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METHYLATED FLAVONOLS IN *LARREA CUNEIFOLIA*

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Key Word Index—*Larrea cuneifolia*; Zygophyllaceae; kaempferol and quercetin methyl ethers; nordihydroguaiaretic acid.

Abstract—Nine of eleven methylated flavonols isolated from *Larrea cuneifolia* Cav. collected in Argentina have been fully characterized. UV, MS and NMR spectral data are presented for the nine substances all of which were identified as methyl ethers of quercetin and kaempferol. Two of the compounds, 7,3′,4′-trimethylquercetin (II) and 3,7,3′-trimethylquercetin (III), are new natural products. The others are 3,7,3′,4′-tetramethylquercetin (I) (retusine), 3,7-dimethylkaempferol (IV), 7,3′-dimethylquercetin (V) (rhamnazin), 3,7-dimethylquercetin (VI) (kumatakenin), 3,3′-dimethylquercetin (VII), 3-methylkaempferol (VIII) and 3′-methylquercetin (IX) (isorhamnetin). Nordihydroguaiaretic acid (XII) was also detected.

INTRODUCTION

THE GENUS *Larrea* (Zygophyllaceae) is composed of four species distributed in the arid and semi-arid areas of North and South America. Three species, *L. cuneifolia* Cav., *L. nitida* and *L. ameghinoi*, are restricted to South America[1,2] while the fourth, *L. divaricata* Cav., has a disjunct distribution with the susp. *tridentata* occurring in North America and the susp. *divaricata* in South America.[3,4]

In connection with an I.B.P. Integrated Research Project entitled 'The Origin and Structure of Ecosystems', we and others have selected an intensive study site north of Tucson, Arizona and a second ecologically and geologically complementary area south of Tucuman, Argentina. Our particular interests concern the investigation of the role of secondary compounds in the evolution of the major plant species in the two subdesert ecosystems. The chemistry of the genus *Larrea* is especially being pursued because the subspecies of *L. divaricata* dominate both the North (subsp. *tridentata*) and South (subsp. *divaricata*) American sites. This paper is the first of a planned series on the chemistry of *Larrea*.

* Most of this work was carried out by the senior author during a 1971 tenure at The University of Texas at Austin.

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RESULTS

In the present communication we report the isolation of eleven methylated flavonols and nordihydroguaiaretic acid[5] (XII) from the tetraploid species *Larrea cuneifolia* Cav. collected in southern Argentina. Nine of the flavonols were fully characterized. Two of the constituents, 7,3',4'-trimethylquercetin (II) and 7,3,3'-trimethylquercetin (III), are new natural products. The other previously known flavonols were identified as 3,7,3',4'-tetramethylquercetin (I)[6,7] (retusine), 3,7-dimethylkaempferol (IV)[8,9] (kumatakenin), 7,3'-dimethylquercetin (V)[10] (rhamnazin), 3,7-dimethylquercetin (VI),[10] 3,3'-dimethylquercetin (VII),[9] 3-methylkaempferol (VIII)[9,11] and 3'-methylquercetin (IX)[9,12,13] (isorhamnetin). Two other

