Flavonoids as Chemotaxonomic Markers for *Erythroxylum ulei*

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Erythroxylum ulei (*E. ulei*), Baptigenin, Genistein, Orobol, Isoflavone

Leaf extracts of *Erythroxylum ulei* O. E. Schulz, produced six *O*-conjugated flavonoids. Three of the flavonoid aglycones were isoflavones, two were isoflavanones and the remainder a flavonol (quercetin). The major glycosides of these flavonols included mono- and dirhamnosides, mono-glucosides and galactosides with either 3', 4', or 7 linkage or a combination thereof. The two isoflavanones, in addition to being glycosylated, possessed a methyl group at position 2 of the **C**-ring. These flavonoids may be used as chemotaxonomic markers for the taxon.

Introduction

Research by early investigators showing the presence of flavonoids in leaves of *Erythroxylum* taxa was summarized by Hegnauer (1981). Thus, flavonoids in leaves and/or various tissues of *Erythroxylum monogynum* Roxb. (Chopra and Ghosh, 1938), *E. capitatum* Baker, *E. corymbosum* Boivin, *E. ferrugineum* Cav., *E. spaeranthum* H. Perr, *E. xerophilum* H. Perr (Bosser and Pernet, 1957; Madagascar species), *Erythroxylum novogranatense* var. *novogranatense* (Morris) Hieron, *Erythroxylum coca* var. *spruceanum* Burck (Paris and Delaveau, 1963) and of *E. coca* (Bate-Smith, 1962) were reported to contain quercetin, kaempferol and quercetin with 3-rutinoside (rutin), 3-glycoside (isoquercetin), and 3-rhamnoside (quercitrin) flavonoids.

Later, Bohm *et al.* (1981) characterized the flavonoid complements of *E. rufum* Cav. and *E. ulei* O. E. Schulz, and concluded that the former taxon contained kaempferol, quercetin-3-O-mono-and -diglycosides and ombuin-3-O-rhamnosylglucoside (7,4'-dimethyl ether), whereas the latter contained kaempferol, quercetin, myricetin 3-O-glycosides and the gallic acid conjugates of the glycosides, naringenin-7-O-glucoside and in fostered tissue, dihydroquercetin. Subsequently, as part of a biosystematic study of cultivated taxa of *Erythroxylum*, Bohm *et al.* (1982) described the flavonoids in *Erythroxylum coca.* var. *coca* Lam (*E. c.* var. *coca*), *E. novogranatense* var. *novogranatense* (*E. n.* var. *novo.*), *Erythroxylum coca* var. *ipadu* Plowman (*E. c.* var. *ipadu*) and *Erythroxylum novogranatense* var. *truxillense* (Rusby) Plowman (*E. n.* var. *truxillense*) and concluded that kaempferol and quercetin 3-O-mono and diglycosides occurred in all taxa, although with variations in glycoside distribution. In a later study, Bohm *et al.* (1988) examined the flavonoids in 13 species of *Erythroxylum* from Brazil and inferred that all species exhibited profiles predicated on flavonol glycosides, with variations in a number of compounds present in profiles of the taxa. In related studies of *Erythroxylum* flavonoids, Raul *et al.* (1985) characterized the flavonoids in *E. argentinum* O. E. Schulz, and showed that the major flavonoids therein were quercetin with 3-rutinoside or 3-rutinoside-5-glucoside and ombuin-3-rutinoside-5-glucoside (its first reported occurrence in nature). Bonefeld *et al.* (1986) characterized flavin-3-ols and additional flavonols in stem tissue of *E. n.* var. *novogranatense* while Chàvez *et al.* (1996) reported the presence of several flavonoids in leaf extracts *E. leal costae* Plowman, where two new flavone glycosides (8-hydroxyluteolin-8-rhamnoside and 6-hydroxyluteolin-6-rhamnoside) were found.

Recently, Johnson *et al.* (1997; 1998) characterized the flavonoids in polar leaf extracts of the four cultivated taxa of *Erythroxylum* (*E. c.* var. *coca* Lam, *E. c.* var. *ipadu* Plowman, *E. n.* var. *novogranatense*, *E. n.* var. *truxillense* [Rusby] Plowman). We concluded that because of the

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uniqueness of the flavonoids within leaf tissue of each taxon they could be used unambiguously as chemotaxonomic markers for the taxa. As a continuation of our research to show the usefulness of leaf flavonoids as chemotaxonomic markers, the current research reevaluates and characterizes flavonoids present in the polar extract of leaves of *E. ulei* to establish their similarity and/or difference from those presence in the four cultivated taxa of *Erythroxylaceae* previously distinguished and to delineate their usefulness as markers for the taxon.

