Pergamon

PII: S0031-9422(96)00617-6

Phytochemistry, Vol. 44, No. 4, pp. 683–685, 1997

0031-9422/97 $17.00+0.00

CINNAMATES AND COUMARINS FROM THE LEAVES OF *MURRAYA PANICULATA*

ATTA-UR-RAHMAN,* MUHAMMAD SHABBIR, S. ZIAUDDIN SULTANI, ABDUL JABBAR and M. IQBAL CHOUDHARY*

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

(*Received in revised form* 10 *August* 1996)

Key Word Index—*Murraya paniculata*; Rutaceae; coumarin; pesudocoumarin; methyl 2-methoxy-5-hydroxycinnamate; 8-(2′-oxo-3′-methyl) butoxy-7-methoxycoumarin.

Abstract—Phytochemical investigations on aerial parts of *Murraya paniculata* have resulted in the isolation of two new natural products, methyl 2-methoxy-5-hydroxycinnamate and 8-(2′-oxo-3′-methyl) butoxy-7-methoxycoumarin along with the two known compounds, methyl 2,5-dihydroxycinnamate and murrayatin, isolated for the first time from this plant. The structures of these compounds were determined by using spectroscopic methods including two-dimensional NMR. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Murraya paniculata (L.) Jack, is a member of family Rutaceae and occurs widely in India, southeast Asia and southern China. The leaves and roots of the plant are used in folk medicine for the treatment of stomach-ache and toothache, as a stimulant and in gout, etc. The leaves possess antibiotic activity against *Mycococcus pyogenes* and *Escherichia coli* [1, 2]. Previous phytochemical studies on this plant have resulted in the isolation of a number of coumarins and carbazole alkaloids [3–5]. The present paper describes the isolation and structure elucidation of two new natural products, methyl 2-methoxy-5-hydroxycinnamate (**1**) and 8-(2′-oxo-3′-methyl) butoxy-7-methoxycoumarin (**2**) along with the two known natural products, methyl 2,5-dihydroxycinnamate (**3**) and murrayatin (**4**), isolated for the first time from *M. paniculata*.

RESULTS AND DISCUSSION

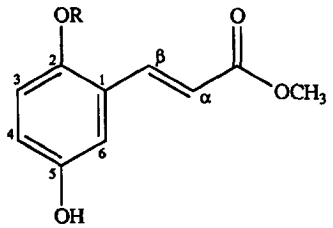
Compounds **1–4** were isolated from the methanolic extracts of the leaves of *M. paniculata* through column chromatography and TLC. Compound **1**, $C_{11}H_{12}O_4$, showed UV absorption maxima at 218, 296 and 324 nm indicating the presence of a cinnamate nucleus [6]. The IR spectrum exhibited strong absorption bands at 1602 (C=C), and 1695 (α, β-unsaturated ester C=O) cm^{-1} and a broad peak at 3520 (OH) cm^{-1}.

The EI-mass spectrum of **1** showed the $[M]^+$, which was also the base peak, at *m/z* 208.0708 ($C_{11}H_{12}O_4$). The ion at *m/z* 193 ($C_{10}H_9O_4$) resulted from the loss of a methyl group from the $[M]^+$. The peak at *m/z* 177.0541 ($C_{10}H_9O_3$) suggested the loss of a OCH_3 group from the $[M]^+$. A molecule of CO was possibly lost from the later giving rise to another prominent peak at *m/z* 149.0571 ($C_9H_9O_2$).

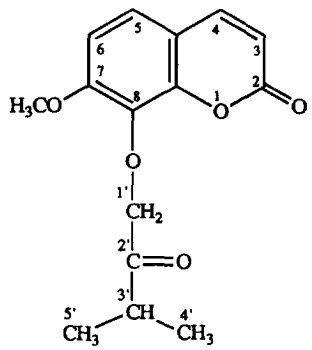
The ^1H NMR spectrum of **1** featured two OCH_3 signals at δ3.75 (ester methoxy) and 3.88 (C-2 aromatic methoxy). Two characteristic doublets in the downfield region at δ6.30 and 7.56 ($J_{\alpha,\beta}$ = 15.9 Hz) corresponded to the *trans*-oriented olefinic protons α and β to a carbonyl function, respectively. A doublet at δ6.93 ($J_{3,4}$ = 8.2 Hz) was due to the C-3 aromatic proton, while the *ortho*-coupled C-4 aromatic proton appeared as a double doublet at δ7.04 ($J_{4,3}$ = 8.2 Hz, $J_{4,6}$ = 2.1 Hz) also showing *meta*-coupling with the C-6 aromatic proton which resonated as a close doublet at δ7.03 ($J_{6,4}$ = 2.1 Hz). Irradiation at δ3.88 (C-2 OCH_3) resulted in 15.9% NOE at δ7.56 (*d*, H-β) and 7.9% NOE at δ6.93 (*d*, H-3). Irradiation at δ7.03 (*d*, H-6) showed NOE of the signals of H-β (13.2%) and H-α (7.5%). These results were consistent with structure **1** established for this new natural product.