TABLE 1. NMR SPECTRA OF *Larrea cuneifolia* FLAVONOL AGLYCONES*

								-OMe			
								C_6D_6			
Compd	H-2'	H-6'	H-3'	H-5'	H-8	H-6	CCl_4	3-OMe	3'-OMe	4'-OMe	7-OMe
I	7·64d (J = 2·5)	7·59dd (J = 2·5) (J = 9·0)	—	6·87d (J = 9·0)	6·48d (J = 2·5)	6·19d (J = 2·5)	3·80–3·94	3·78 (Δ+0·09)	3·57 (Δ+0·30)	3·41 (Δ+0·46)	3·22 (Δ+0·65)
II	7·82d (J = 2·5)	7·77dd (J = 2·5) (J = 9·0)	—	6·97d (J = 9·0)	6·61d (J = 2·5)	6·30d (J = 2·5)	3·91 4·03	—	3·71 (Δ+0·26)	3·49 (Δ−0·48)	3·29 (Δ+0·68)
III	7·78d (J = 2·5)	7·72dd (J = 2·5) (J = 9·0)	—	7·10d (J = 9·0)	6·50d (J = 2·5)	6·41d (J = 2·5)	3·90 4·10	3·82 (Δ+0·18)	3·46 (Δ+0·54)	—	3·25 (Δ+0·75)
IV	8·00d (J = 9·0)	8·00dd (J = 2·5) (J = 9·0)	6·91 (J = 9·0)	6·91d (J = 9·0)	6·50d (J = 2·5)	6·21d (J = 2·5)	3·82 3·97	3·88 (Δ+0·01)	—	—	3·35 (Δ+0·54)
V	7·72d (J = 2·5)	7·55dd (J = 2·5) (J = 9·0)	—	6·83d (J = 9·0)	6·47d (J = 2·5)	6·18d (J = 2·5)	3·80–3·90	—	3·60 (Δ+0·25)	—	3·30 (Δ+0·55)
VI	7·61d (J = 2·5)	7·53dd (J = 2·5) (J = 0·0)	—	6·85d (J = 9·0)	6·32d (J = 2·5)	6·22d (J = 2·5)	3·80–3·95	3·80 (Δ+0·07)	—	—	3·21 (Δ+0·66)
VII	7·70d (J = 2·5)	7·62dd (J = 2·5) (J = 9·0)	—	6·88d (J = 9·0)	6·48d (J = 2·5)	6·20d (J = 2·5)	3·82–4·00	3·83 (Δ+0·08)	3·48 (Δ+0·43)	—	—
VIII	8·08d (J = 9·0)	8·08dd (J = 2·5) (J = 9·0)	6·97 (J = 9·0)	6·97d (J = 9·0)	6·67d (J = 2·5)	6·25d (J = 2·5)	3·85–4·00	3·78 (Δ+0·14)	—	—	—
IX	7·83d (J = 2·5)	7·68dd (J = 2·5) (J = 9·0)	—	6·95d (J = 9·0)	6·58d (J = 2·5)	6·23d (J = 2·5)	3·92	—	3·57 (Δ+0·35)	—	—

* Spectra were recorded in CCl_4 and C_6D_6 (only OMe signals are recorded for this solvent) on a Varian A-60 spectrometer. Values are given in ppm (δ-scale) relative to TMS as an internal standard. Number in parentheses denote coupling constants in Hz. Signals are singlets unless otherwise stated: d (doublet), dd (double doublet).

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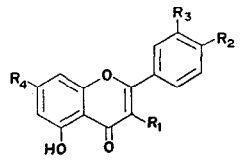
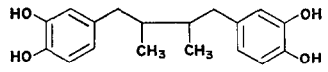


(I) R_1, R_2, R_3, R_4 = OMe
(II) R_1 = OH; R_2, R_3, R_4 = OMe
(III) R_1, R_3, R_4 = OMe; R_2 = OH
(IV) R_1, R_4 = OMe; R_2 = OH; R_3 = H
(V) R_1, R_2 = OH; R_3, R_4 = OMe
(VI) R_1, R_4 = OMe; R_2, R_3 = OH
(VII) R_1, R_3 = OMe; R_2, R_4 = OH
(VIII) R_1 = OMe; R_2, R_4 = OH; R_3 = H
(IX) R_3 = OMe; R_1, R_2, R_4 = OH



(XII)