Materials and Methods

Plant material

Erythroxylum ulei O. E. Schulz (*E. ulei*), leaves (0.05 kg) were harvested from plants grown in the greenhouses at Beltsville, MD, USA, during late summer and fall of 1997. Plants were grown under greenhouse conditions as described by Johnson and Foy (1996) for 2.5 years before leaf harvest. Plants were authenticated by Dr. P. M. Rury, and a voucher specimen was stored in the Weed Science Laboratory at Beltsville Agricultural Research Center, Beltsville, MD, USA, while the living collection is maintained in the greenhouse at Beltsville, MD. Harvested leaves were oven dried (circular air oven at 40 °C) then placed in labeled plastic bags containing four Drierite desiccant bags (30 g/bag; W. A. Hammond Drierite Co., Xenia OH, USA) to ensure that they remained anhydrous during storage. *E. ulei* leaves were analyzed to determine the flavonoid profile as described below.

Isolation of leaf flavonoids

Flavonoids were extracted from oven dried leaves of *E. ulei* as described by Johnson *et al.* (1997). Briefly, two batches (0.025 kg/batch) of *E. ulei* leaves were separately homogenized (anhydrously) in a Waring Blender for 30 sec and placed in two glass beakers containing *ca* 80 ml of 72% aqueous MeOH. The beakers were capped and stored overnight (in darkness) at 21 °C for flavonoid extraction. On the following day the crude extract was filtered through four layers of cheese cloth and the leaf homogenate extracted a second and third time each with 45 ml of 72% MeOH (*ca* 30 min). The extracted fractions were combined, reduced *en vacuo* (45 °C) to *ca* 5 ml, and 25 ml of HPLC grade water added. The flask was gently agitated for 2 min, the concentrate (hue, greenish gray) was decanted, centrifuged at 20,000×*g* for 30 min (4 °C) and the supernatant collected. To the pellet was added 5 ml of HPLC grade H_2O and after re-suspending (*via* vortices), the centrifugation step was repeated two additional times, adding 5 ml HPLC grade water on each repeat. The resultant supernatants after centrifuging were combined, decanted into labeled round bottom flasks and dried *en vacuo* as above. This yielded 2.50 g of residue containing the *E. ulei* flavonoid fraction, which was dissolved in 20 ml of 1% HOAc (aq). This extract was then loaded, in 5 ml fractions, onto a Supelclean ENVI-18, 1 g, SPE Tube (Supelco, Inc., Bellefonte, PA) and subjected to clean-up procedures as follows. After successive washes with 1% HOAc (6 ml), 5% MeOH (4 ml) and 20% MeOH (4 ml) the flavonoids were eluted with 70% MeOH (6 ml). This fraction was then reduced *en vacuo* (45 °C) to dryness, redissolved in 10 ml 1% HOAc (aq), filtered through a 0.2 μm PTFE Whatman filter affixed to a 10 ml syringe (Whatman Laboratory Division, Clinton, NJ, USA), placed into a 15 ml screw cap vial and stored at 4 °C.

HPLC resolution

One ml of the stored flavonoid fraction (above) was transferred to a 1.5 ml amber HPLC autosample vial. The vial was sealed and placed into the autosample carrier of a Hewlett-Packard (H-P) 1090M Liquid Chromatograph equipped with ChemStation, Diode Array detector, Chem-Library (Hewlett-Packard, Avondale PA, USA) and with a Gilson FC 204 fraction collector (Gilson Inc., Middleton, WI, USA) attached to the outlet port of the HPLC. A 100 μl sample was injected onto a Phenomenex Columbus C-8, 25 cm × 10 mm (i.d) 5μ silica/spherisorb semi-prep/ analytical column (Phenomenex, Torrance, CA, USA) for flavonoid separation. The HPLC conditions were: Program: Linear stepwise gradient: Mobile Phase: Solvent A: 100% HPLC grade H_2O: Solvent B: MeOH:HOAc:H_2O (90:5:5): Flow Rate 2 ml/min: Detection: DAD UV at λ_{min}^{MeOH} 230 nm – λ_{max}^{MeOH} 470 nm: Run time 105 min (0.01 min, 25% B;

