Journal of Natural Products
Vol. 51, No. 5, pp. 851-856, Sep-Oct 1988

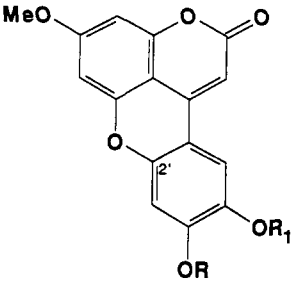
CHEMICAL STUDIES ON MEXICAN PLANTS USED IN TRADITIONAL MEDICINE, VI.[1] ADDITIONAL NEW 4-PHENYLCOUMARINS FROM *EXOSTEMA CARIBAEUM*[2]

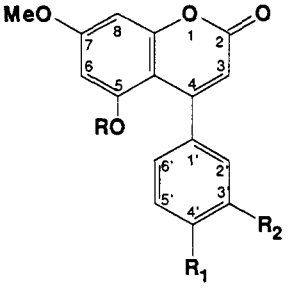
RACHEL MATA,* FERNANDO CALZADA, and MARIA DEL ROSARIO GARCIA

Departamento de Farmacia, División de Bioquímica y Farmacia, Facultad de Química de la Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán 04510, México DF

ABSTRACT.—Six additional 4-phenylcoumarins have been isolated from the MeOH extract of the bark of *Exostema caribaeum*. Their structures were determined by physical and chemical methods as 4′-5′-dihydroxy-7-methoxy-4-phenyl-5,2′-oxidocoumarin [**1**], 5,7,4′-trimethoxy-4-phenylcoumarin [**2**], 5,3′-dihydroxy-7,4′-dimethoxy-4-phenylcoumarin [**3**], 5-*O*-β-D-galactopyranosyl-7,4′-dimethoxy-4-phenylcoumarin [**6**], 5-*O*-β-D-glucopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin [**7**], and 5-*O*-(6″-acetyl)-β-D-galactopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin [**9**]. The last four compounds are new natural products.

In a previous communication we reported the isolation and identification of three 4-phenylcoumarins from the stem bark of *Exostema caribaeum* (Jacq.) Roem. et Schult., a member of the Rubiaceae commonly used in Mexican traditional medicine as an antimalarial agent (1). Continuing with the investigation of the column chromatography fractions obtained as previously described (1), we have now isolated and characterized six additional 4-phenylcoumarins: three glycosides **6**, **7**, and **9**, an oxidocoumarin **1**, and the two simple 4-phenylcoumarins **2** and **3**. Four of these compounds, **3**, **6**, **7**, and **9**, are new natural products.





1 R=R$_1$=H
13 R=Me, R$_1$=H

2 R=Me, R$_1$=OMe, R$_2$=H
3 R=H, R$_1$=OMe, R$_2$=OH
4 R=Ac, R$_1$=OMe, R$_2$=OAc
5 R=Me, R$_1$=R$_2$=OMe
6 R=β-D-galactopyranosyl, R$_1$=OMe, R$_2$=H
7 R=β-D-glucopyranosyl, R$_1$=R$_2$=OH
8 R=tetraacetyl-β-D-glucopyranosyl, R$_1$=R$_2$=OAc
9 R=6″-acetyl-β-D-galactopyranosyl, R$_1$=R$_2$=OH
10 R=tetraacetyl-β-D-galactopyranosyl, R$_1$=R$_2$=OAc
11 R=H, R$_1$=R$_2$=OH
12 R=Ac, R$_1$=R$_2$=OAc
14 R=β-D-galactopyranosyl, R$_1$=R$_2$=OH
15 R=R$_2$=H, R$_1$=OMe
16 R=Me, R$_1$=R$_2$=OMe

[1]For part V see R. Mata, P. Castañeda, Ma. Rayo Camacho and G. Delgado, *J. Nat. Prod.*, **51**, 836 (1988).

[2]Taken in part from the M.S. research work of Maria del Rosario Garcia.