TABLE 2. UV SPECTRA OF *Larrea cuneifolia* FLAVONOL AGLYCONES*

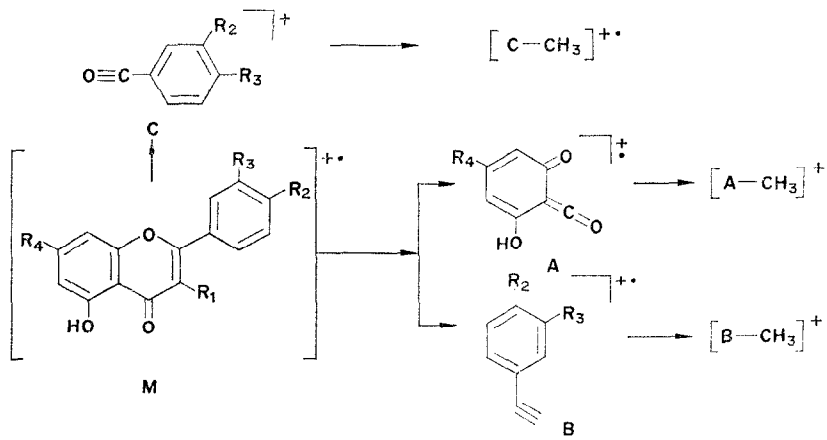
Compd	Methanol (λ_{max}, nm)	NaOMe (λ_{max}, nm)		$AlCl_3$ (λ_{max}, nm)		$AlCl_3$–HCl (λ_{max}, nm)		NaOAc (λ_{max}, nm)		NaOAc–H_3BO_3 (λ_{max}, nm)	
I	352	360		404	Δ:44	402		350		352	
				361		354					
	252	280		294		294		252		252	
	(265)			268		270		(266)		(266)	
II	362	406	Δ:44	420	Δ:58	420				362	
	(308)			364		356					
	(272)	(285)		298		296				250	
	252	263		266		262					
III	354	410	Δ:56	404	Δ:50	404		410		356	
				370		270					
	254	262		298		298		260	Δ:6	254	
	(262)			268		268				(265)	
IV	350	392	Δ:42	398	Δ:46	400		403		349	
				352		347					
	268	268		302		300		264	Δ:−4	266	
		(292)		276		276					
V	370	430	Δ:60	430	Δ:60	430		375		370	
			(dec.)	360		360					
	253	266		265		263		255		255	
		(248)									
VI	360	400	Δ:40	440	Δ:80	405	Δ:45	420		380	Δ:20
						367					
	262	265		278		298		265		262	
						270					
VII	360	410	Δ:50	407	Δ:47	402		378		358	
		(330)		368		363		(320)		(268)	
	(267)	270		300		295		276		255	
	257			270		267					
VIII	348	396	Δ:48	400	Δ:52	396		390		344	
				350		344					
	(282)	275		(294)		(298)		274	Δ:7	266	
	267			267		267					
XI	335	385	Δ:50	368	Δ:33	360		385	Δ:50	322	
				336							
	280	(300)	slow	300		280		(260)			
		260	dec.								
X	336	420	Δ:64	410	Δ:74	410		(320)		(320)	
		330		370		360					
	277	280		305		305		286	Δ:9	282	
				283		285					
IX	366			426	Δ:60	425		408		370	
		dec.		355		357		322		290	
	(268)			302		302		276	Δ:21	272	
	255			265		263				255	

* All UV spectra were recorded using standard procedures.[13]

TABLE 3. MS DATA FOR FLAVONOL AGLYCONES FROM *Larrea cuneifolia*

Compd	$M^{+\cdot}$	M-H (M-1)	M-CH_3 (M-15)	M-H_2O (M-18)	M-HCO (M-29)	M-CH_3O (M-31)	M-CH_3CO (M-43)	$(A^*+H)^+$	$(A\text{-}CH_3)^+$	$B^{+\cdot}$	B-CH_3^+	C^+	C-CH_3^+
I	100†	50	48	5	6	16	57	8				16	7
II	32	16	5	12	16		6	37	13	6	95	100	16
III	100	60	41	8	18	18	59	21	18‡			18‡	
IV	100	85		15	16	10	33	8			—	14	—
V	100	8			6		10		13‡			13‡	
VI	100	80		16	12	6	40	18	14		—	26	—
VII	100	53	47	7	12	14	56	14				19	
VIII§	70	50		13	12	9	24	20	—		—	33	—

* See Scheme 1 for an explanation of the fragment ions A to C.
† Number denotes relative intensity of observed *m/e* ion.
‡ Fragments A-CH_3^+ and C^+ have the same *m/e* values for compounds III and V.
§ The MS of compound IX has been previously discussed (see Ref. 18)



SCHEME 1. MS FRAGMENTATIONS ASSOCIATED WITH RETRO-DIELS-ALDER REACTIONS OF METHYLATED FLAVONOL AGLYCONES.

TABLE 4. CHROMATOGRAPHIC DATA*

Polyamide column		R_fs		Colors	
Fractions	Compd	15% HOAc	TBA	UV	UV/NH_3
3–24	I	0·13	0·85	Purple	Purple
10–17	II	0·04	0·76	Yellow	Fl. yellow
25–36	III	0·14	0·84	Purple	Yellow
36–67	IV	0·06	0·87	Purple	Yellow
62–82	V	0·05	0·75	Yellow	Yellow
75–82	X	0·22	0·78	Purple	Y–green
91–122	VI	0·14	0·81	Purple	Yellow
120–164	VII	0·18	0·82	Purple	Yellow
270–320	VIII	0·21	0·85	Purple	Y–green
347–375	XI	0·14	0·67	Purple	Y–green
430–470	IX	0·03	0·70	Yellow	Yellow

* One-dimensional chromatographs on Whatman No. 1 paper were developed in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and 15% HOAc.

flavonoids were only partially characterized and are designated as compounds X and XI in Tables 2 and 4.