85.00 min, 42% B; 95.00, 65% B; 95.01 min, 25%B; 105.00 min, 25% B). After equilibration, the HPLC chromatogram was divided into six regions, and the primary flavonoid fractions collected by peak elution times (see Table I) where repetitive flavonoid sample injections and peak separations afforded *ca* 300 mg of each flavonoid. The flavonoid fractions (primary peaks) were dried *en vacuo* (45 °C) and aliquots (*ca* 2 mg) stored as above for ^{1}H NMR spectroscopy while the remainder was used for spectral analyses (UV and GC-MS). The classical shift reagents (Mabry *et al.*, 1970; Markham, 1982) were used with compounds (flavonoid peak fractions) **#1** through **#6** for *E. ulei* (Table I).

NMR spectrometry

The stored flavonoid fraction was decanted into a flask, dried *en vacuo* as above, dissolved in 700 µl of MeOD-d_3 99.95 +% D and placed in NMR tubes. The tubes were heat sealed to prevent sample evaporation and/or oxidation during NMR data acquisition and storage. ^{1}H NMR spectra were acquired at 25 °C on a Bruker QE 300 MHz NMR spectrometer. A Mac NMR v.5 program on Power Macintosh 9500/120 was used for data collecting and processing. The proton spectra were determined at 300.6 MHZ with a spectral width of 3100 Hz and 32 scans. Pre-saturation for 1.2 sec at 4.8 ppm virtually eliminated the signal from water in the spectra which otherwise would interfere with the sugar proton peaks. COSY experiments were used to assign and/or confirm intramolecular coupling. Subtraction of spectra from adjacent compounds (HPLC peaks) was used to compare the structural differences and similarities among structural analogues with different HPLC retention times. ^{1}H NMR data are presented in Table II.

EI-MS procedures

Individual *E. ulei* flavonoids from HPLC eluates **#1** – **#6**, were dissolved in 20 µl 1:1 BSTFA: pyridine, pipetted into ampules (sealed), then heated at 60 °C for 1 hour (derivatization) and evaporated to dryness with N_2. Peak samples were individually dissolved in a 1%, 1:1 BSTFA: pyridine mixture. EI spectra were acquired on a Finnigan-MAT TSQ – 70B triple stage mass spectrometer. Acquisition conditions were: Ion Source temp 150 °C: Ionization energy 70 eV: Emission current 200 µA: Scan range *m/z* (rel. int) 100–1600 in 2 sec: Sample introduction *via* direct probe (*ca* 1 to 2 µl): Program: From 50 to 800 °C at 4 °C/sec.

MS data for (derivatized product [-] the derivative) E. ulei peak flavonoid(s)

(EI-MS {probe} 70 eV; rel. int): peak **#1**; 360 [M-245] (59), 437 [M-168] (55), 450 [M-155] (25), 502 [M-103] (90), 559 [M-46] (41), 578 [M-27] (38); peak **#2**; 363 [M-393] (12), 441 [M-315] (11), 455 [M-301] (10), 527 [M-229] (15), 557 [M-199] (100); peak **#3**; 360 [M-216] (89), 403 [M-173] (19), 438 [M-138] (100), 477 [M-99] (61), 517 [M- 59] (38), 536 [M-40] (18); peak **#4**; 349 [M-361] (15), 615 [M-95] (100), 687 [M-23] (19); peak **#5**; 349 [M-223] (18), 438 [M-134] (29), 473 [M-99] (20), 485 [M-87] (100), 550 [M-22] (45); peak **#6**; 349 [M-245] (74), 361 [M-233] (62), 407 [M-187] (28), 421 [M-173] (37), 451 [M-143] (100), 467 [M-127] (38), 481 [M-113] (23), 529 [M-65] (25), 575 [M-19] (61).

