Abietane diterpenoids from *Clerodendrum mandarinorum*

T. Fan[a], Z. Min[b], G. Song[c], M. Iinuma[c,*], T. Tanaka[d]

[a]*Department of Biology, University of Science and Technology of China, Hefei, 230026, China*
[b]*Department of Natural Pharmaceutical Chemistry, China Pharmaceutical University, Nanjing, 210009, China*
[c]*Shanghai Institute of Pharmacy, Shanghai, 200031, China*
[d]*Gifu Prefectural Institute of Health and Environment, 4-6-3 Noishiki, Gifu, 500-8226, Japan*

Abstract

From the stem of *Clerodendrum mandarinorum* Diels (Verbenaceae), three new abietane derivatives, mandarones A, B and C, have been isolated. The structures were characterized as (5*R*,10*S*)-12-hydroxy-8,11,13-abietatriene-37-dione (mandarone A), (16 *S*)-12,16-epoxy-11,14-dihydroxy-17(15 → 16)-*abeo*-abieta-5,8,11,13-tetraene-7-one (mandarone B) and 12,16-epoxy-11,14-dihydroxy-17(15 → 16)-*abeo*-abieta-2,5,8,11,13,15-hexaene-7-one (mandarone C) on the basis of spectral analysis. Mandarones B and C possess a rearranged abietane skeleton which contains a 17(15 → 16)-*abeo*-abietane framework. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Clerodendrum mandarinorum; Verbenaceae; Rearranged abietane; Mandarone A; Mandarone B; Mandarone C

1. Introduction

Clerodendrum mandarinorum Diels is a traditional Chinese medicinal plant known as "Hai Tang". The crude drug has a good reputation in the treatment of infantile paralysis and apoplexy. Following phytochemical study of *Clerodendrum* species (Verbenaceae) (Tian et al., 1993), the chemical constituents in the stems of *C. mandarinorum* were investigated. In this paper the isolation and structural elucidation of three new rearranged abietane derivatives are described.

2. Results and discussion

Repeated column chromatography of a chloroform extract of the stems resulted in isolation of compounds **1–3**.

Compound **1**, mandarone A, obtained as yellow rectangles, showed $[M^+]$ at *m/z* 314.1880 in the high-resolution mass spectrometry (HREIMS), which corresponds to the molecular formula $C_{20}H_{26}O_3$. The IR spectrum showed the presence of hydroxyl (3400 cm[1]) and unsaturated ketone group (1640 cm[1]). The absorption bands at 277 and 344 nm in the UV spectrum also exhibited the presence of a benzene ring and an α, β-unsaturated ketone. The ^{1}H NMR data (Table 1) showed the presence of five methyl groups at δ 0.90 (*s*), 0.95 (*s*), 1.12 (*s*), 1.16 (*d*) and 1.19 (*d*), one hydroxyl group at δ 5.68 (*s*) (disappeared after addition of D_2O) and two aromatic protons at δ 6.78 (*s*) and 7.65 (*s*) to a 1,2,4,5-tetrasubstituted benzene. The chemical shifts are almost identical with those of sugiol (**4**) (Sengupta et al., 1960, Kupchan et al., 1996). The ^{1}H-^{1}H COSY spectrum supported that mandarone A possessed an isopropyl group [a benzylic methine group (δ 3.27) was coupled with two methyl groups at δ 1.16 (*d*) and 1.19 (*d*)] attached to the benzene ring and was an abieta-8,11,13-triene-7-one derivative (Hueso-Rodriguez et al., 1983, Miyase et al., 1977). A double doublet at δ 2.57 assigned to H-6β showed two large coupling constants (J_{gem} = 15.3 Hz and J_{vic} = 10.4 Hz), indicating that the methylene proton and its vicinal partner [H-5α: δ 2.41 (*dd, J_{aa}* = 10.4 Hz, J_{ae} = 7.6 Hz)] has a *trans*-diaxial relationship and that
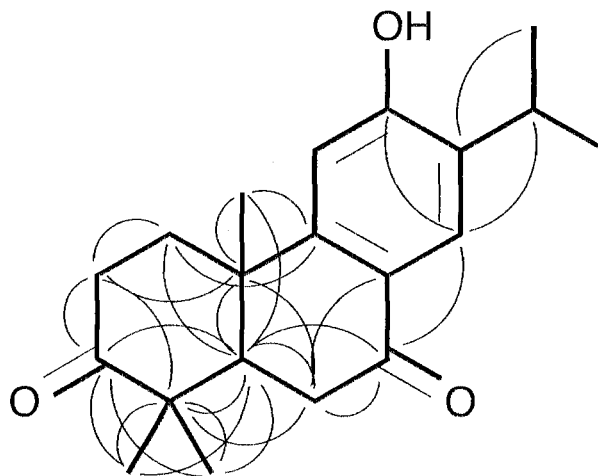
* Corresponding author.

