Iridoid and Phenylpropanoid Glycosides from Orthocarpus spp. Alkaloid Content as a Consequence of Parasitism on Lupinus

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IRIDOID AND PHENYLPROPANOID GLYCOSIDES FROM *ORTHOCARPUS* SPP. ALKALOID CONTENT AS A CONSEQUENCE OF PARASITISM ON *LUPINUS*[1]

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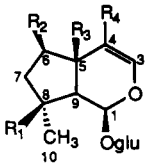
ABSTRACT.—New iridoids were isolated from *Orthocarpus purpurascens* (6β-hydroxyboschnaloside [**4**]) and *Orthocarpus attenuatus* (8-deoxylamiol [**6**] and 5,8-bisdeoxylamiol [**7**]), while the novel phenylpropanoid glycoside 2-*O*-acetylrossicaside A [**8**] was found in *Orthocarpus densiflorus* var. *gracilis*. Known iridoid and phenylpropanoid glycosides were also isolated from these species and from *O. densiflorus* var. *densiflorus* and *Orthocarpus erianthus*. Surprisingly, *Orthocarpus lithospermoides* var. *bicolor* appeared to be devoid of iridoids but contained large amounts of echinacoside, a phenylpropanoid glycoside not found in any other *Orthocarpus*. The results appear to be in general agreement with recent suggestions that *O. purpurascens*, *O. attenuatus*, *O. densiflorus*, and *O. erianthus* be moved from *Orthocarpus* to *Castilleja*. The lamiols in *O. attenuatus* are highly anomalous for the Scrophulariaceae, and they could arise from root parasitism of *O. attenuatus* on lamiol-containing species of the Labiatae. Pyridine monoterpene alkaloids, found in other *Orthocarpus*, were not encountered, but quinolizidine alkaloids from root parasitism on *Lupinus* species were isolated.

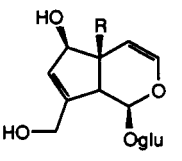
The first chemical examination of species from the western U.S. genus *Orthocarpus* (Scrophulariaceae: Castillejinae) involved four species belonging to Section Orthocarpus and showed a remarkable internal consistency in iridoid content (1), dominated by euphroside [**1**]. The genus *Orthocarpus* has been considered to be polyphyletic and hence not properly constituted (2). Recently the suggestion has been made to keep species of the Section Orthocarpus as the only members of the genus and to remove all other *Orthocarpus* species to *Castilleja* or to the new genus *Triphysaria* (3). We undertook a chemical examination of some *Orthocarpus* species from Sections Castillejoides and Cordylanthoides, and subgenus *Triphysaria*, to see if secondary metabolite patterns were in accord with or could confirm the new systematic and evolutionary alignments.

RESULTS

Three species of *Orthocarpus* Section Castillejoides (*Orthocarpus attenuatus* Gray, *Orthocarpus densiflorus* var. *densiflorus* Benth., and *Orthocarpus densiflorus* var. *gracilis* (Benth.) Keck, and three populations of *Orthocarpus purpurascens* Benth.) were very simi-



1 $R_1=R_3=OH$, $R_2=H$, $R_4=CHO$
2 $R_1=OH$, $R_2=R_3=H$, $R_4=COOMe$
4 $R_1=R_3=H$, $R_2=OH$, $R_4=CHO$
5 $R_1=R_2=OH$, $R_3=H$, $R_4=Me$
6 $R_1=H$, $R_2=R_3=OH$, $R_4=Me$
7 $R_1=R_3=H$, $R_2=OH$, $R_4=Me$



3 R=Oglu
9 R=H

[1]Chemistry of the Scrophulariaceae, 18. For Part 17, see R.L. Arslanian and F.R. Stermitz, *Phytochemical Analysis*, **2**, in press (1991).

lar to each other in major iridoid content. All, except one population of *O. purpurascens*, contained mussaenoside [**2**] and melittoside [**3**] as the two iridoids of highest concentration. Two of the *O. purpurascens* populations were unique in that they contained a new iridoid, 6β-hydroxyboschnaloside [**4**], and this was major in one population. Small amounts of the lamiol derivatives 5-deoxylamiol [**5**] (a known compound) and 8-deoxylamiol [**6**] and 5,8-bisdeoxylamiol [**7**] (novel compounds) were isolated from *O. attenuatus*.