The second compound **2**, $C_{15}H_{16}O_5$, was isolated as an amorphous solid. Its UV spectrum showed absorptions at 256 and 321 nm. The IR spectrum exhibited strong absorptions at 1600 (C=C), 1705 (ketone C=O) cm^{-1} and 1725 (lactone C=O). The EI-mass spectrum of compound **2** showed the $[M]^+$ at *m/z* 276.0992 ($C_{15}H_{16}O_5$). The base peak at *m/z* 191 ($C_{10}H_7O_4$) was due to the loss of fragment C_5H_9O from the $[M]^+$. The ion at *m/z* 175.0368 ($C_{10}H_7O_3$) was due to the loss of $C_5H_9O_2$ from the $[M]^+$.

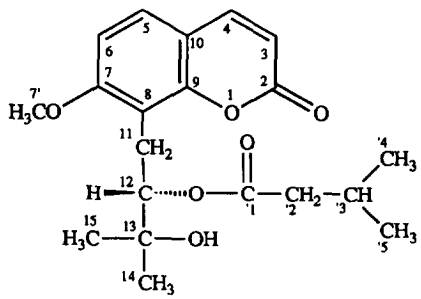
*Authors to whom correspondence should be addressed.



1 R = CH_3
3 R = H



2



4

The ^1H NMR spectrum of the compound **2** featured a doublet at δ6.19 ($J_{3,4}$ = 9.5 Hz) which was assigned to the C-3 olefinic proton. It showed a vicinal coupling with the C-4 olefinic proton which resonated at δ7.60 ($J_{4,3}$ = 9.5 Hz). The C-5 aromatic proton resonated doublet at δ6.82 ($J_{5,6}$ = 8.6 Hz) due to *ortho*-coupling with the C-6 aromatic proton at δ7.34 ($J_{6,5}$ = 8.6 Hz). The *O*-methyl substituent at C-7 resonated at δ3.85 as a singlet and showed a NOE with the C-6 aromatic proton in the NOESY spectrum. The C-1′ methylene protons of O-8 side chain appeared as a singlet at δ3.98. A multiplet at δ2.79 was due to the C-3′ methine proton coupled with a six-proton doublet at δ1.18 (J = 6.9 Hz) which was ascribed to the two methyl groups.

The ^{13}C NMR spectrum of **2** showed the resonances of all 15 carbon atoms. The DEPT spectra revealed the presence of five methine, one methylene and three methyl carbons, while the difference between the broad-band and DEPT spectra indicated the presence of six quaternary carbons in the molecule. The down-field signals at δ164.3 and 200.5 were assigned to the lactone and C-2′ ketonic carbonyl carbons, respectively.

The HMQC and HMBC spectra were very useful for the structural assignments. The direct $^1H/^{13}C$ chemical shift correlations for all protonated carbons were deduced from the HMQC spectrum. In the HMBC spectrum of **2**, the C-1′ methylene protons (δ3.98) showed long-range heteronuclear interactions, with the C-8 and C-9 aromatic carbons (δ155.4 and 154.9). The C-2′ carbonyl carbon (δ200.5) showed correlation with the C-3′ methine proton (δ2.79 m), which in turn, showed long-range $^1H/^{13}C$-correlation with the two methyl carbons (δ18.4 and 18.6). These spectroscopic studies led to structure **2** for this new coumarin.

Methyl 2,5-dihydroxycinnamate (**3**) and murrayatin (**4**) were earlier isolated from *Azadirachta indica* [7] and *Murraya exotica* [8], respectively. This is the first report of their isolation from the leaves of *M. paniculata*. Their spectral data are presented in the experimental section.

EXPERIMENTAL

General. Mass spectra were recorded on a Varian MAT 312 double focusing mass spectrometer connected to DEC PDP 11/34 computer system. The NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 and AM-400 NMR spectrometers with TMS as int. standard. The IR spectra were recorded on a Jasco IRA-1 IR spectrophotometer. The UV spectra were recorded on a Shimadzu UV 240 instrument. The optical rotations were measured on a Polatronic D polarimeter. Purity of the samples was checked by TLC on silica gel (G-254) precoated plates.

