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Terpenes from leaves of *Guarea macrophylla* (Meliaceae)

João Henrique G. Lago [a], Cláudia B. Brochini [a], Nídia F. Roque [b,*]

[a] *Instituto de Química, Universidade de São Paulo, Cp 26077, CEP 05599-970, São Paulo - SP, Brazil*
[b] *Instituto de Química, Universidade Federal da Bahia, CEP 40170-290, Salvador - BA, Brazil*

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Abstract

The dichloromethane extract from the leaves of *Guarea macrophylla* (Meliaceae) was submitted to adsorption chromatography. *Guai-6-en-10β-ol, isopimara-7, 15-dien-2α-ol* and *cycloarta-23,25-dien-3-one* were isolated and identified by spectroscopic data, mainly by ^1H and ^{13}C NMR analyses; eight other known terpenoids were also isolated. © 2000 Elsevier Science Ltd. All rights reserved.

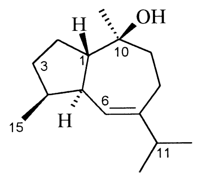
Keywords: Guarea macrophylla; Meliaceae; Terpenoids

1. Introduction

Guarea macrophylla Vahl. subsp. Tuberculata (Meliaceae) is a tree which grows in Brazil from Rio Grande do Sul to Minas Gerais States and in the Amazon (Corrêa, 1984). Species of *Guarea* produce a wide variety of secondary metabolites including meliacines, triterpenes, diterpenes and sesquiterpenes (Mootoo et al, 1992; Furlan et al., 1993; Furlan et al., 1996, Núñez and Roque, 1999). As part of our studies on this genus, the composition of the dichloromethane extract of the leaves of *G. macrophylla* was examined. This paper describes the isolation and identification of eleven terpenes (**1**–**11**), including three new ones, *guai-6-en-10β-ol, (5) isopimara-7,15-dien-2α-ol* (**9**) and *cycloarta-23,25-dien-3-one* (**11**) from the leaves of the plant.

2. Results and discussion

Column chromatography on silica gel of the dichloromethane extract of the leaves from *G. macrophylla* Vahl. subsp. Tuberculata and spectrometric analysis of the fractions resulted in the identification of one monoterpene: α-terpineol (**1**), four sesquiterpenes: aromadendran-1α-ol (palustrol, **2**), alloaromadendran-10α-ol (ledol, **3**), 10-aromadendren-4β-ol (spathulenol, **4**) and guai-6-en-10β-ol (**5**), five diterpenes: 8,13-oxy-labd-14-ene (manoyl oxide, **6**), labda-8,13E-dien-15-ol (**7**), isopimara-7,15-dien-3-one (**8**) isopimara-7,15-dien-3β-ol (**9**), and isopimara-7,15-dien-2β-ol (**10**), one triperpene: cycloarta-23,25-dien-3-one (**11**) and sitosterol (**12**).



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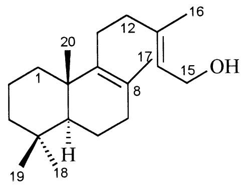

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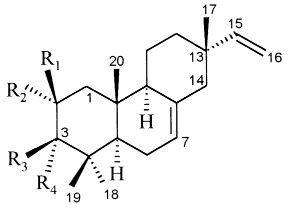
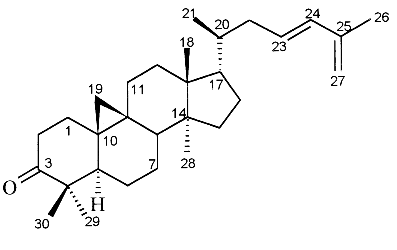


8 $R_1 = R_2 = H; R_3 = R_4 = O;$
9 $R_1 = R_2 = R_4 = H; R_3 = OH;$
10 $R_1 = R_3 = R_4 = H; R_2 = OH;$



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The monoterpene **1**, the sesquiterpenes **2**, **3** and **4** and the diterpenes **6** and **8** were identified by analysis of

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* Corresponding author. Fax: + 5-011-55-712-355166.
E-mail address: nifroque@ufba.br (J.H.G. Lago).