O. attenuatus and *O. purpurascens* contained the phenylpropanoid glycoside verbascoside, while it was absent from both *O. densiflorus* varieties. *O. densiflorus* var. *gracilis* did, however, contain large amounts of the new phenylpropanoid glycoside 2-acetylrossicaside A [**8**]. *O. attenuatus* and *O. densiflorus* var. *densiflorus* contained *p*-hydroxyphenethyl glucoside (salidroside) and adenosine.

Orthocarpus erianthus var. *erianthus* Benth. and *Orthocarpus erianthus* var. *roseus* Gray (from the subgenus *Triphysaria*) were essentially identical to each other chemically, with mussaenoside [**2**] as the major iridoid, but also with large amounts of aucubin [**9**] and verbascoside.

The iridoids gardoside methyl ester, 8-*epi*-loganin, 8-*epi*-loganic acid, geniposide, geniposidic acid, and ixoroside were isolated in minor or trace amounts from several of the species.

Orthocarpus lithospermoides var. *bicolor* (Heller) Jeps. (from Section Cordylanthoides) was found to be devoid of iridoids but contained large amounts of the rare phenylpropanoid glycoside echinacoside as well as salidroside.

Each of the species was probed for the presence of pyridine monoterpene alkaloids, which sometimes accompany iridoids and were found previously in *Orthocarpus* (1). None could be found. Small portions of each of the *Orthocarpus* species were analyzed for other alkaloids, but only *O. attenuatus* was positive. When larger amounts of *Orthocarpus* were analyzed, along with suspected host plants for these hemiparasites, quinolizidine alkaloids could be detected. The *Orthocarpus* species and collection, the presumed host, and the major alkaloid isolated from both were: *O. attenuatus* (FRS 338) near *Lupinus bicolor* var. *microphyllus* (Wats.) D. Dunn (FRS 339), thermopsine and *N*-methylcytisine; *O. purpurascens* (FRS 335) near *Lupinus densiflorus* Benth. var. *palustrus* (Kell.) C.P. Sm. (FRS 337), *N*-methylcytisine; *O. purpurascens* (FRS 335 and FRS 317) near *Eschscholzia californica*, no alkaloids; *O. erianthus* var. *erianthus* (FRS 334) near *Lupinus nanus* ssp. *nanus* Dougl. in Benth. (FRS 334A), thermopsine; *O. erianthus* var. *roseus* (FRS 332) near *L. nanus* ssp. *nanus* (FRS 333A), thermopsine; *O. erianthus* var. *roseus* (FRS 333) near *Lupinus succulentus* Dougl. ex Koch (FRS 333B), tetrahydrorhombifoline.

STRUCTURE ELUCIDATIONS.—6β-Hydroxyboschnaloside [**4**] gave a combustion analysis consistent with a $C_{16}H_{24}O_9$ formulation and showed 16 resonances in the ^{13}C-nmr spectrum (Table 1). These were essentially identical to those of boschnaloside (4) except that the 30.3 ppm resonance for C-6 of boschnaloside was replaced by one at 76.5 ppm, while C-5 was shifted from 30.6 to 39.5 ppm and C-7 from 32.9 to 41.1 ppm. A DEPT spectrum showed the proper multiplicities for the assignments. The ^1H-nmr spectrum (Table 2) was also consistent with **4** and particularly comparable to that (5) of penstemonoside, the analogue with a COOMe group in place of the CHO. The stereochemistry was also assigned by nOe experiments. Irradiation at 0.94 ppm (H-10) gave enhancements of 3.8% at 5.66 ppm (H-1), confirming the α configuration for the C-8 methyl, 4.2% at 2.50 ppm (H-8), and 2.5% at 1.38 ppm (H-7α). In turn, irradiation of H-8 gave a 1.7% enhancement at 1.71 ppm (H-7β), and the H-7α signal was also enhanced (4.1%) when the 4.19 ppm signal (H-6) was irradiated.