Table 1
^{1}H NMR spectral parameters of compounds **1**, **2** and **3** (400 MHz).

Compounds **1** and **3** were measured in DMSO-d_6 and 2 was in CDCl$_3$. [a–d]Assignment may be reversed. [e]Rapidly exchangeable with D$_2$O. [f]Slowly exchangeable with D$_2$O

H	1	2	3
1α	1.43 (*dt*)	1.62 (*dd*)	1.52 (*dd*)
1β	2.17 (*m*)	3.26 (*dd*)	3.32 (*dd*)
2α (2)	2.36 (*m*)	1.84 (*m*)	5.54 (*m*)
2β	2.53 (*m*)	2.21 (*dd*)	
3α (3)		1.26 (*dd*)	5.25 (*d*)
3β		2.32 (*dd*)	
5α	2.41 (*dd*)		
6α (6)	2.51 (*dd*)	6.17 (*s*)	6.29 (*s*)
6β	2.57 (*dd*)		
11	6.78 (*s*)		
14	7.65 (*s*)		
15α (15)	3.27 (*m*)	3.36 (*dd*)	6.43 (*q*)
15β		2.77 (*dd*)	
16α		5.11 (*ddq*)	
Me-16	1.16 (*d*)[a]		
Me-17	1.19 (*d*)[a]	1.61 (*d*)	2.43 (*d*)
Me-18	0.90 (*s*)[b]	1.42 (*s*)[c]	1.90 (*s*)[d]
Me-19	0.95 (*s*)[b]	1.38 (*s*)[c]	1.92 (*s*)[d]
Me-20	1.12 (*s*)	1.74 (*s*)	1.62 (*s*)
OH-11		6.67 (*s*)[c]	6.60 (*s*)[e]
OH-12	5.68 (*s*)[e]		
OH-14		12.44 (*s*)[f]	14.12 (*s*)[f]
J(Hz)			
1α, 1β	12.8	13.3	16.5
1α, 2α	9.6	8.9	8.6
1α, 2β	9.6	8.8	
1β, 2α	3.8	3.6	4.2
1β, 2β	6.2	6.1	
2α, 2β	15.6	15.2	
2α, 3α (2,3)			8.1
5α, 6α	7.6		
5α, 6β	10.4		
6α, 6β	15.3		
15α, 15β		15.2	
15α, 16α		8.9	
15β, 16α		7.6	
15, 17			1.2
16α, 17		5.9	

Fig. 1. CH long-range correlations in the COLOC spectrum of **1**.

C-4, to the contrary another ketone at C-7 (δC 195.7) was correlated with the methylene group at C-6 [δ_H 2.51 (*dd*) and 2.57 (*dd*)] and the aromatic proton at C-14 [δ_H 7.65 (*s*)] which was also correlated with C-15 (δC 26.3). In the ^{13}C NMR spectrum (Table 2), 20 signals including two carbonyl carbons (C-3 and C-7), four non-protonated sp[2] carbons (C-8, C-9, C-12 and C-13), three sp[3] methylene carbons (C-1, C-2 and C-6), and a sp[3] methine carbon (C-5) were observed. Although the chemical shifts of these carbons as well as the other carbons were similar to those of **4**, the location of the saturated ketone group in mandarone A was not at C-1, but at C-3, because the ketone caused

Table 2
^{13}C NMR spectral data of compounds **1**, **2** and **3** (100 MHz)[a]

C	1	2	3
1	34.7	29.3	28.9
2	37.7	18.9	110.8
3	199.6	41.2	102.5
4	47.2	35.6	41.3
5	49.2	165.9	156.7
6	32.9	117.6	146.6
7	195.7	190.4	188.7
8	109.2	108.7	109.4
9	132.6	141.4	137.2
10	40.9	40.7	42.1
11	125.0	132.5	130.8
12	160.1	155.6	154.3
13	122.6	111.3	110.5
14	156.0	155.0	153.9
15	26.3	33.9	101.7
16	22.2	81.9	147.8
17	22.6	22.4	14.1
18	25.4	29.8	12.2
19	19.1	28.8	18.1
20	21.3	27.5	23.9

[a] Compounds **1** and **3** were measured in DMSO-d_6 and **2** was in CDCl$_3$.