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their ^1H and ^{13}C NMR data and comparison with literature values (Almqvit et al., 1975; Bohlmann et al., 1975; Caputo et al, 1976; Iwabuchi et al. 1989; Faure et al. 1991; Weenen et al., 1991) respectively.

Sesquiterpene **5** was obtained as a colourless oil. The FABMS showed a quasi molecular ion signal at 205.1900 $[M+H\text{-}H_2O]^+$ and the ^{13}C NMR spectrum displayed 15 signals suggesting the structure of a hydroxysesquiterpene ($C_{15}H_{26}O$). The ^{13}C NMR spectral data (Table 1) indicated the presence of four methyls, four methylenes, four methines as well as one trisubstituted double bond and one hydroxylated quaternary carbon in the molecule of **5**. Comparison of these data with those of the guaiane sesquiterpenes, alismol and alismoxide (Yoshikava et al., 1992), suggested that **5** has a guaiane skeleton with a double bond at C-6 and a hydroxyl group at C-10. Its ^1H NMR spectrum showed the presence of a methyl group linked to a quaternary carbinolic carbon and an olefinic hydrogen (Table 1). The difference between the ^{13}C NMR spectroscopic data of **5** and those of alismoxide could be explained by the effects of the second hydroxyl at C-4 in the last compound. The proton bearing carbon signals were assigned by analysis of the HMQC spectrum and the correlations observed in the HMBC spectrum (Table 1) confirmed the planar structure of **5**, as *guai-6-en-10-ol*. The overlapping of many signals at δ 2.15 in the ^1H NMR spectrum made the analysis of the NOESY spectrum of this compound very difficult. To confirm the stereochemistry of **5** as being the same as alismoxide, ^1H NMR spectra using the shift reagent $Eu(fod)_3$ were obtained. These showed that the $\Delta\delta$ for H-6, H-12/H-13 and H-15 were 1.12, 0.42 and 0.86 ppm respectively, suggesting that the methyl group at C-4 is located at the same side of the molecule as the hydroxyl group. These observations confirm the structure of the guaiane **5** as *guai-6-en-10β-ol*. This compound has been isolated from the leaves of *Guarea guidonia* (Brochini et al., 1999), and its characterisation is herein described for the first time.

The EIMS spectrum associated with the data of NMR spectra suggested that **7** is an oxigenated diterpene with a molecular formula of $C_{20}H_{34}O$. The signals observed in the ^{13}C NMR spectra, δ 65.2, an oxygenated CH_2, δ 126.0, C and δ 140.2, C, a tetrasubstituted double bond and δ 140.8, C and δ 122.7, CH, a trisubstituted double bond, suggested a labdane skeleton for **7**. The comparison of the ^1H NMR spectral data for **7** with those reported in the literature (Caputo et al., 1976) indicated the structure as *labda-8,13E-dien-15-ol*. The mutual coupling between H-14 and H-15 observed in the ^1H-^1H COSY spectrum and the coupling between H-15 and C-15 observed in the HMQC spectrum are in agreement with the structure of the diterpene. The ^{13}C NMR spectral data, not previously described in the literature, are shown in Table 2.

The molecular formula $C_{20}H_{30}O$ of **8** was determined by the analysis of the EIMS spectrum and ^{13}C NMR spectral data. The presence of a carbonyl was confirmed by IR spectrometry (1710 cm^{-1}) and ^{13}C NMR spectrum, which showed a signal at δ 216.9 (Table 2). The occurrence in the ^1H NMR spectrum of one *dd* at δ 5.80 ($J=17.3$ and 10.3 Hz, H-15) and two *dd* at δ 4.94 ($J=17.3$ and 1.5 Hz, H-16a) and δ 4.87 ($J=10.3$ Hz and 1.5 Hz, H-16b) associated with the presence of a broad doublet at δ 5.41 ($J=5.9$ Hz, H-7) and the presence of four singlets corresponding to methyl groups, suggested a pimarane diterpene skeleton and defined the position

Table 1
^1H and ^{13}C NMR data and correlations observed in the HMBC for sesquiterpene **5** (500* and 125** MHz, δ, $CDCl_3$)

