CONSTITUENTS OF *CHRYSOTHAMNUS PANICULATUS* 3: 3,4,5-TRICAFFEOYLQUINIC ACID (A NEW SHIKIMATE PREAROMATIC) AND 3,4-, 3,5- AND 4,5-DICAFFEOYLQUINIC ACIDS

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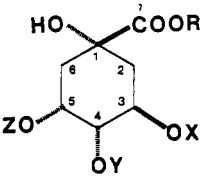
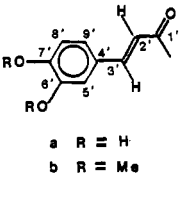
ABSTRACT.—A methanol extract of *Chrysothamnus paniculatus* (Compositae) gave, upon separation of the phenolic acid fraction followed by methylation and chromatography, methylated derivatives of 3,4,5-tricaffeoylquinic acid (**1a**), which is new, and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids (**2a-4a**, respectively), which had been previously described. ^{13}C-nmr and ms data are reported for the latter three compounds for the first time.

Earlier work on ether and ethyl acetate extracts of *Chrysothamnus paniculatus* (Gray) Hall (Compositae) gave a series of grindelane diterpenoids (1-3). We now report the presence, in the methanol extract of this plant, four shikimate prearomatics: 3,4,5-tricaffeoylquinic acid (**1a**), which is new, and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids (**2a**, **3a** and **4a**, respectively), which had been found in coffee beans (4) and various other plants (5). These four natural products were not separated but were converted into their methylate derivatives **1b-4b**, which were separated chromatographically and characterized by ^{1}H-nmr (table 1), ^{13}C-nmr (table 2), and mass spectroscopy (scheme 1), as described below. The absence of methoxyl absorption in the ^{1}H-nmr spectrum of the acidic fraction prior to methylation confirmed that the naturally occurring compounds were **1a-4a**.



	X	Y	Z
1	Caf	Caf	Caf
2	Caf	Caf	H
3	Caf	H	Caf
4	H	Caf	Caf



The ^{1}H-nmr absorptions of H-3, H-4, and H-5 were easily recognized in the spectra of **2b-4b** by their very different splitting patterns, and the chemical shifts of these protons then served to distinguish the three compounds from one another: the upfield proton is H-5 in **2b**, H-4 in **3b**, and H-3 in **4b** (6). Peaks in the ^{13}C-nmr spectra were assigned with the aid of off-resonance decoupling (all compounds), ^{1}H-^{13}C decoupling (**2b** only), and comparisons of the spectra of 2b-4b. As expected, the removal of

TABLE 1. ^{1}H-nmr shifts (δ) and coupling constants (J, in Hz) for **1b-4b** in $CDCl_3$.

	1b	2b	3b	4b
H-2α	2.45	2.51	2.33	2.32
H-2β	2.20	2.09	2.11	2.11
H-3	5.83	5.78	5.61	4.52
H-4	5.35	5.21	3.93	5.06
H-5	5.74	4.44	5.51	5.68
H-6α	2.31	2.25	2.33	2.32
H-6β	2.38	2.25	2.20	2.23
H-2′	6.25,6.25,6.42	6.23,6.36	6.34,6.41	6.30,6.35
H-3′	7.59,7.60,7.68	7.58,7.65	7.67,7.70	7.63,7.64
H-5′	6.96,7.01,7.11,	~7.00,~7.00	7.06,7.06	6.99,~7.04
H-8′	6.78,6.83,6.86	6.87,6.87	6.78,6.81	6.80,6.84
H-9′	7.01,7.07,7.10	~7.00,~7.00	7.11,7.11	~7.04,7.07
MeO	3.82,3.82,3.88,3.89, 3.90,3,93,3,95	3.82,3.87,3.87, 3.88,3.88	3.80,3.92,3.92, 3.92,3.95	3.83,3.84,3.89 3.90,3.90
$J_{2\alpha,2\beta}$	13.4	~13	13.2	13.2
$J_{2\alpha,3}$	~3	3.2	3.4	~3
$J_{2\beta,3}$	10.7	~11	10.9	10.4
$J_{3,4}$	9.9	10.0	~10.9	9.3
$J_{4,5}$	3.4	~3	3.6	3.3
$J_{5,6\alpha}$	~3	~3	3.6	3.5
$J_{5,6\beta}$	3.5	~3	3.6	3.5
$J_{6\alpha,6\beta}$	~16	[a]	~15.5	~15
$J_{2',3'}$	15.9.15.9,15.9	16.0,16.0	16.0,16.0	15.9,15.9
$J_{5',9'}$	2.0,2.0,2.0	[a]	1.9,1.9	[a]
$J_{8',9'}$	8.4,8.4,8.4	[a]	8.4,8.4	8.5,8.5

