Phytochemistry, Vol. 31, No. 3, pp. 1061–1062, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

CYTOTOXIC NAPHTHOQUINONES FROM *MANSOA ALLIACEA*

HIDEJI ITOKAWA,* KOUJI MATSUMOTO, HIROSHI MORITA and KOICHI TAKEYA

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan

(*Received in revised form* 27 *July* 1991)

Key Word Index—*Mansoa alliacea*; Bignoniaceae; wood; naphthoquinone; cytotoxicity.

Abstract—A new cytotoxic naphthoquinone, 4-hydroxy-9-methoxy-α-lapachone was isolated from *Mansoa alliacea* along with 9-methoxy-α-lapachone. Its structure was elucidated by spectroscopic, chemical and exciton chirality methods.

INTRODUCTION

The known bioactive quinone constituents of the Bignoniaceae are lapachol, which exhibits potent antitumour activity [1], and α-lapachone, which is a potent inhibitor of reverse transcriptase activity as well as showing eukaryotic DNA-dependent DNA-polymerase activity [2]. In the course of our study on antitumour and cytotoxic principles from the medicinal plants of South America, we found that the alcoholic extract of *Mansoa alliacea* showed cytotoxic activity against V-79 cells. *Mansoa alliacea* has been used as a medicinal plant having antirheumatic properties in the upper Amazon basin in Peru. Chromatographic purification guided by cytotoxic activity led us to isolate two cytotoxic principles, 9-methoxy-α-lapachone (**1**) and 4-hydroxy-9-methoxy-α-lapachone (**2**). We deal with the structural elucidation of **2** and the cytotoxic activities of both.

RESULTS AND DISCUSSION

The methanol extract of *M. alliacea* was fractionated by partitioning between methylene chloride and water. The cytotoxic activity was concentrated in the methylene chloride fraction, whose chromatographic purification by successive silica gel and medium pressure LC gave two quinones (**1** and **2**) as cytotoxic principles.

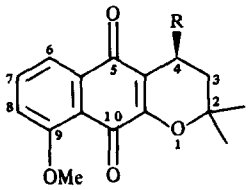
Compound **1** was obtained as a yellow powder and was identical with 9-methoxy-α-lapachone by comparison with the spectral data in the literature [3].

Compound **2** was obtained as yellow powder whose molecular formula was $C_{16}H_{16}O_5$ (HR-MS). The IR and UV spectral data of **2** indicated the presence of a quinone similar to 9-methoxy-α-lapachone [1680 cm^{-1}; 245 nm (48 100), 270 (25 500), 385 (8100)] except for the hydroxyl absorption band [3540 cm^{-1}]. In the ^1H NMR spectrum, a proton (δ4.93) attached to a carbon bearing a hydroxyl moiety is coupled with methylene protons (δ2.00 and 2.10). In addition, signals from three aromatic protons (δ7.72, 7.64 and 7.23) with ABC-type coupling pattern, methoxyl protons (δ3.96) and two singlet methyl moieties (δ1.41 and 1.53) were observed. The position of the methoxyl moiety was assigned to C-9 by comparison of the ^{13}C chemical shift of the attached carbon with that of compound **1**. The position of the hydroxyl moiety was found to be at C-4 by comparison of the chemical shift values with those of 4-hydroxy-α-lapachone [4]. All ^{13}C resonance signals can be correlated with the structure as shown in Table 1. The exciton chirality method [5] using a *p*-bromobenzoate derivative was applied to the determination of absolute configuration at C-4. Based on the positive Cotton effect at 248 nm in the CD spectrum of the *p*-bromobenzoate, the configuration was confirmed to be *S*.

Compounds **1** and **2** showed cytotoxic activities against V-79 cells with IC_{50} values of 5.6 and 6.0 μg ml^{-1}, respectively.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (100.6 MHz, TMS as int. standard)

C	1	2	C	1	2
2	78.05	79.66	10	184.24	185.82
3	31.36	39.61	11	26.44	26.67
4	16.37	60.00	12	26.44	27.09
5	178.66	178.45	4a	117.70	118.26
6	118.72	118.71	5a	134.40	134.17
7	134.82	135.27	9a	119.13	119.08
8	117.22	117.67	10a	155.30	154.74
9	159.82	160.06	OMe	56.44	56.48



R

1 H

2 OH

3 *p*-bromobenzoate of **2**

EXPERIMENTAL

General. Mp: uncorr. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were measured in $CDCl_3$, TMS as int. standard. Silica gel CC was carried out on Wakogel C-200 at amounts equivalent to 100 times the sample amount. Each final purification was made with MPLC

Plant material. The wood of *Monsoa alliacea* was collected in Iquitos, Peru, in June 1990. It was identified by Dr F. Ayala Flores (Director del Herbarium Amazonense, Peru Amazon University, Iquitos, Peru).