Position	^1H[a*] (multiplicity, J/Hz)	^{13}C**	Long range coupling
1	1.90	51.2	1.33, 1.41
2	1.60/1.76	23.9*[a]	2.15
3	1.60/1.33	33.1	
4	2.15	37.2	
5	2.15	43.8	1.90, 1.33
6	5.46 (*d*, 3.3)	124.0	2.15, 1.90
7	–	148.2	
8	1.90/2.15	25.1*[a]	1.41, 1.76, 2.15
9	1.41/1.76	42.6	1.90, 2.15
10	–	76.0	1.41, 1.76, 1.90, 2.15
11	2.15	37.6	
12	0.93*[a] (*d*, 6.6)	21.2[¶,a]	2.15
13	0.94*[a] (*d*, 6.9)	21.3[¶,a]	2.15
14	1.18	21.4	1.41, 1.76, 1.90
15	0.84 (*d*, 7.5)	15.2	

[a] *,¶: Value may be reversed in the columns.

Table 2
^{13}C NMR spectroscopic data for diterpenes **7** and **9** (125 MHz, δ, $CDCl_3$)

Position	7	9
1	37.1	37.9
2	19.1	23.1
3	41.9	79.2
4	33.4	38.6
5	51.9	50.0
6	19.2	27.4
7	33.6	121.4
8	126.0	135.4
9	140.2	51.9
10	39.1	no[a]
11	26.8	20.1
12	40.2	36.2
13	140.8	no
14	122.7	45.9
15	59.5	150.3
16	16.4	109.2
17	19.5	21.5
18	33.4	28.3
19	20.2	15.6
20	21.7	14.9

[a] No: not observed.

of the double bonds at C-7 and C-15. The carbonyl group was located at C-3 because of the occurrence of two *ddd* at δ 2.70 (J = 14.3, 14.7 and 5.2 Hz, H-2_{ax}) and δ 2.24 (J = 14.7, 3.7 and 3.7 Hz, H-2_{eq}) in the ^{1}H NMR spectrum. The structure of this diterpene was defined as *isopimara-7,15-dien-3-one*. The proton bearing carbon signals were assigned by the HMQC spectrum. The NMR spectral data of synthetic **8** have already been reported in the literature (Ceccherelli, 1984) but this is its first description as a natural product.

The ^{13}C NMR spectra data showed that diterpene **9** has an oxygenated methine at δ 79.2 (Table 2). The presence of three *dd* at δ 5.80 (J = 17.4 and 10.8 Hz, H-15), δ 4.92 (J = 17.4 and 3.6 Hz, H-16a) and at δ 4.86 (J = 10.8 and 3.6 Hz, H-16b), associated with the occurrence of four singlets, corresponding to the methyl groups in the ^{1}H NMR spectrum suggested a pimarane diterpene skeleton. The position of the hydroxyl group at C-3 was determined by analysis of its ^{1}H NMR spectrum which showed one *dd* at δ 3.25 (J = 11.2 and 4.5 Hz). The comparison of the ^{1}H NMR data of **9** with those reported in the literature (Polonsky et al., 1970; Ceccherelli et al., 1985) suggested its structure as being *isopimara-7,15-dien-3β-ol*. The ^{1}H-^{1}H COSY as well as the HMQC spectra confirmed this assumption. This compound has been described only as a synthetic product and its ^{13}C NMR data, which had not previously been described, are shown in Table 2.

The structure of **10** was determined by comparison of its NMR spectral data (Table 3) with those of **8** and **9**. The occurrence of three *dd* at δ 5.81 (J = 17.4 and 10.7 Hz, H-15), at δ 4.93 (J = 17.5 and 1.5 Hz, H-16a) and at δ 4.87 (J = 10.7 and 1.5 Hz, H-16b) associated with the presence of a broad doublet at δ 5.38 (J = 3.4 Hz) suggested a pimarane diterpene skeleton. The equatorial position of the hydroxyl group at C-2 was defined by the observation of a *tt* at δ 3.86 (J = 11.5 and 3.9 Hz) suggesting an axial hydrogen with two neighbouring methylene groups, only possible at C-2. The stereochemistry of C-2 was confirmed by the comparison of the coupling constant observed and the literature data (Ceccherelli, et al., 1986) which describes the occurrence of a pentete at δ 4.20 (J = 6.0 Hz) for H-2 of *isopimara-7,15-dien-2β-ol*. The structure of **10** was thus defined as *isopimara-7,15-dien-2α-ol*. The proton bearing carbon signals were assigned by HMQC spectrum (Table 3). The EIMS and the elemental analysis confirmed the proposed structure.

