FLAVONOIDS OF *ERYTHROXYLUM RUFUM* AND *ERYTHROXYLUM ULEI*

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ABSTRACT.—The total flavonoid complements of *Erythroxylum rufum* and *E. ulei* have been determined. *Erythroxylum rufum* contains kaempferol and quercetin-3-*O*-mono- and diglycosides and ombuin-3-*O*-rhamnosylglucoside. Ombuin is quercetin-7,4'-dimethyl ether and has been found in other *Erythroxylum* species. *Erythroxylum ulei* makes kaempferol, quercetin, and myricetin 3-*O*-glycosides, the gallic acid conjugates of the glucosides, naringenin-7-*O*-glucoside and, in cultivated material, dihydroquercetin. Four specimens of *E. rufum* were examined and found to be identical, while different patterns of flavonoids were seen in different collections of *E. ulei*.

Recent interest in the chemistry of *Erythroxylum* (Erythroxylaceae) has centered on alkaloidal constituents of a number of species (1,2,3,4). A review of earlier literature including alkaloids, essential oils, and polyphenolic compounds was presented by Hegnauer (5). Botanical and ethnobotanical descriptions of "coca" by Plowman (6,7) have appeared, and a recent paper by Duke and coworkers (8) reported studies of the nutritional value of coca as used by indigenous peoples of South America.

Although some attention has been paid to the flavonoid chemistry of *Erythroxylum*, no systematic studies of the genus have been published. Paris and Delaveau (9) identified rutin and quercetin-3-*O*-glucoside from *E. coca* var. *novagranatense* Morris and *E. coca* var. *spruceanum* Burck, while Bate-Smith (10) found quercetin, kaempferol, and cyanidin in hydrolyzed extracts of *E. coca* (no variety mentioned). Flavonoids have been detected in *E. monogynum* Roxb. and in several species from Madagascar (12). In a recent study by Bohm and coworkers (13) the total flavonoids of *E. coca* var. *coca*, *E. novogranatense* var. *novogranatense* and *E. novogranatense* var. *truxillense* were studied as part of a biosystematic examination of these ethnobotanically important taxa. Kaempferol and quercetin-3-*O*-mono- and diglycosides occurred in all taxa, although the distribution of specific glycosides varied. Traces of triglycosides were also seen occasionally. The rare quercetin-7,4'-d-i-*O*-methyl ether (ombuin) 3-*O*-rutinoside was found in both varieties of *E. novogranatense*.

Additional species of *Erythroxylum* have been made available to us through the collections of Dr. Timothy Plowman and colleagues. This paper describes the flavonoid chemistry of two of these: *E. rufum* Cav. and *E. ulei* O. E. Schulz.

RESULTS

The flavonoids of *E. rufum* consist of a series of 3-*O*-glycosides of kaempferol, quercetin, and ombuin. Kaempferol and quercetin exist as a mixture of monoglycosides comprising glucosides, galactosides, arabinosides, xylosides, and rhamnosides, and as rhamnosylglucosides that are chromatographically indistinguishable from the rutinosides. Ombuin occurred as a 3-*O*-rhamnosylglucoside that was identical in all respects with the 3-*O*-rutinoside isolated originally from *E. novogranatense* (13).

Four specimens of *E. rufum* were examined in this study; all showed identical patterns of flavonoid spots by thin layer chromatography. We observed similar homogeneity of flavonoid pattern in the three taxa examined in our earlier study (13).

Erythroxylum ulei elaborates a more complex array of flavonol derivatives than seen in *E. rufum* and can be further distinguished from the latter by the accumulation of a flavanone and, under certain conditions, a dihydroflavonol.

The monoglycosides identified were kaempferol, quercetin, and myricetin-3-*O*-glucosides, their corresponding gallic acid conjugates, kaempferol and quercetin-3-*O*-arabinosides, xylosides, and rhamnosides, and the 7-*O*-glucoside of the flavanone naringenin. The position of attachment of the gallic acid residue on the flavonol glucosides was not firmly established, but the compounds had the same thin layer chromatographic and color behavior as the 6″-gallyl derivatives of the flavonol glucosides seen in *Tellima grandiflora* by Collins and coworkers (14) and in *Heuchera* species by Wilkins and Bohm (15) and Wells and Bohm (16). Kaempferol, quercetin, and ombuin-3-*O*-rhamnosylglucosides (probably the rutinosides) occurred along with a small amount of a quercetin-3-*O*-diglucoside whose structure was not pursued. Also present in cultivated material of *E. ulei* was a substantial amount of dihydroquercetin that appeared only as the aglycone.