8-Deoxylamiol [**6**] had the formula $C_{16}H_{26}O_9$ (hrfabms), and 16 resonances were

observed in the ^{13}C-nmr spectrum (Table 1). These resonances were comparable to those reported (6) for 5-deoxylamiol, except that the C-5 resonance was now at 75.2 and the C-8 resonance at 30.8. Expected changes were also noted at C-7 and C-10, consistent with loss of the C-8 OH group. The 60 MHz ^{1}H nmr spectrum resonances at H-1, H-3, H-6, H-9, and H-11 for lamiol (7) corresponded with those for **6** while the 1.17 ppm singlet resonance for H-10 of lamiol was replaced by a doublet at 0.84 for H-10 of

TABLE 1. ^{13}C-nmr Spectral Data (D_2O) for 6β-Hydroxyboschnaloside [**4**], 8-Deoxylamiol [**6**], and 5,8-Bisdeoxylamiol [**7**] (75 MHz, ppm, *J* in Hz).

Carbon	Compound		
	4	**6**	**7**
C-1	97.8	95.0	95.5
C-3	165.7	137.2	134.5
C-4	122.8	114.1	114.2
C-5	39.5	75.2	45.0
C-6	76.5	75.5	76.2
C-7	41.1	39.4	41.6
C-8	32.7	30.8	32.8
C-9	41.2	48.1	41.9
C-10	16.3	12.3	16.5
C-11	195.2	16.5	15.9
C-1′	99.3	98.9	98.7
C-2′	73.4	73.3	73.5
C-3′	76.4	76.2	76.5
C-4′	70.4	70.5	70.4
C-5′	77.2	77.0	77.0
C-6′	61.5	61.5	61.5

6. Decoupling experiments allowed assignment of the ^{1}H-nmr spectrum (Table 2). The indicated stereochemistries at C-6 and C-8 were determined by irradiating H-6 (3.96 ppm), which enhanced the H-7α resonance at 1.36 ppm, and the H-10 doublet, which enhanced the H-7α and H-1 resonances.

5,8-Bisdeoxylamiol [**7**] was isolated as a clean 5:1 mixture with mussaenoside [**2**]. Subtraction of the resonances for **2** yielded the ^{13}C- and ^{1}H-nmr data for **7** (Tables 1 and 2). The only major difference between the ^{13}C spectra of **6** and **7** was at C-5 (change from 75.2 to 45.0 ppm), with minor changes at C-9 and C-10. ^{1}H-nmr assignments were by proton decoupling (8) and carbon assignments by an HETCOR experiment (8).

2-*O*-Acetylrossicaside A [**8**] showed 37 resonances in the ^{13}C-nmr spectrum (Table 3) and examination of these as well as the ^{1}H-nmr spectrum (Table 3) suggested that **8** was a monoacetate of rossicaside A (9). Acetylation produced a peracetate which was identical (^{1}H- and ^{13}C-nmr spectra and tlc) with a standard sample of the peracetate of rossicaside A (9). COLOC and *J*-selective INEPT experiments (8) were necessary to establish the position of the acetyl group in **8**. Initially, C-α (71.7 ppm) and an attached proton (4.06 ppm) of the aglycone were established from a HETCOR spectrum. In a *J*-selective INEPT experiment, irradiation of the 4.06 ^{1}H resonance resulted in signals at 131.7, 35.8, and 101.5 ppm in the ^{13}C-nmr spectrum. These could be assigned to C-1 and C-β of the aglycone and C-1 of glucose 1, respectively. With the resonance for H-1 established, the COSY experiment showed that a resonance at 4.86 ppm could be assigned to H-2. COLOC correlations were found between this proton at 4.86 ppm and

TABLE 2. ^{1}H-nmr Spectral Data (D_2O) for 6β-Hydroxyboschnaloside [**4**], 8-Deoxylamiol [**6**], and 5,8-Bisdeoxylamiol [**7**] (300 MHz, ppm, *J* in Hz).