Table 3
^{1}H and ^{13}C NMR spectroscopic data for the diterpene **10** (500* and 125** MHz, δ, $CDCl_3$)

Position	^{1}H* (multiplicity, J/ Hz)	^{13}C**
1	2.15/2.12	49.1
2	3.86 (*tt*, 11.5, 3.9)	65.2
3	1.78/1.75	51.3
4	–	37.4
5	no[a]	49.8
6	no	23.3
7	5.38 (*br d*, 3.4)	121.6
8	–	135.4
9	1.13	52.0
10	–	34.7
11	1.56	20.3
12	1.37/1.51	36.1
13	–	36.8
14	1.97/1.93	46.0
15	5.81 (*dd*, 17.4, 10.7)	150.3
16	4.87 (*dd*, 10.7, 1.5)/4.93 (*dd*, 17.5, 1.5)	109.3
17	0.94	21.5
18	0.87	33.6
19	0.91	23.6
20	0.97	15.8

[a] No: not observed.

The ^{13}C NMR spectrum of **11** (Table 4) indicated the occurrence of thirty carbon atoms in the molecule including a carbonyl group at δ 216.5, four olefin carbons at δ 129.5 (CH), δ 134.1 (CH), δ 142.2 (C) and δ114.0 (CH_2) and seven methyl groups. The presence in the ^{1}H NMR spectrum of two *d* at δ 0.57 (J = 4.3 Hz) and at δ 0.79 (J = 4.0 Hz) suggested the occurrence of a cycloartane triterpene. This substance had its structure determined by comparing its ^{13}C NMR spectroscopic

Table 4
^{1}H and ^{13}C NMR spectroscopic data for triterpene **11** (500* and 125** MHz, δ, $CDCl_3$)

Position	^{1}H* (multiplicity, J/Hz)	^{13}C**
1	1.49/1.82	33.4
2	2.71 (*td*, 13.9, 6.5)/2.30 (*ddd*, 14.1, 4.3, 2.6)	37.5
3	–	216.5
4	–	50.2
5	1.70	48.4
6	0.94	21.6
7	1.90/1.32	28.2
8	1.59	47.9
9	–	21.1
10	–	26.0
11	1.37/1.15	25.9
12	1.34	35.6
13	–	45.4
14	–	48.8
15	1.65	32.7
16	2.07	26.7
17	1.59	52.2
18	1.01	18.1
19	0.79 (*d*, 4.0)/0.57 (*d*, 4.3)	29.6
20	1.49	36.8
21	0.89 (*d*, 6.5)	18.5
22	1.82/2.27	39.7
23	5.65 (*ddd*, 15.3, 8.4, 6.5)	129.5
24	6.12 (*d*, 15.6)	134.1
25	–	142.2
26	1.85	18.8
27	4.86	114.0
28	0.91	19.3
29	1.05	22.2
30	1.10	20.8

data with those of the cycloartane triterpene *cycloart-23-en-25-ol-3-one* (Furlan et al., 1993). It was thus found that both compounds have a double bond at C-23. However, **11** does not have an hydroxyl group at C-25 but instead another double bond between C-25 and C-26. Therefore, **11** was defined as the new triterpene *cycloarta-23,25-dien-3-one*. The proton bearing carbon signals were assigned by HMQC spectrum (Table 4). The EIMS and the elemental analysis agree with the proposed structure.

3. Experimental

3.1. General

Silica gel 60 (Merck) was used for chromatography (63–200 μm) for CC and silica gel plates F254 for TLC (Riedel-deHaën).