Results and Discussion

Leaf flavonoid chemistry

Methanolic extracts from leaves of *E. ulei* separated by semi-preparative HPLC (see Materials and Methods) afforded major six peaks that were well resolved (Fig. 1; Table I). After collecting sufficient peak fractions from the extract (*ca* 300 mg of each flavonoid) the collected fractions were again HPLC separated (as above) with UV absorbance wavelengths (λ_{min} and λ_{max}; Materials and Methods) maintained and monitored to ensure that the eluting peaks were flavonoids (data not presented). After peak separation, acquired peak UV spectra data were compared with those for authentic or the aglycone (parent) portion of the flavonoid stored in the Chem-library of the HPLC data system, and with those detailed by Mabry *et al.* (1970) and Markham (1982) using the classical shift reagents. Analyses of UV absorbance maximum and spectral shifts (spectra analyses and wavelength shifts comparison) showed that the six peaks collected were flavonoids.

Retention times for the methanolic extracted flavonoids of *E. ulei* are shown in Fig. 1 and Table I. Of the six flavonoid glycosides extracted from *E. ulei* leaf tissue, the aglycone moieties in-

Table I. Analytical HPLC retention times for flavonoids from the MeOH leaf extracts of five *Erythroxylum* taxa.

Peak	R_t [min]	Compound	Peak	R_t [min]	Compound
		E.ulei			*E. n.* var. *novogranatense*
1	32.6	✦Bap-3',4',5'-OH-2,5-OH-7-di-✱Rha-▲isoflav	1	16.5	♦Lu-3'-OH-4'H-3-tri-AcRha
2	42.9	❡Gen-4'-■Glu-2H-5-OH-7-di-Rha-isoflav	2	17.7	Lu-3'-OEt-4'H-3-Rha
3	45.4	●Oro-3',4'-OH-2,5-OH-7-di-Rha-isoflav	3	21.0	K-3'H-4'-OH-3-tri-AcRha-7-tri-Ac□Gal
4	47.8	Oro-3'-OH-4'-Gal-5-OH-7-di-Rha-2-☆Met-◇isoflavan	4	27.1	K-3'H-4'-OEt-7-Gal
5	54.5	Gen-4'-Gal-3H-5-OH-7-Rha-2-Met-isoflavan	5	28.2	K-3'H-4'-OH-3-Rha-7-Gal
6	101.3	†Que-3'-OH-4'-Rha-3,5-OH-7-Rha			
		E. c. var. *coca*			*E. c.* var. *ipadu*
1	21.1	*Eri-3',4'-OH-7-tri-◊AcRha	1	21.1	‴Tax-3',4',5-OH-3H-7-tetr-AcRha
2	24.0	Eri-3'-§OEt-4'-AcRha	2	24.0	Que-4'-OH-3,5-OH-3',7-tetr-di-AcRha
3	24.7	Eri-3',4'-OH-7-AcRha	3	24.7	Tax-3',3,5-OH-4',7-tetr-di-AcRha
4	26.1	Eri-3'-OEt-4'-OH-7-AcRha	4	26.1	Tax-3'H-3,5-OH-4',7-tetr-Ac-di-Rha
5	27.3	Eri-3'-OEt-4'-OH-7-tri-AcRha	5	27.1	Tax-3',5,7-OH-4',3-tetr-AcRha
6	30.3	Eri-3',4'-OEt-7-tri-Ac-di-Rha	6	30.3	Tax-3',5-OH-4',3,7-tetr-Ac-tri-Rha
		E. n. var. *truxillense*			
1	19.3	Que-4',5-OH-3'-Rha-3-Glu-7-AcRha			
2	22.0	○Fis-3',5-H-4'-OEt-3,7-Ac-di-Rha			
3	26.4	‡K-3'H-4'-OEt-5-OH-3,7-Ac-di-Rha			
4	38.5	1,2 dihydrokaemp-4'-OEt-3,7-Ac-di-Rha			

*Eri = Eriodictyol
♦Lu = Luteolin
□Gal = Galactosyl
○Fis = Fisetin
❡Gen = Genistein
▲isoflav = isoflavone
◊Ac = Acetyl
§OEt = Ethoxy
†Que = Quercetin
‴Tax = Taxifolin
●Oro = Orobol
◇isoflavan = isoflavanone
✱Rha = Rhamnosyl
‡K = Kaempferol
■Glu = Glucosyl
✦Bap = Baptigenin
☆Met = Methyl

