Notes

Phenylpropanoid Glycosides from *Penstemon serrulatus*

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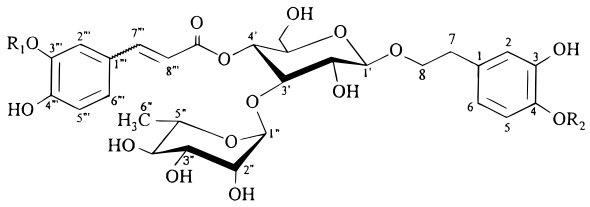
Two new phenylpropanoid glycosides named *cis*-martynoside (**1**) and *cis*-leucosceptoside A (**3**) were recognized in cell suspension cultures of *Penstemon serrulatus* Menz. The structures of these compounds were determined on the basis of ^{1}H NMR spectral data.

Phenylpropanoid glycosides are a group of water soluble natural products widely distributed in the plant kingdom.[1] These compounds are not specific to any plant organ and occur also in callus and suspension cultures. Large amounts of phenylpropanoid glycosides (up to 16% dry) have been isolated from cell suspension cultures of *Syringa vulgaris* L. (Oleaceae), and verbascoside was the main component of a hydroxyphenylethanol glycoside fraction.[2] This class of compound shows antibacterial,[3] antifeedant,[4] cytotoxic,[5] and enzyme inhibitory[6,7] activities.

In the course of our chemical and biological studies on cultures of *Penstemon serrulatus* Menz. (Scrophulariaceae) several known[8] and new[9] compounds have been isolated. Here, we describe new cis isomers of phenylpropanoid glycosides obtained from suspension cultures of *P. serrulatus*.

Methanolic extracts of dried cell suspensions of *P. serrulatus* yielded three phenylpropanoid glycoside fractions, which were isolated on a Si gel column and subjected to spectroscopic (MS, NMR) and chemical analysis. They consisted of a 1:3 mixture of *cis*- (**1**) and *trans*-martynoside (**2**), a 1:2 mixture of *cis*- (**3**) and *trans*-leucosceptoside A (**4**), and *cis*- (**5**) and *trans*-verbascoside (**6**) (a *cis*/*trans* ratio of 1:6). Amounts of phenylpropanoid glycosides produced by the culture in 15 days were quite significant. For example, 1 g of dry weight contained 17 mg of **1** and **2**, 25 mg of **3** and **4**, and 203 mg of **5** and **6** (or 29 mg of **5** and 174 mg of **6**, because these isomeric verbascosides were well separated).

Acid hydrolysis of each fraction gave two sugar residues, D-glucose and L-rhamnose, which were identified by paper chromatography followed by polarimetry. Ferulic acid[10] was identified by ^{1}H NMR in the products of alkaline hydrolysis of **1** and **2**. LSIMS data (molecular weight and fragmentations of molecular ion), together with the results of chemical degradation, supported molecular formula $C_{31}H_{40}O_{15}$ for **1** and **2**. The ^{1}H NMR (500 MHz) spectrum in general consisted of two sets of signals well separated in the aromatic and olefinic regions. The chemical shifts and coupling constants assigned to the major isomer matched the known data for *trans*-martynoside (**2**).[10,11] The ^{13}C NMR spectrum of **1** and **2** supported this conclusion.[10,11] The



1 $R_1 = R_2 = CH_3$; 7‴ - 8‴ = *cis*
2 $R_1 = R_2 = CH_3$; 7‴ - 8‴ = *trans*
3 $R_1 = CH_3$, $R_2 = H$; 7‴ - 8‴ = *cis*
4 $R_1 = CH_3$, $R_2 = H$; 7‴ - 8‴ = *trans*
5 $R_1 = R_2 = H$; 7‴ - 8‴ = *cis*
6 $R_1 = R_2 = H$; 7‴ - 8‴ = *trans*

minor isomer was identified as the previously unknown *cis*-martynoside (**1**) based on the smaller ^3J (HC═CH) coupling (13.0 vs. 15.9 Hz) and significant upfield shifts of the olefinic protons.[12] Both isomers had the same configurations at anomeric carbons: β for D-glucose [J (H1′− H2′) = 7.9 Hz] and α for L-rhamnose [J (H1″−H2″) = 1.8 Hz]. The positions of two methoxy groups in the aromatic rings of the acid moiety and the aglycon in **1** and **2**, as well as their assignments to a particular ring, were concluded from the NOE difference spectra.

