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IRIDOID GLUCOSIDES OF *PENSTEMON AMBIGUUS*[1]

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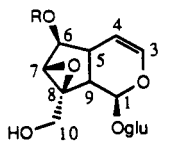
ABSTRACT.—Two new iridoid glycosides, 6″(*Z*)-nemoroside [**4**] and ambiguuside, 6-*O*-[6″(*S*)-hydroxy-2″,6″-dimethyl-2″(*E*)-7″-octadienoyl]catalpol [**5**], were isolated from *Penstemon ambiguus* (Scrophulariaceae). The known iridoids catalpol, specioside, and nemoroside were also isolated as were two known phenylpropanoid glycosides, verbascoside and martynoside. Structural and stereochemical relationships of **4** and **5** to previously known iridoid esters of foliamenthic and menthiafolic acid are discussed.

Penstemon ambiguus Torr. (Scrophulariaceae) is a unique *Penstemon* species of the southwestern United States. It is one of only two members of the section Ambiguii, the other being *Penstemon thurberi* Torr., a taxon which at one time was considered a subspecies of *P. ambiguus*. *P. ambiguus* occurs mainly in western Texas, New Mexico, southern Utah, Arizona, and northern Chihuahua, Mexico. It is generally displaced by *P. thurberi* in the western part of the range and into southern California. Distinguishing characteristics are its shrub-like growth ("bush penstemon"), phlox-like flowers ("moth penstemon"), and linear grass-like leaves. In a continuation of our studies in the Scrophulariaceae, we identified the iridoid glycosides of *P. ambiguus* in a northern New Mexico population belonging to var. *laevissimus* (Keck) N. Holmgren (1).

RESULTS AND DISCUSSION

A crude iridoid extract from dried, whole plant material was subjected to vlc (H_2O, then MeOH). The H_2O fraction yielded catalpol [**1**]. Extensive purification of the



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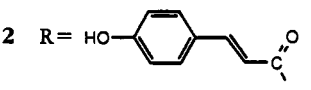
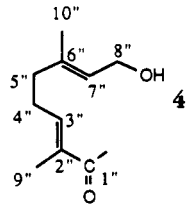
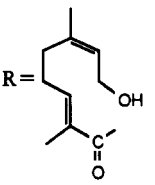
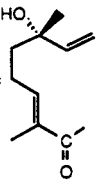
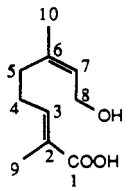
2 R= 

3 R= 

4 R= 

5 R= 



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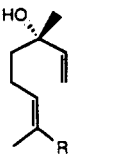

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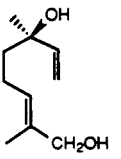
8 R=CH_2OH



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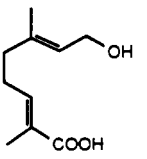

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[1]Part 16 in the series Chemistry of the Scrophulariaceae. For Part 15, see K.M. L'Empereur and F.R. Stermitz, *J. Chem. Ecol.*, **16**, 1495 (1990).

MeOH fraction gave four iridoids which, on the basis of ^{1}H- and ^{13}C-nmr signals, were identified as esters of **1**. Differences between the ^{1}H-nmr spectra of the esters and that of catalpol were the H-6 resonances, which were shifted between 1.12 and 1.03 ppm downfield, indicating that the ester linkage involved the OH group at the C-6 carbon. The ^{1}H-nmr spectrum of one catalpol ester isolate showed two two-proton aromatic doublets typical of a para-substituted phenyl group. Two doublets at 7.67 and 6.38 ppm (J = 15.9 Hz) indicated a coumaroyl moiety, allowing identification of this compound as specioside [**2**].

The ^{13}C-nmr spectra of three additional iridoids showed the presence of 25 carbons in each. The signals for catalpol [**1**] were clearly distinguished in each spectrum (Table 1) while the presence of ten additional signals indicated monoterpene units. Further analysis of ^{13}C and DEPT spectra revealed the presence of a hydroxyl group, two double bonds, two methyl groups, and a carbonyl functionality in the terpene side chain of each iridoid. ^{13}C- and ^{1}H-nmr spectra for one ester showed all signals in good agreement with literature data for nemoroside [**3**].

