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SESQUITERPENE DIMER AND TRIMER FROM *CHLORANTHUS JAPONICUS**

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Key Word Index—*Chloranthus japonicus*; Chloranthaceae; lindenane skeleton; sesquiterpene trimer; sesquiterpene dimer; 2D NMR.

Abstract—A novel sesquiterpene trimer, trishizukaol A, consisting of three lindenane units was isolated from the roots of *Chloranthus japonicus*. The structure was elucidated by 1D- and 2D-NMR methods. A structurally related sesquiterpene dimer was also found in the same plant. © 1997 Elsevier Science Ltd

INTRODUCTION

In the course of our continuing search for sesquiterpenes in plants of the Chloranthaceae, sesquiterpene lactones having an unusual lindenane skeleton have been isolated from *Chloranthus japonicus* Sieb. [2–4] and *C. serratus* Roem. et Schult. [4, 5]. The lindenane sesquiterpenes are characteristic constituents of the chloranthaceous plants [6]. Recently, a series of dimeric lindenanes named shizukaols were isolated from *Chloranthus* spp [1, 7–9]. A further survey of the chemical constituents of *C. japonicus* yielded a novel lindenane trimer, trishizukaol A (**1**), along with a structurally related dimer, shizukaol J (**2**). Although a number of lindenane dimers have been isolated from the chloranthaceous plants, this is the first report of the isolation of a trimeric lindenane sesquiterpene.

RESULTS AND DISCUSSION

Trishizukaol A (**1**) was assigned the molecular formula $C_{48}H_{54}O_{12}$ (FD-MS; $[M]^+ = m/z$ 822 and NMR: Table 1). The ^1H NMR spectrum of **1** showed three high-field signals (δ_H 0.12, 0.32 and 0.36) characteristic of H-2β of the lindenane skeleton. The HH COSY showed three sets of proton networks of a 1,2-disubstituted cyclopropane ring (δ_H 0.36, 0.92, 1.90 and 1.95; 0.32, 1.03, 1.95 and 1.97; 0.12, 0.80, 1.60 and 1.86). Their chemical shifts and coupling pattern were quite similar to those found in H-1, 2 and 3 of the previously isolated lindenanes [1–5, 7–9]. Therefore, **1** seemed to be a trimer of a lindenane sesquiterpene. The molecular formula of **1** corresponded to a homotrimer of a basic lindenatriene ($C_{16}H_{18}O_4$, **3**) which is a component of all shizukaols [1, 7–9] previously found as lindenane dimers in *Chloranthus*. The presence of three each of the quaternary methyls (δ_H 0.89, 0.99 and 1.04; δ_C 15.2, 16.9 and 17.4), methoxyls (δ_H 3.45, 3.57 and 3.58; δ_C 51.8, 52.0 and 52.2), ester carbonyls (δ_C 171.7, 172.9 and 176.4) and ketones (δ_C 198.5, 201.3 and 201.8) strongly supported this proposal. The connectivity of the three units was determined by long-range CH correlation experiments such as HMBC and COLOC (Table 2). In unit A, one of the characteristic cyclopropyl methylene protons (δ_H 0.36, H-2β) was correlated to a quaternary carbon (δ_C 58.5) in HMBC. This quaternary carbon was assigned to C-10 by additional long-range CH correlations with a singlet methyl (δ_H 1.04, H-14) and a carbinol methine (δ_H 3.92, H-9). C-10 also correlated with an olefinic proton (δ_H 7.33) which had a correlation with a carbonyl (δ_C 201.3) assignable to C-8 by a cross peak with H-9. Hence, the olefinic proton had to be assigned to H-6. H-6 was also correlated with a quaternary sp^2 carbon (δ_C 147.1, C-4) which had correlations with H-2β and an allylic methyl (δ_H 2.01, H-15). Thus unit A was a lindena-4,6-diene system. A quaternary carbon, C-11 (δ_C 48.4), was assigned by correlations with H-6 and a singlet methyl (δ_H 1.69, H-13). C-11 also showed correlation with a pair of non-equivalent methylene protons (δ_H 2.53 and 3.35, H_2-15′). These protons had further correlations with two quaternary sp^2 carbons (δ_C 147.4, C-4′ and 140.5, C-5′) and a cyclopropyl methine (δ_C 29.9, C-3′). The latter methine had to be included in another cyclopropane unit characteristic of the lindenane skel-

*Part 11 in the series 'Studies on the Chemical Constituents of Chloranthaceae Plants'. For Part 10, see ref. [1].

