PERGAMON

Phytochemistry 54 (2000) 909–912

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Lignans from *Mosla scabra*

Qi Wang[a], Christian Terreaux[a], Andrew Marston[a], Ren-Xiang Tan[b], Kurt Hostettmann[a,*]

[a]*Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne-Dorigny, Switzerland*
[b]*Department of Biological Sciences and Technology, Nanjing University, Nanjing 210093, People's Republic of China*

Received 4 October 1999; received in revised form 9 March 2000

Abstract

Two new cyclobutane-type lignans, named moslolignans A and B, together with two known ones, andamanicin and magnosalin, were isolated from the whole plant of *Mosla scabra*. Their structures were established as 1β*,2β*,3α*,4α*-1,2-dimethyl-3-(3-methoxy-4,5-methylene-dioxyphenyl)-4-(2,4,5-trimethoxyphenyl)-cyclobutane and 1β*,2β*,3α*,4α*-1,2-dimethyl-3-(2,5-dimethoxy-3,4-methylenedioxyphenyl)-4-(2,4,5-trimethoxyphenyl)-cyclobutane by spectroscopic methods. This is the first report of naturally-occurring cyclobutane-type lignans with asymmetrical substitutions.

Keywords: *Mosla scabra*; Lamiaceae; Cyclobutane-type lignan; Moslolignan A; Moslolignan B; Andamanicin; Magnosalin

1. Introduction

Mosla scabra (Thunb.) C.Y. Wu and H.W. Li is a well-branched small annual herb endemic to eastern China and is used in Chinese traditional medicine for the treatment of heatstroke, common cold, fever and chronic bronchitis (Jiangsu New Medical College, 1985). Except for the comparative analysis of the essential oil constituents of some *Mosla* species (Zhang and Xu, 1989), no chemical investigation has been reported on this plant. Therefore, the study of the dichloromethane extract of *M. scabra* was undertaken, which led to the isolation of simple phenylpropanoids, α,β-asarone, asaronaldehyde and isoapiol, triterpenes, ursolic acid and tormentic acid, and four cyclobutane-type lignans, moslolignans A (**1**), B (**2**), andamanicin (**3**) and magnosalin (**4**), the former two as novel natural products.

2. Results and discussion

Both compounds **3** and **4** gave similar mass spectra showing characteristic peaks at *m/z* 208 (base peak) together with the $[M]^+$ signal at *m/z* 416. Their NMR spectra, however, displayed only half of the signals expected for such a molecular weight, indicating a symmetrical nature in both molecules. Analysis of these data and comparison with literature verified their structures as andamanicin (**3**) and magnosalin (**4**), isolated previously from *Piper sumatranum* and *Magnolia salicifolia*, resp. (Malhotra et al., 1990; Kikuchi et al., 1983). Compound **4**, heterotropan (**5**) and the synthetic asymmetrical derivative **6** (Yamamura et al., 1978) were synthesized by photochemical dimerization of β-asarone (Kadota et al., 1987).

Moslolignan A (**1**) was obtained as a colorless gum. The molecular ion observed in the EI mass spectrum at *m/z* 400, together with its ^{1}H- and ^{13}C-NMR data, suggested the molecular formula $C_{23}H_{28}O_6$, with ten degrees of unsaturation. Its ^{1}H-NMR spectrum displayed four isolated aromatic protons at δ 6.71, 6.47, 6.25 and 6.18, one methylenedioxy group at δ 5.79

* Corresponding author. Tel.: +41-21-6924561; fax: +41-21-6924565.
E-mail address: kurt.hostettmann@ipp.unil.ch (K. Hostettmann).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00105-9

(2H), which was in agreement with the ^{13}C-NMR signal at δ 101.53, four methoxyl groups at δ 3.72, 3.68, 3.66 and 3.65 (12H), two doublets for methyl groups at δ 1.19 and 1.14 (each 3H). The remaining multiplets, integrated as four protons, showed resonances at δ 3.74, 3.37, 2.94 and 2.71. In the ^{13}C-NMR spectrum, 12 aromatic carbons were attributed to two phenyl rings, four carbons to the methoxyl groups, and two signals at δ 15.5 and 15.3 to two methyl groups (Table 1). Therefore, a four-membered ring could be deduced to fulfil the requirement of unsaturation degrees, with the four remaining carbons. The ^{1}H–^{1}H COSY and HSQC experiments confirmed the presence of the 1,2-dimethyl-cyclobutane structure in the molecule. The substitution patterns of both phenyl rings were determined by observation of aromatic protons signals in the ^{1}H-NMR spectrum, as well as HMBC correlations between H-2″ and C-3, H-6″ and C-3 as well as H-6′ and C-4.

