Journal of Natural Products
Vol. 50, No. 3, pp. 485-489, May-Jun 1987

NEW IRIDOID GLUCOSIDES FROM *CASTILLEJA* AND *BESSEYA*: 6-HYDROXYADOXOSIDE AND 6-ISOVANILLYLCATAPOL[1]

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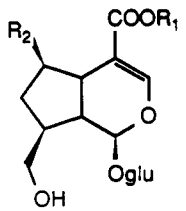
In south-central Colorado checkerspot butterflies (*Euphydryas anicia*) utilize two species of Scrophulariaceae as host plants:*Castilleja integra* Gray and *Besseya plantaginea* (James) Rydb. Following an earlier lead of Bowers (1), we have shown (2) that adult butterflies contain catalpol and macfadienoside, iridoid glucosides obtained through larval consumption of the host plants. The present work describes details of the host plant iridoid glucoside isolations and structure determinations, including the assignment of structures to two new iridoids, 6-β-hydroxyadoxoside and 6-isovanillylcatalpol.[2] Because *Castilleja linariifolia* Benth. and *Besseya alpina* (Gray) Rydb. host *Euphydryas* species at other Colorado locations, we have also assessed the iridoid glucoside content of these alternate host plants.

Eight iridoid glucosides were isolated and identified from a methanolic extract of the *C. integra* aerial parts: adoxoside [**1**], adoxosidic acid [**2**], 6-β-hydroxyadoxoside [**3**], shanzhiside methyl ester [**4**], shanzhiside [**5**], catalpol [**6**], macfadienoside [**7**], and 8-*epi*-loganic acid [**8**]. Of these, **7** was the major iridoid. The crude iridoid fraction also yielded verbascoside (acteoside). From *C. linariifolia* we isolated **6** as the major component followed by **1** and then **2**, **5**, and **8** as minor components. Tlc evidence was obtained for verbascoside, but it was not isolated. *B. plantaginea* yielded **6**, **8**, veronicoside [**9**], 6-isovanillylcatalpol [**10**], verproside [**11**], and mussaenoside [**12**], with **6** and particularly its esters **9-11** being the major isolates. *B. alpina* was similar in iridoid content, yielding **6**, **9**, **10**, and **11**, but also aucubin. Of these, **6** and aucubin were the major iridoids. Neither of the *Besseya* species contained verbascoside.

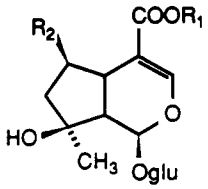
The identification of 6-β-hydroxyadoxoside proceeded as follows. Fab hrms established the molecular formula as $C_{17}H_{26}O_{11}$. The ir spectrum showed a carbonyl at 1695 cm^{-1}. In the ^1H-nmr spectrum the presence of an H-3 resonance (7.49 ppm, not coupled to an H-4 proton) along with an OCH_3 resonance at 3.73 ppm and an H-1 resonance at 5.48 ppm established the C-4 carbomethoxy functionality in the pyran ring. A pair of doublet of doublets at 3.60 and 3.64 ppm for the C-10 CH_2 were only slightly shifted from those of adoxoside [**1**] as was the H-9 proton resonance at 2.33 ppm. The C-10 ^{13}C resonance (65.9 ppm) of **3** was virtually identical with that (7) of **1** (66.1). These data establish the C-8 β-configuration. Adoxoside shows seven proton resonances below 3 ppm, while only five were observed for **3**. This, along with the appearance of a new resonance at 4.21 ppm, indicated substitution of an OH group at either C-6 or C-7. Placement at C-6 was established by a proton decoupling experiment. Irradiation of the resonance at 4.21 ppm resulted in the H-5 doublet of doublets at 2.90 ppm collapsing to a doublet. The stereochemistry of the OH group at C-6 was assigned on the basis of the $J_{5,6}$ coupling value and on chemical shift correlations with similar compounds. The dihedral

[1]Paper 9 in the series Chemistry of the Scrophulariaceae. For Paper 8, see: M.R. Roby and F.R. Stermitz, *J. Nat. Prod.*, **49**, 368 (1986).

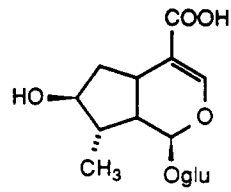
[2]In Stermitz *et al.* (2), we reported this component as the known amphicoside (6-vanillylcatalpol) based upon incomplete evidence.



