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FLAVONOID ANALYSIS OF OENOTHERA SPECIOSA (ONAGRACEAE)

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ABSTRACT—Foliar flavonoids were identified from 20 populations of *Oenothera speciosa* representing a major part of its geographical range, including areas where it is predominantly diploid (n 7) and tetraploid (n = 14). Nine flavonol glycosides and one flavonol sulfate based on kaempferol, quercetin and myricetin were found, six reported in this species for the first time. This is an unusually high number of compounds for a single species of Onagraceae. No geographical or cytological correlation was observed in the occurrence of the flavonol compounds. Two compounds occurred uniformly throughout all of the samples. The remainder occurred sporadically throughout the range of *O. speciosa*.

Oenothera speciosa Nutt. traditionally has been treated as a member of sect. *Hartmannia* (Spach) Endl. (Munz, 1965; Raven and Parnell, 1970). Crossing experiments (Raven, Parnell, and Wagner, in prep.) have shown *O. speciosa* to be the only member of one of four subgroups. *Oenothera speciosa* is also morphologically distinctive in its rhizomatous weedy habit, sharply nodding inflorescences, and the sterile, hollow and cylindrical lower part of the capsule. For these reasons *O. speciosa* is currently treated as a member of the monotypic section *Xylopleurum* (Spach) Endl. Populations of *O. speciosa* from north Texas northward are usually diploid (n = 7) and have flowers that open at sunset, whereas populations from central Texas southward, previously recognized as *O. delessertiniana* Steud. (Munz 1965), are typically tetraploid (n = 14) and have rose purple flowers that open near sunrise (Raven and Parnell 1970). Intensive field studies by Raven and Parnell (1970) suggested that morphological and cytological correlations did not always hold true and that these populations were best treated as a single species, with no further subdivision.

This paper, as part of an extensive overall study of flavonoids in *Oenothera*, reports on the flavonoids of *O. speciosa*. Previous studies of *O. speciosa* include a report of three aglycones (myricetin, quercetin, and kaempferol) by Zinsmeister and Bartl (1971) and a report of four flavonol glycosides, based upon myricetin and quercetin, by Howard, Mabry and Raven (1972). The latter study prsented results from the analyses of two populations of *O. speciosa*, one diploid and one tetraploid. Three of the glycosides, quercetin 3-0-rhamnoside, myricetin 3-0-rhamnoside, and myricetin 3-0-galactoside, were common to each of the populations but the fourth compound, the unusual myricetin 3-methyl ether, 3′-0-glucoside, was present only in the diploid population. The emphasis of our study was to determine whether flavonoid profiles, especially with respect to the presence of the 3′-0-glucoside, might be geographical and thus correlate with the two cytotypes of *O. speciosa*.

TABLE 1—Distribution of ten flavonol glycosides in 20 populations of *Oenothera speciosa*. K = kaempferol, Q = quercetin, M = myricetin, glu = glucose, gal = galactose, ara = arabinoise, + = present, 0 = not detected. Vouchers at MO; collectors are as follows: A = Averett; B & R = Berry & Rodriguez; N = Nixon; R = Rowell; S = Shaw; W = Webster.

Sample Site and voucher	1. K-3-0-glu	2. Q-3-0-rha	3. Q-3-0-ara	4. M-3-0-gal	5. M-3-0-rha	6. M-3-0-ara	7. Q-3, 7-diglucuronide	8. M-3, 7-diglucuronide	9. M-3-OCH_3, 3'-0-glu	10. Q-3-0-sulfate
OKLAHOMA:										
Bryon Co. (*A 1121*)	+	+	0	+	+	0	0	0	+	0
Ottawa Co. (*A 1118*)	+	+	0	+	+	0	0	0	+	0
Ottawa Co. (*A 1119*)	+	+	0	0	+	0	0	0	0	0
TEXAS:										
Bastrop Co (*S 2120*)	+	+	+	+	+	+	+	0	+	0
Bee Co. (*B&R 2278*)	+	+	0	+	+	+	+	0	+	+
Brazos Co. (*S&W 2112*)	+	0	+	+	+	0	+	+	+	+
Brazos Co. (*S&W 2132*)	+	+	+	+	+	+	+	+	+	+
Brazos Co. (*S&W 2124*)	+	+	0	+	+	+	+	0	0	+
Coke Co. (*R 16232*)	+	+	0	+	+	+	+	0	+	+
Comal Co. (*S 2112*)	+	+	+	+	+	+	+	0	+	+
Comal Co. (*S 2105*)	+	+	+	+	+	+	+	0	+	0
Grimes Co. (*S&W 2127*)	+	+	+	+	+	+	+	+	+	+
Hays Co. (*S 2119*)	+	+	+	+	+	+	+	+	+	+
Hays Co. (*S 2116*)	+	+	+	+	+	+	+	+	+	+
Hays Co. (*S 2114*)	+	+	0	+	+	+	0	0	+	+
Hays Co. (*S 2101*)	+	+	0	0	+	+	0	0	0	+
Rusk Co. (*N 9455*)	+	+	+	+	+	+	+	+	+	+
Rusk Co. (*N 9456*)	+	+	+	+	+	+	0	+	0	+
Rusk Co. (*N 9457*)	+	+	+	+	+	+	+	+	0	0
Rusk Co. (*N 9458*)	+	+	+	+	+	+	+	0	+	+
% occurrence of compounds	100	95	60	85	100	80	70	40	75	70

