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ORTHOSIPHOL D AND E, MINOR DITERPENES FROM *ORTHOSIPHON STAMINEUS*

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Key Word Index—*Orthosiphon stamineus*; Labiatae; orthosiphol D; orthosiphol E; pimarane diterpene.

Abstract—From the aerial parts of *Orthosiphon stamineus*, two new diterpenes, orthosiphols D and E were isolated together with the known rosmarinic acid, sinensetin, scutellarein tetramethyl ether, salvigenin and orthosiphols A and B. The structures of the new compounds were elucidated mainly by spectroscopic methods.

INTRODUCTION

Kumis-kuching (whole plant of *Orthosiphon stamineus* BENTH.) is widely used as a diuretic. Some constituents of this plant, such as monoterpenes [1], hexoses [2], organic acids [2, 3], triterpenes [4], saponins [5], flavonoids [6–8], rosmarinic acid [9], chromene [10], and orthosiphols A, B and C [11] have been reported. During the course of our investigation on the biologically active constituents of Labiatae plants, we examined the constituents of the aerial part of *Orthosiphon stamineus* cultivated in Okinawa Prefecture and isolated two new diterpenes, orthosiphols D (**1**) and E (**2**) together with the known compounds, orthosiphol A (**3**), orthosiphol B (**4**) [11], sinensetin (**5**) [8], scutellarein tetramethyl ether (**6**) [6], salvigenin (**7**) [12] and rosmarinic acid (**8**) [13]. This paper describes the structure elucidation of the new diterpenes.

RESULTS AND DISCUSSION

Orthosiphol D (**1**), $[\alpha]_D$ −96° (MeOH) was obtained as an amorphous powder and the molecular formula was determined as $C_{31}H_{36}O_9$ based on its HR mass spectrum. It contained two acetoxyl groups [δ1.96 and 2.03 (each 3H, *s*); δ20.1 and 21.1 (each *q*), 168.9 and 169.2 (each *s*)], a benzoyloxyl group [δ7.48 (2H, *t*, J=7.7 Hz), 7.62 (1H, *br t*, J=7.7 Hz) and 8.06 (2H, *dd*, J=7.7 and 1.3 Hz); δ133.5 (*s*), 128.6 (2C, *d*), 129.8 (2C, *d*), 129.8 (*s*) and 165.7 (*s*)], a vinyl group [δ5.96 (1H, *dd*, J=17.6 and 11.1 Hz) (H_b), 4.96 (1H, *d*, J=17.6 Hz) (H_f) and 5.16 (1H, *d*, J=11.1 Hz) (H_e); δ115.8 (*t*) and 140.0 (*s*)], an isolated carbonyl group [δ208.4], four tertiary methyl groups [δ1.14, 1.16 and 1.17, 1.50 (each 3H, *s*); δ19.7, 21.5, 25.9 and 26.7 (each *q*)] and a tertiary hydroxyl group [δ2.93 (1H, *br d*, J=1.3 Hz); δ75.2 (*s*)], an α,β-unsaturated carbonyl group [δ6.61 (1H, *s*) (H_a); δ142.3 (*d*), 142.2 (*s*) and 196.2 (*s*)] and two secondary carbinyl groups [δ5.47 (1H, *br t*, J=2.8 Hz) (H_d) and 5.74 (1H, *br t*, J=6.4 Hz) (H_c); δ69.2 and 70.5 (each *d*)] as partial structures based on its ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra. The ^{13}C NMR spectrum of orthosiphol D (**1**) further showed signals due to two methylene groups, two methine groups and three quaternary carbon atoms. These spectral data, coupled with the consideration on the co-existence of orthosiphols A (**3**) and B (**4**), suggested that orthosiphol D is tricyclic and has a pimarane skeleton as a basic skeleton. The structure between C-9 and C-13 (partial structure **A**) was elucidated by following the cross peaks H_h [δ2.77 (1H, *dd*, J=7.9 and 1.3 Hz)]→H_c→H_i [δ2.61 (1H, *dd*, J=15.7 and 6.6 Hz)]→H_k [δ2.24 (1H, *dd*, J=15.7 and 1.1 Hz)] in the ^{1}H–COSY spectrum. Another partial structure **B** consisting of the C-5–C-7 portion of the molecule was also elucidated by following cross peaks H_d→H_m [δ1.93 (1H, *m*)]→H_e[δ2.17 (1H, *m*)]→H_j [δ2.33 (1H, *dd*, J=13.2 and 1.9 Hz)]. The location of an isolated carbonyl group and a tertiary hydroxyl group was elucidated at C-14 and C-8, respectively, based on the analysis of the results obtained from ^{1}H–^{13}C long range COSY (J=10 Hz) (Fig. 1). Namely, cross peaks due to two and three bond couplings for the carbon (δ_C 75.2) having a tertiary hydroxyl group were observed from the proton signals due to H_d (H-7) and H_m (H_1-6). On the other hand, cross peaks for the carbon (δ208.4) were observed from the proton signals due to H_b, H_k, and that at δ1.16 (H_3-17). The stereochemical correlation between the H-9 and OH-8 was also suggested to be the *trans*-orientation from the observation of the cross peaks between H-9 and the hydroxyl group at C-8 via W-letter interaction. Thus,