Proton	Compound		
	4	**6**	**7**
H-1	5.66 d 2.3	5.50 bs	5.31 d 1.7
H-3	7.38 bs	6.05 d 1.4	5.93 bs
H-5	2.81 d 8.7		2.36 bd 8.3
H-6	4.19 t 2.3	3.96 t 3.7	4.19 bm
H-7α	1.38 ddd 14.0, 9.5, 4.6	1.36 ddd 13.4, 8.5, 4.6	1.43 ddd 13.4, 8.9, 4.7
H-7β	1.71 bdd 14.0, 6.8	1.68 ddd 13.4, 7.1, 3.2	1.60–1.74 m
H-8	2.50 m	2.40–2.57 m	2.33–2.46 m
H-9	2.65 dt 9.2, 2.3	2.45 bs	2.52 ddd 10.1, 8.4, 1.6
H-10	0.94 d 7.2	0.84 d 6.8	0.91 d 7.1
H-11	9.05 s	1.51 d 1.4	1.49 bs
H-1′	4.75	4.59 d 8.0	4.60 d 8.2
H-2′	3.16 dd 9.2, 8.3	3.19 dd 9.3, 8.0	3.16 dd 8.9, 8.2
H-3′	3.41 dd 9.2, 9.1	3.40 dd 9.3, 8.9	3.39 dd 9.3, 8.9
H-4′	3.30 dd 9.5, 9.1	3.29 dd 9.6, 8.9	3.28 dd 9.3, 9.0
H-5′	3.40 m	3.40 m	3.34 m
H-6′	3.85 dd 12.3, 1.8	3.82 dd 12.3, 1.9	3.81 dd 12.4, 1.8
	3.63 dd 12.3, 5.7	3.62 dd 12.3, 5.7	3.62 dd 12.4, 5.5

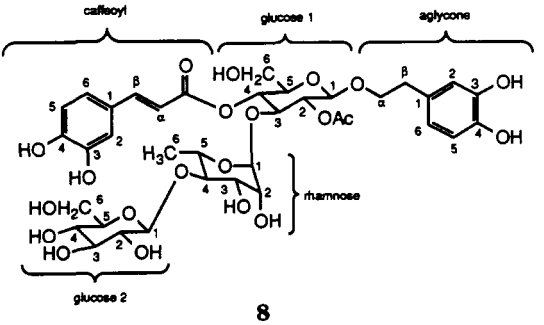
^{13}C-nmr signals at 171.5, 101.5, 80.0, and 75.0 ppm. Since the 171.5 ppm signal could only be due to the C=O of the acetyl group, the correlation of that resonance to H-2 proved the placement of the acetate at C-2. The COLOC experiment also established the connectivities of the various other moieties, in confirmation of the previous degradative studies on rossicaside A (9).

DISCUSSION

None of the species studied contained **1**, the major iridoid of species from the Section Orthocarpus, nor had we previously (1) found **2** or **3** in any of the Section Orthocarpus species. Mussaenoside [**2**] is a common constituent of *Castilleja* species (10), while melittoside [**3**] was recently found (T. Ianiro and F.R. Stermitz, unpublished results) in *Castilleja foliolosa* and a monomelittoside ester was reported (11) from *Castilleja wightii*. The minor or trace iridoids are also typical of *Castilleja*. Thus, this work and the previous study (1) provide additional evidence for the suggested (3) removal of *O. purpurascens*, *O. attenuatus*, and *O. densiflorus* to the genus *Castilleja*, which emphasizes their difference from those remaining in *Orthocarpus*. The iridoids **2** and **9** from *O. erianthus* are also typical of *Castilleja*, but this is the first isolation of **9** from any of the *Orthocarpus* species. Removal of this taxon from the new *Orthocarpus* also seems chemically justified, but the presence of typical *Castilleja* iridoids provides no additional grounds for separation of this taxon from *Castilleja* into a new *Triphysaria* genus. This must remain a separation based on morphology and biology, at least until further related *Orthocarpus* are studied. *O. lithospermoides*, which was in the old Section Cordylanthoides, was remarkable in being the first species in any of the Scrophulariaceae that we have studied which lacks iridoids. The presence of echinacoside is also anomalous. Species of the old *Orthocarpus* Section Cordylanthoides have morphological connections to the genus *Cordylanthus*, and knowledge of the iridoid content in this as yet unstudied taxon would be valuable.

We have previously studied alkaloid uptake from host plants by the hemiparasitic perennial genera *Castilleja* and *Pedicularis* (12–15), and the present work extends such

TABLE 3. Nmr Data (CD_3OD) for Monoacetylrossicaside A [**8**].