METHODS—Dried leaf material from 20 populations of *Oenothera speciosa* was examined for flavonoids. Populations from the general range of both diploid and tetraploid cytotypes were examined. Leaf material was extracted overnight with 85% aqueous methanol, the resulting extract examined by two-dimensional paper chromatography. Some extracts were examined also by TLC (polyamid and cellulose). In some cases, flavonoids were crudely separated prior to paper chromatography by column chromatography on Sephaex LH 20 with methanol as described by Hiermann, Exner and Averett (1978). For structural elucidation, 10-15 replicate chromatograms were run and the isolated compounds cut from the paper for further purification and analysis. The approximately quantity of leaf material used was 0.5-1.0 g for general screening, 5-10 g for replicate chromatograms, and 20-30 g for column chromatography. Identification of the glycosides, their aglycones, and sugars was done as previously described (Averett et al., 1978; 1979). Glucuronides were identified according to procedures outlined by Markham (1982). In addition, most of the aglycones, sugars and authentic reference compounds were run by circular thin-layer chromatography as described by Exner et al. (1977).

RESULTS AND DISCUSSION—Ten flavonols were identified from the 20 populations of *Oenothera speciosa*, six (with asterisks) for the first time in the species: 1) kaempferol 3-0-glucoside*, 2) quercetin 3-0-rhamnoside*, 3) quercetin 3-0-arabinoside, 4) myricetin 3-0-galactoside, 5) myricetin 3-0-

rhamnoside, 6) myricetin 3-0-arabinoside*, 7) quercetin 3, 7-diglucuronide*, 8) myricetin 3, 7-diglucuronide*, 9) myricetin 3-methyl ether 3′-0-glucoside, and 10) quercetin 3-0-sulfate*. Compounds 2,4,5, and 9 were those reported by Howard et al. (1972). The remainder are previously unreported for *O. speciosa* but, except for the diglucuronides, are known elsewhere in the genus (Averett, Huang, and Wagner unpubl.). Compounds 1 and 5 occur in all of the populations and compound 2 is absent from only one. The remainder were present in some populations and absent in others without any noticeable geographical correlation. The distribution of the compounds among the 20 populations examined is given in Table 1.

Only flavonols, one of the three classes of foliar flavonoid compounds reported for the family Onagraceae, were found in *Oenothera speciosa*. It is notable, however, that the largest number of flavonol compounds known from a single species of Onagraceae occurs in *O. speciosa*, and that 37% of the flavonol compounds reported for the family by Averett and Raven (1984) occur in this species. Flavonols, the most ubiquitous class of flavonoids in the family, occur in all seven tribes (Averett and Raven 1984). Thus, the interesting feature here is primarily the number of compounds present in this relatively specialized monotypic section. The unusual flavonol, myricetin 3-methyl ether, 3′-0-glucoside, first reported by Howard, et al. (1972) from one diploid population of *O. speciosa*, occurred in 75% of the populations of *O. speciosa* sampled. These samples represent much of the native range of the species and the Texas samples are from the part of the range where tetraploids predominate. Thus there does not appear to be any geographical or cytological correlation with the occurrence of this unusual flavonol. The compound occurs, in addition to *O. speciosa*, in the related sections *Hartmannia*, *Kneiffia* (Spach) Endl., and *O. canescens* Torr. & Frém. of sec. *Gauropsis* (Torr. & Frém.) W. L. Wagner (Averett, Huang, and Wagner unpubl.) but is not known to be present in any other group of plants.

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