Mp was uncorr. NMR spectra were recorded at 125 for ^{13}C and 500 MHz for ^{1}H (Brucker DRX-500). The spectra were measured in $CDCl_3$ (Aldrich) as solvent and as internal standard; δ values are expressed in ppm. EIMS were obtained at 70 eV (INCOS 50 Finnigan-Mat-quadrupole); Optical rotations were measured in $CHCl_3$ in a digital polarimeter JASCO DIP-370 (Na filter, $\lambda = 588$ nm); IR spectra were obtained as KBr pellets in a Perkin-Elmer Infrared Spectometer model 1750; Elemental analysis were obtained in a Perkin-Elmer Elemental Analyser model 2400 CHN.

3.2. Plant material, extraction and isolation

The leaves of *G. macrophylla* (1200 g) were collected at the Universidade de São Paulo on 17 November 1998, in São Paulo city, São Paulo State, Brazil. The plant material was identified by Prof. Dr. José Rubens Pirani, from the Instituto de Biociências at the Universidade de São Paulo, São Paulo, Brazil. A voucher specimen is deposited in the herbarium of this Institute.

The air-dried plant material (410 g) was extracted with CH_2Cl_2 three times. The crude extract (17.3 g) was applied to a silica gel column, using solvent gradient (hexane-CH_2Cl_2-EtOAc-MeOH) to yield twenty fractions. The first two were constituted of waxy material. Fraction 3 was separated by CC with CH_2Cl_2 and yielded **6** (345 mg) and **8** (256 mg). Fraction 6 was submitted to chromatography on silica gel and eluted by CH_2Cl_2 to afford **10** (66 mg) and **2** (97 mg). Fraction 7 yielded, by CC with CH_2Cl_2:Me_2CO (9:1), **9** (30 mg) and **5** (31 mg). Fraction 8 was submitted to chromatography on silica gel, eluted with hexane-EtOAc (8:2) to afford **7** (21 mg), **10** (27 mg) and (19 mg) of a mixture of **3** and **4**. Fraction 10 was applied to a silica gel column, eluted with hexane-EtOAc (7:3) to yield **12** (58 mg) and a mixture of **12** and **1** (13 mg). Fraction 11 and 14, after CC with CH_2Cl_2:Me_2CO (9:1) as mobile phase yielded of **11** (25 mg and 80 mg), respectively.

(1S,4S*,5R*,10S*)-guai-6-en-10-ol* (**5**): Colourless oil, $[\alpha]_D = +13°$ ($CHCl_3$, c0.20); IR (KBr) ν_{max}, cm^{-1}: 3403, 2959, 2928, 2862, 1710, 1460, 1378, 1191, 1121, 1090; ^{1}H and ^{13}C NMR data are given in Table 1; EIMS (70eV): 222 (not observed), 204(36), 161 (100), 121(63), 105(84), 81(68), 55(48); Found C, 80.93; H, 11.68%. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%;

3.2.1. (2S*)-Isopimara-7,15-dien-2-ol (**10**)

White crystals, mp: 40–43°C; $[\alpha]_D = +100°$ ($CHCl_3$, c0.13); IR (KBr) ν_{max}, cm^{-1}: 3432, 2922, 2851, 1714, 1668, 1639, 1463, 1438, 1379, 1033, 910, 738; ^{1}H and ^{13}C NMR data are given in Table 3; EIMS (70 eV): 288 (12), 255 (29), 213 (27), 187 (30), 177 (16), 163 (16), 159 (23), 149 (27), 145 (33), 135 (35), 123 (36), 119 (49), 109 (52), 107 (64), 95 (67), 91 (68), 79 (63), 71 (54), 69 (78), 55(100); Found C, 83.21; H, 10.99%. $C_{20}H_{32}O$ requires C, 83.27; H, 11.18%

3.2.2. Cycloarta-23,25-dien-3-one (**11**)

White crystals, mp: 54 — 57°C; $[\alpha]_D = +14°$ ($CHCl_3$, c0.50); IR (KBr) ν_{max}, cm^{-1}: 2937, 1707, 1609, 1461, 1379, 1112, 966, 881. ^{1}H and ^{13}C NMR data are given in Table 4. EIMS (70 eV): 422 (no), 326 (3), 290 (1), 213 (12), 173 (9), 157 (7), 149 (8), 123 (7), 109 (12), 105 (13), 86 (63), 84 (100), 69 (22), 57 (25), 48 (90), 43 (60), 41 (37). Found C, 84.81; H, 11.30%, $C_{30}H_{48}O$ requires: C, 84.84; H, 11.39%;

Acknowledgements

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References

Almqvit, S., Enzell, C.R., Wehrli, F.W., 1975. Carbon-13 NMR studies of labdane diterpenoids. Acta Chem. Scand. Ser B 29, 695–720.

