Phytochemistry, Vol. 27, No. 3, pp. 833 837, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

FLAVONOID VARIATION IN *ERYTHROXYLUM*

B. A. BOHM, T. LOO, K. W. NICHOLLS and T. PLOWMAN*

Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 2B1, Canada; *Department of Botany, Field Museum of Natural History, Chicago, IL 60605, U.S.A.

(*Received* 24 *June* 1987)

Key Word Index—*Erythroxylum*; *Erythroxylaceae*; flavonoids; flavonol glycosides; variation; chemical races.

Abstract—Thirteen species of *Erythroxylum* from Brazil were studied for their flavonoids. All exhibit profiles based upon flavonol glycosides. Predominant flavonols are kaempferol, quercetin and 7,4′-dimethylquercetin. *O*-Methylated chalcones and flavanones were identified in *E.* cf. *loefgrenii*. This represents the first report of chalcones from the genus. Variation was observed in the number of compounds present in the profiles, the nature of the flavanols present, and in the degree of glycosylation of the flavonols. In the case of *E. vacciniifolium* three distinct flavonoid profiles were seen in specimens from different regions of Brazil.

INTRODUCTION

Erythroxylum P. Browne is a genus of more than 200 species [1] of shrubs and small trees that occurs widely throughout tropical and subtropical regions. The largest number of species is found in the New World, but the genus is well represented in Africa, southeast Asia and Melanesia. Three species are also known in the Australian flora. Most attention in the genus has been focused on *E. coca* and related taxa which have been cultivated for thousands of years [2]. While most attention has been directed toward the chemical [3], pharmacological [4–6], and ethnobotanical [2] features of the cultivated taxa, studies have recently been undertaken with a view toward applying chemical data to arrive at a better understanding of relationships within the genus as a whole. It is the purpose of this paper to illustrate the variety of flavonoid patterns that one can expect in the genus. We describe the flavonoid chemistry of 13 species previously not reported in the literature.

RESULTS AND DISCUSSION

Despite its size and widespread distribution in the tropics of both hemispheres, *Erythroxylum* has received comparatively little attention. Interest in the cocaine-bearing taxa is a notable exception. Reports of flavonoids in members of the genus have been few and for the most part, at least until recently, incomplete. Extensive collections in South America coupled with the availability of material from other parts of its range have allowed us to undertake a systematic survey of *Erythroxylum* for flavonoids.

The chemotaxonomy of the Erythroxylaceae has been reviewed by Hegnauer [7, 8] and does not need to be repeated in any detail here. Published reports of flavonoids from *Erythroxylum*, however, have been summarized in Table 1. Hegnauer [8] concluded, on the basis of the few reports available to him, that the prominant flavonoid compounds in the family were flavonols. The results obtained in the current study, which are presented in Table 2, along with published information, support his conclusion. Table 3 summarizes the occurrence of aglycones and glycosylation types that have so far been found in the genus (including the present work). Kaempferol accumulates in 15 of the 19 species for which relevant data are available. Quercetin accumulates in 17, ombuin (7,4′-dimethylquercetin) in 16, and myricetin in only one. Other flavonoid types occur much less commonly: dihydroflavonols were observed in only two species, dihydrokaempferol in *E. squamatum* (present study), and dihydroquercetin in a specimen of *E. ulei* grown under cultivation [9]. Several changes in the flavonoid profile of *E. ulei* grown under cultivation were observed in that study as well.

Flavanones were observed in one of the species examined in the present study. We identified 7-methylnaringenin and 7,4′-dimethylnaringenin 5-glucoside from *E.* cf. *loefgrenii*. It is interesting to note that the substitution pattern of the second compound, including the positioning of its two methyl groups, corresponds to the arrangement of substituents in ombuin. 7-Methylnaringenin was observed only as the aglycone which suggests that it might have been formed as an artifact from the corresponding chalcone, 2′,4,6′-trihydroxy-4′-methoxychalcone 2′-*O*-glucoside, which was also isolated from *E.* cf. *loefgrenii*. A second chalcone, 2′,3,4,6′-tetrahydroxy-4′-methoxychalcone 2′-*O*-glucoside, was also isolated from this taxon and identified during the course of this study.

