (2.9), 281 (2.8) nm; eims m/z (rel. int.): 177 (3.5), 162 (61), 159 (89), 158 (64), 157 (39), 156 (29), 130 (100), 128 (40), 103 (35), 77 (45); ^1H and ^{13}C nmr see Table 3.

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LITERATURE CITED

1. D.D. Keck, *Proc. Calif. Acad. Sci.*, **16**, 517 (1927).
2. T.I. Chuang and L.R. Heckard, *Brittonia*, **34**, 89 (1982).
3. T.I. Chuang and L.R. Heckard, *Am. J. Bot.*, **70**, 877 (1983).
4. F.R. Stermitz, G.H. Harris, and W. Jing, *Biochem. Syst. Ecol.*, **14**, 499 (1986).
5. D.R. Gardner, J. Narum, D. Zook, and F.R. Stermitz, *J. Nat. Prod.*, **50**, 485 (1987).
6. P. Kooiman, *Acta Bot. Neerl.*, **19**, 329 (1970).
7. A. Bianco, C.C. Bonini, M. Guiso, C. Iavarone, and C. Trogolo, *Tetrahedron Lett.*, 4829 (1978).
8. K. Vangehr, P. Luger, and H. Paulsen, *Chem. Ber.*, 2609 (1980), and references therein.
9. M.L. Scarpati and M. Guiso, *Gazz. Chim. Ital.*, **99**, 1150 (1969).
10. P. Junior, *Planta Med.*, **55**, 81 (1989).
11. W.D. Stohrer, *Angew. Chem., Int. Ed. Engl.*, **22**, 613 (1983), and references therein.
12. O. Stricher and O. Salama, *Helv. Chim. Acta*, **64**, 78 (1981).
13. A. Bianco, M. Guiso, C. Iavarone, M. Massa, C. Trogolo, J. Oguakwa, and A. Francesconi, *Gazz. Chim. Ital.*, **112**, 199 (1982).
14. Y. Takeda, H. Nishimura, and H. Inouye, *Phytochemistry*, **14**, 2647 (1975).
15. F. Bailleul, A. Rabaron, M. Koch, and P. Delaveau, *Planta Med.*, **37**, 316 (1979).
16. H. Kobayashi, H. Karasawa, T. Miyase, and S. Fukushima, *Chem. Pharm. Bull.*, **33**, 3645 (1985).
17. G.A. Cordell, in: "The Alkaloids." Ed. by R.H.F. Manske, Academic Press, New York, 1977, Vol. 16, Chapter 8, pp. 431-510.
18. D. Gournelis, A. Skaltsounis, F. Tillequin, M. Koch, J. Pusset, and S. Labarre, *J. Nat. Prod.*, **52**, 306 (1989).

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