The signals at δ 15.5 and 15.3 in the ^{13}C-NMR spectrum suggested that both the methyl groups are in *cis* orientation (Eliel and Pietrusiewicz, 1980). Moreover, the correlations between Me-1, H-1 and H-4 as well as Me-2, H-2 and H-3 in the NOE difference spectrum showed the orientation and substitution patterns in the cyclobutane moiety. Therefore, the structure of moslolignan A (**1**) was assigned as 1S*,2R*,3R*,4S*-1,2-methyl-3-(3-methoxy-4,5-methylenedioxyphenyl)-4-(2,4,5-trimethoxyphenyl)-cyclobutane.

Table 1
^{1}H- and ^{13}C-NMR (500 and 125 MHz, respectively) spectral data of **1** and **2** in $CDCOCD_3$

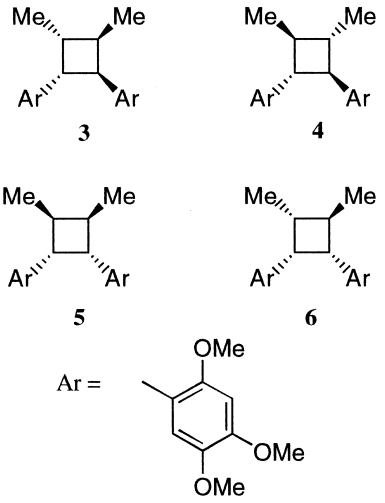
Position	**1** δ_H (*J* in Hz)	**1** δ_C	**2** δ_H (*J* in Hz)	**2** δ_C
1	2.94 *m*	33.25	2.78 *m*	34.72
2	2.71 *m*	35.58	2.74 *m*	35.39
3	3.37 *m*	51.04	3.77 *m*[d]	43.51[b]
4	3.74 *m*	44.42	3.77 *m*[d]	43.62[b]
CH_3-1	1.15 *d* (6.5)	15.28	1.16 *d* (7.0)[d]	15.38[a]
CH_3-2	1.19 *d* (6.5)	15.50	1.14 *d* (7.0)[d]	15.42[a]
1′	–	121.76	–	122.30
2′	–	152.87	–	153.00
3′	6.47 *s*	98.72	6.49 *s*	99.08
4′	–	149.31[a]	–	149.18
5′	–	143.82[b]	–	143.80
6′	6.71 *s*	114.68	6.75 *s*	114.65
1″	–	137.68	–	128.45
2″	6.18 *d* (1.5)	108.46	–	137.37
3″	–	143.79[b]	–	139.22[c]
4″	–	133.72	–	135.58
5″	–	149.09[a]	–	139.44[c]
6″	6.25 *d* (1.5)	102.60	6.27 *s*	107.82
OCH_2O	5.79 *d* (1.0)	101.53	5.84 *d* (1.0)	101.92
	5.79 *d* (1.0)		5.84 *d* (1.0)	
CH_3O-2′	3.65 *s*	56.15	3.58 *s*	56.36
CH_3O-4′	3.73 *s*	56.43	3.77 *s*	56.41
CH_3O-5′	3.67 *s*	57.43	3.64 *s*	57.22
CH_3O-2″	–	–	3.57 *s*	59.92
CH_3O-3″	3.68 *s*	56.53	–	–
CH_3O-5″	–	–	3.66 *s*	57.04

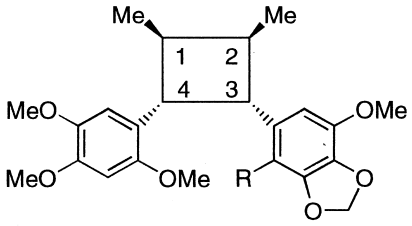
[a–c] Assignments in the same column may be interchangeable.
[d] Overlapping or partially overlapping signals.

