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SOME IRIDOID GLUCOSIDES, INCLUDING THE NEW 6-DEOXYCATALPOL, FROM INDIAN PAINTBRUSH SPECIES RELATED TO *CASTILLEJA MINIATA*[1]

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ABSTRACT.—Two red Indian paintbrush species, *Castilleja miniata* and a purported hybrid of *C. miniata* and *C. rhexifolia*, were found to contain very similar iridoid glucosides: aucubin, catalpol, 8-epiloganin, gardoside methyl ester, mussaenoside, and shanzhiside methyl ester, along with the new iridoid 6-deoxycatalpol. The *C. miniata* also contained bartsioside. The purported hybrid of *C. rhexifolia* and *C. miniata* was found to be devoid of alkaloids, although it was previously shown that *C. rhexifolia* contains pyrrolizidines and *C. miniata*, quinolizidines.

One of the most difficult problems in the systematics of *Castilleja* (Scrophulariaceae) is the delimiting of a number of red-bracted taxa that are morphologically intermediate between *Castilleja rhexifolia*, which usually occurs at 10,000 feet or above in the Rocky Mountains, and *Castilleja miniata* Dougl., a species occurring at lower altitudes. The intermediate taxa have been labeled (1) "*C. rhexifolia* aff. *miniata*" or "*C. miniata* aff. *rhexifolia*" and often have been shown (1,2) to have chromosome counts different from each other and/or from *C. rhexifolia* or *C. miniata*. Recent divergence and allopolyploidy have been suggested (1,3) to account for the closeness of these taxa. We have been applying plant alkaloid and iridoid analyses in an attempt to differentiate *Castilleja* populations and also to explore interactions with hosted specialist insects.

In previous work on this group of *Castilleja*, we found *C. rhexifolia* of northern Colorado to be dominated in its alkaloid content by senecionine (an unsaturated macrocyclic ester pyrrolizidine), while its sympatric "*C. rhexifolia* aff. *miniata*" taxon contained a minute trace of senecionine as its only unsaturated pyrrolizidine and was dominated by saturated diester pyrrolizidine alkaloids: sarracine and substituted platynecines (4). *C. miniata* from northwest Wyoming was found to contain only quinolizidine alkaloids (5). Extensive iridoid glucoside analyses (6,7) delimited a group of high-altitude species related to *C. rhexifolia* from more distant relatives, such as *Castilleja integra* and *Castilleja linariifolia*, but did not separate the *C. rhexifolia* group.

In the present work, we describe iridoid analyses of the northwest Wyoming *C. miniata*, as well as iridoid and alkaloid analyses of a related low-altitude taxon occurring in northwest Montana.

RESULTS

The Wyoming *C. miniata* population studied for iridoid content was the same for which we described (5) the alkaloid content. The Montana taxon was intermediate and could be described (L.R. Heckard, University of California, Berkeley, private communication, 1983) as either "*C. rhexifolia* aff. *miniata*" or "*C. miniata* aff. *rhexifolia*." A series of known iridoids was obtained from each, but both also yielded a new iridoid glucoside, 6-deoxycatalpol (**1**).[2] This compound was in particularly high concentration in the Montana taxon, from which material was isolated for the following characterization.

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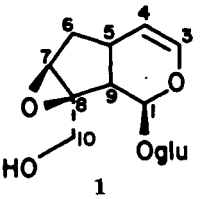
[1]Paper 7 in the series "Chemistry of the Scrophulariaceae." For paper 6, see Harris *et al.* (7).

[2]NOTE ADDED IN PROOF: This iridoid was also recently reported from *Utricularia australis*: S. Damtoft *et al.*, *Phytochemistry*, **24**, 2281 (1985).

High resolution fabms of the isolated foamy substance, $[\alpha]^{24}D=-34°$ (c 0.94 EtOH), established the molecular formula as $C_{15}H_{22}O_9$ (calcd for $C_{15}H_{22}O_9Na^+$:369.116160, found: 369.116156). All 15 carbons were observed in the ^{13}C-nmr spectrum, and their assignments were confirmed by an INEPT experiment. All protons (except four in the sugar region) could be assigned in the 360 MHz ^1H-nmr spectrum after proper decoupling experiments. The data are given in Table 1.