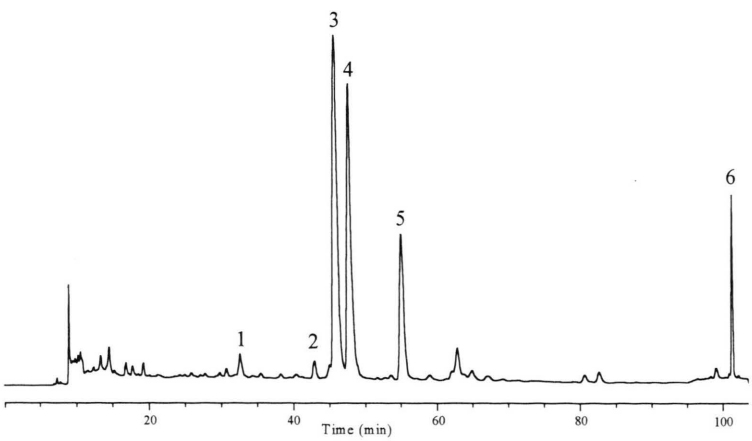

Fig. 1. HPLC chromatograph of flavonoids in the polar leaf extract from dried leaves of *E. ulei* separated semi-preparatively.

cluded three isoflavones (peaks **#1**, **#2**, and **#3;** Fig. 1), two isoflavanones (peaks **#4** and **#5**; Fig. 1), and a flavonol (quercetin; peak **#6**; Fig. 1). The compound types for the three *O*-conjugates of isoflavones of *E. ulei* (peaks **#1**, **#2**, **#3;** Fig. 2), were: 3', 4', 5'-trihydroxy-2, 5- dihydroxy-7-dirhamnosylisoflavone (baptigenin; peak **#1**; Fig. 2), 4'-glucosyl-2H-5-OH-7-dirhamnosylisoflavone (genistein; peak **#2**; Fig. 2), 3',4'-dihydroxy-2,5-dihydroxy-7-dirhamnosylisoflavone (2-hydroxy orobol; peak **#3**; Fig. 2). For the two *O*-conjugated isoflavanones, 3'-OH-4'-galactosyl-5-OH-7-dirhamnosyl-2-methylisoflavanone (2, 3-dihydro-2-methyl orobol; peak **#4;** Fig. 2), and 4'-galactosyl-5-OH-7-rhamnosyl-2-methylisoflavanone (2,3-dihydro-2-methyl genistein; peak **#5**; Fig. 2). And for the flavonol quercetin, 3'-OH- 4'-rhamnosyl-3, 5-dihydroxy-7-rhamnosyl (peak **#6**; Fig. 2). Of note was the absence of kaempferols in leaf tissue of *E. ulei* in the current study, which was present in the polar leaf tissue extracts from two of the four alkaloid bearing taxa of *Erythroxylum* (*E. n.* var. *novogranatense* and *E. n.* var. *truxillense*; Johnson *et al.*, 1997; 1998) as well as the previous work of Bohm *et al.* (1981). We recognize that there are differences (quantitative) in flavonoid compositions of leaf tissue harvested from greenhouse, field and/or feral grown *Erythroxylum*. However, our analyses of leaf tissue upon receipt of the taxon did not contain kaempferol. Therefore, the absence of kaempferol from leaf tissue of *E. ulei* is unknown, unless it is indicative of a relationship to the ancestral taxon, *E. c.* var. *coca*, (Bohm, *et al.*, 1982) since both contain flavanones (Johnson *et al.*, 1997).

Chemistry

Figure 1 shows the profile of flavonoid elution from extracted leaf tissue of *E. ulei* separated