the junction between rings A and B was in *trans*-configuration. A spin system composed of two adjacent methylene groups was located between a fully substituted sp[3] carbon and one of the ketone groups, because a large geminal coupling ($J = 15.6$ Hz) was observed between the two protons. The results obtained from the COLOC spectrum (Fig. 1) led to the following structural conclusions: the methyl group at C-20 [δ_H 1.12 (*s*)] had a long-range correlation with C-1 (δ_C 34.7), C-5 (δ_C 49.2) and C-9 (δ_C 132.6), and two methyls at C-18 [δ_H 0.90 (*s*)] and C-19 [δ_H 0.95 (*s*)] were correlated to C-5 (δ_C 49.2) and C-3 (δ_C 199.6). A ketone at C-3 (δ_C 199.6) had long-range correlations with the methylene protons at C-2 [δH 1.43 (*dt*) and 2.17 (*m*)] and two geminal methyl groups at

strong deshielding effects on the C-2 and C-4 at β-carbon. Except for the configuration of an asymmetric center at C-10, the structure of mandarone A was thus determined. The negative cotton effect at 305 nm and positive one at 358 nm in the CD spectrum showed that the structure had the same abietane absolute configuration as candelbrone (Narr & Chandran, 1976) which is signed as 5R,10 S-configuration. Ring A has a boat conformation caused by the presence of a ketone group at C-3. Therefore, the structure of mandarone A is characterized as (5R,10 S)-12-hydroxy-8,11,13-abietatriene-37-dione (**1**).

Compound **2**, mandarone B, obtained as yellowish rectangles, showed [M$^+$] at *m/z* 328.1673 in HREIMS which corresponds to the molecular formula $C_{20}H_{24}O_4$. Its UV and the NMR data (Tables 1 and 2) were in partial agreement with those of **1**. The differences were that only one ketone group was observed in the ^{13}C NMR spectrum and four protons assigned at H_2-1 and H_2-2 in **1** were substituted with six protons which were attributable to three adjacent methylene groups in mandarone B, indicating that a ketone at C-3 in **1** did not exist in mandarone B. The methylene group at C-6 and the methine group at C-5 in **1** was not found in the ^{1}H NMR spectrum of **2**; instead a down-field signal at δ 6.17 (*s*) was observed, which showed that an olefinic proton was connected with the ketone group at C-7. Mandarone B possesses a partial structure which is rarely seen in an abietane diterpenoid, namely the presence of ring D, deduced as follows. One of the spin systems was based on a benzylic group (C-15) [two protons at δ 3.36 (*dd*, J_{gem} = 15.2 Hz, J_{vic} = 8.9 Hz and δ 2.77 (*dd*, J_{gem} = 15.2 Hz, J_{vic} = 7.6 Hz) coupled with a methine proton (C-16) at δ 5.11 observed in a double doublet of quartet (J_1 = 8.9 Hz, J_2 = 7.6 Hz, and J_3 = 5.9 Hz) which was connected to a secondary methyl group (C-17) at δ 1.61 (*d*, *J* = 5.9 Hz). This partial structure is due to an α-methyl dihydrofuran condensed with an aromatic ring that is completely substituted, because the proton resonance of the methine at C-16 (δ 5.11) and the methyl group at C-17 (δ 1.61) were observed in a lower field than those of lanugon S (δ 4.33 and 1.32) (Schmid et al., 1982). The ^{1}H NMR spectrum of **2** showed the presence of two phenolic hydroxyl groups (δ 6.67 and 12.44), indicating that a 1,2,4-trioxygenated benzene ring existed in **2**. The oxygen atom of the dihydrofuran moiety must be located at C-12, since no shifts were observed in the UV spectrum when measured in the presence of boric acid and sodium acetate, showing that mandarone B had no *ortho*-diphenolic hydroxyl group. One of the phenolic groups was strongly chelated with the ketone (δ 12.44) and could be located at C-14, which was further supported when an alternative structure with a dihydrofuran oxygen at C-14 was ruled out. An oxygen-

bearing carbon at δ 81.9 could be assigned to C-16 by the CH COSY spectrum (Table 2) which also supported the formation of the α-methyl dihydrofuran. The stereochemistry at C-16 was determined by CD spectrum as compared with that of teuvincenone B (Carreira et al., 1990). As a negative absorption at 297 nm in the CD curve was found, the methyl group at C-16 was S-configuration. The ^{1}H NMR spectral data of an α-methyldihydrofuran unit in **2** were identical with those of teuvincenone B. Therefore the structure of mandarone B was shown to be (16 S)-12,16-epoxy-11,14-dihydroxy-17(15 → 16)-*abeo*-abieta-5,8,11,13-tetraene-7-one (**2**).