1 R_1=CH_3, R_2=H
2 R_1=R_2=H
3 R_1=CH_3, R_2=OH



4 R_1=CH_3, R_2=OH
5 R_1=H, R_2=OH
12 R_1=CH_3, R_2=H



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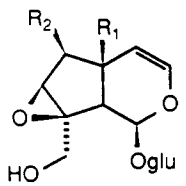
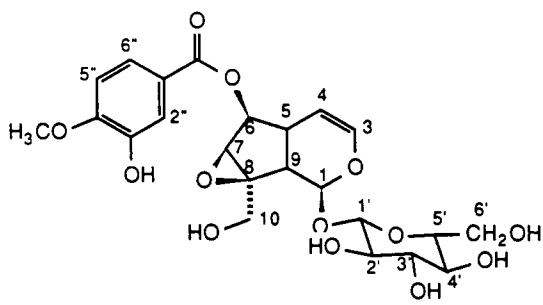


6 R_1=H, R_2=OH
7 R_1=R_2=OH
9 R_1=H, R_2=benzoyloxy
11 R_1=H, R_2=3,4-dihydroxybenzoyloxy



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angles between the C-5H and C-6H bonds for α-and β-substituted OH groups are 5° and 125°, respectively, as measured on a Dreiding model. $J_{5,6}$ values for α- or β-substituted hydroxy compounds should, therefore, be approximately 9 Hz or 4 Hz. Decoupling experiments showed the value of $J_{5,6}$ to be 3.4 Hz, suggesting a β-substituted OH group. Additional evidence was from an exact fit with three key resonances that have been previously used (3) to assign this stereochemistry. In the β series H-6 is near 4.2 ppm, but at 4.5 for the α series, H-1 is near 5.5 ppm for the β series but at 5.2 ppm for the α series; finally, H-3 appears near 7.5 ppm for the β series but near 7.7 ppm for the α series. All three resonances observed for **3** (4.21 for H-6, 5.48 for H-1, and 7.49 for H-3) conform to expectations for the β series and not for the α series. For comparison we had samples of dihydrocornin and penstemonoside (both with β-OH groups at C-6), and the three pertinent resonances were virtually identical in **3**, dihydrocornin, and penstemonoside. Data from the ^{13}C-nmr spectrum was also consistent with a 6-β-hydroxy substituent, since the C-1 resonance was at 93.5 ppm. Data compiled for similar compounds have shown C-1 to be near 100 ppm for the α-configuration and near 94 ppm for β-substitution (7).

The identification of 6-isovanillylcatalpol was as follows. Elemental analysis established the molecular formula as $C_{23}H_{28}O_{13}$. In addition to the typical catalpol resonances, the ^{1}H-nmr (360 MHz) spectrum showed signals above 6.9 ppm characteristic of a 1,3,4-trisubstituted aromatic system. The presence of a methoxy signal at 3.83 ppm and a shift in the uv spectrum with added OH^- suggested either a 4-hydroxy-3-methoxy (vanillyl) or 3-hydroxy-4-methoxy (isovanillyl) ester of catalpol. NOe experiments showed enhancements between the OMe and H-5″, and hydrolysis of **10** gave isovanillic acid and catalpol. That the acylation with isovanillic acid was at C-6 was evident by the down field shift of H-6 from 4.00 ppm in catalpol to 5.11 ppm in **10**.

We had previously (4,5) isolated and identified **6** and aucubin from other

plants. Standard samples of **4**, **9**, **11**, and **12** were obtained and proved to be identical with our isolates by glc of the trimethylsilyl derivatives, tlc, 360 MHz ^{1}H nmr, and, in some cases, ^{13}C nmr. Standard samples of verbascoside and acteoside were identical with our isolate by tlc and ^{1}H-nmr spectra. Compound **1** was acetylated and this derivative compared with a standard by ^{1}H nmr. A standard of **7** could not be obtained, but our ^{13}C- and ^{1}H-nmr spectra were comparable to those in the literature (6) and in accord with the structure, particularly in comparison with the spectral data for **6**. Literature data was particularly incomplete for the acids **2**, **5**, and **8**. Their structures, however, were assured by ^{1}H-nmr spectra compared to those for the methyl esters **1**, **4**, and 8-*epi*-loganin. Where high field ^{1}H-nmr spectra have not previously appeared in the literature, these data are given in the Experimental.

The varied iridoid content in the two *Castilleja* species as compared with that of the *C. sulphurea-rhexifolia-miniata* group (5), which lack the macfadienoside or adoxoside derivatives, indicates that iridoid content will help in delimiting sections or other species groups in the *Castilleja*.