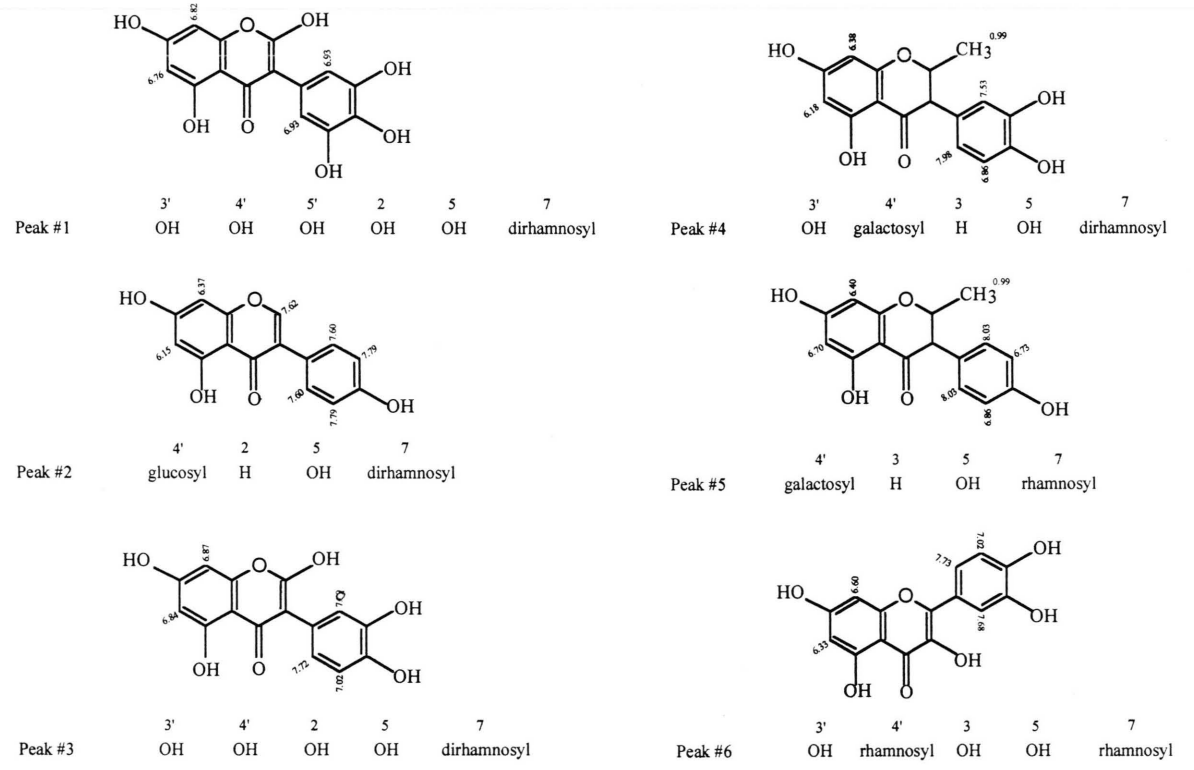

Fig. 2. Primary structures of the parent flavonoids present in the polar leaf fraction extracted from dried leaf tissue of *E. ulei.* Compounds were identified as follows: **peak #1,** Baptigenin; **peak #2,** Genistein; **peak #3,** Orobol; **peak #4,** 2-methyl Orobol; **peak #5,** 2-methyl Genistein; **peak #6,** Quercetin. Numbers (#) correspond to the peak numbers in Fig. 1.

semi-preparatively by HPLC. Peak retention times for the six flavonoids were well resolved with no overlapping or shouldering. Close peak retention time (i. e., peaks **#3** and **#4**) was due to structural conjugation and differences in aglycone substitution on the parent flavonoid, and was resolved by ^{1}H NMR spectroscopy (Table II) as detailed in our previous study (Johnson *et al.*, 1997; 1998). The flavonoids of *E. ulei* differed from flavonoids in the four cultivated taxa of *Erythroxylum* in that the prevalent constituents in *E. ulei* were isoflavonoids, while those of the four cultivars mostly flavonols (see Johnson *et al.*, 1997; 1998). It should be noted that retention on the HPLC column is based on hydrophobicity; the more polar the compound, the earlier it elutes. The retention order of HPLC peaks can be explained after the assignments of chemical structures is made (see Johnson *et al.*, 1998). Because it has five free **-OH** groups and is a disaccharide, each with corresponding sugar **-OH** groups, the compound in peak **#1** elutes first and from its chemical structure would be predicted to elute first. Interestingly, an aromatic ring attached to the **3**-position consistently had shorter retention than the same structure at the **2**-position. With these structures, the number and site of the sugar conjugation influences the sequence of elution, but the chemical structure of an individual sugar (rhamnose, glucose or galactose) appears less important. Disaccharides have shorter retention than monosaccharides at the same site. Each significantly different retention time corresponds to a significant change in chemical structure and/or conjugation from a previous or later peak. Which compounds are most closely structurally related (and which are structurally most different) can be readily discerned from the NMR experiments and confirmed by MS only by correctly assigning chemical structures to all relevant HPLC peaks.