Extraction and isolation. The wood (550 g) of *M. alliacea* was crushed and extracted with hot MeOH. The concd extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble fraction was subjected to silica gel CC with *n*-hexane–EtOAc (stepwise elution). The fraction eluted with *n*-hexane–EtOAc (7:3) was further purified by MPLC with toluene–EtOAc (4:1 or 7:3) to obtain compound **1** (137 mg) and **2** (60 mg).

9-*Methoxy-α-lapachone* (**1**). Yellow powder, mp 156–158°; EI-MS *m/z* (rel. int.): 272 $[M]^+$ (100), 257 (93), 216 (69), 189 (94); IR ν_{max}^{KBr} cm^{-1}: 2950, 1680, 1620, 1590, ^{1}H NMR ($CDCl_3$) δ: 1.41 (6H, *s*), 1.79 (2H, *t*, J = 6.6 Hz), 2.57 (2H, *t*, J = 6.6 Hz), 3.97 (3H, *s*), 7.22 (1H, *br d*, J = 8.5 Hz), 7.61 (1H, *t*, J = 8.0 Hz), 7.73 (1H, *dd*, J = 0.9, 8.0 Hz).

4-*Hydroxy-9-methoxy-α-lapachone* (**2**). Yellow powder, mp 133–135°, $[\alpha]_D$ +26.8° (MeOH; *c* 0.44); EI-MS *m/z* (rel. int.): 288 $[M]^+$ (84) (calcd for $C_{16}H_{16}O_5$: 288.0996, Found: 288.1028), 232 (76), 204 (64), 186 (20), 176 (100) UV λ_{max}^{EtOH} (ε): 220 (37 700), 245 (48 100), 270 (25 500), 385 (8100); IR ν_{max}^{KBr} cm^{-1}: 3540, 3000, 2950, 1680, 1620, 1585, 1290; ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, *s*), 1.53 (3H, *s*), 2.00 (1H, *dd*, J = 6.4, 14.2 Hz), 2.10 (1H, *dd*, J = 6.4, 14.2 Hz), 3.96 (3H, *s*), 4.93 (1H, *t*, J = 6.4 Hz), 7.23 (1H, *dd*, J = 1.0, 8.0 Hz), 7.64 (1H, *t*, J = 8.0 Hz), 7.72 (1H, *dd*, J = 1.0, 8.0 Hz).

p-*Bromobenzoyl ester of compound* **2**. A soln of **2** (10 mg) and *p*-bromobenzoyl chloride (30 mg) in pyridine (1 ml) was stirred overnight, then poured into H_2O, and extracted with $CHCl_3$. The product was purified by silica gel MPLC eluting with toluene–EtOAc (7:3) to give the *p*-bromobenzoyl ester of **2** (8 mg) as a yellow powder, mp 115–117°; EI-MS *m/z* (rel. int.): 472 $[M]^+$ (8), 470 $[M]^+$ (7), 287 (57), 231 (68), 182 (96), 180 (100), 157 (34), 155 (32); CD (MeOH): $\Delta\varepsilon_{248}$ +4.86; ^{1}H NMR ($CDCl_3$) δ: 1.53 (3H, *s*), 1.57 (3H, *s*), 2.18 (1H, *dd*, J = 5.1, 15.4 Hz), 2.29 (1H, *dd*, J = 2.6, 15.4 Hz), 4.00 (3H, *s*), 6.32 (1H, *dd*, J = 2.6, 5.1 Hz), 7.25 (1H, *dd*, J = 1.0, 7.6 Hz), 7.65 (1H, *t*, J = 7.6 Hz), 7.68 (2H, *d*, J = 8.7 Hz), 7.72 (1H, *dd*, J = 1.0, 7.6 Hz), 7.99 (2H, *d*, J = 8.7 Hz).

Assay of cytotoxic activities using V-79 cells. See previous paper [6].

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