EXPERIMENTAL

Plant identifications, general isolation methods, instrumentation, and a detailed description of the *B. plantaginea* and *B. alpina* iridoid isolations were given previously (2). Following is a description of isolations from *C. integra* (CSU Voucher No. 66099). The analysis of *C. linariifolia* (CSU Voucher No. 65243) was performed similarly.

Whole, above-ground, dried plant material (75 g) of *C. integra* afforded 2.3 g of a crude iridoid mixture (2) also containing about 20% sugars. Flash chromatography of the crude over Si gel (200 ml, 7:3, $CHCl_3$-MeOH) provided two fractions: A (500 mg, a mixture of six iridoids, mainly the less polar ones) and B (1.5 g, a mixture of sugars and polar iridoids). Fraction B was chromatographed on an anion exchange resin (Bio-Rex 5, chloride form), eluting with 295 ml of H_2O, to yield five equal fractions. Fractions 1 and 2 contained sugars and neutral iridoids, the latter better isolated from Fraction A. Fractions 3, 4, and 5 yielded 90 mg of three acidic iridoids. These were separated by mplc (Si gel, 6:4:0.2, $CHCl_3$-MeOH-H_2O followed by 1:1, $CHCl_3$-MeOH and, finally, pure MeOH) to afford **2** (6 mg) from the first solvent elution, **8** (7 mg) from the second, and **5** (32 mg) from the pure MeOH fraction. Mplc of Fraction A (Si gel, 300 ml, 8:2:0.2, $CHCl_3$-MeOH-H_2O; 300 ml, 7:3:0.2, $CHCl_3$-MeOH-H_2O; 300 ml, 65:35:0.2, $CHCl_3$-MeOH-H_2O; and 300 ml, 1:1, $CHCl_3$-MeOH) resulted in 8 pooled fractions which were evaporated to give **1** (71 mg, fraction 1), **4** (12 mg, fraction 2), **3** (10 mg, fraction 3), a mixture of **3** and verbascoside (50 mg, fraction 4), a mixture of verbascoside and **6** (30 mg, fraction 5), **2** (12 mg, fraction 6), **7** (76 mg, fraction 7), and **8** (15 mg, fraction 8). An additional 10 mg of **3** was obtained by repeating the above separation, but neither of the isolates containing **3** were completely pure. The combined 20 mg of crude **3** was purified by C18 reverse phase preparative hplc (20% MeOH at 5 ml/min, 240 nm detection, Beckman 331 system) to afford 4 mg of pure **3**. The above procedure did not allow isolation of pure verbascoside. This was best accomplished by a separate isolation. From 105 g of plant material a 3 g crude iridoid-sugar-verbascoside mixture was obtained by MeOH extraction and residue trituration. Flash chromatography of this on Sephadex LH20 (MeOH) gave 440 mg of impure verbascoside which was rechromatographed on a gravity Sephadex LH20 column (MeOH) to give 300 mg of pure verbascoside.

Identifications of isolated iridoids were as given below. ^{1}H-nmr spectra were at 360 MHz (D_2O, HDO 4.73 ppm). Glc was of the trimethylsilyl derivatives.

ADOXOSIDE [**1**].—^{1}H nmr of the pentaacetate was compared with a standard from S.R. Jensen and B.J. Nielsen (8). ^{13}C-nmr spectral comparison was with literature (7) values. ^{1}H nmr of **1**: 1.32 (1H, m, H-7), 1.42 (1H, m, H-6), 1.80 (1H, m, H-7), 1.96 (1H, br.dd, 6.0, 13.7, H-9), 2.08 (2H, m, H-6,8), 2.83 (1H, br q, 7.0, 14.3, H-5), 3.28 (1H, dd, 8.1, 9.2, H-2′), 3.35 to 3.45 (3H, H-3′,4′,5′), 3.53 (1H, dd, 7.5, 11.0, H-10), 3.58 (1H, dd, 7.0, 11.0, H-10), 3.70 (1H, dd, 5.5, 12.4, H-6′), 3.70 (3H, s, -OMe), 3.88 (1H, dd, 2.1, 12.4, H-6′), 4.78 (1H, d, 8.1, H-1′), 5.23 (1H, d, 6.0, H-1), 7.48 (1H, d, 1.0, H-3).