8

^{1}H nmr (300 MHz)				^{13}C nmr (75 MHz)			
aglycone				aglycone			
H-α	4.06	dt	9.6, 6.1	C-α	71.7		
	3.62	m		C-β	36.1		
H-β	2.68	m		C-1	131.7		
H-2	6.63	d	2.0	C-2	117.1		
H-5	6.66	d	8.1	C-3	144.4		
H-6	6.51	dd	8.1, 2.0	C-4	145.8		
caffeoyl				C-5	116.2		
H-α	6.24	d	15.9	C-6	121.2		
H-β	7.58	d	15.9	caffeoyl		acetate	
H-2	7.05	d	2.0	CO	168.0	$OCOCH_3$	171.5
H-5	6.78	d	8.3	C-α	114.4	$OCOCH_3$	20.8
H-6	6.95	dd	8.3, 2.0	C-β	148.2	rhamnose	
glucose 1				C-1	127.5	C-1	102.7
H-1	4.51	d	8.1	C-2	115.3	C-2	71.3
H-2	4.86	dd	9.5, 8.1	C-3	146.6	C-3	71.8
H-3	4.00	dd	9.5, 9.2	C-4	149.6	C-4	82.9
H-4	4.99	dd	9.2, 9.2	C-5	116.5	C-5	69.1
H-5	3.58	m		C-6	123.3	C-6	18.5
H-6	3.72	dd	9.4, 3.2	glucose 1		glucose 2	
	3.48	dd	9.4, 9.4	C-1	101.5	C-1	105.3
rhamnose				C-2	75.0	C-2	75.6
H-1	4.79	d	1.6	C-3	80.0	C-3	77.6
H-2	3.64	dd	2.8, 1.6	C-4	70.4	C-4	72.3
H-6	1.15	d	6.0	C-5	75.8	C-5	77.9
glucose 2				C-6	62.0	C-6	62.6
H-1	4.46	d	7.9				
H-2	3.07	dd	9.0, 7.9				
acetate	1.97	s					

studies to annual species of *Orthocarpus* growing on *Lupinus*. Because of the density of root masses from many plants in the neighborhood of the collected *Orthocarpus*, we were not able to dig specimens and search for the rather fragile haustorial connections between the *Orthocarpus* and the suspected host plant, but the comparable alkaloid patterns in the host and parasite provide evidence for the suspected parasitism. Although *E. californica* contains benzophenanthridine alkaloids in the roots, none could be found in *O. purpurascens* growing intermixed with *E. californica*. In greenhouse studies, we have not been able to culture hemiparasites on *E. californica*, so it is possible that it cannot serve as a host.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were obtained on Bruker ACE-300 (300/75 MHz), Bruker WP-270 (270/67 MHz), and Bruker WP-200 (200 MHz) spectrometers. DEPT, HETCOR, COSY, COLOC, and nOe experiments were performed on the ACE-300, and *J*-selective INEPT experiments on a Bruker AM-500. Other instruments used were: ir, Perkin Elmer 1600 FT-IR; uv, Varian DMS 80; optical rotation, Rudolph Research Autopol III; eims and NH_3 cims, VG Micromass 16F. High-resolution and fabms spectra were obtained from the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska and the Center for Advanced Instrumentation, University of California–Davis. Elemental analyses were performed by Desert Analytics, Tuscon, Arizona.

Hplc was performed using a Beckman System 110B Solvent Delivery Module and 163 Variable Wavelength Detector. A Beckman Altex Ultrasphere ODS (25 cm × 10.0 mm i.d., 5 μm p.d.) semi-preparative column was used. Hplc solvents were distilled and filtered through Rainin Nylon-66 45 mm filters. Tlc utilized 0.25 mm and 2.0 mm Si Gel 60 F-254 (Merck) and 0.2 mm octadecylsilane reversed-phase $KC_{18}F$ (Whatman) plates. Iridoid visualization was achieved with uv radiation and acidic *p*-anisaldehyde spray solution followed by heating. Vacuum liquid chromatography (vlc) and flash columns utilized Si Gel 60 230–400 mesh (Merck) or a prepared C18 reversed-phase derivative.