Base hydrolysis of a mixture of **3** and **4** yielded cis and trans isomers of ferulic acid, which were identified by ^{1}H NMR.[12−14] Acid hydrolysis afforded 2-(3,4-dihydroxyphenyl)ethanol (^{1}H NMR)[13] in addition to D-glucose and L-rhamnose. The ^{1}H NMR spectrum of **3** and **4** clearly showed the presence of **3** together with its known[13,14] trans isomer **4** (a 1:2 mixture), and the ^{13}C NMR spectrum contained all resonances of **4**.[13,14]

The last fraction contained known *cis*- (**5**) and *trans*-verbascosides (**6**) as revealed by ^{1}H and ^{13}C NMR spectra.[12,15]

The *cis*/*trans* ratios of **1** and **2**, **3** and **4**, and **5** and **6** were established by comparing ^{1}H NMR integrals for all respective well-separated multiplets (at least four pairs of signals for each compound). The quantitative ^{1}H NMR data were in good agreement with those obtained for isomeric verbascosides **5** and **6** by HPLC. NMR spectroscopy was more valuable than HPLC because *cis*/*trans* ratios for **1** and **2** and for **3** and **4** could be easily estimated.

To demonstrate that the high concentrations of cis isomers were specific for *P. serrulatus*, the ratio of **5** to **6** was studied (by HPLC) for cultures grown under various conditions. The same ratio (1:6) was found for the cell

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suspension grown in darkness. UV irradiation (365 nm) of the suspension for 15, 30, and 120 min did not influence the ratio, whereas the total content of verbascoside increased. For example, after UV irradiation for 120 min, the cells produced ca. 250 mg of verbascoside per 1 g of dry weight. This was a twofold increase in comparison to the control sample (cells grown in darkness). Verbascosides (**5** and **6**) could be stress compounds (phytoalexins) of *P. serrulatus*, as verbascoside was a confirmed phytoalexin for *Rehmannia glutinosa* Libasch. var. *purpurea*.[16] The **5**/**6** ratio was constant (1:6) throughout a 30-day growing period of the culture.

Although every effort was made to protect the phenylpropanoid glycosides from daylight, light-induced *trans*/*cis* interconversion of geometrical isomers could not be excluded as a possible source of cis isomers. For this reason the configurational stability of **6** was studied. Pure **6** isomerized to a 2:3 mixture of **5** and **6** after irradiation (365 nm) of its MeOH-d_4 solution for 30 min. A trans/cis isomerization was also observed when a MeOH-d_4 solution of **6** was left in continuous fluorescent light. After 30 min the cis/trans ratio reached 1:5, while after 24 h a 1:3 mixture was observed. When **6** was left in MeOH-d_4 solution in daylight, it slowly isomerized to a 1:9 cis/trans mixture after 10 days. However, solid **6** remained configurationally homogeneous after UV irradiation for 2 h.

The relatively slow formation of **5** from **6** in daylight and the lack of isomerization of solid **6** even after UV irradiation indicate that cis isomers of phenylpropanoid glycosides were produced by *P. serrulatus*. Low concentrations of **5** were previously found in leaves of *Lantana camara* L. var. *aculeata* (Verbenaceae)[15] and *Stachus sieboldii* L. (Lamiaceae).[12]

Experimental Section

General Experimental Procedures. Mass spectrometer, Finnigan MAT. Molecular fragmentations of the samples were achieved by bombardment with cesium ions; matrix, glycerol. ^{1}H NMR, 500 MHz, with TMS as an internal standard. Chemical shifts are expressed in δ (ppm), and J values are in Hz. Polarimeter, Perkin−Elmer 241 MC. Column chromatography, polyamide (Roth) and Si gel 60 (70−230 mesh, Merck); TLC, Si gel 60 F_{254} (Merck) prepared plates with the solvent system $CHCl_3$−MeOH−H_2O (75:25:2, v/v/v) for phenylpropanoid glycosides. Paper chromatography (Whatman Chr 1) solvent system, *n*-BuOH−HOAc−H_2O (4:5:1, v/v/v, upper phase) for sugars. Spots were detected by (a) UV; (b) 2% methanolic $FeCl_3$; (c) ceric sulfate in 65% H_2SO_4 (heated to 120° for 15 min); (d) vanillin − H_2SO_4; (e) 3,5-dinitrobenzoic acid in 2% Na_2CO_3 for sugars; (f) aniline phthalate reagent for sugars. After spraying with (d), (e), and (f), the plates were heated for 2−5 min at 100 °C. HPLC, Beckmann, computer system−System Gold; detection UV−vis, λ = 334 nm; column ODS (analytical or semipreparative); mobile phase, analysis, 0.4 *M* H_3PO_4−CH_3CN (4:1, v/v); flow 1 mL/min; separation of **5** and **6**, MeOH−H_2O (3:1, v/v); flow 1 mL/min.