The ether-soluble material* (aglycones) yielded by chromatography over polyamide[13] compounds I–XI (see Table 4). The NMR spectra of the trimethylsilyl ethers of the two new natural products II and III indicated they were trimethyl ethers of quercetin (see Table 1 for NMR assignments). As expected, compounds II and III yielded quercetin upon demethylation with pyridinium hydrobromide.[14]

Comparison of the NMR spectrum in benzene-d_6[15] with that obtained in CCl_4 for II indicated the presence of methoxyls at the 7,3′ and 4′-positions (upfield shifts of 0·26, 0·48 and 0·68 ppm, respectively). That the compound was a flavonol and unsubstituted at positions 3 and 5 was evident from its yellow color when examined as a chromatographic spot (in UV light) and from UV spectra (Table 2). The MS data for the trimethyl ether (Table 3) supported structure II: parent peak at *m/e* 344 (MW for $C_{18}H_{12}O_7$) and *m/e* peaks at 326 (12%; $M-H_2O$) and 315 (16%, corresponding to the loss of 29 mass units, HCO)[16,17] (see Table 3, compound II).

The second new flavonol, III, also contained three methoxyl groups (by NMR) but only two of the OMe signals shifted upfield to 0·54 and 0·75 ppm (Table 1) when the spectrum was recorded in benzene-d_6. This result indicated that two of the positions 7,3 and 4′ were substituted. The UV spectrum in the MeOH–NaOMe confirmed that the 7 and 3′ positions were methoxylated (bathochromic shift of 56 nm for Band I with an increase in intensity is diagnostic for the presence of a free 4′-OH).[13] Since the flavonol is dark purple when viewed in UV, the 3-position must be substituted by the third methoxyl;[13] thus the NMR signal at δ 3·82 (in benzene-d_6) can be assigned to the OMe group at position 3. The MS data were in agreement with the 3,7,3′-trimethylquercetin structure: parent peak at *m/e* 344 (MW for $C_{18}H_{18}O_7$) and strong *m/e* peaks at 329 (41%; $M-CH_3$) and *m/e* 301 for the loss of $M-COCH_3$ from the C-3 position[16] (see Table 3, compound III).

NMR, UV and MS data are presented in Tables 1–3, respectively, for all nine methylated flavonols (compounds I–IX) as well as nordihydroguaiaretic acid (XII). Kingston[16] in his review of the MS of methylated flavonols points out that the most common fragment ions observed are those of M-15 (CH_3), M-18 (H_2O), M-19 (H_3O^+), M-28 (CO), M-29 (COH), and M-43 ($COCH_3$). These types of fragmentations are indeed present in all of the methylated flavonols of *Larrea cuneifolia*; in addition, the usual fragment ions from retro-Diels-Alder reactions were also observed (Table 3 and Scheme 1).

EXPERIMENTAL

All voucher specimens are deposited in the University of Buenos Aires Herbarium, Buenos Aires, Argentina. Air-dried and ground leaf material of *Larrea cuneifolia* Cav. (collected on Route 3, KM 1193, in the province of Rio Negro, Argentina; JHH and RAP, No. 8523) was extracted with 80% aqueous methanol and the extract was filtered and concentrated. The aqueous solution was extracted with Et_2O in a continuous liquid–liquid extractor. The ether extracts, containing only methoxylated flavonols and nordihydroguaiaretic acid, were combined and taken to dryness *in vacuo*. The greenish syrup (9 g) was chromatographed over polyamide (200 g packed in the elution solvent); the column was eluted with Egger's solvent ($CHCl_3$–MeOH–MeCOEt–2,4-pentanedione, 20:10:5:1). Fractions of 15 ml were collected (see Table 4), spotted on

* The aqueous methanol-soluble material appears to be rich in flavonoid glycosides all of which are presently under investigation.

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one-dimensional chromatograms and developed in the appropriate solvents. Fractions (430–470) contained a mixture of compound IX and nordihydroguaiaretic acid.

The NMR spectrum of nordihydroguaiaretic acid (XII) displayed the following signals: aromatic protons (6) at 6·55–6·91; methylene protons (6) at 1·9–2·9; two secondary methyls at 0·85 (J = 7 Hz). MS showed the following peaks: parent peak at m/e 302 (26·2%) and strong m/e peaks at, 151 (5·2%), 137 (12·1%), 123 (base peak), 69 (13·1%).

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