In a recent study of *E. novogranatense*, Bonefeld *et al.* [10] reported the isolation and characterization of (+)-catechin 3-rhamnoside. This is the first tannin to be described in detail from a member of the genus although the presence of tannins in these plants was recognized by Bate-Smith [11] who observed procyanidins in *E. coca* and by Bezanger-Beauquesne *et al.* [12] who showed that *E. laurifolium* has high concentrations of tannin in the bark (*ca* 10%) and in the leaves (7–11%). Although no structural studies were reported, these workers [12] stated that the compounds were condensed tannins and that they were accompanied by flavan-3-ols and flavan-

Table 1. Summary of published reports of flavonoids in *Erythroxylum* presented in chronological order

Taxon	Compound (s)	Reference
E. monogynum Roxb.	'flavonoids'	[19]
E. capitatum Baker	"	[20]
E. corymbosum Boivin	"	[20]
E. ferrugineum Cav.	"	[20]
E. sphaeranthum H. Perr.	"	[20]
E. xerophilum H. Perr.	"	[20]
E. coca Lam.	Km, Qu	[11]
E. coca Lam.	Rutin, Qu 3 Glc	[21]
E. laurifolium Lam.	Rutin, Qu 3-Rha	[12]
E. rufum Cav. and *E. ulei* O. E. Schulz	Km, Qu and Om mono-, and diglycosides, naringenin, DHQu, My	[9]
E. coca Lam. var. *coca* and var. *ipadu*	Km and Qu mono- and diglycosides (not rutinosides)	[9]
E. novogranatense (Morris) Hieron. var. *novogranatense*	Km, Qu and Om mono-glycosides and rutinosides	[9]
E. novogranatense var. *truxillense* (Rusby) Plowman	Km, Qu and Om mono-glycosides and rutinosides	[9]
E. argentinum O. E. Schulz	Qu, Om glycosides	[14]
E. novogranatense Hieron.	(+)-catechin Rha	[10]

Madagascar species—no structures reported.

Km = kaempferol, Qu = quercetin, Om = ombuin, My = myricetin, DHQu = dihydroquercetin, Glc = glucoside, Rha = rhamnoside.

Table 2. The distribution of flavonoids in 13 Brazilian *Erythroxylum* species

Taxon	Flavonol										Glycosides											Number of flavonoids present
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
E. barbatum	+			+		+			+				+		+							6
E. campestre						+			+	+			+	+	+	+						7
E. cuspidifolium				+					+													2
E. daphnites				+		+		+					+									4
E. engleri											+	+	+									3
E. cf. *loefgrenii*	+		+			+		+			+	+	+									11†
E. pruinosum	+		+			+		+	+		+	+	+									8
E. squamatum	+					+							+		+							5‡
E. suberosum 9366	+				+	+	+	+	+						+	+						9
E. suberosum 9927				+		+		+							+	+						5
E. subracemosum	+					+					+	+	+									5
E. subrotundum													+									1
E. tenue	+		+								+		+				+					5
E. vacciniifolium 9985	+		+			+		+	+		+	+	+									8
E. vacciniifolium 10131	+	+	+	+	+	+			+	+							+	+	+			11
E. vacciniifolium 12717			+			+		+					+							+	+	6

*A = kaempferol 3-glucoside, B = kaempferol 3-galactoside, C = kaempferol 3-rhamnoside, D = kaempferol 3-arabinoside, E = kaempferol 3-xyloside, F = quercetin 3-glucoside, G = quercetin 3-galactoside, H = quercetin 3-rhamnoside, I = quercetin 3-arabinoside, J = quercetin 3-xyloside, K = kaempferol 3-rutinoside, L = quercetin 3-rutinoside, M = ombuin 3-rutinoside, N = kaempferol 3-glucosylxyloside, O = quercetin 3-glucosylxyloside, P = quercetin 3-glucosyl arabinoside, Q = kaempferol 3-glucoside-7-rhamnoside, R = kaempferol 3-arabinoside-7-rhamnoside, S = quercetin 3-rhamnoside-7-glucoside, T = quercetin 3,7-dirhamnoside, U = quercetin 3-glucosylglucoside.