Plant Material. Suspension culture was initiated from anther-derived calli of *P. serrulatus*, as described previously.[17] The suspension culture was cultivated on a rotary shaker (100 rpm) in the liquid Schenk and Hildebrandt medium,[18] supplemented with 0.1 mg/L of 2,4-dichlorophenoxyacetic acid and 2 mg/L of benzylaminopurine. The cells were transferred every 14 days to a fresh medium. The suspension cultures were grown under continuous fluorescent light (2.4 W/m^2) or in the darkness at 26 ÷ 28 °C.

HPLC. Dried cells of suspension cultures (0.5 g) were extracted with boiling MeOH (2 × 50 mL) for 1 h. The solvent was evaporated in vacuo, and the green residue was dissolved in MeOH (100.0 mL). Aliquots (10 μL) were injected into the column. Retention times: **1** and **2**, 24.0 min; **3** and **4**, 13.6 min; **5**, 7.1 min; **6**, 9.0 min.

Isolation. In all stages of the procedure, extracts were protected from daylight. Dried material (100 g) of suspension culture was extracted (2 × 2.5 L) with boiling MeOH for 2 h. MeOH was evaporated in vacuo, and the resulting green gum was suspended in hot (80 °C) H_2O. The aqueous solution was left for 24 h, then filtered and extracted with petroleum ether (bp 60−80 °C) until the extracts were colorless. The aqueous phase was concentrated in vacuo to give a dark brown oil (35 g), which was chromatographed on polyamide (150 g) (column length 78 cm, i.d. 3.5 cm) using H_2O and a H_2O−MeOH mixture (1:1, v/v). The fractions were monitored by TLC. The aqueous fractions containing iridoids and those containing phenylpropanoid glycosides were combined and concentrated in vacuo. The phenylpropanoid glycoside fraction (16 g) was chromatographed on Si gel (150 g, column length 80 cm, i.d. 3.5 cm) eluted with $CHCl_3$−MeOH−H_2O (75:25:2,v/v/v). Fractions of ca. 15 mL were collected and checked by TLC. Fractions 6−20, 22−29, and 30−67 were pooled as fractions of martynosides **1** and **2** (100 mg), leucosceptosides A **3** and **4** (29 mg), and verbascosides **5** and **6** (300 mg), respectively. These were further purified on a Si gel column using the same solvent system.

***cis*-Martynoside** (**1**): NMR data obtained from the spectrum of a 1:3 mixture of **1** and **2**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.87 (1H, d, J = 2.0 Hz, H-2‴), 7.16 (1H, dd, J = 8.5, 2.0 Hz, H-6‴), 6.94 (1H, d, J = 13.0 Hz, H-7‴), 6.82 (1H, d, J = 8.2 Hz, H-5), 6.77 (1H, d, J = 8.4 Hz, H-5‴), 6.73 (1H, d, J = 3.0 Hz, H-2), 6.68 (1H, dd, J = 8.2, 3.0 Hz, H-6), 5.79 (1H, d, J = 13.0 Hz, H-8‴), 5.16 (1H, d, J = 1.8 Hz, H-1″), 4.89 (1H, t, J = 9.6 Hz, H-4′), 4.36 (1H, d, J = 7.9 Hz, H-1′), 4.09−4.03 (2H, m, H-8), 3.92 (1H, dd, J = 3.4, 1.8 Hz, H-2″), 3.82 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.76 (1H, t, J = 9.1 Hz, H-4′), 3.75−3.70 (2H, m, H-8), 3.71−3.52 (2H, m, H-3″, H-5″), 3.40 (1H, dd, J = 9.1, 8.0 Hz, H-2′), 3.32−3.28 (1H, m, H-4″), 2.86−2.80 (2H, m, H-7), 1.16 (3H, d, J = 6.2 Hz, H-6″); LSIMS (positive mode) m/z 675 [M + Na]$^+$ (4.3), (negative mode) m/z 651.2 [M − H]$^-$; NOE data, irradiation at δ 3.89 produced 4.9% intensity enhancement at δ 7.20, irradiation at δ 3.81−14.5% produced enhancement at δ 7.87, and irradiation at δ 3.8−4.9% produced enhancement at δ 6.82.