Peak **#2** is a structural analog of **#1** with three fewer **-OH** groups and the 4' **-OH** is conjugated with glucose (Figs. 1 and 2). Peak **#3** is also a structural analog of **#1** with one less **-OH** group on the **B**-ring. Peaks **#4** and **#5** are structural analogs, again differing in only in an **-OH** group on the **B**-ring (Figs. 1 and 2). Peak **#6** differs from all other peaks, because the **B**-ring substitution is at position **2** instead of position **3** (Fig. 2).

The set of peaks which includes **#1**, **#2** and **#3** is different from those of **#4** and **#5** by two structural features. In **#1**, **#2** and **#3**, the bond at **C2-C3** is a double bond, whereas in **#4** and **#5**, it is a single bond. In addition, the substitution at the **2** position is an **-OH** in **#1** and **#3**, but a methyl group in **#4** and **#5** (Fig. 2). This suggests more complicated biochemical pathways are required within the plant for the synthesis of **#4** and **#5**. Structural analogs unique to specific species could offer insight into the type of plant enzymes which enable these

Table II. ^{1}H NMR data for leaf flavonoids of *Erythroxylum ulei* **#1**, **#2**, **#3**, **#4**, **#5**, **#6** in MeOD-d_3 +99.5% D.

	Peak Number					
Proton	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**
2'	6.93	7.60	7.69	7.53	8.03	7.68
3'	OH	7.79	OH	OH	6.73	OH
4'	OH	OH	OH	OH	OH	OH
5'	OH	7.79	7.02	6.86	6.86	7.02
6'	6.93	7.60	7.72	7.98	8.03	7.73
2	OH	7.62	OH	2.41	2.40	–
				2.01	2.01	
3	–	–	–	–	–	–
6	6.76	6.15	6.84	6.18	6.70	6.33
8	6.82	6.37	6.87	6.38	6.40	6.60
Sugar	3.54	5.07	3.47	5.67	5.85	3.89
H-1	3.36	3.51	3.41	4.31	3.81	3.59
		3.38		3.57		
Sugar	1.09	1.16	1.31	1.25	1.28	1.29
CH_3	1.14	1.09	1.06	1.14	0.986	1.06
H2 – H6	3.1–4.0	3.0–3.9	3.1–4.0	3.1–4.0	3.1–4.0	3.1–4.0
Acetyl	1.95	1.95	1.91	1.92	1.92	1.92
C2-Methyl	–	–	–	0.99	0.99	–

corresponding chemical transformations to occur. Further, because of hydroxylation of **#1** and **#2** at position **5** of the **A-ring**, (Cassady *et al.*, 1990) they are currently under scrutiny as chemotherapeutic and chemopreventive agents.

Cognate flavonoid chemistry

It should be noted that *E. ulei* does not contain the alkaloid benzoylmethylecgonine (cocaine) or other ecgonine derived alkaloids in leaf tissue as do the four cultivated taxa (*E. c.* var. *coca*, *E. c.* var. *ipadu*, *E. n.* var. *novogranatense* and *E. n.* var. *truxillense*; Plowman and Rivier, 1983; Johnson, E. L., 1997, unpublished data). The current study was our first examination of flavonoids in leaf tissue from a non-alkaloid-bearing taxon of *Erythroxylum* for use as chemotaxonomic markers (i. e., *E. ulei*). However, this study is not the first assessment of flavonoids within the leaf tissue of *E. ulei*. Flavonoids within leaf tissue of the taxon were first characterized by Bohm *et al.* (1981), however, it was stated in their investigation "that the position of attachment on gallic acid residues was not firmly established and that several of the flavonoids, 'probably the rutinosides,' occurred along with small quantities of quercetin-3-*O*-diglucoside whose structures were not pursued." Thus, in the current study, we reexamined the flavonoids in leaf tissue of *E. ulei* that were grown under greenhouse conditions for 2.5 years (before flavonoid analyses) for the reasons that follow: (*i*) to ensure that the plants were acclimated to the new growth environment, thus minimizing flavonoid variations occurring among plants; (ii) to assure that the leaf flavonoid profiles did not differ from those of the first analyses, i.e. within leaves, upon receipt of the taxon and (iii) to assure that all major components present in the polar leaf extract after semi-preparative HPLC separation, were confirmed by UV and EI-MS as flavonoids and subsequently, structurally identified by ^{1}H NMR spectroscopy so that differences in O-conjugation and/or occurring structures on the parent flavonoid(s) were unambiguously defined.