† Includes chalcones and flavanones, see text.

‡ Includes a dihydroflavonol, see text.

Table 3. Occurrence of aglycones and glycosylation types in *Erythroxylum* including published and present work

Taxon [Ref.]†	Flavonoid* K	Q	M	O	H	C	F	M	D	T	35	37
E. argentinum [14]		+		+				+	+	+	+‡	
E. barbatum	+	+		+				+	+			
E. campestre		+		+				+	+			
E. coca var. *coca* [3]	+	+						+	+			
E. coca var. *ipadu* [3]	+	+						+	+			
E. cuspidifolium	+	+						+				
E. daphnites	+	+		+				+	+			
E. engleri	+	+		+					+			
E. laurifolium [12]		+		+				+	+			
E. cf. *loefgrenii*	+	+		+		+	+	+	+			
E. novogranatense var. *novo.* [3]	+	+		+				+	+	+§		
E. novo. var. *truxillense.* [3]	+	+		+				+	+	+§		
E. pruinosum	+	+		+				+	+			
E. rufum [9]	+	+		+				+	+			
E. squamatum	+	+		+	+			+	+			
E. suberosum	+	+						+	+			
E. subracemosum	+	+		+				+	+			
E. subrotundum				+					+			
E. tenue	+			+				+	+			+
E. ulei [9]	+	+	+	+	+		+	+	+			
E. vacciniifolium 9985	+	+		+				+	+			+
E. vaccinifolium 10131	+	+						+				+
E. vacciniifolium 12717	+	+		+				+	+			+

*K = kaempferol, Q = quercetin, M = myricetin, O = ombuin, H = dihydroflavonol, C = chalcone, F = flavanone, M = monoglycoside, D = diglycoside, T = triglycoside, 35 = 3,5-glycosylation, 37 = 3,7-glycosylation.

†Reference to published data; others are from present work.

‡This is a 3-diglycoside-5-monoglycoside.

§This is a 3-triglycoside.

3,4-diols. Okuda *et al.* [13] reported the presence of 0.3% of the elagitannin, geraniin, in *E. coca* leaves.

Variation in the nature of the glycosidic derivatives of the flavonoids of the genus has also been observed. The majority of flavonols occur as 3-*O*-monoglycosides with glucosides, galactosides, arabinosides, xylosides, and rhamnosides known, and as 3-*O*-diglycosides with various combinations of those sugars. Not all taxa have the same arrangements, however. Whereas kaempferol and quercetin occur as both mono- and diglycosides (myricetin was seen only as a monoglucoside), ombuin has not been observed so far as a monoglycoside. In all taxa studied in the present work, ombuin was observed only as the 3-rutinoside while in *E. argentinum* it was reported as the 3-rutinoside-5-glucoside [14]. We observed traces of what appeared to be 3-*O*-triglycosides in both varieties of *E. novogranatense* [3] but owing to limited amounts of material we were unable to establish the structures. An additional glycosylation type was observed in the present study: two collections of *E. vacciniifolium* exhibited flavonol 3,7-diglycosides. *Erythroxylum tenue* also yielded a small amount of a 3,7-diglycoside of kaempferol but we were able to determine only that it gave glucose and rhamnose on hydrolysis; the positions of attachments of the sugars were not determined.