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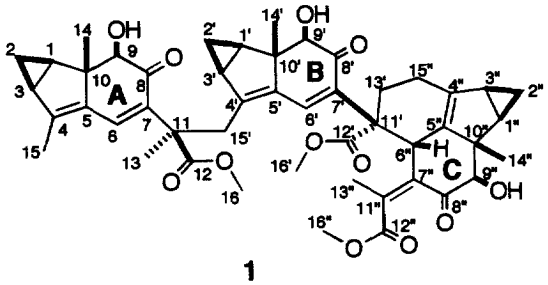

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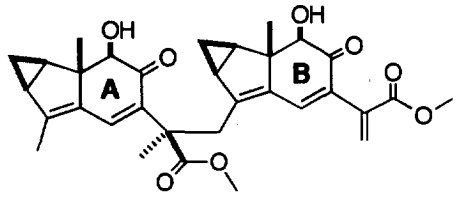

2

eton, namely, unit B. In the same manner as above, the lindena-4,6-diene system was also characterized for unit B and a linear connectivity between C-11 of unit A and C-15′ of unit B was clarified by the HMBC correlations of H-15′ with C-7, 11, 12 and 13. On the other hand, unit C was found to be a lindena-4,7(11)-diene system, as shown for most shizukaols [1, 7, 8], by the HMBC data (Table 2). Connectivity between the units B and C was not very easy to determine. In unit B, a quaternary carbon at δ_C 52.7 was assigned to C-11′ by the HMBC correlations with H-6′ (δ_H 6.98). Thus C-11′ was connected to unit C since it appeared as a quaternary sp^3 carbon. This carbon was also correlated with non-equivalent methylene protons (δ_H 1.99 and 2.33) which were assigned to H_2-13′. The total unsaturation index of the molecule is 22 and thus the units B and C had to be connected by formation of a ring. Therefore, C-13′ was also connected to unit C. In the case of unit C, the lindena-4,7(11)-diene structure needed connection at both C-6″ and C-15″. Two non-equivalent methylene protons of H_2-15″ at δ_H 1.99 and 2.44 were severely overlapped with the signals of H_2-13′. However, their complex multiplet nature strongly indicated the presence of *J* couplings between H_2-15″ and H_2-13′, which indicated the C-15″/C-13′ connection since H_2-15″ would be rather simple AB doublet pairs if C-15″ was connected to the quaternary C-11′. The NOEs of H-6″ with H-6′ and H-13′ supported this connectivity though sufficient HMBC correlations were not available between H-6″ and the carbons in the unit B. Hence, the connectivity between the units B and C was established. The relative stereochemistry of **1** was determined by analysis of the NOE data (Fig. 1). The typical NOE correlations characteristic of lindenanes such as H-2β/H-14, H-1/H-9 and H-6/H-15 were also found in the three units in **1** though, for clarity they are not shown in Fig. 1. The stereochemical relationships of the units A, B and C are tentatively assigned by the inter-unit NOEs shown in Fig. 1. The absolute configuration was also proposed through the biogenetic relationships with other *Chloranthus* lindenanes mentioned below. This is the first isolation of a trimeric lindenane. The manner of connection between the three units is unprecedented in the previously isolated lindenane dimers [1, 7–11]. Signal broadening of the 12 carbons in the NMR (Table 1) was observed possibly due to the complex nature of the total structure.