The other esters were characterized as the new iridoids **4** and **5** as follows. Hrfabms established the molecular formulas as $C_{25}H_{36}O_{12}$. Uv data indicated that the acyclic terpene side chains in both compounds possessed an α,β-unsaturated carbonyl functionality. The ^{13}C-nmr data for **4** were very similar to those for **3** (Table 1) with some important exceptions. The C-10″ methyl and the C-5″ methylene signals were shifted +7.3 and −7.6 ppm from those of **3**. These resonances are similar to those reported for dihydrofoliamenthin (2) and 6-*O*-nerol-8-oyl-antirrinoside (3), where the 6″–7″ double bond is in the *Z* conformation, rather than the *E* as in **3**. The *E* configuration of the 2″–

TABLE 1. ^{13}C-nmr Data (75.5 MHz, CD_3OD) of **1**, **3**, **4**, and **5**.

Carbon	Compound			
	1	**3**	**4**	**5**
C-1	95.3	95.0	95.0	95.0
C-3	141.7	142.4	142.4	142.3
C-4	104.0	102.9	102.9	102.9
C-5	39.1	36.7	36.5	36.6
C-6	79.5	81.6	81.6	81.5
C-7	62.5	60.1	60.1	60.1
C-8	66.2	66.8	66.8	66.8
C-9	43.5	43.1	43.1	43.1
C-10	61.6	61.3	61.2	61.2
C-1′	99.7	99.7	99.6	99.6
C-2′	74.8	74.8	74.8	74.7
C-3′	78.5	78.6	78.6	78.5
C-4′	71.7	71.8	71.7	71.6
C-5′	77.6	77.7	77.6	77.6
C-6′	62.9	62.9	62.9	62.8
C-1″		169.3	169.3	169.4
C-2″		128.6	128.7	128.2
C-3″		144.1	143.9	145.0
C-4″		28.1	28.3	24.6
C-5″		39.1	31.5	41.6
C-6″		138.4	138.6	73.6
C-7″		125.7	126.6	145.8
C-8″		59.4	59.1	112.5
C-9″		12.5	12.5	12.4
C-10″		16.2	23.5	27.8

3″ double bond in **4** was assured due to the very close ^{13}C-nmr chemical shift values for the C-10″ methyl in **3** and **4**. In the *Z* conformation the C-9″ methyl would have been observed at about 9 ppm lower field. Examples of this have been documented for tiglic and angelic acids (4). Therefore, **4** is the 6″–7″ *Z* isomer of **3**, or 6″(*Z*)-nemoroside. Previously known other iridoid esters of 8-hydroxy-2,6-dimethyl-2(*E*),6(*Z*)-octadienoic acid [**6**] were foliamenthin (5–7) and an antirrinoside analogue (3). Complete ^{1}H- and ^{13}C-nmr assignments (Table 1) were aided by DEPT, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C HETCOR experiments. DEPT and ^{13}C spectra indicated that **5** differed from **3** and **4** in that it possessed a quaternary hydroxyl group and a terminal double bond. Basic hydrolysis of **5** gave a monoterpene acid whose optical rotation and ^{1}H- and ^{13}C-nmr signals were in agreement with literature data (8) for 6(*S*)-hydroxy-2,6-dimethyl-2(*E*)-7-octadienoic acid [**7**]. Both **7** and its *Z* isomer were isolated from *Kickxia spuria* (8), and the H-3 resonance of the *Z* isomer was reported to be 0.17 ppm upfield from that of **7**. We assigned the stereochemistry at C-6 as *S* by comparing the optical rotation of the reduction product of **7**, (+)-1-hydroxylinalool [**8**], to that reported (9) for its enantiomer **9**. Thus, **5** is 6-*O*-[6″(*S*)-hydroxy-2″,6″-dimethyl-2″(*E*)-7″-octadienoyl]catalpol, given the trivial name ambiguuside. Other iridoid and secoiridoid esters of **6** (3,5,7), **7** or its enantiomer (6,10,11), and **10** (12–16) have previously been isolated, but this is the first report of esters from all three acids in the same species.