Moslolignan B (**2**) was also obtained as a colorless gum. The molecular formula of $C_{24}H_{30}O_7$ was deduced by combination of ^{1}H- and ^{13}C-NMR data and $[M]^+$ at *m*/*z* 430 in the EI-MS spectrum. Great similarity was observed in its NMR spectra when compared to moslolignan A (**1**), suggesting that they both belong to the same chemical class. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of one more methoxyl group compared to **1**. Among the five methoxyl groups, only one had a low field shift (δ 59.92) due to a di-*ortho*-substitution while the other four were not di-*ortho*-substituted and possessed chemical shifts around δ 56–57 (Miura et al., 1978). HMBC revealed the correlations between H-6′, H-6″ and C-4/C-3. Additionally, the absence of interactions between signals of δ 3.57 (2″-OMe) and 3.66 (5″-OMe) in NOESY showed that the two methoxyl groups were not in *ortho* position. The observation of NOESY interactions between Me-1 and H-1, Me-2 and H-2, and between the overlapping signals of Me-1/Me-2 and the superposed signals of H-3/H-4, as well as the lack of NOE effects between H-1/H-2 and H-3/H-4 suggested the same relative configuration for **2** as moslolignan A (**1**). Moslolignan B (**2**) was therefore characterized as 1S*,2R*,3R*,4S*-1,2-methyl-3-(2,5-dimethoxy-3,4-methylenedioxyphenyl)-4-(2,4,5-trimethoxyphenyl)-cyclobutane. No attempt was made to determine the absolute configuration of the cyclobutane ring in (**1**) and (**2**). However, their low optical rotation values suggest that they may be present as a racemic mixture.

Naturally-occurring compounds with symmetrically substituted cyclobutane ring reported previously from higher plants and marine organisms (Casapullo et al., 1994; Rahman et al., 1998; Lu and Foo, 1999 and references cited therein; Ngadjui et al., 1989) probably originate biogenetically from dimerization of two halves due to their cooccurrence with their potential monomer precursors. Andamanicin (**3**), magnosalin (**4**) and heterotropan (**5**) (Yamamura et al., 1978) represent the only three naturally-occurring lignans possessing a symmetrically substituted cyclobutane ring known to date. While moslolignans A **1** and B (**2**) reported herein are the first natural asymmetrically substituted lignans on the cyclobutane ring, their biogenetic formation is of great interest.

Magnosalin (**4**) was reported to possess significant anti-inflammatory effects using the pouch granuloma method in mice (Kimura et al., 1985). Biological testing of related compounds and a more detailed phytochemical study of *M. scabra* are currently underway.





1 R = H

2 R = OMe

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Inova-500 spectrometer at 500 and 125 MHz, respectively, in acetone-d_6 using TMS as int. std. EI-MS (70 eV) spectra were measured on a Finnigan-MAT TSQ-700 triple stage quadrupole instrument. UV spectra were measured in MeOH on a Perkin Elmer Lambda 20 spectrophotometer, and optical rotation ($[\alpha]_D$) on a Perkin Elmer 241 MC polarimeter at r.t.

3.2. Plant material

The flowering whole plant of *M. scabra* was collected in September, 1996 in a farmland near Hekou Village, Wujin County, Jiangsu, China and was identified by Dr. Lixing Zhang, Nanjing University. A voucher specimen is deposited both in the Herbarium of Nanjing University and at the Institute of Pharmacognosy and Phytochemistry, University of Lausanne (No. 97004).

3.3. Extraction and isolation

Air dried and ground whole plants (120 g) were successively extracted for 24 h at r.t. with CH_2Cl_2 (3 × 1000 ml) and MeOH (3 × 1000 ml) to yield 8.20 g CH_2Cl_2 extract and 4.60 g MeOH extract. The CH_2Cl_2 extract (8.10 g) was subjected to silica gel CC (1 kg), eluting with petroleum ether–EtOAc–MeOH mixtures of stepwise increasing polarities (8:1:0 to 0:1:0 to 0:0:1) to give 12 fractions. Fraction 3 was rechromatographed over silica gel with $CHCl_3$–petroleum ether (3:2), then separated by CPC with *n*-hexane-*t*-BuOMe–MeCN 5:1:5 (upper phase as mobile phase) to give isoapiol (15 mg). The other fractions were filtered on Sephadex LH-20 gel ($CHCl_3$–MeOH 1:1) to eliminate pigments and separate phenolic compounds from terpenoids. The resulting phenolic part of fraction 5 gave α- and β-asarone (50 mg) as inseparable isomers, asaronaldehyde (14 mg) and **3** (7 mg) after repeated silica gel CC. By similar treatments, the phenolic fractions of fractions 7, 8, 10 yielded **1** (7 mg), **2** (10 mg), (**4**) (34 mg), respectively. Ursolic acid (79 mg) and tormentic acid (21 mg) were also isolated from the terpenic part of fractions 9 and 10.