TABLE 1. ^{1}H-nmr Chemical Shifts of Compounds **3**, **4**, **6**, **7**, **9**, **10**, and **12** (80 MHz, TMS as internal standard).[a]

Proton	Compound							
	3[b]	**4**[b]	**6**[c]	**7**[d]	**9**[c]	**9**[e]	**10**[b]	**12**[b]
H-3	5.95 s	6.04 s	5.92 s	5.87 s	5.76 s	6.17 s	6.15 s	6.07 s
H-6	6.28 d (3)	6.81 d (3)	6.53 d (3)	6.56 d (3)		6.98 d (3)	6.65 d (3)	6.82 d (3)
H-8	6.51 d (3)	6.42 d (3)	6.62 d (3)	6.61 d (3)		6.61 d (3)	6.53 d (3)	6.48 d (3)
H-2′		7.12 d (3)	7.20 d (8)	6.80 d (3)		7.33 d (3)		
H-5′			6.87 d (8)	6.82 d (8)		7.10 d (8)	7.15	7.10–7.30 m
H-6′	6.97–6.99 m	7.04–6.94 m	7.20 d (8)	6.55 d (8,3)	6.50–6.80 m	6.98 dd (8,3)	7.20 m	
H-3′	—	—	6.87 d (8)	—	—	—	—	—
7-OMe	3.83 s	3.86 s	3.84 s	3.85 s	3.75 s	3.77 s	3.85 s	3.83 s
4′-OMe	3.98 s	3.88 s	3.84 s	—	—	—	—	—
C-5	—	1.55 s	—	—	—	—	—	1.59 s
3′-OAc	—	2.32 s	—	—	—	—	2.28 s	2.30 s
4′-OAc	—	—	—	—	—	—	2.28 s	2.30
C-2″–C-6″	—	—	—	—	1.96 s	2.095 s	1.85 s 1.92 s 1.99 s 2.18 s	—
H-1″	—	—	4.54 d (8)	4.70 d (8)	4.63 d (8)			
H-2″–H-6″			4.03–3.0 m	3.10–4.60 m	2.90–3.6 m	4.05–5.25 m	3.29–5.30 m	—

[a]Coupling constants (Hz) in parentheses.
[b]$CDCl_3$ as solvent.
[c]DMSO-d_6 as solvent.
[d]DMSO-d_6/$CDCl_3$ as solvent.
[e]Pyridine-d_5 as solvent.

5-*O*-(6″-acetyl)-β-D-Galactopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin [**9**] had the composition $C_{24}H_{24}O_{11}$ (elemental analysis). Treatment with Ac_2O/pyridine afforded the hexaacetyl derivative **10**. The presence of a 4-phenylcoumarin skeleton in the molecule was easily deduced by the uv and ir spectra along with the highly diagnostic resonance of the H-3 proton (Table 1) (1,2). The ^{1}H-nmr spectra in pyridine-d_5 exhibited, in addition to the sugar and H-3 signals, an ABC system for a trisubstituted aromatic ring [δ 7.33 (d, J = 3 Hz), δ 7.10 (d, J = 8 Hz) δ 6.87 (dd, J = 8,3 Hz)], an AB system attributed to two mutually meta located protons [signals at δ 6.98 and δ 6.61], and peaks for a methoxyl (δ 3.77) and a nonaromatic acetate (δ 2.09) group. The ^{13}C-nmr spectra (Table 2) confirmed all the above mentioned functionalities, and the signals at δ 100.8, 72.81, 72.66, 70.06, 68.22, and 63.52 strongly supported the presence of one unit of 6″-acetylgalactose in the molecule. The assignment of the sugar was accomplished by comparing the ^{13}C-nmr peaks of **9** with those of other aromatic galactosides (1,3,4), and by considering that the acylation of a sugar generally produces a downfield shift of about 2 ppm in the signal of the acylated carbon, accompanied by upfield shifts of ca. 1–3 ppm in the resonances of adjacent carbons (4). The hydrolysis of **9** with 1 N HCl afforded galactose (tlc) and an aglycone that was characterized as 3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin [**11**], whose spectral parameters were previously described (1). The presence of a noticeable shifted upfield signal for an acetate at δ 1.59 in the ^{1}H nmr of the triacetyl derivative of the aglycone **12** (1) and the absence of such a signal in the ^{1}H nmr of **10** (Table 1) were consistent with the attachment of the sugar moiety at C-5 (1,2). Finally, the β-*O*-glyco-

TABLE 2. ^{13}C-nmr Spectra of Compounds **6**, **7**, and **9** (DMSO-d_6, TMS as internal standard).