Bohlmann, F., Zeisberg, R., Klein, E., 1975. ^{13}C NMR spektren von monoterpenen. Org. Mag. Res. 7, 426–432.

Brochini, C.B., Núñez, C.V., Moreira, I.C., Chaves, M.H., Martins, D., Roque, N.F., 1999. Identificação de componentes de óleos voláteis: análise espectroscópica de misturas de sesquiterpenos. Química Nova 22, 37–40.

Caputo, R., Mangoni, L., Monaco, P., Pelosi, L., Previtera, L., 1976. Neutral diterpenes from *Araucaria bidwilli*. Phytochemistry 15, 1401–1402.

Ceccherelli, P., Curini, M., Marcotullio, M.C., 1985. 3β,19-oxidoisipimara-7,15-diene as intermediate in the conversion of virescenol B into isopimara-7,15-dien-19-ol. J. Chem. Soc. Perkin Trans. I, 2173–2175.

Ceccherelli, P., Curini, M., Coccia, R., Diamantini, M., 1984. A cyclopropanol derivative as an intermediate for the preparation of A-homopimaradiene. J. Chem. Perkin Trans. I 8, 1693–1695.

Ceccherelli, P., Curini, M., Marcotullio, M.C., 1986. Iodohyfrins and tetrahydrofurans from lead tetraacetate-iodine oxidation of terpenenic alcohols. J. Org. Chem. 51, 1505–1509.

Correa, M.P., 1984. Dicionário de Plantas Úteis e das Exóticas Cultivadas, Vol. 1.

Mootoo, B.S., Játiva, C., Tinto, W.F., Reynolds, W.F., McLean, S., 1992. Ecuadorin, a novel tetranortriterpenoid of *Guarea kunthiana*: structure elucidation by 2-D NMR spectroscopy. Can. J. Chem. 70, 1260–1264.

Faure, R., Ramanoelina, A.R.P., Rakatonirany, O., Bianchini, J.P., Gaydou, E.M., 1991. Two-dimensional nuclear magnetic resonance of sesquiterpenes. 4*-Application to complete assignment of ^{1}H and ^{13}C NMR spectra of some aromadendrane derivative. Mag. Res. in Chem. 29, 969–971.

Furlan, M., Roque, N.F., Wolter Filho, W., 1993. Cycloartane derivatives from *Guarea trichilioides*. Phytochemistry 32, 1519–1611.

Furlan, M., Lopes, M.N., Fernandes, J.B., Pirani, J.R., 1996. Diterpenes from Guarea trichilioides. Phytochemistry 41, 1159–1161.

Iwabuchi, H., Yoshikura, M., Kamisako, W., 1989. Studies on the sesquiterpenoids of *Panax ginseng* C. A. Meyer. Chem. Pharm. Bull 37, 509–510.

Nuñez, C.V., Roque, N.F., 1999. Sesquiterpenes from steam bark from *Guarea guidonia* (Meliaceae). J. Essent. Oil Res. 11, 439–440.

Polonsky, J., Baskevitch, Z., Bellavitta, N.C., Cecherrelli, P., 1970. Structures des virescenols A et B, métabolites d'*Oospora virescens* (Link) Wallr. Bull. Soc. Chim. de France 5, 1912–1918.

Weenen, H., Nkunya, M.H.H., Mgani, Q.A., Postumus, M.A., Warbel, R., Achenbach, 1991. Tanzanene, a spiro benzopyranyl sesquiterpene from *Uvaria tanzaniae*. J. Org. Chem. 56, 5865–5867.

Yoshikawa, M., Hatakeyama, S., Tanaka, N., Fukuda, Y., Murakami, N., Yamahara, J., 1992. Orientalols A, B and C, sesquiterpene constituents from chinese *Alismatis rhizoma* and revised strucutres of alismol and alismoxide. Chem. Pharm. Bull 40 (9), 2582–2584.