Application of the trivial names foliamenthic acid and menthiafolic acid for **6** and **7**, respectively, ambiguity concerning the double bond configuration of **6** (5), and in subsequent years either failure to assign C-6 stereochemistry or the use of improper optical rotation comparisons for this assignment have made literature in the area somewhat of a maze. Some progress has been made in straightening this out (2), but care must still be taken in interpreting literature data. One additional possible complication, that of isomerization of acids such as **7** or iridoids containing this moiety to the more stable allylic alcohol structure, does not appear to have been addressed, although the acid-catalyzed isomerization is well known (6,17). We warmed **5** in a variety of dilute acids at 50° for 30 min, but saw no changes in the methyl resonances of the nmr spectrum as would be expected for the allylic rearranged isomer. More extensive heating at 80° resulted in cleavage of the sugar and decomposition. Hence, isolation under low temperature extraction conditions of iridoids containing the **7** or **9** moieties will not result in isomerization. We did not test for isomerization under extended hot Soxhlet extraction procedures which are sometimes employed.

One additional isolate was characterized as verbascoside, by comparison with previously isolated samples, and a second differed from verbascoside only by the additional presence of two aromatic methoxy groups. Placement of these was facilitated by HETCOR and ^{13}C-nmr experiments which allowed identification of the compound as martynoside (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Varian DMS 80 spectrophotometer. Exact mass spectra were obtained by the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln. ^{1}H- (300 MHz), ^{13}C- (75.5 MHz), DEPT, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr experiments were carried out on a Bruker ACE-300 spectrometer in CD_3OD. The middle of the septet was set at 3.30 ppm. Tlc was accomplished on 0.25-mm precoated plates of Si gel 60 F-254 (Merck) or reversed-phase octadecylsilane (C_{18}) F-254 (Whatman) and visualized using a solution of *p*-anisaldehyde. Hplc was carried out using a Beckman 110B solvent delivery module with a Beckman 163 variable wavelength detector and an Altex Ultrasphere-ODS, semipreparative, reversed-phase (C_{18}) column. Optical rotations were carried out using an Rudolf Research Autopol III.

PLANT COLLECTION.—*P. ambiguus* var. *laevissimus* was collected at Cactus Hill, 3.7 miles south of Bernal, San Miguel County, New Mexico. A voucher (FRS 323) was deposited in the Colorado State Uni-

versity Herbarium, and the identity of the specimen was confirmed by D.M. Wilken, Department of Biology, Colorado State University.

ISOLATIONS.—Whole, ground, air-dried *P. ambiguus* (2.1 kg) was extracted at room temperature for 24 h using 16 liters of MeOH. The extract was evaporated in vacuo, and the crude extract was partitioned between 400 ml of CH_2Cl_2 and 400 ml of H_2O. The aqueous layer was back-extracted with CH_2Cl_2, placed (in three portions) on a reversed-phase vlc column (60 ml fritted glass funnel 4.5 × 5.0 cm), and eluted with 400 ml of H_2O followed by 400 ml of MeOH. Evaporation of the aqueous fraction gave 50 g of residue, 500 mg of which was purified by reversed-phase vlc and then hplc [H_2O-MeOH (9:1)] to give 124 mg of a white solid which was identified as **1** [R_f 0.64, Si gel, CH_2Cl_2-MeOH (6:4)]. The MeOH fraction was reduced in volume to 50 ml and filtered through 50 g of activated charcoal. The charcoal was rinsed with MeOH, and the rinses were combined, evaporated in vacuo, and placed on a Si gel flash column, which was eluted with 500 ml of MeOH-CH_2Cl_2 (1:1). Evaporation gave 28 g of a light brown foam which contained catalpol esters and phenypropanoids. One fourth (7 g) of the mixture was separated using reversed-phase vlc (32 × 3.7 cm). Fraction 3 (70% MeOH/H_2O) and fraction 4 (80% MeOH/H_2O) were combined and separated by hplc [MeOH-H_2O (1:1)] to yield 204 mg of **5** [R_f 0.18, reversed-phase Si gel, H_2O-MeOH (6:4)], 26 mg of **3** (R_f 0.14) and 44 mg of **4** (R_f 0.14). The rest (21 g) of the mixture was likewise separated, first by reversed-phase vlc and then by hplc, to give 3 mg of verbascoside [R_f 0.80, Si gel, CH_2Cl_2-MeOH (6:4)] and 8 mg of specioside [**2**] [R_f 0.20, reversed-phase Si gel H_2O-MeOH (6:4)]. Preparative tlc [CH_2Cl_2-MeOH (8:2)] gave 11 mg of martynoside (R_f 0.24).

