pigment of *Salix purpurea* bark [3]. This paper deals with the presence of D-fructose in two flavonoid glycosides from commercial tea. A naringenin glycoside was identified in fresh tea leaves and a quercetin glycoside was shown to be present only in the processed leaf.

Flavonoid glycosides (FG1 and FG2) were isolated from aqueous extracts of commercial tea (TE'ATI, black tea) by preparative PC. The aglycones were obtained by acid hydrolysis (2N HCl; 2 hr at 100°) and identified as quercetin and naringenin by PC (three solvents) and UV spectroscopy.

The sugars attached to flavonoid glycosides were obtained by controlled acid hydrolysis (10% HOAc; 3,5 hr under reflux) and identified by PC (six solvents) and colour reactions with diphenylamine-*p*-anisidine [4]. Both glycosides gave glucose and fructose and FG1 in addition gave a dissacharide. This latter sugar (R_G 0·64) was unaffected by β-glucosidase; on acid hydrolysis it gave glucose and fructose and no intermediate could be detected during degradation. This compound is thus a fructosylglucose. In order to ascertain whether these two flavonoid glycosides are artifacts, fresh tea leaves were extracted with water; from the aqueous extracts the product FG2 was isolated by band chromatography and identified by UV spectroscopy and PC (three solvents); the identification was confirmed by acid hydrolysis. Since compound FG1 was absent from aqueous extracts of fresh tea leaves, it is presumably a product of tea processing.

REFERENCES

1. Kameswaramma, A. and Seshadri, T. R. (1947) *Proc. Indian Acad. Sci.* **25A**, 43.
2. Shakhova, M. F. (1971) *Rast. Resur.* **7**, 407.
3. Bridle, P., Stott, K. G. and Timberlake, C. F. (1973) *Phytochemistry* **12**, 1103.
4. Bailey, R. W. (1962) *J. Chromatog.* **8**, 57.

Phytochemistry, 1976, Vol. 15, pp. 440–441 Pergamon Press. Printed in England.

FLAVONOL METHYL ETHERS FROM *ERICAMERIA DIFFUSA*

LOWELL E. URBATSCH,* TOM J. MABRY,* MASAKAZU MIYAKADO,† NOBUO OHNO† and HIROSUKE YOSHIOKA†

*The Cell Research Institute and Department of Botany, The University of Texas at Austin, TX 78712 U.S.A.

†The Pesticide Department, Institute for Biological Science, Sumitomo Chemical Company Ltd, Takarazuka, Hyogo, Japan

(*Received* 15 *August* 1975)

Key Word Index—*Ericameria diffusa*; Compositae; kaempferol and quercetin methyl ethers.

Two new flavonol aglycones, kaempferol 3,5,7-trimethyl ether (**1**) and quercetin 3,3',4'-trimethyl ether (**2**), were characterized from *Ericameria diffusa* (Compositae) in connection with our chemosystematic study of the genus *Ericameria*. In addition, ten known aglycones were detected in this same species: galangin 3-methyl ether (**3**), kaempferol 3,4'-dimethyl ether (**4**), kaempferol 3,7-dimethyl ether (**5**), kaempferol 3-methyl ether (**6**), quercetin