TABLE 1. Nmr Assignments for 6-Deoxycatalpol (**1**)



1

Carbon	^{13}C (CH_3OD)	^1H (D_2O)
1	95.25	5.08 d (9.1 Hz)
3	141.14	6.33 dd (5.8, 1.8)
4	105.93	5.06 dd (5.8, 4.2)
5	32.46	2.47 m
6	36.14	2.33 dd (14.1, 7.9)
		1.56 dd (14.1, 9.9)
7	61.04	3.63 br s
8	69.25	—
9	44.40	2.49 dd (9.1, 7.4)
10	62.15[a]	4.33 d (13.3)
		3.76 d (13.3)
1′	100.20	4.87 d (7.9)
2′	75.06	3.35-3.52 m
3′	78.64[b]	3.35-3.52 m
4′	71.99	3.35-3.52 m
5′	78.03[b]	3.35-3.52 m
6′	63.11[a]	3.89 br d (12.2)
		3.73 dd (12.2, 4.0)

[a,b]Can be interchanged.

The assignments of Table 1 were also made by comparison with the same data (6,8) for catalpol. Nearly all of the ^{13}C- and ^1H-resonances for **1** were identical with those of catalpol, and only specific differences need be emphasized. In the ^{13}C-nmr spectrum, catalpol exhibits a resonance at 78.7 ppm for the C-6 methine carbon. This is missing in the spectrum for **1**, which, instead, has a methylene resonance at 36.14 ppm. This is the significant difference between the ^{13}C spectra. In catalpol, the proton at C-6 appears in the ^1H-nmr spectrum as a doublet of doublets (J=8.1, 1.0) at 4.00 ppm. This resonance is missing in the spectrum of **1**. It is replaced by a doublet of doublets at 2.33 and 1.56 ppm. As in catalpol (6), there is an unusually large $J_{1,9}$ of 9.1 Hz. The C-7 proton in catalpol is a broad singlet at 3.56 ppm, and it is still a singlet (3.63 ppm) in **1**. Models indicate that the dihedral angles between the two C-6 protons and the C-7 proton are identical, and, hence, the otherwise unexpected observance of the C-7 proton as a singlet is explained. This is further confirmed by comparison with the ^1H-nmr spectrum of galiroside (5-hydroxy-6-deoxycatalpol). Here, the C-7 proton was also reported (9) as a broad singlet.

The total iridoid content analyses for the two taxa are given in Table 2. Relative

TABLE 2. Individual Iridoid Content as Percentage of Total Iridoids

Iridoid	Plants				
	"*Castilleja miniata* aff. rhexifolia" (Montana)			*Castilleja miniata* (Wyoming)	
	leaf	stem	inflorescence	leaf and stem	inflorescence
Aucubin	3	27	14	32	40
Bartsioside	—	—	—	4	9
Catalpol	32	41	67	15	47
6-Deoxycatalpol	16	22	12	3	4
8-Epiloganin	15	3	2	12	—
Gardoside methyl ester	7	3	—	6	—
Mussaenoside	14	2	3	5	—
Shanzhiside methyl ester	13	2	2	23	—

amounts were determined by glc of the isolated and subsequently TMS-derivatized mixture.

The quinolizidine alkaloid content of *C. miniata* was previously reported (5). All plant parts of "*C. miniata* aff. *rhexifolia*" from Montana were analyzed for alkaloid content, but no alkaloids were encountered.

DISCUSSION

The total iridoid spectrum of both plants is similar to that of the *C. rhexifolia* group (7) with the exception of the presence of 6-deoxycatalpol in both and the small amount of bartsioside in *C. miniata*. Because this is the first discovery of 6-deoxycatalpol among the nine *Castilleja* species we have now examined and because it is present only in these two, it may represent a marker for *C. miniata* and its related taxa. Of interest also is the low concentration or lack of carbomethoxy-substituted iridoids (the last four entries of Table 2) in stems and inflorescences as compared to leaves, where they are major components. Decarboxylated iridoids (the first four entries) are secondary in the biosynthetic pathway and were found to be the almost exclusive components of stems and inflorescences.