ADOXOSIDIC ACID [**2**].—No standard (9) was available. The ^{1}H-nmr spectrum was virtually identical to that of **1** except for lack of COOMe methyl resonances and the expected shift in the H-3 resonance: 1.32 (1H, m, H-7), 1.44 (1H, m, H-6), 1.77 (1H, m, H-7), 1.96 (2H, m, H-6,9), 2.07 (1H, m, H-8), 2.85 (1H, br q, H-5), 3.26 (1H, dd, 8.0, 9.1, H-2′), 3.30 to 3.45 (3H, H-3′,4′,5′), 3.51 (1H, dd, 7.2, 10.0, H-

10), 3.57 (1H, dd, 6.5, 10.0, H-10), 3.69 (1H, dd, 5.6, 12.4, H-6′), 3.87 (1H, dd, 2.1, 12.4, H-6′), 4.75 (1H, d, 8.0, H-1′), 5.24 (1H, d, 5.0, H-1), 7.15 (1H, d, 1.2, H-3).

6-β-HYDROXYADOXOSIDE [3].—A foamy solid, $[\alpha]^{27}D-83.4°$ (*c* 0.43 MeOH). Hrfabms 407.1550; calc for $C_{17}H_{26}O_{11}\cdot H^+$ 407.1553; ^{13}C nmr (D_2O) 35.7 (C-7), 41.0, 41.6, 42.6 (C-5 and/or C-8 and/or C-9), 52.6 (OMe), 61.5 (C-6′, used as reference peak), 65.9 (C-10), 70.4 (C-4′), 73.4 (C-2′), 76.4 (C-3′ or C-6), 76.9 (C-6 or C-3′), 77.2 (C-5′), 97.3 (C-1), 99.5 (C-1′), 109.4 (C-4), 153.5 (C-3), 170.5 (C=O); ^1H nmr 1.37 (1H, m, H-7), 2.12 (2H, m, H-7,8), 2.33 (1H, m, H-9), 2.90 (1H, br.dd, 3.4, 7.8, H-5), 3.25 (1H, dd, 8.1, 9.3, H-2′), 3.35 to 3.50 (3H, H-3′,4′,5′), 3.60 (1H, dd, 6.1, 11.0, H-10), 3.64 (1H, dd, 6.3, 11.0, H-10), 3.70 (1H, dd, 5.7, 12.3, H-6′), 3.73 (3H, s, OMe), 3.90 (1H, dd, 2.1, 12.3, H-6′), 4.21 (1H, br.dd, 3.7, 7.8, H-6), 4.77 (1H, d, 8.1, H-1′), 5.48 (1H, d, 3.9, H-1), 7.49 (1H, d, 1.3, H-3); ir 3361, 1695, 1634, 1297, 1076 cm^{-1} (thin film, NaCl).

SHANZHISIDE METHYL ESTER [4].—Glc of the trimethylsilyl derivative, tlc, and ^1H-nmr spectrum were compared to a standard sample from H. Inouye. Literature (10) spectral data are available.

SHANZHISIDE [5].—No standard sample was available. ^1H nmr 1.22 (3H, s, H-10), 1.79 (1H, dd, 6.7, 13.1, H-7), 2.03 (1H, dd, 6.2, 13.1, H-7), 2.60 (1H, dd, 2.0, 10.6, H-9), 2.90 (1H, dd, 3.6, 10.6, H-5), 3.21 (1H, dd, 8.1, 9.4, H-2′), 3.30 to 3.45 (3H, H-3′,4′,5′), 3.66 (1H, dd, 5.8, 12.4, H-6′), 3.88 (1H, dd, 2.1, 12.4, H-6′), 3.96 (1H, m, H-6), 5.48 (1H, d, 2.0, H-1), 7.00 (1H, d, 1.3, H-3). A 60 MHz ^1H nmr spectrum was published (11).

CATALPOL [6] AND AUCUBIN.—Glc of the trimethylsilyl derivatives, tlc, and ^1H-nmr spectrum were compared to previous isolates (4).

MACFADIENOSIDE [7].—No standard sample was available. ^{13}C- and ^1H-nmr spectra were compared with literature values (6).

8-*EPI*-LOGANIC ACID [8].—No standard sample available. ^1H nmr 0.96 (3H, d, 7.3, H-10), 1.85 (1H, m, H-6), 1.95 (1H, m, H-6), 2.14 (1H, m, H-8), 2.67 (1H, dt, 2.3, 9.2, H-9), 2.98 (1H, m, H-5), 3.25 to 3.45 (4H, H-2′,3′,4′,5′), 3.68 (1H, dd, 5.9, 12.6, H-6′), 3.88 (1H, dd, 1.6, 12.6, H-6′), 3.81 (1H, m, H-7), 5.51 (1H, d, 2.5, H-1), 7.22 (1H, s, H-3). A 60 MHz ^1H-nmr spectrum was published (12).