Table 1. NMR Spectra of *Ericameria diffusa* flavonol aglycones

Compound	H-2'	H-6'	H-3'	H-5'	H-4'	H-8	H-6	3-OMe	5-OMe	OMe in CCl_4 C_6D_6 7-OMe	3'-OMe	4'-OMe
1*	7·88*dd*	7·88*dd*	6·88*dd*	6·88*dd*	—	6·83*d*	6·44*d*	3·85	3·71	3·82	—	—
	(*J* 2·0)†	(*J* 2·0)	(*J* 2·0)	(*J* 2·0)				—	—	—		
	(*J* 8·5)	(*J* 8·5)	(*J* 8·5)	(*J* 8·5)		(*J* 2·5)	(*J* 2·5)					
2	7·53*d*	7·68*dd*	—	6·88*d*	—	6·40*d*	6·11*d*	3·82	—	—	3·80	3·80
	(*J* 2·0)	(*J* 2·0)		(*J* 6·0)		(*J* 2·0)	(*J* 2·0)	(Δ + 0·03)			(Δ + 0·26)	(Δ + 0·32)
		(*J* 8·5)										
3*	signals overlapping 7·55–7·95					6·44*d*	6·23*d*	3·81	—	—	—	—
						(*J* 2·0)	(*J* 2·0)	—				
4	7·99*dd*	7·99*dd*	6·90*dd*	6·90*dd*	—	6·49*d*	6·28*d*	3·82	—	—	—	3·82
	(*J* 2·0)	(*J* 2·0)	(*J* 2·0)	(*J* 2·0)		(*J* 2·5)	(*J* 2·5)	(Δ + 0·02)				(Δ + 0·38)
	(*J* 9·0)	(*J* 9·0)	(*J* 9·0)	(*J* 9·0)								
5	7·95*dd*	7·95*dd*	6·85*dd*	6·85*dd*		6·43*d*	6·15*d*	3·83	—	3·83	—	—
	(*J* 2·0)	(*J* 2·0)	(*J* 2·0)	(*J* 2·0)		(*J* 2·5)	(*J* 2·5)	(Δ + 0·02)		(Δ + 0·51)		
	(*J* 8·5)	(*J* 8·5)	(*J* 8·5)	(*J* 8·5)								
7	7·55*d*	7·55 *d*	—	6·79*d*	—	6·41*d*	6·11*d*	3·82	—	3·82	—	3·82
	(*J* 2·0)	(*J* 2·0)		(*J* 5·5)		(*J* 2·0)	(*J* 2·0)	(Δ + 0·01)		(Δ + 0·37)		(Δ + 0·31)
		(*J* 5·5)										
8	7·54*d*	7·60*dd*	—	6·83*d*	—	6·41*d*	6·09	3·87	—	—	—	3·85
		(*J* 2·0)										
	(*J* 2·0)	(*J* 5·0)		(*J* 5·0)		(*J* 2·0)	(*J* 2·0)	(Δ + 0·05)				(Δ + 0·41)
9	7·66*d*	7·43*dd*	—	6·85*d*	—	6·45	6·17	3·84	—	3·78	—	—
	(*J* 2·0)	(*J* 2·0)		(*J* 8·5)		(*J* 2·5)	(*J* 2·5)	(Δ + 0·01)		(Δ + 0·45)		
		(*J* 8·5)										

* Spectra were recorded for the underivatized flavonoid in $DMSO_6$. All other compounds were trimethylsilated and spectra were recorded in CCl_4 and C_6D_6 (only OMe signals are given for the latter solvent). Values are given in ppm (δ-scale) relative to TMS. † Numbers in parentheses denote coupling constants in Hz. Signals are singlets unless otherwise noted: *d* (doublet), *dd* (double doublet).

3,7,4′-trimethyl ether (**7**), quercetin 3,4′-dimethyl ether (**8**), quercetin 3,7-dimethyl ether (**9**), isorhamnetin (**10**), quercetin 3-methyl ether (**11**), and quercetin (**12**).

The MS spectrum of the new kaempferol methyl ether **1** exhibited a molecular ion at *m/e* 328 in accord with a flavonol containing one hydroxyl and three methoxyl groups; the NMR spectrum in DMSO confirmed the presence of these groups and exhibited signals for 6 aromatic protons: two sets of two-proton doublets (J 8·5 Hz) at δ 7·88 and 6·88 for a typical kaempferol-type B-ring [1] and two sets of one-proton doublets (J 2·5 Hz) at δ 6·44 and 6·73 were typical for protons at C-6 and C-8, respectively [1]. The presence of a hydroxyl group at C-4′ was confirmed by a Band I bathochromic shift of 43 nm with increased intensity in NaOMe relative to Band I in MeOH [1]; this result established that the methoxyl groups are at 3, 5 and 7 since these are the only positions available. On a paper chromatogram this compound appears blue when observed in UV light (366 nm) characteristic for flavonols substituted at both C-3 and C-5 [1].