Four different collections of *E. ulei* were examined in this study. Collections 3754 and 5653 exhibited identical flavonoid patterns and yielded all of the compounds described in the preceeding paragraph except for dihydroquercetin. Chromatograms of material of collections 4846 and 5960 were also identical to each other but showed a simpler pattern than that seen in the other samples. Noteworthy was the absence of myricetin, gallylated glucosides of all three flavonols, and quercetin-3-*O*-diglucoside. A further source of variation was observed when the flavonoids of freshly harvested leaves of *E. ulei*, collection No. 4846, were examined. The pattern of flavonoids was even simpler than that of the dried specimen of this number: quercetin and kaempferol arabinosides and xylosides were absent, but a compound identified as dihydroquercetin was present in relatively large amounts. Since 60 g (fresh weight) of collection 4846 was examined, compared to about 1.5 g of the dry sample, the absence of the pentosides appears to be real. Clearly, cultivation of this specimen of *E. ulei* had a significant effect upon the organism's flavonoid synthetic capacities.

Although the sampling of *E. ulei* from nature is limited, it is clear that no simple pattern of flavonoid distribution exists. Collections 4846 and 5960, the chemically more impoverished specimens, originated from sites in central Peru, while collection 3754 came from a site in southern Peru, and collection 5653 originated from central Colombia. Wider samplings would be desirable as would a better understanding of the influence of growth conditions on the biosynthesis

TABLE 1. Distribution of flavonoids in *Erythroxylum rufum* and *Erythroxylum ulei*.[a]

Species / Flavonoid	*E. rufum* 1399, 4767 4768, 8210	*E. ulei* (*wild*) 3754 5653	*E. ulei* (*wild*) 4846 5960	*E. ulei* (Cult.) 4846
Kaempferol-3-*O*-glucoside	+	+	+	+
-galactoside	+	+	+	+
-arabinoside	+	+	+	−
-xyloside	+	+	+	−
-rhamnoside	+	+	+	+
-glucoside-X″-gallate	−	+	−	−
Quercetin-3-*O*-glucoside	+	+	+	+
-galactoside	+	+	+	+
-arabinoside	+	+	+	−
-xyloside	+	+	+	−
-rhamnoside	+	+	+	+
-glucoside-X″-gallate	−	+	−	−
Myricetin-3-*O*-glucoside	−	+	−	−
-glucoside-X″-gallate	−	+	−	−
Kaempferol-3-*O*-rhamnosylglucoside	+	+	+	+
Quercetin-3-*O*-rhamnosylglucoside	+	+	+	+
Ombuin-3-*O*-rhamnosylglucoside	+	+	+	+
Quercetin-3-*O*-glucosylglucoside	−	+	−	−
Naringenin-7-*O*-glucoside	−	+	+	−
Dihydroquercetin	−	−	−	+

[a]Numbers refer to collection numbers, see table 2. (+) means present; (−) means absent.

TABLE 2. Sources of Plant Material.[a]

Erythroxylum rufum Cav.

T. Plowman and E. E. Davis, Nos. 4767, 4768: PERU, Dept. Cuzco, Prov. La Convencion, 1975.
R. T. Martin, No. 1399: PERU, Dept. Cuzco, Prov. La Convencion, 1966.
T. Plowman, G. Davidse, N. A. Rosa, C. S. Rosario and M. R. dos Santos, No. 8210: BRAZIL, State of Goiás, Municipio Presidente Kennedy, 1980.

Erythroxylum ulei O. E. Schulz

T. Plowman, R. Jaramillo and L. Jacobs, No. 3754: COLOMBIA, Dept. Tolima, 1976.
T. Plowman and E. W. Davis, No. 4846: PERU, Dept. Cuzco, Prov. La Convencion, 1975.
T. Plowman and H. Kennedy, No. 5653: PERU, Dept. Junin, Prov. Tarma, 1976.
T. Plowman, No. 5960: PERU: Dept. Huanuco, Prov. Leoncio Prado, 1976.

[a]Voucher specimens at ECON.

of flavonoids in this species.

The distribution of flavonoids observed in this study is summarized in table 1.