Carbon atom	Compound		
	6	**7**	**9**
2	160.00 s	159.65 s	159.46 s
3	112.31 d	112.14 d	112.28 d
4	156.35 s	156.35 s	156.37 s
4a	103.26 s	103.48 s	103.50 s
5	155.54 s	155.54 s	155.36 s
6	98.36 d	98.27 d	98.94 d
7	162.69 s	162.80 s	162.66 s
8	95.14 d	94.29 d	95.05 d
8a	155.97 s	155.86 s	155.67 s
1′	129.80 s	130.35 s	130.44 s
2′	129.37 d	114.69 d	114.76 d
3′	114.20 d	144.05 s	144.13 s
4′	157.60 s	145.73 s	145.75 s
5′	114.20 d	115.72 d	115.54 d
6′	129.37 d	119.24 d	119.17 d
7-MeO	55.91 q	55.96 q	55.8 q
4′-MeO	55.91 q	—	—
1″	100.90 d	100.01 d	100.80 d
2″	69.78 d	73.03 d	70.06 d
3″	73.30 d	76.33 d	72.66 d
4″	68.05 d	69.56 d	68.22 d
5″	75.85 d	77.15 d	72.81 d
6″	60.46 t	60.73 t	63.52 t
CH_3-C=O	—	—	20.35 q
CH_3-C=O	—	—	170.12 s

sidic linkage was inferred both from the coupling constant value (d, $J = 8$ Hz) observed for the anomeric proton (δ 4.63) in the ^{1}H nmr of **9** in DMSO-d_6 and from the enzymatic hydrolysis of the deacetylated galactoside with a β-galactosidase.

5-*O*-β-D-glucopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin [**7**] possesses a molecular formula of $C_{22}H_{22}O_{11}$ (elemental analysis). The spectral properties of this compound were very similar to those of **9**, differing mainly in the ^{13}C- and ^{1}H-nmr signals for the sugar portion, which was readily assigned to glucose (3). Upon acid (2 N H_2SO_4) and enzymatic hydrolysis (β-glucosidase), **7** yielded glucose and **11**. The positioning of the sugar and the nature of the glycosidic linkage were established as described for **9**.

5-*O*-β-D-galactopyranosyl-7,4′-dimethoxy-4-phenylcoumarin [**6**] had a molecular formula of $C_{23}H_{24}O_{10}$. The hydrolysis with β-galactosidase afforded galactose (tlc) and aglycone **15**, which upon methylation with ethereal CH_2N_2 yielded **2**. The ^{1}H-nmr spectrum (Table 1) was similar to those of **7** and **9**; however, for the 4-aryl protons two doublets (2H each) ortho coupled at δ 7.20 and 6.87, rather than the ABC system, were observed. Also, the singlet at δ 3.84 (6H) suggested the presence of two methoxyl groups instead of one. The ^{13}C nmr (Table 2) was consistent with the presence of galactose (1,3,4), the 4-aryl ring's being para substituted with a methoxyl group, and the location of the sugar moiety at C-5. The observed chemical shifts for the aromatic carbons were in good agreement with those calculated using as a model 7-methoxy-4-phenylcoumarin (5). The β-anomeric center was deduced in the same way as for **7** and **9**.