The second new flavonol was shown to be quercetin 3,3′,4′-trimethyl ether (**2**); a molecular ion at *m/e* 344 indicated the presence of two hydroxyl and three methoxyl groups. The NMR spectrum of its trimethylsilyl ether confirmed the presence of three methoxyl groups (δ 3·80–3·82) [1]. In addition, NMR signals were observed for five aromatic protons in accord with a quercetin-type flavonol (δ 7·53, 6·88 and 7·68 for the B-ring and 6·11 and 6·40 for the A-ring protons) [1]. The presence of a hydroxyl group at C-5 was evident since the compound appears as a purple spot on a paper chromatogram when viewed in UV light (366 nm) [1]. The presence of a second hydroxyl group at C-7 was indicated by a bathochromic shift (22 nm) of Band II in NaOAc relative to Band II in MeOH [1]. The three remaining available positions for substituents, C-3, C-3′, and C-4′, must therefore, contain the three methoxyl groups; the UV spectral data are consistent with these assignments for the methoxyl groups (see Table 2). The NMR benzene-induced shifts for methoxyl groups indicated the following: C-3, +0·03; C-3′, +0·26; C-4′, +0·32. Compounds **3**–**12** were also found to occur as natural products in *E. diffusa*. The compounds reported in this paper were isolated from two different populations; the results indicate that the populations differ somewhat in their flavonoid complements. All of the aforementioned compounds except **1** and **3** were detected in population *1184* (by the group at the Univ. of Texas) while only compounds **1** and **3**–**6** were detected in population *1154* (by the group at Sumitomo Chemical Co., Ltd.).

Tables 1 and 2 contain UV and NMR data, respectively, for compounds not previously recorded.

Table 2. UV spectra of seven *Ericameria diffusa* flavonol aglycones

Compound	MeOH (λ_{max}, nm)	NaOMe (λ_{max}, nm)	$AlCl_3$ (λ_{max}, nm)	$AlCl_3$–HCl (λ_{max}, nm)	NaOAc (λ_{max}, nm)	NaOAc–H_3BO_3 (λ_{max}, nm)
1	336	379	336	414	336	336
	263	(293)	263	340	263	263
	(257)*	264	(257)	(308)	(257)	(257)
				264		
				(258)		
2	351	380	401	399	392	351
	267	313	356	352	314	267
	254	275	297	(300)	276	259
			276	275		
			270	262		
4	347	371	396	397	369	348
	(296)	296	344	341	297	(299)
	266	275	301	292	275	267
			276	276		
5	348	396	398	397	388	350
	(294)	(296)	350	346	(292)	(298)
	266	266	302	299	266	265
		256	275	275	(260)	
7	352	388	404	400		354
	(267)	263	364	358		(268)
	254		298	(298)		254
			(277)	(272)		
			268	268		
8	351	384	399	397	371	352
	266	314	352	360	318	265
	252	276	298	(298)	274	252
			(274)	275		
				262		
9	359	407	441	402	407	380
	294	(298)	332	362	(296)	259
	(266)	267	(299)	298	262	
	256		276	(276)		
				267		

* Numbers in parentheses denote shoulders.

EXPERIMENTAL

Voucher specimens (*Urbatsch, Hartman, Umber 1154* collected from Mexico, Baja California, 68 miles S of La Paz and *Urbatsch, Hartman, Umber 1184* collected from Mexico, Baja California, 50 miles N of Villa Insurgentes) are deposited in the Lundell Herbarium (LL) at the University of Texas at Austin. The isolation and purification procedures employed here were previously described [2]. The major MS fragments *m/e* and their percentages for **1** are: $M^{+\cdot}$ 328 (100%), M-1 (88%), M-15 (32%), 299 (12%), 150 (7%), 121 (15%); for **2** the MS data are: $M^{+\cdot}$ 344 (100%), M-1 (75%), M-15 (65%), M-18 (18%), 313 (55%), 301 (80%), 283 (27%), 271 (39%), 257 (25%).

Acknowledgements—This research was supported by the National Science Foundation (Grants GB-40362 and BMS 71-01088) and the Robert A. Welch Foundation (Grant F-130).

REFERENCES

1. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, Heidelberg.
2. Urbatsch, L. E., Bacon, J. D. and Mabry, T. J. (1975) *Phytochemistry* **14**, 2279.