Bohm *et al.* (1981) concluded that *E. ulei* elaborated a more complex array of flavonol derivatives than the taxon *E. rufum* Cav., which contained a series of 3-*O*-glycosides of kaempferol, quercetin and ombuin, while in *E. ulei*, there was an aggregation of flavanones and under some conditions (not detailed) a dihydroflavonol. However, in the current study we found the prevalent flavonoids present in the leaf tissue of *E. ulei* to be *O*-conjugated isoflavononones (Fig. 2), differing from the findings of Bohm *et al.* (1981). In addition, the monoglycosides in the *E. ulei*, leaf extracts identified by Bohm *et al.* (1981), as kaempferol, myricetin-3-O-glucosides, ombuin-3-O-rhamnosylglucosides and their corresponding gallic acid conjugates were not found in the polar leaf extracts in the current study. However, if the flavan group are associated with tannins (Bezanger-Beauques *et al.*, 1965), then the isoflavanols **#4** and **#5** are relatives thereof.

The absence of kaempferol from extracts of *E. ulei* leaf tissue is of interest. This is because it is absent in leaf tissue of *E. c.* var. *coca* and *E. c.* var. *ipadu* grown in Hawaii, Bolivia and Peru, but present in leaf tissue of *E. n.* var *novogranatense* and *E. n.* var. *truxillense* (Johnson *et al.*, 1997; 1998). This may indicate that *E. ulei* is more closely related to *E. c.* var. *coca* that to *E. n.* var. *novogranatense*. Indeed, *E. c.* var. *coca* does contain an array of the flavanone, eriodictyol (Johnson *et al.*, 1997; 1998), however, it should be recognized that in regards to the absence of kaempferol, its absence in *E. ulei*, does not make it structurally equivalent to the taxon *E. c.* var. *coca* (see Table I).

Quercetin was present in the polar extracts of leaf tissue from *E. ulei*, as a 4', 7-O-rhamnosyl conjugate (Fig. 2; peak **#6**), in concordance with the findings of Bohm *et al.* (1981). However, in the current study, we found *O*-4' and *O*-7 glycoside linkages to quercetin rather than the reported *O*-3-glycosides (Bohm *et al.* (1981). Moreover, in the taxa we have examined thus far using flavonoids as chemotaxonomic markers, quercetin appeared more ubiquitous among the *Erythroxylum* taxa than kaempferol (Johnson *et al.*, 1997; 1998). This finding is also consistent with that of Bohm *et al.* (1988), however, their presence within taxa examined hitherto, differs (see Table I and Johnson *et al.*, 1997; 1998).

The isoflavanones, (Fig. 2; peaks **#4** and **#5**), in addition to 4'-galactosyl and a 7- mono-and-dirhamnosyl conjugation, contained a methyl group at position **#2** on the **C**-ring, a finding not previously reported for the taxon.

Whether flavonoid patterns of *Erythroxylum* taxa (as in Fig. 1) are strictly constitutive (and therefore qualitatively insensitive to environmental changes) or whether they are affected by altered pH, light or other environmental parameters remains an unanswered question. If the latter, the changes seem more likely to vary in the absolute and relative amounts of the individual flavonoids, not in their qualitative pattern. Preliminary observations indicate diminished flavonoid patterns for *Erythroxylum* taxa grown under controlled environments and greenhouse as compared to field-grown plants. Thus, for any one of the tested taxa, the same flavonoids seem always present. The flavonoid composition continues thus far, to be a useful marker for plant identity. As mentioned, how environmental conditions impact flavonoid composition of *Erythroxylum* is unknown. Understanding those effects could also provide useful information essential to identifying the conditions under which the plant has been growing. We are currently investigating environmental factors that may influence flavonoid biosynthesis of the *Erythroxylum* taxa in our laboratory.

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