5,3′-Dihydroxy-7,4′-dimethoxy-4-phenylcoumarin [**3**], had the composition $C_{17}H_{14}O_6$ (ms). Its spectral properties revealed remarkable, albeit partial, similarities with those of aglycone **11**. Thus, the eims contains intense peaks at *m/z* $[M]^+$ 314 (base peak), $[M-28]^+$ 286 (64.8), $[M-28-15]^+$ 271 (37), 196. The ^{1}H nmr in $CDCl_3$ (Table 1) exhibited the typical signal for H-3 (δ 5.95, s), an AB system for H-8 and H-6 (δ 6.51 and 6.28, d, 3 Hz), and resonances for two methoxyl groups (δ 3.98 and δ 3.83). In this case, however, the signals attributable to the 4-aryl-ring protons appeared as a complex set that could not be resolved even when the solvent was substituted by C_5N_5D. Therefore, the substitution pattern of the pendant ring was established by chemical correlation with **11**; methylation with CH_2N_2/Et_2O of **11** and **3** afforded the same tetramethyl derivative **16** (1). Acetylation with Ac_2O/pyridine gave the diacetyl derivative **4**. The presence of an upfield acetate signal at δ 1.55 in the ^{1}H nmr of **4** was indicative of a free hydroxyl group at C-5 in **3**. Also, this spectrum allowed us to conclude that one of the methoxyl groups was at C-7 because, upon acylation, only one of the branches of the AB system shifted downfield significantly. Finally, the relative disposition of the hydroxyl and methoxyl groups in the 4-aryl ring was ascertained by the chemical conversion of **3** into 5′-hydroxy-7,4′-dimethoxy-4-phenyl-5,2′-oxidocoumarin [**13**] by treatment with alkali (MeOH/KOH) in the presence of K_3 [$Fe(CN)_6$]. The physical and spectral parameters of the reaction product were identical to those of the natural product **13** (1).

Compound **2** was previously isolated from *Coutarea hexandra* Jacq., and its properties were in good agreement with those previously described (2). Compound **1** was previously synthesized from **11** by treatment with KOH/MeOH; thus, identification by comparison with an authentic sample was possible (1). Also, **1** was isolated from *Coutarea latiflora* Sessé et Moc. ex DC. (6).

During the revision of this paper, the isolation and structure elucidation of compound **7** were also reported by Aquino *et al.* (7). It is worthwhile to mention that the ir spectrum of our aglycone **11** and that of 5,2′,5′-trihydroxy-7-methoxy-4-phenylcoumarin, previously reported by Reher and co-workers (8,9), are identical, indicating that their compound was misidentified. Aquino *et al.* (7) arrived at the same conclusion

just by comparing the ^{1}H-nmr data in CD_3COCD_3 of **11** with those described by Reher *et al.* (8).

Compounds **14**, **11**, and **13** were evaluated as antimicrobial agents. Compounds **11** and **13** were inactive. However, compound **14** showed moderate activity against *Candida albicans* (MIC = 12.5 μg/ml). Compounds **3**, **6**, **7**, and **9** will be evaluated by IOCD as potential antimalarial agents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected; ir spectra were recorded on a Nicolet FT-IR instrument; uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer with MeOH as solvent and ^{1}H-nmr spectra on a Varian FT spectrometer (80 MHz) in $CDCl_3$ $CDCl_3$/DMSO-d_6, or pyridine-d_5 solutions with TMS as internal standard. ^{13}C-nmr spectra were taken in a 50 MHz instrument; mass spectra were determined on a Hewlett-Packard 5985-B spectrometer.

EXTRACTION AND FRACTIONATION.—For the extraction, preliminary fractionation, and separation procedures see Mata *et al.* (2).

ISOLATION OF 5,7,4′-TRIMETHOXY-4-PHENYLCOUMARIN [**2**].—Fractions 14–17, eluted with $CHCl_3$-EtOH (99.5:0.5), of the initial column (1) were rechromatographed on Si gel (7 g); elution was started with hexane and then with increasing amounts of $CHCl_3$. Fractions 10–13 eluted with hexane-$CHCl_3$ (70:30) yielded 90 mg (0.007% of the dry wt) of **2**: mp 150–152° [lit. (2) mp 151–152°]. ^{1}H-nmr, uv, and ms data were identical to those previously described (2).

ISOLATION OF 5,3′-DIHYDROXY-7,4′-DIMETHOXY-4-PHENYLCOUMARIN [**3**].—From fractions 38–50, also eluted with $CHCl_3$-EtOH (99.5:0.5) (1), crystallized 155 mg (0.012% yield) of **3**, mp 225–226°; uv λ max (MeOH) 196, 252, 328 nm; ir ν max (KBr) 3460, 1690, 1620, 1515, 1440, 1370, 1350, 1335, 1285, 1200, 1050, 835, 810 cm^{-1}; eims *m/z* (rel. int.) 314 (100), 286 (64.8), 271 (37.7).