One additional modification of glycoside structure was seen in *E. ulei* in our earlier study [9]. Kaemferol, quercetin and myricetin 3-glucosides were accompanied in two of the wild collections studied by gallic acid derivatives. Again, limited amounts of material precluded detailed studies but chromatographic behaviour of these compounds resembled that of the 6″-*O*-galloylglucosides of the flavonols that we observed in *Tellima grandiflora* (Saxifragaceae) [15].

There is also a considerable degree of variation with regard to the complexity of total flavonoid profiles seen in the speices studied. In the number of compounds present, taxa vary from *E. subrotundum*, with only a single compound, to *E. vacciniifolium* (10131) and *E.* cf. *loefgrenii* both of which afforded eleven compounds. Numbers of compounds identified from each species are recorded in Table 2.

Most of the taxa examined in the present study had a variety of both aglycones and glycosylation types. A few of the taxa exhibited profiles that merit comment. Thus, *E. subrotundum* appears to accumulate only ombuin rutinoside. *Erythroxylum engleri* accumulates only rutinosides but has the full complement of normal aglycones, namely, kaempferol, quercetin and ombuin. *Erythroxylum tenue* is distinguished by having kaempferol and ombuin glycosides with no detectable quercetin derivatives, while *E. cuspidifolium* is noteworthy in that it accumulates only kaempferol and quercetin 3-arabinosides.

In our study of *E. coca* and *E. novogranatense* [3] we noted that the flavonoid profiles were invariant. Three further comparisons were available in the current study.

Table 4. Abbreviated collection data

Taxon	Coll. No.	State of Brazil
E. barbatum O. E. Schultz	9211	Goiás
E. campestre St. Hil.	9999	Dist. Federal
E. cuspidifolium Mart.	10114	Rio de Janeiro
E. daphnites Mart.	8330	Goiás
E. engleri O. E. Schultz	9960	Dist. Federal
E. cf. *loefgrenii* C. Diogo	12821, 12823	Bahia
E. pruinosum O. E. Schulz	8400	Goiás
E. squamatum Sw.	9212	Goiás
E. suberosum St. Hil.	9366	Maranhao
E. suberosum St. Hil.	9927	Dist. Federal
E. subracemosum Turcz.	9213	Bahia
E. subrotundum St. Hil.	12824	Bahia
E. tenue Plowman	12812	Bahia
E. vacciniifolium Mart.	9985	Dist. Federal
E. vacciniifolium Mart.	10131	Rio de Janeiro
E. vacciniifolium Mart.	12717	Ceará

All numbers are Plowman collections.

Vouchers are deposited at Field Museum and various Brazilian herbaria.

Two collections of *E.* cf. *loefgrenii* gave qualitatively and quantitatively identical patterns as judged by TLC comparisons. The two specimens of *E. suberosum* studied had the same diglycosides but differed markedly in the complexity of their monoglycoside fractions. Specimen No. 9366 exhibited seven compounds, including the complete array of quercetin derivatives plus two kaempferol glycosides. We found only one kaempferol and two quercetin 3-monoglycosides in No. 9927. None of the three kaempferol 3-monoglycosides was held in common between these two collections of *E. suberosum*. The most striking example of intraspecific variation, however, was observed within *E. vacciniifolium*. Three specimens of this taxon were analysed, No. 9985, which came from swampy sites near Brasilia, No. 10131, which came from montane forests near Rio de Janeiro, and No. 12717, which was collected in northeastern Brazil and represents the typical form of the species. Reference to Table 2 shows that only two compounds, kaempferol 3-rhamnoside and quercetin 3-glucoside, are held in common by the three. Specimen No. 10131 does not appear to make ombuin whereas the other two specimens do. Significant differences can be seen in the nature of diglycoside profiles in general. Specimen No. 9985 has the simplest diglycoside profile in that it has only rutinosides. The typical form of *E. vacciniifolium* (No. 12717) has only one rutinoside (of ombuin) but does exhibit quercetin 3,7-diglucoside and quercetin 3,7-dirhamnoside. The Rio de Janeiro specimen (No. 10131) lacks 3-diglycosides altogether but does exhibit three 3,7-diglycosides, thus at least having a glycosylation type in common with the typical form. These two specimens, 10131 and 12717, exhibit mutually exclusive 3,7-diglycoside profiles. It would be of interest to examine a larger number of collections of *E. vacciniifolium* in order to judge the true extent of these flavonoid 'races'. Morphological differences between plants from these three areas are comparatively minor. They are currently considered as geographical or ecological components of the same polymorphic species.