VERONICOSIDE [9].—Glc of the trimethylsilyl derivative, ^{13}C- and ^1H-nmr spectrum were compared with the literature (13) and a standard sample (O. Sticher).

6-ISOVANILLYLCATALPOL [10].—Colorless needles from acetonitrile, mp 162-166°; *Anal.* found: C, 53.99; H, 5.55; calc. for $C_{23}H_{28}O_{13}$: C, 53.91; H, 5.51; $[\alpha]^{22}D-165.8$ (*c*=0.69 MeOH); ir 1709, 1297, 1223 cm^{-1} (KBr); uv λ max (MeOH) 223, 262, 297; λ max (MeOH+NaOH) 221, 239, 273, 322; ^1H nmr 2.61 (1H, m, H-5), 2.68 (1H, m, H-9), 3.30 to 3.45 (4H, H-2′,3′,4′,5′), 3.71 (1H, dd, 4.8, 12.5, H-6′), 3.78 (1H, d, 13.3, H-10), 3.83 (2H, m, H-6′,7), 3.86 (3H, s, OMe), 4.26 (1H, d, 13.3, H-10), 4.85 (1H, d, 8.0, H-1′), 5.03 (1H, dd, 4.6, 5.8, H-4), 5.11 (1H, dd, 1.1, 8.0, H-6), 5.12 (1H, d, 9.6, H-1), 6.35 (1H, dd, 1.5, 6.0, H-3), 6.97 (1H, d, 8.7, H-5″), 7.39 (1H, d, 2.0, H-2″), 7.54 (1H, dd, 2.0, 8.7, H-6″). Two separate hydrolysis experiments were used to identify catalpol and isovanillic acid. Base hydrolysis was accomplished by adding 100 mg of **10** to 30 ml 1M NaOH and refluxing for 1 h. The pH was adjusted to 1 with H_2SO_4, and the solution was washed three times with 20 ml $CHCl_3$. The $CHCl_3$ was evaporated in vacuo to give isovanillic acid shown by hplc and tlc comparison with standard samples of isovanillic and vanillic acids. Catalpol was obtained by hydrolysis similar to that described by Bobbit (16). 100 Mg of **10** was added to 5 ml of strong base anion-exchange resin (Bio-Rad AG1-X8) and 15 ml H_2O at 80° for 2 h. The solution was filtered and evaporated to give catalpol by tlc comparison with a standard. Acetylation of **10** formed a heptaacetate whose ^1H-nmr spectrum was very similar but not identical to that of a standard sample (A. Zaman) of amphicoside (6-vanillylcatalpol) heptaacetate.

VERPROSIDE [11].—Glc, tlc, and ^1H-nmr spectrum were compared with the literature (15) and a standard sample (O. Sticher).

MUSSAENOSIDE [12].—Glc, tlc, and ^1H-nmr spectrum were compared with a standard sample (H. Inouye). ^1H nmr 1.26 (3H, s, H-10), 1.44 (1H, m, H-7), 1.69 (2H, m, H-7,6), 2.20 (1H, m, H-8), 2.32 (1H, dd, 2.7, 9.7, H-9), 3.08 (1H, m, H-5), 3.22 (1H, dd, 8.1, 9.3, H-2′), 3.30 to 3.45 (3H, H-3′,4′,5′), 3.69 (1H, dd, 5.8, 12.4, H-6′), 3.69 (3H, s, OMe), 3.87 (1H, dd, 2.1, 12.4, H-6′), 4.75 (1H, d, 8.1, H-1′), 5.51 (1H, d, 2.8, H-1), 7.41 (1H, s, H-3).

VERBASCOSIDE (ACTEOSIDE).—^{13}C- and ^1H-nmr spectra were compared with standard samples of verbascoside (R. Wylde) and acteoside (S. Kobayashi).

Spectra and complete details of all comparisons are available in a thesis (17).

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (Grant BSR-8505988). High resolution ^{1}H-nmr spectra were obtained at the Colorado State University Regional NMR Center funded by NSF Grant CHE8208821 and the Hrfabms at the Midwest Center for Mass Spectrometry, University of Nebraska (NSF Grant CHE8211164). We also thank those cited in the Experimental (S.R. Jensen, B.J. Nielsen, H. Inouye, O. Sticher, R. Wylde, and S. Kobayashi) for standard samples.

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Received 29 September 1986