IDENTIFICATIONS.—High-field ^{1}H- and ^{13}C-nmr spectra in comparison with literature values were used to identify **2** (18), **3** (19), and martynoside (14). Compound **1** and verbascoside were compared to standard samples (^{1}H nmr and tlc).

Compound **4**.—Oil: $[\alpha]^{25}D$ −134.7° (*c* = 0.49, EtOH), uv λ max (H_2O) 217 sh nm; hrfabms $[M+H]^+$ 529.2290 (calcd for $C_{25}H_{37}O_{12}$, 529.2285); ^{1}H nmr δ 6.82 (1H, ddq, $J_{3'',4''}$ = 7.4, $J_{3'',9''}$ = 1.5, H-3″), 6.36 (1H, dd, $J_{3,4}$ = 5.8, $J_{3,5}$ = 1.5, H-3), 5.41 (1H, ddq, $J_{7'',8''}$ = 6.9, $J_{7'',10''}$ = 1.5, H-7″), 5.15 (1H, d, $J_{1,9}$ = 9.1, H-1), 4.95 (1H, dd, $J_{6,5}$ = 8.1, $J_{6,7}$ = 1.2, H-6), 4.94 (1H, dd, $J_{4,3}$ = 5.8, $J_{4,5}$ = 4.4, H-4), 4.79 (1H, d, $J_{1',2'}$ = 7.9, H-1′), 4.15 (1H, d, $J_{10d,10u}$ = 13.2, H-10d), 4.06 (2H, dd, $J_{8'',7''}$ = 6.9, $J_{8'',10''}$ = 1.0, H-8″), 3.91 (1H, dd, $J_{6'd,6'u}$ = 12.0, $J_{6'd,5'}$ = 2.0, H-6′d), 3.82 (1H, d, $J_{10u,10d}$ = 13.2, H-10u), 3.66 (1H, br s, H-7), 3.63 (1H, dd, $J_{6'u,6'd}$ = 12.0, $J_{6'u,5'}$ = 6.5, H-6′u), 3.35 (1H, m, H-5′), 3.33 (1H, m, H-3′), 3.25 (1H, m, H-2′), 3.22 (1H, m, H-4′), 2.60 (1H, dd, $J_{9,1}$ = 9.1, $J_{9,5}$ = 7.7, H-9), 2.58 (1H, m, H-5), 2.35 (2H, m, H-4″), 2.23 (2H, m, H-5″), 1.86 (3H, br s, H-9″), 1.77 (3H, br s, H-10″).

Compound **5**.—Oil: $[\alpha]^{25}D$ −56.2° (*c* = 2.52, MeOH); uv λ max (MeOH) 219 nm; hrfabms $[M+H]^+$ 529.2295 (calcd for $C_{25}H_{37}O_{12}$, 529.2285); ^{1}H nmr δ 6.84 (1H, ddq, $J_{3'',4''}$ = 7.5, $J_{3'',9''}$ = 1.2, H-3″), 6.35 (1H, dd, $J_{3,4}$ = 6.0, $J_{3,5}$ = 1.5, H-3), 5.82 (1H, dd, $J_{7'',8''d}$ = 17.4, $J_{7'',8''u}$ = 10.8, H-7″), 5.22 (1H, dd, $J_{8''d,7''}$ = 17.4, $J_{8''d,8''u}$ = 1.6, H-8″d), 5.15 (1H, br d, $J_{1,9}$ = 9.1, H-1), 5.05 (1H, dd, $J_{8''u,7''}$ = 10.8, $J_{8''u,8''d}$ = 1.6, H-8″u), 4.95 (1H, dd, $J_{6,5}$ = 7.6, $J_{6,7}$ = 1.0, H-6), 4.93 (1H, dd, $J_{4,3}$ = 6.0, $J_{4,5}$ = 4.6, H-4), 4.78 (1H, d, $J_{1',2'}$ = 7.9, H-1′), 4.15 (1H, d, $J_{10d,10u}$ = 13.2, H-10d), 3.92 (1H, dd, $J_{6'd,6'u}$ = 11.9, $J_{6'd,5'}$ = 1.9, H-6′d), 3.81 (1H, d, $J_{10u,10d}$ = 13.2, H-10u), 3.66 (1H, d, $J_{7,6}$ = 1.0, H-7), 3.63 (1H, dd, $J_{6'u,6'd}$ = 11.9, $J_{6'u,5'}$ = 6.5, H-6′u), 3.33 (1H, m, H-5′), 3.31 (1H, m, H-3′), 3.25 (1H, m, H-2′), 3.23 (1H, m, H-4′), 2.62 (1H, dd, $J_{9,1}$ = 9.1, $J_{9,5}$ = 7.7, H-9), 2.55 (1H, m, H-5), 2.25 (2H, m, H-4″), 1.84 (3H, d, $J_{9'',3''}$ = 1.2, H-9″), 1.62 (2H, m, H-5″), 1.27 (3H, s, H-10″).

