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# PENSTEMONOSIDE AND OTHER IRIDOIDS FROM *CASTILLEJA RHEXIFOLIA*. CONVERSION OF PENSTEMONOSIDE TO THE PYRIDINE MONOTERPENE ALKALOID RHEXIFOLINE[1]

MARK R. ROBY and FRANK R. STERMITZ*

*Department of Chemistry, Colorado State University, Fort Collins, CO 80523*

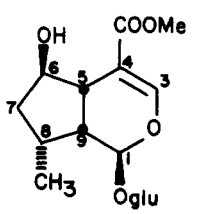
ABSTRACT.—Blossom heads of *Castilleja rhexifolia* yielded the iridiod glucosides penstemonoside, catalpol, and aucubin in approximately equal amounts. Penstemonoside was converted to its aglycone with β-glucosidase, and the aglycone was transformed into the pyridine monoterpene alkaloid rhexifoline by treatment with $NH_3$. The conversion of penstemonoside into rhexifoline corroborates the rhexifoline structure proposed from spectroscopic data and also establishes its absolute stereochemistry.

In the previous paper (1), we reported the isolation and structure characterization of a new pyridine monoterpene alkaloid, rhexifoline, from *Castilleja rhexifolia* Rydb. (Scrophulariaceae). The structure was assigned solely on the basis of spectral data. The present study describes the isolation of the iridiod glucosides penstemonoside, catalpol, and aucubin from the same source and the chemical conversion of penstemonoside to rhexifoline.

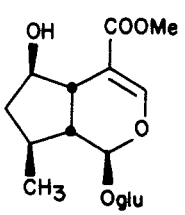
## RESULTS

Extraction of *C. rhexifolia* blossom heads (bracts, flowers, seeds) and subsequent chromatographic separation yielded approximately 0.2% each of penstemonoside, aucubin, and catalpol. Because larvae of the plume moth *Platyptilia pica*, which are hosted by *C. rhexifolia*, are mainly borers that feed on seeds, a comparison was made of iridoid content in seed, leaf, and stem samples. Aucubin and catalpol content was similar in all three, while penstemonoside content was three to four times higher in seeds and leaves than in stems.

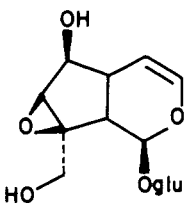
Penstemonoside, **1**, was identified by cmr and 360 MHz pmr spectra in comparison with the literature (2) and by similar data plus the mass spectrum of the pentaacetate. A standard sample of the C-8 epimer, dihydrocornin (**2**), was also available and proved not to be identical (360 MHz pmr spectrum) with the isolated **1**. Standard samples of catalpol (**3**) and aucubin (**4**) were identical with our isolates by tlc and cmr and 360 MHz pmr spectra. The previously unpublished high-field pmr spectra of the four iridoid glucosides and penstemonoside pentaacetate are given in Table 1 with assignments. One comment is of interest in regard to the catalpol spectrum. The unusually large (3) $J_{1,9}$ of 9.8 Hz suggests that these protons are dipseudoaxial, rather than



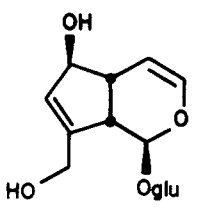

**1**
penstemonoside




**2**
dihydrocornin




**3**
catalpol




**4**
aucubin

[1]Paper 4 in the series "Chemistry of the Scrophulariaceae." For paper 3, see M.R. Roby and F.R. Stermitz (1).

TABLE 1. High-Field Pmr Spectra of Iridoids

| | 1[a] | 1OAc[b] | 2[c] | 3[c] | 4[c] |
|---|---|---|---|---|---|
| H-1 | 5.58, d ($J$=2.5) | 5.35, d (2.7) | 5.47, d (3.0) | 5.02, d (9.8) | 5.19, d (5.0) |
| H-3 | 7.48, d (0.9) | 7.40, d (0.9) | 7.41, d (1.1) | 6.33, dd (6.0, 1.7) | 6.21, dd (6.2, 1.8) |
| H-4 | — | — | — | 5.08, dd (6.0, 4.6) | 5.03, dd (6.2, 3.5) |
| H-5 | 2.88, br.d | 2.88, br.d | 2.82, br.d | 2.25, m | 2.70, m |
| H-6 | 4.23, m | 5.25, m | 4.11, m | 4.00, dd (8.1, 1.0) | 4.45, m |
| H-7d | 1.80, m | 2.29, m | 2.22, quin. | 3.56, br.s | 5.78, m |
| H-7u | 1.50, ddd (14, 9.8, 4.2) | 1.52, m | 1.21, sep. (~14, 8.8, 5.4) | — | — |
| H-8 | 2.58, m | 2.50, m | 1.86, br.quin. | — | — |
| H-9 | 2.71, td (11.7, 9.3, 2.5) | 2.69, td | 2.12, td | 2.58, dd (9.8, 7.7) | 3.06, br.t |
| $CH_3$ | 1.02, d (7.2) | 1.04, d (7.2) | 1.08, d (6.7) | — | — |
| H-10d | — | — | — | 4.21, d (13.2) | 4.26, d (15.2) |
| H-10u | — | — | — | 3.70, d (13.2) | 4.19, d (15.2) |
| $OCH_3$ | 3.75, s | 3.71, s | 3.72, s | — | — |
| H-1′ (glu) | 4.76, d | 4.86, d (8.1) | 4.74, d | 4.81, d (8.0) | 4.70, d (8.0) |
| H-2′ | 3.25, dd (9.3, 8.1) | 4.98, br.t | 3.23, dd (9.2, 8.3) | 3.3 | 3.26, dd (9.3, 8.0) |
| H-3′ | | 5.10, br.t | | | |
| H-4′ | 3.39-3.51 | 5.22, br.t | 3.32-3.5 | 3.25-3.5 | 3.2-3.6 |
| H-5′ | | 3.74, br.t | | | |
| H-6′d | 3.92, dd (12.3, 2.0) | 4.29, dd (12.4, 4.4) | 3.89, dd (12.3, 1.9) | 3.84, br.d | 3.83, dd (12.3, 1.8) |
| H-6′u | 3.72, dd (12.3, 5.7) | 4.17, dd (12.4, 2.4) | 3.69, dd (12.3, 5.8) | 3.66, dd (12.3, 5.5) | 3.65, dd (12.3, 5.8) |
| OAc | | 1.91, 2.01, 2.038, 2.042 2.10 | | | |