Shizukaol J (**2**) was assigned the molecular formula of $C_{32}H_{36}O_8$ by FD-MS ($[M]^+$ *m/z* 548) and NMR (Table 1). The molecular formula indicated **2** to be a homo-dimer of the lindenatriene **3**. Detailed analysis of the 1D and 2D NMR data including HMBC experiments (Table 2) yielded a combined structure for **2** corresponding to units A and B of **1**. The ^1H and ^{13}C NMR assignments of **2** were well related to those of **1** as shown in Table 1. The terminal structure of the unit B of **2** was shown to be a vinylidene group (δ_H 5.77 and 6.26, both *s*, H-13′; δ_C 128.3(CH_2), C-13′ and 136.3(*Cq*), C-11′) in place of the connection to another lindenane unit as in **1**. The higher field proton signal of H-13′ (δ_H 5.77) was assigned to the *endo* proton since it showed a NOE with H-6′ (δ_H 6.90). Hence, the structure of **2** was determined. The stereochemistry was determined by the NOE interactions similar to those of **1** (Fig. 1).

The plausible biogenetic relationships of **1**–**3** are shown in Scheme 1. Subtraction of a proton from C-13 of the lindenatriene **3** and successive double bond migration results in a carbanion at C-15 which then attacks C-11 of another molecule of **3** to give the dimer **2**. Finally, a Diels–Alder type cycloaddition between the terminal methylene of **2** and the C-15/C-4/C-5/C-6 diene of the third molecule of **3** completes the structure of the trimer **1**. So far, more than 10 lindenane dimers containing **3** as a structural unit have been isolated from *Chloranthus*. All attempts to isolate monomeric **3**, however, have failed. Structural variations and biogenetic correlations [9] of such lindenane dimers suggest that these compounds are synthesized in plant cells.

EXPERIMENTAL

^1H and ^{13}C NMR: $(CD_3)_2CO$ or $CDCl_3$, chemical shifts relative to residual signals of each solvent: $(CD_3)_2CO$, δ_H 2.04 and δ_C 29.8; $CDCl_3$, δ_H 7.23 and δ_C 77.0. Air-dried roots (1.6 kg) of *C. japonicus* were extracted with Et_2O at room temp. The extracts were washed with 5% $NaHCO_3$ and chromatographed over silica gel using a hexane–Et_2O and then Et_2O–Me_2CO gradient. The Et_2O–Me_2CO (7:3) eluate was subjected to silica gel prep. TLC developed with Et_2O–MeOH (25:1). A characteristic yellow fluorescent band was scraped off and further purified by silica gel prep. TLC ($CHCl_3$–MeOH, 13:1) to give **1** (28 mg) as a yellow viscous gum. The ether eluate of the first silica gel