ALKALINE HYDROLYSIS OF **5**.—Compound **5** (109 mg) was dissolved in 5 ml of 2 N NaOH and allowed to stand overnight. The solution was extracted with Et_2O to remove neutral material. Acidification with dilute HCl was followed by extraction with Et_2O. The Et_2O extracts were evaporated in vacuo. Purification of one half of the extract by hplc gave 7 mg of **7**.

Compound **7**.—Colorless oil: $[\alpha]^{25}D$ +14° (*c* = 0.65, $CHCl_3$), ^{1}H nmr ($CDCl_3$) δ 6.82 (1H, br s, H-3), 5.89 (1H, dd, $J_{7,8u}$ = 10.8, $J_{7,8d}$ = 17.3, H-7), 5.21 (1H, dd, $J_{8d,7}$ = 17.3, $J_{8d,8u}$ = 0.7, H-8d), 5.07 (1H, dd, $J_{8u,7}$ = 10.8, $J_{8u,8d}$ = 0.7, H-8u), 2.21 (2H, m, H-4), 1.79 (3H, br s, H-9), 1.62 (2H, m, H-5), 1.29 (3H, s, H-10); ^{13}C nmr δ 173.1 (C-1), 144.5 (C-7), 144.0 (C-3), 112.3 (C-8), 73.1 (C-6), 40.5 (C-5), 28.0 (C-10), 23.7 (C-4), 12.1 (C-9).

REDUCTION OF **7**.—$LiAlH_4$ (20 mg) was added to 5 ml of dry Et_2O. After stirring at room temperature for 10 min a solution containing 12 mg of **7** was added dropwise. The mixture was stirred for 10 min then cooled to 0°. H_2O and then 10% NaOH were added dropwise, after which the solution was stirred overnight. The mixture was filtered, diluted with 5 ml of H_2O, and extracted 3× with Et_2O. The extracts were combined, dried with Na_2SO_4, evaporated in vacuo, and purified by preparative tlc [Si gel, CH_2Cl_2-MeOH (19:1)] to give 0.3 mg of (+)-1-hydroxylinalool [**8**].

Compound **8**.—Colorless oil: $[\alpha]^{25}D$ +5° (c = 0.03, MeOH), ^{1}H nmr ($CDCl_3$) δ 5.90 (1H, dd, $J_{7,8u}$ = 10.8, $J_{7,8d}$ = 17.3, H-7), 5.40 (1H, ddq, $J_{3,4}$ = 5.7, $J_{3,9}$ = 1.3, H-3), 5.19 (1H, dd, $J_{8d,7}$ = 17.3, $J_{8d,8u}$ = 1.3, H-8d), 5.04 (1H, dd, $J_{8u,7}$ = 10.8, $J_{8u,8d}$ = 1.3, H-8u), 3.96 (2H, m, H-1), 2.06 (2H, m, H-4), 1.64 (3H, br s, H-9), 1.56 (2H, m, H-5), 1.27 (3H, s, H-10).

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