Plants.—Collections were as follows: *O. attenuatus* (FRS 342), 10 miles S of Lockwood on Highway G14, Monterey County, CA, March 17, 1988; *O. attenuatus* (FRS 338) and *L. bicolor* var. *microphyllus* (FRS 339), 1 mile N and 1 mile E of Creston, San Luis Obispo Co., CA, March 16, 1988; *O. densiflorus* var. *gracilis* (FRS 344), N side of the highway in Hunter-Ligett Reservation 1 mile E of the border highway to the coast, Monterey County, CA, March 17, 1988; *O. densiflorus* var. *densiflorus*, N of Gavilan College in southern Gilroy, Santa Clara County, CA, by Ray White, March 26, 1988 (16); *O. purpurascens* collection A (17), San Francisco Bay area, CA, by M. Deane Bowers, Department of EPO Biology, University of Colorado, Boulder, May 5, 1986; *O. purpurascens* collection B (FRS 335) and *L. succulentus* (FRS 335), N side of the road halfway between Paso Robles and Creston, San Luis Obispo County, CA, March 16, 1988; *O. purpurascens* collection C (FRS 316), Highway 86 between mile markers 60 and 61, 7.5 miles E of Why, Pima County, AZ, March 14, 1987; *O. purpurascens* (FRS 317), Organ Pipe Cactus National Monument, Pima Co., Arizona, March 14, 1987; *O. lithospermoides* var. *bicolor* (LH 6674), State Road 20 SW of Williams just E of the Lake County line, Colusa County, CA, by Lawrence Heckard, May 17, 1988; *O. erianthus* var. *erianthus* (FRS 327), 3 miles E of Fairfield on Highway 12 E of Travis Air Force Base, Solano County, CA, March 12, 1988; *O. erianthus* var. *erianthus* (FRS 334) and *L. nanus* ssp. *nanus* (FRS 334A), on Turri Road SE of Los Osos, San Luis Obispo, Co., CA, March 15, 1988; *O. erianthus* var. *roseus* (FRS 332) and *L. succulentus* (FRS 333B), State Highway 1 at the Hearst Castle turnoff N of Cambria, San Luis Obispo County, CA, March 15, 1988; *O. erianthus* var. *roseus* (FRS 332) and *L. nanus* ssp. *nanus* (FRS 333A), State Highway 1, mile 58, 200 yds N of the Hearst Castle turnoff, San Luis Obispo Co., CA, March 15, 1988. The identities of all *Orthocarpus* specimens were confirmed by Dieter Wilken, Department of Biology, Colorado State University, Lawrence R. Heckard, Jepson Herbarium, University of California–Berkeley, or Raymond R. White, Department of Biology, City College of San Francisco. *Lupinus* species were identified by L.R. Heckard. Voucher specimens of all collections are deposited at the Colorado State University Herbarium.

ISOLATION OF GLYCOSIDES.—Whole, air-dried, and crushed *O. attenuatus* (24.5 g) were extracted with hexane (24 h), EtOAc (24 h), and MeOH (25 h) successively. The MeOH extract was concentrated in vacuo to yield 3.2 g of residue which was partitioned between H_2O and Et_2O. When concentrated, the aqueous portion afforded 1.99 g of residue. This was dissolved in H_2O and chromatographed (vlc, C18 Si gel, 60 ml fritted glass funnel, 4.3 × 4.3 cm, H_2O/MeOH gradient, 26 20-ml fractions): Fractions 1–4 (0% MeOH), sugars (600 mg); fractions 5–8 (10% MeOH), melittoside (260 mg); fractions 10–12 (15–20% MeOH), a 40-mg mixture rechromatographed (vlc followed by hplc), gardoside methyl ester and 5-deoxylamiol (10 mg); fraction 13 (20% MeOH, 17 mg) rechromatographed (hplc, C18 Si gel, 20% aqueous MeOH), gardoside methyl ester (2 mg); fractions 14–17 (20–30% MeOH, 83 mg), rechromatographed (hplc, C18 Si gel, 25% aqueous MeOH), 8-*epi*-loganin (8 mg), an unstable heptenediol diglycoside (10 mg) not completely identified, 8-deoxylamiol (2.6 mg), mussaenoside (6.2 mg), 5,8-bisdeoxylamiol (2.5 mg); fractions 18 and 19 (30–40% MeOH), mussaenoside (65 mg); fractions 21 and 22 (50% MeOH), verbascoside (186 mg).