[a]360 MHz; $D_2O$, DSS internal standard. The data are also in accord with a 360 MHz nmr provided by O. Sticher.

[b]270 MHz; $CDCl_3$, TMS standard.

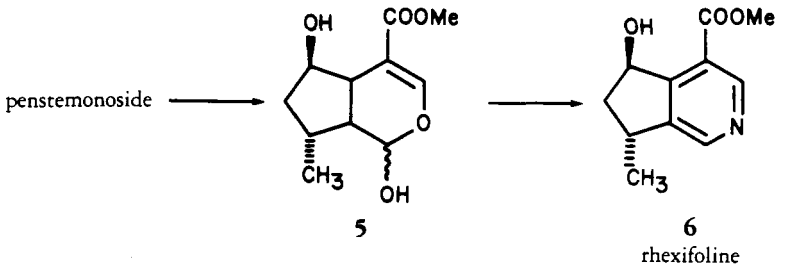
[c]360 MHz; $D_2O$, HDO 4.73 ppm reference (same value as in **1**).

dipseudoequatorial as in penstemonoside, dihydrocornin, and aucubin ($J_{1,9}$=2.5, 3.0, and 5.0 Hz), respectively).

Penstemonoside was cleaved with β-glucosidase to yield a 2:1 epimeric mixture of the aglycone **5** (Scheme 1), which was characterized by mass and 360 MHz pmr spectra. The aglycone epimeric mixture was converted to rhexifoline (**6**) by treatment with methanolic HCl, followed by $NH_3$.

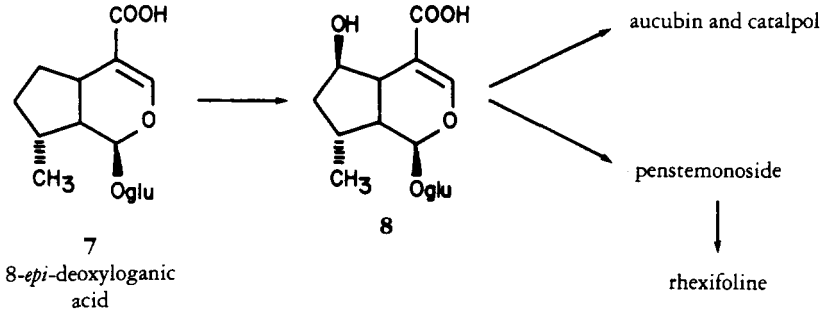
## DISCUSSION

Penstemonoside was recently described (2) from *Penstemon barbatus* and its structure assigned by pmr and cmr. The discovery of penstemonoside in *C. rhexifolia* and its chemical conversion to rhexifoline confirm the relative configuration suggested (1) for rhexifoline and establish its absolute configuration as well. A previous report (4) suggested the presence of catalpol (tlc evidence only) in seeds of *C. rhexifolia*. The inter-




SCHEME 1.

relationship among the three iridiods of *C. rhexifolia* seems clear from recent biosynthetic studies. In several species of the Scrophulariaceae, it was found (5) that 8-*epi*-deoxyloganic acid (**7**) and not 8-deoxyloganic acid was the precursor of aucubin. It was also reported (6) that methylation of the carboxylic acid group blocks the in vivo decarboxylation of 8-*epi*-deoxyloganic acid. Based upon these results, Scheme 2 is likely for the biogenetic pathways in *C. rhexifolia*. Although aucubin and catalpol are undoubtedly the most widespread of all iridiod glucosides (7), the presumed precursor **8** has yet to be reported. Rapid conversion of **8** to **3** and **4** may result in a very small plant pool size of **8**. Alternatively, the increased polarity expected for **8** might simply make it more difficult to isolate, and hence its presence may have been overlooked so far.