MATERIAL AND METHODS

PLANT MATERIAL.—Plant material used in this study was obtained from Dr. Timothy Plowman (Field Museum of Natural History, Chicago). Collection data are presented in table 2. In all cases ca. 1.5 g of dried leaves were available for analysis. In the case of *E. rufum*, where thin layer chromatographic patterns were identical, the four samples were combined for the separation steps. The matching specimens of *E. ulei* were also combined. Fresh leaves of *E. ulei* amounting to 60 g were harvested from a plant cultivated in the University glasshouse.

EXTRACTION AND SEPARATION OF FLAVONOIDS.—Dried leaf samples were extracted repeatedly with hot 80% methanol until the leaves were cleared of pigmentation. Several changes of solvent were used. Fresh leaves were extracted with hot 100% methanol. Extracts were evaporated to dryness *in vacuo*; the residue was slurried with hot water and a diatomaceous earth filter aid and filtered with the aid of suction. These aqueous extracts were exhaustively extracted with water saturated *n*-butanol. The butanol extracts were evaporated to dryness, and the residues were taken up in a small volume of methanol. These extracts were used for two dimensional thin layer chromatography on 20 x 20 cm. plates of Polyamid 6.6. Development in the first direction was accomplished with water-n-butanol-acetone-dioxane (70:15:10:5), while benzene-methanol-methyl-ethyl ketone-water (55:20:22:3) was used for the second direction. To visualize the flavonoids, the plates were sprayed with 0.1% diphenylboric acid ethanolamine complex (Aldrich Chemical Co.) in 1:1 methanol-water and observed under ultraviolet light (366 nm) before and after fuming with ammonia. Full color development required 15 to 30 minutes, after which time the individual specimens were scored for flavonoids.

Separation of individual flavonoids was accomplished by a combination of column chromatographic steps. First, Sephadex LH-20 with methanol-water mixtures yielded flavonoid glycoside classes; partition by chromatography on microcrystalline cellulose (Avicel) with petroleum ether with increasing amounts of ethyl acetate (for monoglycosides) and ethyl acetate with increasing amounts of methylethyl ketone (for diglycosides) followed. The stationary phase was water. Final purifications were accomplished by thin layer chromatography on Polyamid 6.6 with solvents described by Wilkins and Bohm (15).

STRUCTURAL DETERMINATIONS.—Flavonoid structures were determined by standard methods of ultraviolet spectroscopy (17), partial and total hydrolyses with trifluoroacetic acid, and thin layer chromatography performed with compounds from our earlier study (13) as standards. Chromatographic conditions were those of Wilkins and Bohm (13). In the case of the gallylated flavonol glycosides, the presence of gallic acid was confirmed by chromatography against a standard; the following reagents were used for detection: ferric chloride; Gibbs reagent oversprayed with saturated sodium bicarbonate; saturated potassium iodate and heat. The structure of dihydroquercetin was established by proton magnetic resonance (Varian EM-390). In all cases observed spectroscopic data agreed with published spectra (17). Yields of flavonoid glycosides were not determined.

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ERRATA

Mikhail D. Antoun, David Abramson, Richard L. Tyson, Ching-jer Chang, Jerry L. McLaughlin, Garnet Peck and John M. Cassady: Potential Antitumor Agents. XVII. Physalin B and 25,26-Epidihydrophysalin C from *Witheringia coccoloboides*. Vol. **44**, No. 5, pp 579–585.

The second paragraph on p581 should read as follows:

The ms of physalin B showed a strong molecular ion at 510. The ms of compound **2**, on the other hand, showed a very weak molecular ion at 512 and a strong peak at 494 (M^+–18). High resolution ms of this peak at 494.191 (M^+–18, calculated for $C_{28}H_{30}O_8$, 494.194) indicated, together with elemental analysis, a molecular formula of $C_{28}H_{32}O_9$ for compound **2**.

Manuel F. Balandrin and A. Douglas Kinghorn: Characterization of Sweetinine, a Constituent of *Sweetia elegans*, as the *Ormosia* Alkaloid, (±)-6-Epipodopetaline. Vol. **44**, No. 5, pp 619–621.

The ir and pmr (270 MHz) data for homo-sweetinine on p 621 should be as follows:

ir ν max ($CDCl_3$) 2800, 2760 cm^{-1} (trans-bands); pmr (270 MH_2, $CDCl_3$) δ3.43 (2H, s, H_2-24), 3.95 (dd, $J_{10e,10a} = -11.4$, $^4J_{10e,8} = 1.9$, H–10 eq), 5.27 (bd, $^3J_{17,7} = 6.0$, H–17).