Whole, air-dried, and crushed *O. densiflorus* var. *densiflorus* (22.1 g) were extracted with MeOH (24 days). The MeOH was filtered and evaporated in vacuo to yield 4.42 g of a residue which was partitioned between H_2O and CH_2Cl_2. The concentrated aqueous solution afforded 3.63 g of a brown residue, which was chromatographed (vlc, C18 Si gel, 250 ml fritted glass funnel, 6.5 × 5.0 cm, H_2O/MeOH elution gradient, 40 ml fractions): fractions 5–9 (10–20% MeOH), melittoside (350 mg); fraction 10 (20% MeOH, 18 mg) rechromatographed (hplc, C18 column, 15% aqueous MeOH), melittoside, 8-*epi*-loganic

acid, and salidroside; fractions 11–16 (20–30% MeOH), individually rechromatographed similarly, gardoside methyl ester, salidroside, and adenosine in small quantities; fractions 17 and 18 (30–40% MeOH, 75 mg) rechromatographed (hplc, C18 column, 30% aqueous MeOH), mussaenoside, 8-*epi*-loganin, and the heptenediol diglycoside; fractions 19 and 20 (40% MeOH), mussaenoside (80 mg).

Whole, air-dried, and crushed *O. densiflorus* var. *gracilis* (16.8 g) were soaked with MeOH (66 h). The crude extract was 2.83 g, and H_2O/Et_2O partitioning gave 2.26 g in the aqueous layer. Vlc similar to that described above yielded 220 mg melittoside (fractions 4 and 5, 30% MeOH), 100 mg mussaenoside (fractions 7 and 8, 40–50% MeOH), 485 mg 2-acetylrossicaside A (fractions 9–11, 50–60% MeOH), and a trace of gardoside methyl ester purified from fraction 6 (40% MeOH).

Whole, air-dried, and crushed *O. purpurascens* (collection B) (52.0 g) were soaked in MeOH (6 days) to yield 11.2 g of a brown-green residue which was partitioned between H_2O and CH_2Cl_2. Concentration of the aqueous layer afforded 5.94 g of material which was chromatographed (vlc, C18 Si gel, 250 ml fritted glass funnel, H_2O/MeOH elution gradient, 40-ml fractions): fractions 6–10 (15–25% MeOH), melittoside (260 mg); fraction 11 rechromatographed (hplc, C18 Si gel, 20% MeOH), gardoside methyl ester (1.5 mg), mellitoside (trace); fractions 12 and 13 (32% MeOH, 120 mg) rechromatographed (hplc, C18 Si gel, 30% MeOH), 6β-hydroxyboschnaloside (35 mg), mussaenoside (3 mg), geniposide (8 mg), and 8-*epi*-loganin (2.5 mg); fraction 14 (40% MeOH, 100 mg) similarly rechromatographed, gardoside methyl ester (5 mg), 6β-hydroxyboschnaloside (8.5 mg), mussaenoside (3 mg), and a mixture of mussaenoside and geniposide (3 mg); fractions 15–18 (40–60% MeOH, 600 mg) rechromatographed (vlc, Si gel, CH_2Cl_2/MeOH gradient), a mixture of mussaenoside and geniposide with several unidentified aromatic compounds; fractions 19–21 (60–70% MeOH) rechromatographed (vlc, C18 Si gel, H_2O/MeOH gradient), small amounts of verbascoside, mussaenoside, and geniposide. *O. purpurascens* (collection C) was treated in a similar fashion to yield similar amounts of verbascoside, melittoside, mussaenoside, geniposidic acid, and gardoside methyl ester. *O. purpurascens* (collection A) contained 6β-hydroxyboschnaloside as the major iridoid and mussaenoside as a minor component. Due to the small size of this collection, these compounds were identified only through ^{1}H-nmr spectral analysis of the total crude iridoid fraction.

O. lithospermoides var. *bicolor* air-dried plants containing mostly inflorescences and leaves (28.6 g) were extracted with MeOH (6 days). Concentration of the filtered solution yielded 2.97 g of residue. Partitioning this crude extract between H_2O and CH_2Cl_2 afforded 2.11 g of orange residue from the aqueous phase. This was chromatographed (vlc C18 Si gel, 60 ml fritted glass funnel, 4.3 × 4.3 cm, H_2O/MeOH elution gradient, 28 20-ml fractions): fractions 1–4 (0–5% MeOH), sugars; fractions 10 and 11 (15–20% MeOH, 22 mg) rechromatographed (hplc, C18 Si gel, 20% MeOH), salidroside (3 mg); fractions 19–22 (35–40% MeOH), echinacoside (226 mg); fractions 25 and 26 (60% MeOH), yellow, coral-like clumps of 7-*O*-glucosylluteolin precipitate [50 mg; mp 232–236° (dec), 95% EtOH]; fractions 23–26 (50–60% MeOH, 118 mg) rechromatographed (vlc, Si gel, 30 ml fritted glass funnel, 2.4 × 3.2 cm, CH_2Cl_2/MeOH elution gradient, 17 20-ml fractions), fractions 6 and 7 (25% MeOH), a small quantity of verbascoside.