SCHEME 2.

## EXPERIMENTAL

General experimental procedures and plant material descriptions were given previously (1).

ISOLATION.—Blossom heads of *C. rhexifolia* (204 g) were percolated cold with EtOH, which was then evaporated to leave a wet residue that was distributed between $CHCl_3$ and $H_2O$. The $H_2O$ layer was evaporated to leave a wet residue (18 g) that was purified by flash chromatography ($Al_2O_3$, EtOH, and $H_2O$). Twelve fractions were taken of which the first nine showed tlc evidence (*p*-anisaldehyde visualization) for iridoids. These were combined, evaporated, and triturated with MeOH to give 9 g of wet residue after MeOH evaporation. This residue was separated by mplc (Si gel, MeOH)) and the iridoid fractions again combined (3.7 g). The iridoid mixture was separated by a second mplc (Si gel, 7:3 $CHCl_3$-MeOH) with the following results: Fraction 2, 17 mg mostly penstemonoside; Fraction 3, 310 mg penstemonoside; Fractions 4 and 5, 15 mg mostly penstemonoside; Fractions 6, 65 mg aucubin; Fraction 7, 749 mg 1:1 aucubin and catalpol; Fraction 8, 910 mg aucubin, catalpol, sugars, pigments. One-half of Fraction 7 was separated on a preparative $Al_2O_3$ plate (7:3:1, *n*-BuOH-$H_2O$-MeOH) to yield 85 mg of aucubin, 92 mg of catalpol, and 210 mg of a mixture.

Equal amounts of dry seeds, stems, and leaves were defatted with hexane and then treated as above to the point of MeOH trituration and evaporation. Residues were diluted equally and tested by tlc ($Al_2O_3$; 7:3:1, *n*-BuOH-$H_2O$-MeOH). Seed content of iridoids (total and relative amount of each) was similar to that of the blossom heads described above. Aucubin and catalpol content did not vary greatly in any of the samples. Penstemonoside content was, however, three to four times higher in the seeds and leaves than in the stems, where it was the minor component.

IDENTIFICATIONS.—*Aucubin*: tlc, cmr, and 360 MHz pmr compared to a standard sample; mp and optical rotation compared to literature. *Catalpol*: tlc, cmr, and 360 MHz pmr spectra compared to a standard sample; mp and optical rotation compared to literature. *Penstemonoside*: A standard sample was unavailable. Our 360 MHz pmr spectrum was identical with one provided of the standard. The cmr spectrum was identical with that reported (2). The ir and uv spectra and optical rotation were also compared with those in the literature. Only the latter showed a discrepancy: $[\alpha]^{24}D$ −120° (c 1.1 MeOH) compared to the literature value: $[\alpha]^{20}D$ −142 (c .55 MeOH) (2). No mp was reported; we obtained a somewhat undefined mp of 74-85°. Pentaacetate mp 158°. Lit. (2) pentaacetate prepared, but no mp reported.

CONVERSION OF PENSTEMONOSIDE TO RHEXIFOLINE.—Penstemonoside (60 mg) was added to 5 mg of almond β-glucosidase (Sigma Chemical Company) in 3 ml pH 5.5 acetate buffer and allowed to stand 24 h at 35°. The reaction mixture was then extracted with $CHCl_3$ (3×4 ml) and the $CHCl_3$ evaporated to yield 20 mg of aglycone **5** epimeric mixture. Mass spectrum *m/z* 210.0902 (21%) calcd. for $C_{11}H_{14}O_4$ 210.0903, ($M^+$-$H_2O$); 360 MHz pmr: β-isomer 1.10 (3H, d, 7.0 Hz, C-$CH_3$), 1.7-2.0 (2H, m, H-7), 2.25 (1H, m, H-8), 2.5-2.8 (2H, m, H-5 and H-9), 3.77 (3H, s, $OCH_3$), 4.27 (1H, m, H-6), 5.48 (1H, d, 3.1, H-1), 7.41 ppm (1H, d, 1.3, H-3); α-isomer 1.12 (3H, d, 7.2 Hz, C-$CH_3$), 1.7-2.0 (2H, m, H-7), 2.25 (1H, m, H-8), 2.5-2.8 (2H, m, H-5 and H-9), 3.76 (3H, s, $OCH_3$), 4.06 (1H, m, H-6), 4.98 (1H, d, 7.9, H-1), 7.43 (1H, d, 1.0, H-3). The aglycone epimeric mixture (10 mg) was dissolved in 1.0 ml of MeOH to which one drop of HCl had been added. After complete dissolution (blue-green color), anhydrous $NH_3$ gas was bubbled through the solution until the color changed to yellow. The solution was evaporated, triturated with $CHCl_3$, and the $CHCl_3$ evaporated. The residue was purified by preparative tlc (silica gel; 9:1, $CHCl_3$-MeOH) to yield 5 mg of rhexifoline identical by 360 MHz pmr spectrum and direction of optical rotation to that isolated from *C. rhexifolia* (1).

## ACKNOWLEDGMENTS

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