***cis*-Leucosceptoside A** (**3**): NMR data calculated from the spectrum of a 1:2 mixture of **3** and **4**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.88 (1H, d, J = 2.0 Hz, H-2‴), 7.16 (1H, dd, J = 8.3, 2.0 Hz, H-6‴), 6.93 (1H, d, J = 13.0 Hz, H-7‴), 6.77 (1H, d, J = 8.3 Hz, H-5‴), 6.69 (1H, d, J = 2.1 Hz, H-2), 6.67 (1H, d, J = 8.1 Hz, H-5), 6.56 (1H, dd, J = 8.1, 2.1 Hz, H-6), 5.80 (1H, d, J = 13.0 Hz, H-8‴), 5.16 (1H, d, J = 1.7 Hz, H-1″), 4.91 (1H, t, J = 9.1 Hz, H-4′), 4.36 (1H, d, J = 7.9 Hz, H-1′), 4.07−4.01 (2H, m, H-8), 3.93 (1H, dd, J = 3.4, 1.7 Hz, H-2″), 3.77 (1H, t, J = 9.1 Hz, H-3′), 3.886 (3H, s, OCH_3), 3.75−3.69 (2H, m, H-8), 3.64−3.57 (2H, m, H-3″, H-5″), 3.40 (1H, dd, J = 9.0, 8.1 Hz, H-2′), 3.31−3.30 (1H, m, H-4″), 2.81−2.78 (2H, m, H-7), 1.16 (3H, J = 6.2 Hz, H-6″).

Separation of 5 and 6 by HPLC. A solution of a 1:6 mixture of **5** and **6** (11.2 mg) in MeOH (2 mL) was injected into the column in 100-μL aliquots to yield **5** (1.8 mg, 16%, t_R 41.98 min) and **6** (3.8 mg, 34%, t_R 53.23 min). Final purifications of **5** and **6** were accomplished on Si gel columns using $CHCl_3$−MeOH−H_2O (75:25:2, v/v/v) to afford **5** (1.331 mg) and **6** (3.114 mg).

Acid Hydrolysis of Compounds 1−6. The compounds (2−3 mg) were refluxed for 1 h in 2 *M* HCl in MeOH (2 mL); H_2O (2 mL) was then added, and the mixture was extracted with EtOAc. The aqueous layer was passed through Dowex 1 × 8 (120−250 mesh) in acidic form, and then concentrated in vacuo to give a residue identified by comparison with authentic samples of D-glucose (R_f 0.20) and L-rhamnose (R_f 0.32) by paper chromatography with the solvent system *n*-BuOH−HOAc−H_2O.[19]

This procedure was repeated using a mixture of compounds **1**−**6** (1.85 g). The separated monosaccharides were extracted from the paper (Whatman Chr 3) with H_2O (100 mL) at 50 °C for 30 min. The aqueous solutions were concentrated in vacuo

to leave D-glucose (5.1 mg), $[\alpha]^{25}_D$ +49° (*c* 0.51, H_2O, equilibrium) {lit.[20] $[\alpha]^{25}_D$ +52.3° (*c* 8.0, H_2O, equilibrium)}- and L-rhamnose (16.0 mg), $[\alpha]^{25}_D$ +7.8° (*c* 1.6, H_2O, equilibrium) {lit.[20] $[\alpha]^{25}_D$ +8.3° (*c* 10, H_2O, equilibrium)}.

Alkaline Hydrolysis of Compounds 1−6. Solutions of **1**−**6** (20 mg of each) in 3 mL of 1 *M* NaOH were heated at 80 °C for 2 h, then neutralized with 1 *M* HCl, and finally extracted with EtOAc. The EtOAc extract was concentrated in vacuo to give ferulic acid (3.5 mg)[10−12] for **1**−**4** and caffeic acid (2.8 mg)[14,15] for **5** and **6**. The aqueous layer was concentrated in vacuo, and then refluxed for 1 h in 2 *M* HCl in MeOH (2 mL).Then H_2O (2 mL) was added, and the mixture was extracted with EtOAc. The extract was concentrated in vacuo to give 2-(3,4-dihydroxyphenyl)ethanol[13] (2.2 mg) for **3**−**6** and 2-(4-hydroxy-3-methoxyphenyl)ethanol (2.6 mg)[10] for **1** and **2**.

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