[a]These coupling constants were not obtained.

an acyl group causes an upfield shift for the resulting alcoholic carbon atom (β effect) and a downfield shift for the carbons attached to the alcoholic carbon (γ effect). The downfield shift of C-1 in **2b** is probably caused by a change in the hydrogen bonding arrangement between the C-1 OH and the C-5 substituent in the predominant chair form. The downfield shift of C-7 in **4b** indicates the presence of some of the *other* chair form, in which hydrogen bonding can occur between the 3-OH and the C-7 carbonyl group.

The ir and ms of **2b-4b** were virtually identical. The unexpected peaks at *m/z* 776 and 774 in the spectrum of 26 correspond to an extra caffeoyl group and the loss of methanol (scheme 1), which appears to be due to intramolecular acyl migration during heating of the sample for volatilization in the ion source. Some indole alkaloids are known to give peaks well above their molecular ion peaks for similar reasons (7).

The structure 3,4,5-tri(3′,4′-dimethoxy-*E*-cinnamoyl) quinate (**1b**) was assigned to the major component from the methylation on the basis of its spectral properties and by comparison with those of **2b-4b**. The ^{1}H-nmr spectrum (table 1) showed an extra 3′,4′-dimethoxy-*E*-cinnamoyl group to be present, and the chemical shifts of H-3, H-4, and H-5 showed acyl groups to be present at all three of these positions. The ^{13}C-nmr spectrum (table 2) was consistent with structure **1b**. The ir spectrum was virtually indistinguishable from those of **2b-4b**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A description of the analytical procedures has been produced by Jolad *et al.* (8); Hoffmann *et al.* describe the plant material used in this study (1).

TABLE 2. ^{13}C-nmr shifts (δ) for **1b-4b** in $CDCl_3$.

	1b	**2b**	**3b**	**4b**
1	74.4s	75.6	74.5	74.5
2	39.2t	39.4	38.7	41.4
3	67.4d	67.1	71.7	65.5
4	72.2d	74.9	70.4	75.8
5	69.1d	69.1	72.6	69.0
6	36.1t	37.3	35.8	36.5
7	174.7s	174.4	174.8	175.3
1′	166.2,166.3,166.3s	166.4[4],166.8[3]	166.8[3],167.1[5]	166.2[4],166.9[5]
2′	115.1[4],115.3[3],115.6[5]d	115.2[3],115.2[4]	115.4[3],115.4[5]	115.1[4],115.6[5]
3′	145.6[4],145.7[3],145.8[5]d	145.5[4],145.8[3]	145.7[3],145.9[5]	145.6[4],146.0[5]
4′	127.4[3],127.4[4],127.5[5]s	127.2[3],127.24	127.4[3],127.5[5]	127.4[4],127.5[5]
5′	110.0,110.1,110.1d	109.7,109.7	110.1,110.2	110.1,110.2
6′	149.4,149.5,149.5s	149.2,149.2	149.5,149.5	149.5,149.5
7′	151.5,151.5,151.5s	151.3,151.3	151.6,151.6	151.6,151.6
8′	111.3,111.3d	111.0,111.0	111.3,11.4	111.3,111.3
9′	122.9[4],122.9[5],123.1[3]d	122.9[4],123.0[3]	122.8[5],123.1[3]	122.9[4],122.9[5]
CafOMe	6 x 56.0q	4 x 56.0	4 x 56.1	4 x 56.1
CO_2Me	53.2q	53.4	53.1	53.3

[3,4,5]These superscripts designate to which carbon the methylated caffeoyl group is attached, and must be regarded as tentative inasmuch as the differences are quite small.