3.4. 1S*,2R*,3R*,4S*-1,2-Dimethyl-3-(3-methoxy-4,5-methylene-dioxyphenyl)-4-(2,4,5-trimethoxyphenyl)cyclobutane (Moslolignan A (1))

Colorless gum. $[\alpha]_D$: −0.15° (MeOH, *c* 1.63). EI-MS (70 eV) *m/z* (rel. int.): 400 $[M]^+$ (3), 208 (100), 193 (17), 165 (8), 149 (5). UVλ_{max}^{MeOH} nm (log ε): 207 (4.93), 233 (sh, 4.40), 289 (4.01). ^{1}H- and ^{13}C-NMR data (see Table 1).

3.5. 1S*,2R*,3R*,4S*-1,2-Dimethyl-3-(2,5-dimethoxy-3,4-methylenedioxyphenyl)-4-(2,4,5-trimethoxyphenyl)cyclobutane (Moslolignan B (2))

Colorless gum. $[\alpha_D$: −0.43° (MeOH, *c* 1.38). EIMS (70 eV) *m/z*: 430 $[M]^+$ (2), 223 (5), 208 (100), 193 (14), 165 (6). UVλ_{max}^{MeOH} nm (log ε): 207 (4.66), 234 (sh, 4.21), 292 (3.74). ^{1}H- and ^{13}C-NMR data. (see Table 1)

Acknowledgements

Swiss National Science Foundation is gratefully acknowledged for supporting this work.

References

Casapullo, A., Minale, L., Zollo, F., 1994. Four new dimeric peptide alkaloids, anchinopeptolides B–D, and cycloanchinopeptolide C, congeners of anchinopeptolide A, from the Mediterranean marine sponge *Anchinoe tenacior*. Journal of Natural Products 57 (9), 1227–1233.

Eliel, E.L., Pietrusiewicz, K.M., 1980. Carbon-13 NMR spectra of methylated cyclobutanes and ethyl cyclobutanecarboxylates. Organic Magnetic Resonance 13 (3), 193–196.

Jiangsu New Medical College (Eds.), 1985. A Dictionary of Chinese Traditional Medicine. Shanghai Science and Technology Press, Shanghai. pp. 607–608.

Kadota, S., Tsubono, K., Makino, K., Takeshita, M., Kikuchi, T., 1987. Convenient synthesis of magnoshinin, an anti-inflammatory neolignan. Tetrahedron Letters 28 (25), 2857–2860.

Kikuchi, T., Kadota, S., Yanada, K., Tanaka, K., Watanabe, K., Yoshizaki, M., Yokoi, T., Shingu, T., 1983. Isolation and structure of magnosalin and magnoshinin, new neolignans from *Magnolia salicifolia* Maxim. Chemical and Pharmaceutical Bulletin 31 (3), 1112–1114.

Kimura, M., Suzuki, J., Yamada, T., Yoshizaki, M., Kikuchi, T., Kadota, S., Matsuda, S., 1985. Anti-inflammatory effect of neolignans newly isolated from the crude drug "Shin-i" (Flos Magnoliae). Planta Medica 51 (4), 291–293.

Lu, Y., Foo, Y., 1999. Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry 51 (1), 91–94.

Malhotra, S., Koul, S.K., Taneja, S.C., Pushpangadan, P., Dhar, K.L., 1990. A neolignan from *Piper sumatranum*. Phytochemistry 29 (8), 2733–2734.

Miura, I., Hostettmann, K., Nakanishi, K., 1978. ^{13}C-NMR of naturally occurring xanthone aglycones and glycosides. Nouv. J. Chim. 2, 653–657.

Ngadjui, B.T., Lontsi, D., Ayafor, J.F., Sondengam, B.L., 1989. Pachypophyllin and pachypostaudins A and B: three bisnorlignans from *Pachypodanthium staudtii*. Phytochemistry 28 (1), 231–234.

Rahman, A.-U., Khattak, F.K., Nighat, F., Shabbir, M., Hemalal, K.D., Tillekeratne, L.M., 1998. Dimeric tropane alkaloids from *Erythroxylum moonii*. Phytochemistry 48 (2), 377–383.

Yamamura, S., Niwa, M., Nonoyama, M., Terada, Y., 1978. Three novel neolignans from *Heterotropa takaoi* M. Tetrahedron Letters 49, 4891–4894.

Zhang, S., Xu, B., 1989. An analysis of the chemical composition of essential oil of *Mosla* in the Yangtze delta and its bearing on phylogeny. Yunnan Zhiwu Yanjiu 11, 187–192.