Plant material. The fresh leaves of *M. paniculata* (50 kg) were collected from suburban areas of Karachi (Pakistan) in June 1992. The plant was identified by the plant taxonomist Mr Tahir Ali, Department of Botany, University of Karachi, and a voucher specimen (KUH # 63485) was deposited in the Herbarium of the department.

Extraction and isolation. The dried leaves of *M. paniculata* (19 kg) were extracted with MeOH. The methanolic extract (2 l) of the fresh plant material was evapd to leave a gum (500 g). The gum was dissolved in H_2O and extracted first with $CHCl_3$ and then with EtOAc by adjusting the pH (pH 3–8) with HCl and NH_4OH. All these extracts were subjected to CC on silica gel.

Methyl 2-methoxy-5-hydroxycinnamate (**1**). The $CHCl_3$ fr. (12 g), extracted at pH 8.0 was loaded on a silica gel column which was eluted with $CHCl_3$–CH_3OH (93:7) to afford a fraction containing compound **1**. Further purification of **1** was carried out by prep. TLC (silica gel) using $CHCl_3$–CH_3OH (99:1) as

the developing solvent to yield compound **1** (5 mg, 2.6×10^{-5}% yield). UV λ_{max}^{MeOH} nm (log ε): 218 (3.46), 296 (3.54), 324 (3.63). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1602 (C=C), 1695 (ester C=O), 3530 (OH). ^1H NMR (CD_3OD, 400 MHz): δ3.75 (3H, *s*, ester OCH_3), 3.88 (3H, *s*, aromatic OCH_3), 6.30 (1H, *d*, $J_{\alpha,\beta} = 15.9$ Hz, H-α), 6.93 (1H, *d*, $J_{3,4} = 8.2$ Hz, H-3), 7.03 (1H, *d*, $J_{6,4} = 2.1$ Hz, H-6), 7.04 (1H, *dd*, $J_{4,3} = 8.4$ Hz, $J_{4,6} = 2.1$ Hz, H-4), 7.56 (1H, *d*, $J_{\beta,\alpha} = 15.9$ Hz, H-β). EI-MS *m/z* (rel. int.) 208.0708 ([M]$^+$, 100), 193 (17), 177 (58), 149 (30), 145 (17).

8-(*2′-Oxo-3′-methyl*) *butoxy-7-methoxycoumarin* (**2**). The $CHCl_3$ fraction (7 g) was obtained by extraction of the aq. layer at pH 3.0. It was loaded on to a silica gel column (100 g) and the column was eluted with $CHCl_3$–*n*-C_6H_{14} (7:3) to give crude compound **2**. Further purification of **2** on precoated silica gel TLC plates using toluene Me_2CO–*n*-hexane (5:10:85) yielded compound **2** (6 mg, 3.1×10^{-5}% yield) as an amorphous solid. UV λ_{max}^{MeOH} nm (log ε) 256 (3.80), 321 (3.67). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600 (C=C), 1705 (C=O), 1725 (lactone carbonyl). ^1H NMR spectrum ($CDCl_3$, 500 MHz): δ1.18 (6H, *d*, $J = 6.9$ Hz, $2 \times CH_3$), 2.79 (1H, *m*, H-3′), 3.85 (3H, *s*, OCH_3), 3.98 (2H, *s*, H-1′), 6.82 (1H, *d*, $J_{5,6} = 8.6$ Hz, H-5), 6.19, (1H, *d*, $J_{3,4} = 9.5$ Hz, H-3), 7.34 (1H, *d*, $J_{6,5} = 8.6$ Hz, H-6), 7.60 (1H, *d*, $J_{4,3} = 9.5$ Hz, H-4). ^{13}C NMR (100 MHz, $CDCl_3$); δ200.5 (C-2′), 164.3 (C-2), 155.4 (C-8), 154.9 (C-9), 139.4 (C-7), 127.4 (C-6), 113.1 (C-3), 110.3 (C-10), 56.1 (OCH_3), 40.9 (C-3′), 34.7 (C-1′), 18.6 (C-5′), 18.4 (C-4′): EI-MS: *m/z* (rel. int.), 276.0992 ([M$^+$], 925), 205 (10), 191 (100), 175 (20).