EXPERIMENTAL

Plant material. Abbreviated collection data, along with collection numbers and author designations for the taxa examined in this study, are given in Table 4. Detailed collection information is available from T. P. Voucher specimens have been deposited in the Field Museum of Natural History and in major Brazilian herbaria.

Analysis of flavonoids. Isolation and purification of flavonoids was done using procedures described in detail by Wilkins and Bohm [16]. Chromatographic systems were also described in that paper [16]. Structures of flavonoids were determined using standard methodologies [17, 18].

Acknowledgements—The flavonoid work was supported by operating and equipment grants from the Natural Sciences and Engineering Research Council of Canada (to B.A.B.). Field work and specimen collection in Brazil was supported in part by the Field Museum of Natural History and in part by the National Science Foundation (Grant INT78-23341, G. T. Prance, Principal Investigator) through the Projeto Flora Amazônica. Mass spectra were run by Mr Filipe Balza. Technical help was provided by Ms Evelyn Leaf.

REFERENCES

1. Plowman, T. (1984) in *Pre-Columbian Plant Migration* (Stone, D., ed.) Papers of the Peabody Museum of Archaeology and Ethnology **76**, 125.
2. Plowman, T. (1984) *Advances in Economic Botany* **1**, 62.
3. Bohm, B. A., Ganders, F. R. and Plowman, T. (1982) *Syst. Botany* **7**, 121.
4. Fuchs, A. (1978) *Current Anthropology* **19**, 277.
5. Holmstedt, B., Lindgren, J.-E., Rivier, L. and Plowman, T. (1979) *J. Ethnopharmacol.* **1**, 69.
6. Martin, R. T. (1970) *Economic Botany* **24**, 422.
7. Hegnauer, R. (1966) *Chemotaxonomie der Pflanzen*, Band 4, pp. 94–99, 454–455, 489–490. Brikhauser, Basel.

8. Hegnauer, R. (1981) *J. Ethnopharmacol.* **3**, 279.
9. Bohm, B. A., Phillips, D. W. and Ganders, F. R. (1981) *J. Nat. Prod.* **44**, 676.
10. Bonefeld, M., Friedrich, H. and Kolodziej, H. (1986) *Phytochemistry* **25**, 1205.
11. Bate-Smith, E. C. (1962) *J. Linnean Soc., Botany* **58**, 95.
12. Bézanger-Beauquesne, L., Guilbert, N. and Deneck, D. (1965) *Ann. Pharmaceut. Francaises* **23**, 377.
13. Okuda, T., Mori, K. and Hatano, T. (1960) *Phytochemistry* **19**, 547.
14. Inigo, R. P. A. and Pomilio, A. B. (1985) *Phytochemistry* **24**, 347.
15. Collins, F. W. and Bohm, B. A. (1974) *Can. J. Botany* **52**, 307.
16. Wilkins, C. K. and Bohm, B. A. (1976) *Can. J. Botany* **54**, 2133.
17. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, New York.
18. Markham, K. R. (1982) *Techniques of Flavonoid Identification.* Academic Press, New York.
19. Chopra, R. N. and Ghosh, N. N. (1938) *Arch. Pharmaz.* **276**, 340.
20. Bosser, J. and Pernet, R. (1957) *Le Naturaliste Malgache* **9**, 195.
21. Paris, R.-R. and Delaveau, P. (1963) *Compt. Rend.* **256**, 301.