ISOLATION OF PHENOLIC ACID MIXTURE.—The marc from EtOAc extraction process of *C. paniculatus* (3) was extracted exhaustively in a Lloyd extractor with methanol. In a typical run, 130 g of concentrated dark-brown methanol extract was partitioned between *n*-butanol (500 ml) and water (500 ml), and the butanol layer, after extracting once more with water (300 ml), was evaporated to dryness (34 g) under vacuum, dissolved in 5% aqueous Na_2CO_3 (500 ml), and extracted with ether (2 x 400 ml). The alkali layer was carefully acidified (pH 6) with 10% aqueous hydrochloride, and the resulting precipitate was extracted with *n*-butanol (700 ml). The butanol layer was evaporated to dryness (24 g) under vacuum.

ISOLATION OF **1b-4b**.—The above phenolic acid mixture (24 g) was methylated under the conditions described by Timmermann *et al.* (3). The methylated product (19 g) was estimated by hplc [Varian Vista 5000 Fully Automatic Liquid Chromatograph equipped with a UV detector (set at 220 nm)] and microcomputer-based chromatography data system (Varian Vista CD 401): MCH-10 (30 cm x 4 mm) reversed-phase column; mobile phase 50% aqueous methanol to 100% methanol with the flow rate at 2 ml/min to contain 29% **1b**, 13% **2b**, 13% **3b**, and 21% **4b**. It was dissolved in CH_2Cl_2-MeOH (1:1), adsorbed on EM SiO_2-60 and subjected to EMSiO_2-60 column chromatography. The column was eluted with CH_2Cl_2-EtOAc (90:10); and fractions (1000 ml) were collected. **1b** was isolated from fraction 2, **2b** from fraction 3, and **3b** and **4b** from the combined fractions 5 and 6 by preparative tlc using various mixtures of Ch_2Cl_2-EtOAc. Repetition of preparative tlc gave non-crystalline tlc homogeneous **1b-4b**, purity greater than 90% as judged from analytical reversed-phase hplc.

METHYL 3,4,5-TRI(3′,4′-DIMETHOXYCINNAMOYL) QUINATE (**1b**).—Spectral properties: $[\alpha]^{25}D$ −257.7°, c 0.44, MeOH; ir ($CHCl_3$) 3600, 3550, 3020, 2980, 2850, 1735, 1710, 1632, 1600, 1585, 1512, 1463, 1440, 1420, 1300, 1250, 1210, 1152, 1135, 1065, 1020, 975, 840, 800 cm^{-1}; ^{1}H-nmr, table 1; ^{13}C-nmr, table 2; ms, molecular ion peak at *m/z* 776 followed by daughter ion peaks at *m/z* 744 [M-32(MeOH)], *m/z* 586 [M-190(Caf-H)], *m/z* 584 [M-192(Caf+H)], *m/z* 570 [M-206(Caf·O-H)], *m/z* 569 (M-207(Caf·O)], *m/z*568 [M-208(Caf·Oh)], *m/z* 554 [M-222(Caf·OMe)] and *m/z* 509.

Anal. Calcd for $C_{41}H_{44}O_{15}$: Mol. wt., 776.2680. Found: Mol. wt., 776.2725.