The complete lack of alkaloids in the Montana population is striking and supports an independent species designation for this taxon. A more detailed look at morphological characters is probably in order. The Montana taxon is highly branched (similar to some *C. miniata* and not to *C. rhexifolia*) and is considerably taller than the other species where we have so far encountered them. Because of the highly variable alkaloid content we have now demonstrated among various *Castilleja* (7), we are concerned at the moment with identifying allopatric red-bracted populations whose alkaloid and iridoid content as well as chromosome counts are consistent from area to area. This will help to confirm our suspicion that many of the taxa lumped together under the "*C. miniata* aff. *rhexifolia*" (or vice versa) umbrella are worthy of specific designations. It has been suggested recently that allotetraploid species of the fern genus *Asplenium* have originated more than once, and the implications of these processes in the dynamics of evolution have been discussed (10). The *Castilleja* situation in the western United States may represent a similar example.

The Wyoming *C. miniata* taxon might theoretically obtain quinolizidine alkaloids from a host plant since it, like other *Castilleja* and other members of the tribe Rhinanthoidae-Rhinantheae, is a hemiparasite. We previously considered (5) and rejected this possibility. The closest possible host, *Lupinus argenteus*, contained quinolizidines of a

different nature, and our *C. miniata* collection from the Jackson Lake flood plain was not growing directly on *Lupinus* (5). *Castilleja* host plants are found in a very large number of genera and families (11), some alkaloid-containing and some not. In addition, given *Castilleja* populations seem to have more than one host, and some individuals, particularly those in disturbed road banks (4), do not appear to have any root attachments to other plants. Nevertheless, we are currently assessing possible host-plant-determined effects on the variability of secondary metabolites by investigating the alkaloid content of greenhouse-seeded and -grown taxa.

EXPERIMENTAL

The *C. miniata* collection (Colorado State University Herbarium No. 14184) was described previously (5). The "*C. miniata* aff. *rhexifolia*" was collected on June 21, 1981, at the north side of Noxon Dam on the Big Thompson River west of Thompson Falls, Montana, and was identified by L.R. Heckard. A voucher specimen (FRS 198) was deposited in the Jepson Herbarium, University of California, Berkeley. The following describes a typical isolation procedure.

Dried and ground inflorescences (47 g) of "*C. miniata* aff. *rhexifolia*" were defatted with hexane and twice extracted cold with 1000 ml of MeOH. The MeOH was evaporated to a brown foam, distributed between 100 ml H_2O and 150 ml $CHCl_3$. The layers were separated, the aqueous layer reextracted with $CHCl_3$, and the H_2O evaporated in vacuo to leave a foam. This was triturated in a few ml of MeOH and filtered from insoluble sugars. The resultant crude iridoid mixture was passed through an alumina flash column, and eight fractions were collected, eluting with MeOH. Fractions 1-4 (980 mg) contained mainly iridoids, while fractions 5-8 (560 mg) were mixtures of sugars and iridoids. The 980-mg fraction was chromatographed on Si gel ($CHCl_3$-MeOH, 8:2), and three iridoid-containing fractions were obtained: (a) 12 mg, a mixture of mussaenoside and 8-epiloganin, (b) 96 mg, a mixture of mussaenoside, 8-epiloganin, and 6-deoxycatalpol, and (c) 430 mg, a mixture of catalpol and aucubin. Fraction (b) was rechromatographed on C_{18}-Si gel, eluting with H_2O-MeOH (7:3), and three fractions were obtained. The first contained 21 mg of impure 6-deoxycatalpol (**1**), which was further purified by preparative tlc (Si gel; EtOAc-EtOH-heptane-H_2O, 75:20:15:4. Si gel flash column chromatography ($CHCl_3$-MeOH, 9:1 or 6:4) and plc was also used to obtain samples of mussaenoside, 8-epiloganin,. gardoside methyl ester, shanzhiside methyl ester, aucubin, and catalpol from the above extraction or from leaf or stem isolates. These were identified by tlc, glc, and 270 MHz ^{1}H-nmr spectra in comparison with standards and previously isolated compounds (6,7). Similar procedures were used to separate and identify the iridoids from *C. miniata* (Wyoming). The only additional iridoid, bartsioside, was compared with a standard (12) by glc, tlc, and 360 MHz ^{1}H nmr.

For the quantitative data of Table 2, the crude iridoid mixture (see above) was derivatized with Tri-Sil Z for 20 min at 80°. Quantitative glc analysis was then carried out on a Hewlett-Packard 5890A instrument using a 5% phenylmethyl silicone (DB-5) capillary column (280° isothermally FID detection). The percentages were obtained by comparing the integral for an individual iridoid to the total integrals for all iridoids.

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