ISOLATION OF 4′,5′-DIHYDROXY-7-METHOXY-4-PHENYL-5,2′-OXIDOCOUMARIN [**1**].—Further chromatography of fractions 150–295 eluted with $CHCl_3$-EtOH (90:10) (1) on Si gel (46 g) using $CHCl_3$-MeOH (95:5) as eluent allowed the isolation of a yellow crystalline powder. Subsequent recrystallizations from MeOH yielded 18.9 mg (0.0015% yield) of **1**, mp>300°, identical to a standard sample previously synthesized (1).

ISOLATION OF 5-*O*-(6″-ACETYL)-β-D-GALACTOPYRANOSYL-3′,4′-DIHYDROXY-7-METHOXY-4-PHENYLCOUMARIN [**9**].—From fractions 296–316 eluted with $CHCl_3$-EtOH (90:10) crystallized 545.9 mg (0.043% yield) of **9**, mp 215–220°; uv λ max (MeOH) 218, 258, 328 nm; ir ν max (KBr) 3467, 3409, 3395, 2918, 1730, 1695, 1536, 1614, 1442, 1355, 1258, 1072, 1047 cm^{-1}. *Anal.* calcd for $C_{24}H_{24}O_{11}$: C 57.14, H 4.76, found C 56.99, H 4.73.

ISOLATION OF 5-*O*-β-D-GLUCOPYRANOSYL-3′4,′-DIHYDROXY-7-METHOXY-4-PHENYLCOUMARIN [**7**].—The yellow residue obtained from fractions 400–442 [eluted with $CHCl_3$-EtOH (90:10)] of the original column was further purified by recrystallization from MeOH to yield 903.6 mg (0.071% of the dry wt) of **7**, mp 237–238°; uv λ max (MeOH) 218, 259, 329 nm; ir ν max (KBr): 3500, 2900, 1700, 1618, 1430, 1360, 1310, 1230, 1090, 1040. *Anal.* calcd for $C_{22}H_{22}O_{11}$: C 57.14, H 4.76, found C 56.95, H 4.80.

ISOLATION OF 5-*O*-β-D-GALACTOPYRANOSYL-7,4′-DIMETHOXY-4-PHENYLCOUMARIN [**6**].—The residue left after the isolation of compound **7** was combined with fractions 443–462, and after repeated recrystallizations from MeOH, 50 mg (0.0039% yield) of **6**, mp 217–221°, was obtained; uv λ max (MeOH) 212, 326 nm; ir ν max (KBr) 3500, 3200, 1690, 1671, 1614, 1593, 1512, 1444, 1367, 1252, 1205, 1120, 1068, 831 cm^{-1}. *Anal.* calcd for $C_{23}H_{24}O_{10}$: C 60.00, H 5.21, found C 59.90, H 5.19.

ACID HYDROLYSIS OF **7** AND **9**.—Compound **7** (56.2 mg) was refluxed for 3 h with 2 ml of 2 N H_2SO_4. The aglycone **11** (13.3 mg) precipitated from the acid solution, and it was monitored as previously described (1). The physical and spectral properties of **11** were identical to those previously reported (1); glucose was identified (tlc) in the acid solution by comparison with an authentic sample. Compound **9** (100 mg) was dissolved in 200 ml of 2 N HCl. After refluxing for 3 h, **11** (50.4 mg) precipitated from the acid solution. In this case galactose was identified as the sugar component by tlc.

ENZYMATIC HYDROLYSIS OF **6** WITH CELLULOSE.—To 8 mg of **6** were added 1 ml of H_2O and 16 mg of cellulose (Sigma Type I). The mixture was incubated at 36° for 72 h. After usual workup, the

aglycone was separated by preparative tlc using as solvent system $CHCl_3$-MeOH (8:2). The separated substance (4 mg) was dissolved in MeOH and treated with CH_2N_2 in Et_2O, yielding 3 mg of **2**.