Methyl 2,5-*dihydroxycinnamate* (**3**). Compound **3** was isolated from the $CHCl_3$ extract (12 g) obtained at pH 8.0. The column was packed with silica gel (100 g) and eluted with $CHCl_3$–CH_3OH (93:7) to yield a semi-pure compound **3**. Crystals of **3** were washed successively with petrol (bp 40–60°), Et_2O and Me_2CO to yield pure compound **3** (3 mg, 1.5×10^{-5}% yield). UV λ_{max}^{MeOH} nm: 242, 291, 320. IR ν_{max}^{KBr} cm^{-1}: 1595 (C=C), 1664 (ester carbonyl), 3130 (CH, aromatic), 3310 (OH). ^1H NMR (CD_3OD, 400 MHz):δ3.87 (3H, *s*, ester OCH_3), 6.43 (1H, *d*, $J_{\alpha,\beta)} = 15.8$ Hz, H-α), 6.91 (1H, *d*, $J_{3,4} = 8.3$ Hz, H-3), 7.00 (1H, *dd*, $J_{4,3} = 8.3$ Hz, $J_{4,6} = 2.1$ Hz, H-4), 7.05 (1H, *d*, $J_{6,4} = 2.1$ Hz, H-6), 7.42 (1H, *d*, $J_{\beta,\alpha} = 15.8$ Hz, H-β). EI-MS *m/z* (rel. int.) 194 ([M]$^+$, 100), 177 (37), 149 (18).

Murrayatin (**4**). Compound **4** was obtained from the $CHCl_3$ fr. (7 g) obtained by extraction of aq. layer at pH 3.0. It was loaded onto a silica gel column and the column was eluted with $CHCl_3$–*n*-hexane (1:4) to give compound **4**. Further purification by using precoated silica gel TLC plates in Et_2O–*n*-hexane (1:9) yielded compound **4** (5 mg, 2.6×10^{-5}% yield). $[\alpha]_D^{25} = 56°$ (EtOH). UV λ_{max}^{MeOH} nm: 257, 321. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1595 (C=C), 1705 (ester C=O), 3350 (OH). ^1H NMR ($CDCl_3$, 400 MHz):δ0.65 (3H, *d*, $J_{4',3'} = 6.5$ Hz, H-4′), 0.71 (3H, *d*, $J_{5',3'} = 6.5$ Hz, H-5′), 1.30 (3H, *s*, H-15), 1.34 (3H, *s*, H-14), 1.86 (3H, *m*, H-2′ and H-3′), 3.02 (1H, *dd*, $J_{11a,11b} = 13.7$ Hz, $J_{11a,12} = 10.5$ Hz, H-11a), 3.22 (1H, *dd*, $J_{11a,11b} = 13.7$ Hz, $J_{11b,12} = 2.4$ Hz, H-11b), 3.92 (3H, *s*, OCH_3), 5.15 (1H, *dd*, $J_{12,11a} = 10.5$ Hz, $J_{12,11b} = 2.4$ Hz, H-12), 6.22 (1H, *d*, $J_{3,4} = 9.4$ Hz, H-3), 6.80 (1H, *d*, $J_{6,5} = 8.6$ Hz, H-6), 7.30 (1H, *d*, $J_{5,6} = 8.6$ Hz, H-5), 7.58 (1H, *d*, $J_{4,3} = 9.4$ Hz, H-4). EI-MS *m/z* (rel. int.): 362 ([M]$^+$, 100), 304 (4), 261 (10), 219 (100), 189 (18).

Acknowledgements—This research was indirectly supported through ONR research grant to A. R. We are grateful to Mr Tahir Ali, plant taxonomist, for identification of plant material.

REFERENCES

1. *The Wealth of India*, Vol. VI ed. B. N. Sastri. Council of Scientific and Industrial Research, New Delhi, 1962, p. 446.
2. Lily, E. M., *Medicinal Plants of East and Southeast Asia*. M.I.T. Press, Cambridge, 1980, p. 367.
3. Talapatra, S. K., Dutta, L. N. and Talapatra, B., *Tetrahedron Letters*, 1973, 5005.
4. Ramstad, E., Lin, W. C., Lin, T. J. and Koo, W.-Y., *Tetrahedron Letters*, 1968, 811.
5. Dreyer, D. L., *Journal of Organic Chemistry*, 1968, **33**, 3574.
6. Scott, A. I., *Ultraviolet Spectra of Natural Products*. Pergamon Press, Oxford, 1964, p. 119.
7. Ara, I., Siddiqui, B. S., Faizi, S. and Siddiqui, S., *Phytochemistry*, 1989, **28**, 1177.
8. Barik, B. R., Dey, K. A. and Chatterjee, A., *Phytochemistry*, 1983, **22**, 2273.