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2**

		1 (acetone-d_6)		**2** (chloroform-*d*)	
		δC	δ_H	δ_C	δ_H
Unit A	1	24.8	1.90 *m*	24.2	1.93 *m*
	2	13.2	α 0.92 *ddd* (8,8,4)	13.0	α 0.94 *m*
			β 0.36 *ddd* (4,4,3)		β 0.38 *ddd* (4,4,3)
	3	29.3	1.95 *m*	28.9	1.87 *m*
	4	147.1	—	146.8	—
	5	136.8	—	135.7	—
	6	137.4	7.33 *s*	136.4	7.10 *s*
	7	136.6	—	135.2	—
	8	201.3	—	200.5	—
	9	82.1	3.92 *d* (4)	80.9	3.95 *s*
	10	58.5	—	57.5	—
	11	48.4	—	47.6	—
	12	176.4	—	176.0	—
	13	23.9	1.69 *s*	23.3	1.66 *s*
	14	16.9	1.04 *s*	16.5	1.06 *s*
	15	15.1	2.01 *s*	15.1	1.96 *s*
	16	52.2	3.58 *s*	52.3	3.74 *s*
	OH	—	4.18 *d* (4)	—	—
Unit B	1′	26.8	1.97 *m*	26.0	2.05 *m*
	2′	13.9	α 1.03 *ddd* (8,8,4)	13.2	α 0.96 *m*
			β 0.32 *ddd* (4,4,3)	—	β 0.30 *ddd* (4,4,3)
	3′	29.9	1.95 *m*	29.4	1.84 *m*
	4′	147.4	—	147.8	—
	5′	140.5	—	140.1	—
	6′	137.8*	6.98 *s*	137.9	6.90 *s*
	7′	139.5*	—	131.6	—
	8′	198.5	—	198.7	—
	9′	80.7	4.02 *d* (3)	80.0	4.27 *s*
	10′	56.8	—	56.5	—
	11′	52.7*	—	136.3	—
	12′	172.9*	—	166.5	—
	13′	34.9*	α 1.99 *m*	128.3	*endo* 5.77 *s*
			β 2.33 *m*		exo 6.26 *s*
	14′	17.4	0.89 *s*	17.3	0.94 *s*
	15′	36.3	α 2.53 *d* (14)	36.0	α 2.57 *d* (14)
			β 3.35 *d* (14)		β 3.35 *d* (14)
	16′	51.8*	3.45 *s*	52.3	3.66 *s*
	OH′	—	4.07 *d* (3)	—	—
Unit C	1″	26.5	1.86 *ddd* (8,7,4)		
	2″	15.4	α 0.80 *ddd* (8,8,4)		
			β 0.12 *ddd* (4,4,3)		
	3″	23.8	1.60 *ddd* (7,6,4)		
	4″	140.9*	—		
	5″	134.6*	—		
	6″	43.8*	3.55 *m*		
	7″	135.8*	—		
	8″	201.8*	—		
	9″	79.9	4.21 *br s*		
	10″	52.2	—		
	11″	139.9*	—		
	12″	171.7	—		
	13″	19.0	1.69 *s*		
	14″	15.2	0.99 *s*		
	15″	24.6	α 1.99 *m*		
			β 2.44 *m*		
	16″	52.0	3.57 *s*		
	OH″	—	3.87 *br s*		

* Broadening signals.

Table 2. Long-range CH correlations found in compounds **1** and **2***

	C	**1** (acetone-d_6) H	**2** (chloroform-*d*) H
Unit A	1	2α, 2β, 9, 14	9, 14
	2	—	—
	3	2α, 2β, 15	15
	4	1, 2α, 2β, 3, 6, 15	6, 15
	5	1, 3, 14, 15	14, 15
	6	—	—
	7	6, 9, 13, 15′α	6, 13, 15′α
	8	6, 9, OH	6
	9	1, 14, OH	—
	10	1, 2α, 2β, 3, 6, 9, 14, OH	6, 9, 14
	11	6, 13, 15′α, 15′β	6, 13, 15′α, 15′β
	12	16, 15′β	13, 16, 15′β
	13	15′α, 15′β	15′α, 15′β
	14	9	9
	15	—	—
	16	—	—
Unit B	1′	2′β, 9′, 14′	9′, 14′
	2′	—	—
	3′	2′α, 2′β, 15′α, 15′β	15′α, 15′β
	4′	2′α, 2′β, 6′, 15′α, 15′β	6′, 15′α, 15′β
	5′	1′, 3′, 14′, 15′α, 15′β	14′, 15′α, 15′β
	6′	—	—
	7′	6′, 9′, 13′α	6′, 13′*endo*, 13′*exo*
	8′	6′, 9′	6′, 9′
	9	1′, 14′, OH′	—
	10′	1′, 2′α, 2′β, 3′, 6′, 9′, 14′	6′, 9′, 14′
	11′	6′, 13′β, 15″β	6′, 13′*endo*, 13′*exo*
	12′	13′β, 16′	13′*endo*, 13′*exo*, 16′
	13′	—	—
	14′	9′	9′
	15′	13	13
	16′	—	—
Unit C	1″	2″α, 2″β, 14″	
	2″	—	
	3″	2″α, 2″β	
	4″	13′β, 1″, 2″α, 2″β, 15″β	
	5″	1″, 3″, 14″	
	6″	13′α, 13′β	
	7″	13″	
	8″	—	
	9″	1″, 14″	
	10″	1″, 2″α, 2″β, 9″, 14″, OH″	
	11″	13″	
	12″	13″, 16″	
	13″	—	
	14″	—	
	15″	13′α, 13′β	
	16″	—	

* Determined by HMBC and/or COLOC experiments.

chromatography was fractionated by successive silica gel prep. TLC ($CHCl_3$–MeOH, 50:1 and then hexane–EtOAc, 2:1) to yield **2** (1.6 mg) as a yellow oil.