Whole, air-dried *O. erianthus* var. *erianthus* (100 g) were extracted with MeOH twice for a total of 3 days. The combined extracts yielded 13 g of residue which was partitioned between H_2O and Et_2O to yield 11 g of residue from the H_2O. Of this residue, 3.1 g was dissolved in H_2O and chromatographed (vlc, C18 Si gel, 250 ml fritted glass funnel, 6.5 × 4.1 cm, H_2O/MeOH elution gradient, 14 50-ml fractions): fractions 1–3 (0–10% MeOH), mostly sugars; fractions 5 and 6 (20–30% MeOH), aucubin (110 mg); fraction 8 (40% MeOH), gardoside methyl ester (38 mg); fraction 9 (40% MeOH), a mixture of mussaenoside and 8-*epi*-loganin (107 mg); fraction 10 (50% MeOH), a 2:1 mixture of mussaenoside and verbascoside (101 mg); fractions 11 and 12 (60% MeOH), verbascoside (363 mg). *O. erianthus* var. *roseus* was treated similarly and yielded the same iridoids in approximately the same relative concentrations.

ISOLATION OF ALKALOIDS.—*Orthocarpus* and *Lupinus* above-ground plant parts were crushed into 10% aqueous $NaHCO_3$, mixed thoroughly with toluene–*n*-BuOH (1:1), and allowed to stand for 48 h. The mixture was filtered and the organic layer extracted with 0.5 N HCl. The acidic solution was made basic to pH 10 with NaOH and extracted with $CHCl_3$. The organic layer was dried and evaporated, and the residue examined by tlc (iodoplatinate visualization) and 300 MHz ^{1}H-nmr spectroscopy.

COMPOUND IDENTIFICATIONS.—High-field ^{1}H- and ^{13}C-nmr spectra were compared with published data to identify geniposidic acid pentaacetate (18), melittoside (19), 8-*epi*-loganin (20), geniposide (21), aucubin (22), gardoside methyl ester (23) and 7-*O*-glucosylluteolin (24,25). The ^{13}C-nmr spectrum of the last (not previously reported) was consistent with the structure. Geniposidic acid was identified by comparison of the ^{1}H-nmr spectrum with that of geniposide. Identified by tlc, ^{1}H nmr, and, when appropriate, ^{13}C-nmr comparison with our previous isolates were aucubin (26), verbascoside (27), mussaenoside (28), gardoside methyl ester (10), 8-*epi*-loganic acid (27), tetrahydrorhombifoline (28), *N*-methylcytisine (15), and thermopsine (15). Echinacoside (29) and rossicaside A dodecaacetate (9) were identified by tlc and ^{1}H- and ^{13}C-nmr comparison with standard samples.

NEW ISOLATES.—6β-Hydroxyboschnaloside [**4**]: solid, mp 100–105°; uv λ max (MeOH) 249 nm; $[\alpha]^{24}D$ −79.7° (*c* = 3.1, H_2O); C 52.21, H 6.82 (calcd for $C_{16}H_{24}O_9 \cdot 0.5\ H_2O$, C 52.03, H 6.82). 8-Deoxylamiol [**6**]: noncrystalline solid; $[\alpha]^{26}D$ −196.2° (*c* = 0.21, MeOH); hrfabms $[M]^+$ 361.1519 (calcd for $C_{16}H_{25}O_9$, 361.1521). 5,8-Bisdeoxylamiol [**7**]: oil, 5:1 mixture with mussaenoside; $[\alpha]^{26}D$ −132° (*c* = 0.25, MeOH); hrfabms $[M]^+$ 345.1533 (calcd for $C_{16}H_{25}O_8$, 345.1531). 2-*O*-Acetylrossicaside A [**8**]: noncrystalline solid; $[\alpha]^{23}D$ −132° (*c* = 0.20, MeOH).

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