METHYL DI(3′,4′-DIMETHOXYCINNAMOYL) QUINATES (**2b-4b**).—Spectral properties; $[\alpha]^{25}D$ (in MeOH) −125.3° (**2b**), c 1.78; −203.9° (**3b**), c 0.85; −87° (**4b**), c 0.73; ir ($CHCl_3$) indistinguishable from one another and **1b**; ^{1}H-nmr, table 1; ^{13}C-nmr, table 2; ms, scheme 1.

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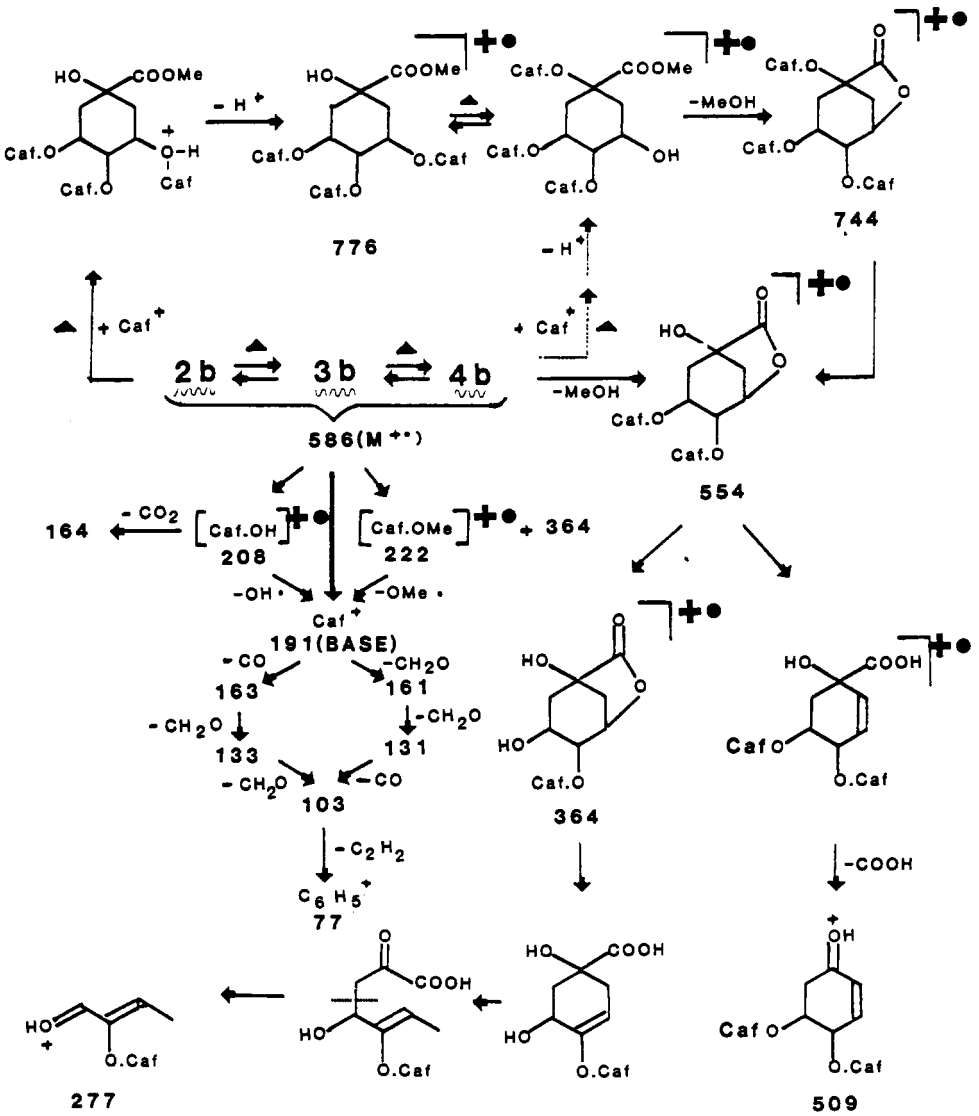

SCHEME 1. Major significant ions (*m/z* ratios) in the mass spectra of **2b-4b**.

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