Trishizukaol A (**1**). Yellow gum, $[\alpha]_D^{25} -27°$ ($CHCl_3$; *c* 1.10). FD-MS *m/z* (rel. int.): 822 $[M]^+$ (100), 790 (30), 548 (17), 274 (15).

Shizukaol J (**2**). Yellow oil, $[\alpha]_D^{25} +62°$ ($CHCl_3$; *c* 0.20) FD-MS *m/z* (rel. int.): 548 $[M]^+$ (100); EI-MS *m/z* (rel. int.): 548 $[M]^+$ (16), 275 (43), 247 (44), 243 (58), 215 (80), 43 (100).

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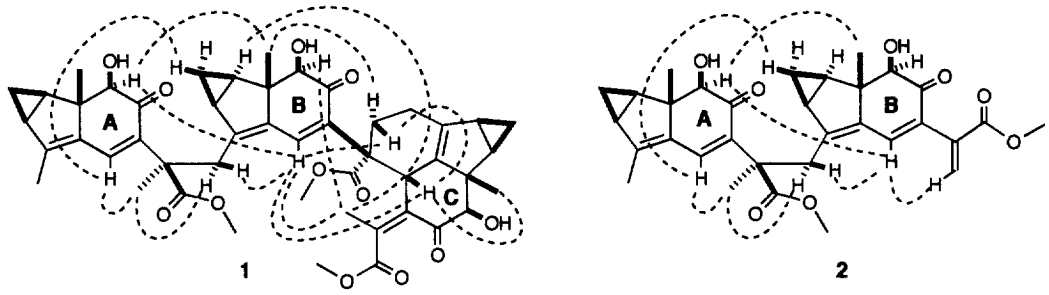
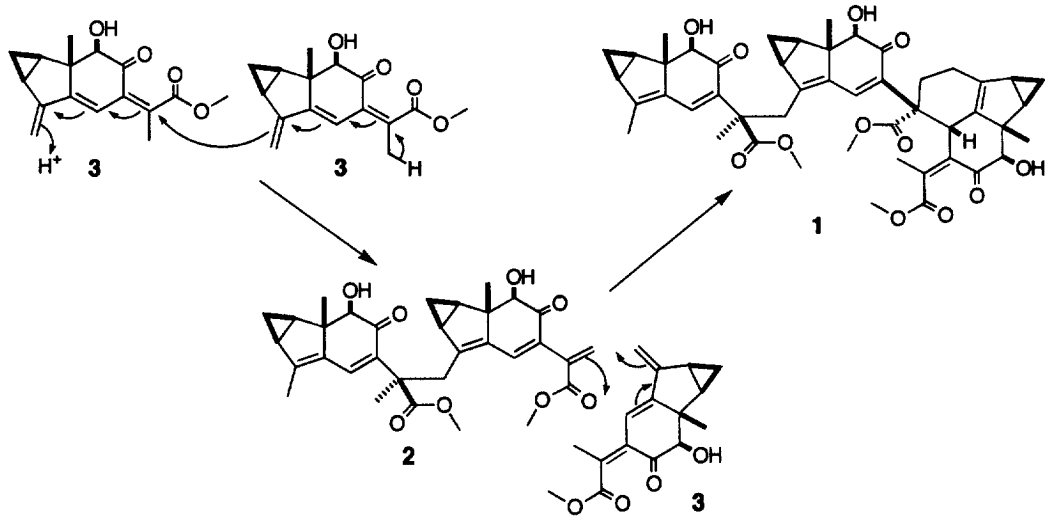


Fig 1. Some of the NOE correlations in trishizukaol A (**1**) and shizukaol J (**2**).



Scheme 1. Plausible biogenetic route for the formation of trishizukaol A (**1**) and shizukaol J (**2**) from **3**.

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