Compound **3**, mandarone C, obtained as orange needles, showed [M$^+$] at *m/z* 324.1361 in HREIMS which corresponds to the molecular formula $C_{20}H_{20}O_4$. The presence of a *para*-dihydroxyphenyl moiety which was conjugated with a ketone group (C-7) was revealed by the UV spectral data. The ^{1}H NMR spectrum showed that mandarone C had a same chromophore as **2**. Differences were the existence of two double bonds, in ring A and D, which was supported by the presence of a spin system corresponding to four protons assigned to H_2-1, H-2 and H-3, indicating that a double bond formation occurred between C-2 and C-3. The down-field shift of H-1β (δ 3.32) showed no double bond formation at C-1. Another double bond was formed between C-15 and C-16, which was revealed by the disappearance of α-methyl dihydrofuran spin system in **2**. An olefinic proton at δ 6.43 and a methyl group at δ 2.43 were assigned to H-15 and C-17-Me, respectively. Their chemical shifts were identical to those of teuvincenone F which has a same 2-methyl-benzo[b]furan moiety in ring D (Cuadrado et al., 1992), which was supported by comparison of the chemical shift on the furan β-proton (H-15) [δ 6.43 (*q*, *J* = 1.2 Hz)] with the α-proton values of a furan for the natural or synthesized tanshinone [δ 7.20-7.60 (*q*, *J* = 1.1–1.3 Hz, H-16) (Haiza et al., 1990). The structure of mandarone C was therefore characterized as **3**.

3. Experimental

Plant material. Stems of *Clerodendrum mandarinorum* Diels cultivated in Lushan Plant Garden of Jiangxi, China were collected.

Extraction and isolation. The naturally dried and pulverized stems of *C. mandarinorum* (5 kg) were extracted with hot EtOH three times. The solvent was removed in vacuo to yield crude extract (180 g). H_2O was added to the crude extract and the mixture was agitated thoroughly to form a suspension, which was extracted with CHCl$_3$. After evaporation, the CHCl$_3$ extract (80 g) was subjected to column chromatog-

raphy on silica gel eluted with an EtOAc-petroleum ether gradient to give **1** (12 mg), **2** (20 mg) and **3** (15 mg), respectively.

Compound **1** *[mandarone A, (5R10 S)-12-hydroxy-8,11,13-abietatriene-3,7-dione]*. mp. 212–214° (CHCl3), yellowish rectangles. HREIMS m/z: 314.1880 for $C_{20}H_{26}O_3$ (calcd 314.1882), $[\alpha]_D$ −80.1° (CHCl$_3$, $c = 0.0162$), CD: 221 (0), 245 (−3.79), 268 (0), 274 +0.29), 277 (0), 305 (−4.51), 333 (0), 358 (+5.48) nm, IR ν_{max}^{KBr}: 3400 (OH), 1640, 1620 (ketone), 1600, 1580, 1450, 1380, 1020 cm^{-1}, UV λ_{max}^{MeOH}: 277, 344 nm, ^{1}H NMR and ^{13}C NMR spectral data are listed in Tables 1 and 2.

Compound **2** *[mandarone B, (16 S)-12,16-epoxy-11,14-dihydroxy-17(15 → 16)-abeo-abieta-5 ,8,11,13-tetraene-7-one]*. mp. 201–202° (CHCl3), yellowish rectangles. HREIMS m/z: 328.1673 for $C_{20}H_{24}O_4$ (calcd 328.1675), $[\alpha]_D$ −123.2° (CHCl$_3$, $c = 0.0178$), CD: 236 (0), 252 (+2.39), 266 (0), 297 (−2.52), 320 (0), 341 (+4.85), 365 (0) nm, IR ν_{max}^{KBr}: 3300 (OH), 1700, 1680 (ketone), 1600, 1580, 1460, 1280, 980 cm^{-1}, UV $\lambda_{max}^{MeOH.}$: 258, 314, 373 nm, ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

Compound **3** *[mandarone C, 12,16-epoxy-11,14-dihydroxy-17(15 → 16)-abeo-abieta-2,5,8,1 1,13,15 -hexaene-7-one]*. mp. 223–224° (CHCl3), orange needles. HREIMS m/z: 324.1361 for $C_{20}H_{24}O_4$ (calcd 328.1362), $[\alpha]_D$ +75.5° (CHCl$_3$), $c = 0.0171$), IR ν_{max}^{KBr}: 3400 (OH), 1600 (ketone), 1610, 1575, 1480, 1320, 1010 cm^{-1}, ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2, respectively.

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