ENZYMATIC HYDROLYSIS OF COMPOUNDS **6** AND **7**.—Compounds **6** and **7** and deacylated **9** (1 mg in each case) were treated with 0.5 ml of H_2O and 1 mg of enzyme (β-galactosidase in the case of **6** and **9** and β-glucosidase in the case of **7**). The mixtures were incubated for 48 h at 36°. The completeness of the hydrolysis was monitored by tlc. β-D-Galactose was readily identified by tlc in the hydrolysates of **6** and **9**. β-D-Glucose was present in the hydrolysis products of **7**.

PREPARATION OF THE METHYL ETHER DERIVATIVE **5**.—To 20 mg of **3** dissolved in MeOH was added an excess of CH_2N_2 in Et_2O. The reaction mixture was left overnight, and after pulling out the solvent 12.1 mg of **5**, mp 153–155°, was obtained. This compound was identified by comparison with an authentic sample (1).

ACETYLATION OF **9**, **7**, AND **3**.—To a solution of **3**, **7**, and **9** (50 mg of each in 0.5 ml of pyridine was added 0.5 ml of Ac_2O; the mixtures were left at room temperature for 24 h, and after usual workup the acetyl derivatives **10**, **8**, and **4**, respectively, were obtained. Compound **10** (61.7 mg) was identified with an authentic sample (1). Compound **8** (40 mg): mp 90–95°; ir ν max (KBr) 2930, 1755, 1620, 1370, 1225, 1170, 1110, 1070, 910 cm^{-1}. Compound **4** (71 mg): mp 162–163°; ir ν max (KBr) 2940, 1758, 1621, 1369, 1224, 1158, 1070, 915 cm^{-1}; eims *m/z* (rel. int.) 398 (14.9), 356 (39.8), 314 (100), 286 (38.1), 43 (39.0).

CONVERSION OF **3** TO **13**.—To a solution of **3** (10 mg) in 10% KOH/MeOH solution (5 ml) was added 1 ml of 0.08 M $K_3Fe(CN)_6$. After 3 h, the mixture was neutralized with 0.1 N HCl, extracted with three 30-ml portions of EtOAc. After usual workup of the organic phase, 5 mg of **13** were obtained. Compound **13** was identified by comparison with the natural product previously obtained (1).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the following people: Dra. Rosalinda Contreras and Mr. Guillermo Uribe who provided facilities and assistance necessary to conduct the nmr analysis; Mr. Alejandro Correa from Negromex S.A. for the recording of uv and ir spectra; the staff of the mass spectrometry laboratories of the Instituto de Quimica, UNAM, and Dr. Guillermo Delgado for his continuous help. Partial support from IOCD is also acknowledged. The antimicrobial testing results were obtained in the Medicinal Chemistry Laboratories of Kansas University, Lawrence, Kansas, in a biotesting program organized by IOCD.

LITERATURE CITED

1. R. Mata, F. Calzada, M.R. Garcia, and M.T. Reguero, *J. Nat. Prod.*, **50**, 866 (1987).
2. G. Delle Monache, B. Botta, A.S. Neto, and R. Alves de Lima, *Phytochemistry*, **22**, 1657 (1983).
3. K.R. Markham, H.D. Zinsmeister, and R. Mues, *Phytochemistry*, **17**, 1601 (1978).
4. K.R. Markham. "Techniques of Flavonoid Identification," Academic Press, London, 1982, p. 82.
5. A. Pelter, R.S. Ward, and T. Gray, *J. Chem. Soc., Perkin Trans. 1*, 2475 (1976).
6. G. Reher and L. Kraus, *J. Nat. Prod.*, **47**, 172 (1984).
7. R. Aquino, M. D'Agostino, F. De Simone, and C. Pizza, *Phytochemistry*, **27**, 1827 (1988).
8. G. Reher, L.J. Krauss, V. Sinwell, and W.A. Koning, *Phytochemistry*, **22**, 1524 (1983).
9. G. Reher, "Untersuchungen der Rinde von *Coutarea latiflora* DC. (Rubiaceae) und aderer copalchi Rinden," Ph.D. Thesis, University of Hamburg, Hamburg, 1982, p. 45.

Received 8 February 1988