FLAVONOIDS OF *MARAH OREGANUS*

K. W. NICHOLLS and B. A. BOHM*

Botany Department, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

ABSTRACT.—The flavonoids of *Marah oreganus* (Cucurbitaceae) comprise kaempferol and quercetin 3-O-glucosides, 3,7-di-O-glucosides, 3-O-glucoside-7-O-rhamnosides, 3-O-diglucosides, and triglycosides with substitution at both positions 3 and 7. The full structures of these latter compounds were not established. Four samples of *Marah* from widely spaced collections in its range were chromatographically identical. This represents the first detailed report of flavonoids from a member of the tribe Cyclanthereae (*sensu* Jeffrey).

The North and Central American genus *Marah* Kell. contains six species (1) of which *M. oreganus* (T. & G.) Howell is by far the most common and widespread representative. It occurs west of the Cascades from Vancouver Island south to coastal California; inland it may be found along the Snake River into eastern Oregon (2). As part of a general survey of flavonoid chemistry of plants of western North America we undertook a study of *M. oreganus*.

The closest relative of *Marah* appears to be *Echinocystis* (1). Hutchinson (3) places both genera in the Sicyoideae whereas Jeffrey (4) treats them as members of the tribe Cyclanthereae.

The Cucurbitaceae, with perhaps 90 genera and 700 species (5), has received comparatively little attention from flavonoid chemists. The economically important melon *Cucumis melo* (tribe Melotrieae) was found to contain apigenin and luteolin 6-C-diglucosides (6) while Saunders and McClure (7) isolated orientin, a luteolin 7-O-diglycoside, and quercetin 3-O-mono- and diglycosides from *C. maxima*. The flowers of pumpkin, *Cucurbita pepo*, have been shown by Itokawa and coworkers (8) to accumulate rhamnazin 3-O-rutinoside, isorhamnetin 3-O-rutinoside, and isorhamnetin 3-O-rutinoside-4'-O-rhamnoside. *Luffa echinata* (tribe Beninceae) accumulates chrysoeriol and its 7-O-glucoside and 7-O-apioglucoside (9). In a recent report of flavonoids of all members of the genus *Luffa*, Schilling (10) described two groups of species: *L. graveolens* and *L. operculata* are characterized by flavonol 3-O-glucosides while *L. acutangula*, *L. aegyptica*, and *L. echinata* are characterized by flavone 7-O-glucosides. Furthermore, Schilling (10) noted that each species exhibits a unique flavonoid profile. Kaempferol 3,7-di-O-rhamnoside. (kaempferitin) was isolated from *Trichosanthes cucumeroides* (tribe Trichosantheae) by Nakaoki and Morita (11), and Hegnauer (12) reports the presence of kaempferol and quercetin in *Echinocystis wrightii* (tribe Cyclanthereae). This present work is the first report of flavonoids of *Marah*; indeed, it is the first detailed report of flavonoids in the tribe Cyclanthereae (*sensu* Jeffrey).

RESULTS

The flavonoids of *Marah oreganus* consist of a series of kaempferol and quercetin glycosides. Both compounds occur as 3-O-glucosides, 3,7-di-O-glucosides, 3-O-glucoside-7-O-rhamnosides, 3-O-diglucosides, and as 3,7-substituted triglycosides. Partial hydrolysis of the triglycosides yielded the corresponding flavonol 3,7-di-O-glucosides and rhamnose. The position of attachment of the rhamnose group was not determined owing to limited material. The mono- and diglycosides dominated the flavonoid profile. Yields were not determined. Four specimens of *M. oreganus* were examined in this study, one in detail and three by 2D thin layer chromatography. The patterns in all cases were identical.[1]

[1]Two specimens of *Marah fabaceus* (Naud.) Greene (BAB–1623 from Alameda Co., and BAB–1627 from San Mateo Co., California) have also been analyzed for flavonoids. Kaempferol and quercetin were found as the 3-O-glucosides, 3-O-rutinosides, 3-O-rutinoside-7-O-glucosides, and, in trace amounts, as 3,7-triglycosides which gave glucose and xylose on acid hydrolysis. Flavonoid patterns from the two collections were identical.

MATERIALS AND METHODS

PLANT MATERIAL.—One bulk collection was made from the field: seventy-seven grams (dry weight) of material was collected from Patrick's Point State Park, Humboldt Co., California (*Bohm & Nicholls 1470*). Small samples (0.10 g) were taken from herbarium specimens: Trinity National Forest, Trinity Co., California, (*Sharsmith 4379*); Saddle Mountain, Clatsop Co., Oregon, (*Bohm & Schofield 1317*); and Fulford Harbour, Saltspring Island, B.C., (*Ashlee s.n., 1958*). All vouchers are in UBC.

EXTRACTION AND SEPARATION OF FLAVONOIDS.—The bulk sample of dried leaves was extracted repeatedly with hot 80% methanol until the leaves were cleared of pigment. Several solvent changes were needed. The pooled extract was evaporated to dryness *in vacuo*, the residue was slurried with hot water and diatomaceous earth filter aid, and filtered with the aid of suction. The aqueous extract was exhaustively extracted with water-saturated *n*-butanol. The *n*-butanol extract was evaporated to dryness and the residue was taken up in a small volume of methanol. This was used for the 2D thin layer chromatography on a 20 x 20 cm plate of Polyamid DC6.6. Development in the first direction employed water-*n*-butanol-acetone-dioxane (70:15:10:5) while a mixture of benzene-methanol-methylethyl ketone-water (55:20:22:3) was used for the second direction. To visualize the flavonoids, the plates were sprayed with a 0.1% solution of diphenylboric acid ethanolamine complex (Aldrich Chem. Co.) in 1:1 methanol-water followed by observation under uv light (366 nm) before and after fuming with ammonia. Full color development required 15 to 30 minutes after which the individual specimens were scored for flavonoids. The herbarium specimens were extracted in a small volume of 80% methanol, the solution was concentrated to dryness, and the residue was taken up in a few drops of methanol and applied to Polyamid plates and developed as before.

Separation of individual flavonoids for structural studies was accomplished by a combination of column chromatographic steps. First, Sephadex LH-20 (Pharmacia), using methanol-water mixtures, was employed to yield flavonoid glycoside classes. Partition chromatography on microcrystalline cellulose (Avicel) with water as the stationary phase was used to separate the constituent glycosides of the monoglycoside fraction (elution with increasing amounts of ethyl acetate in petroleum ether). The diglycoside and triglycoside fractions were fractionated similarly except that water-saturated ethyl acetate with increasing amounts of methylethyl ketone was used to resolve the diglycosides, and water-saturated methylethyl ketone was used to resolve the triglycoside fraction. Final purifications were accomplished by tlc and solvent systems described by Wilkins and Bohm (13).

STRUCTURAL DETERMINATIONS.—Flavonoid structures were determined by standard methods of uv and pmr spectroscopy (14), partial and total hydrolysis using trifluoroacetic acid, and tlc against known compounds when available. All standards used had been established by pmr spectroscopy. Uv spectra were determined on a Unicam SP8-100 instrument; pmr spectra were taken on a Varian EM-390 instrument.

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