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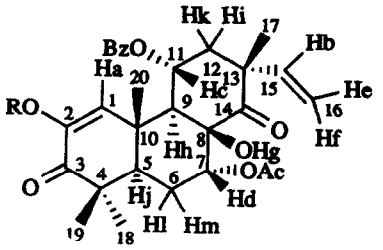
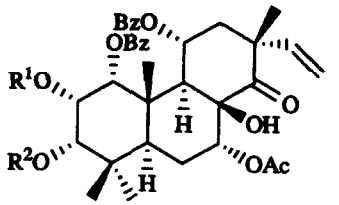
Table 1. ^{1}H NMR data of orthosiphols D (**1**) and E (**2**) (measured in $CDCl_3$ at 400 MHz)

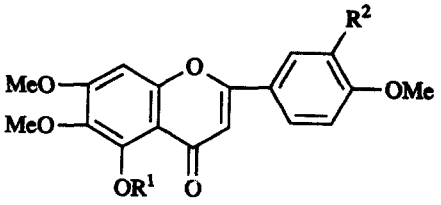
H	1	2
1	6.61 (*s*)	6.26 (*s*)
5	2.33 (*dd*, J = 13.2 and 1.9 Hz)	2.24 (*br s*, J = 13.5 Hz)
6	1.93 (*m*)	1.92 (*br d*, J = 13.7 Hz)
	2.17 (*m*)	2.13–2.22
7	5.47 (*br t*, J = 2.8 Hz)	5.47 (*br s*)
9	2.77 (*dd*, J = 7.9 and 1.3 Hz)	2.72 (*d*, J = 7.7 Hz)
11	5.74 (*br t*, J = 6.4 Hz)	5.79 (*br t*, J = 6.3 Hz)
12	2.24 (*dd*, J = 15.7 and 1.1 Hz)	2.13–2.22
	2.61 (*dd*, J = 15.7 and 6.6 Hz)	2.63 (*dd*, J = 15.8 and 5.8 Hz)
15	5.96 (*dd*, J = 17.6 and 11.1 Hz)	5.97 (*dd*, J = 17.7 and 10.7 Hz)
16	4.96 (*d*, J = 17.6 Hz)	4.93 (*d*, J = 17.7 Hz)
	5.16 (*d*, J = 11.1 Hz)	5.13 (*d*, J = 10.7 Hz)
17	1.16 (*s*)	1.17 (*s*)
18	1.14 (*s*)	1.14 (*s*)
19	1.17 (*s*)	1.17 (*s*)
20	1.50 (*s*)	1.49 (*s*)
OH	2.93 (*br d*, J = 1.3 Hz)	2.89 (*m*), 5.87 (*m*)
Ac	1.96 (s), 2.03 (*s*)	2.01 (*s*)
Bz	7.62 (1H, *br t*, J = 7.7 Hz)	7.62 (*t*, J = 7.7 Hz)
	7.48 (2H, *t*, J = 7.7 Hz)	7.50 (2H, *t*, J = 7.7 Hz)
	8.06 (2H, *dd*, J = 7.7 and 1.3 Hz)	8.11 (2H, *br d*, J = 7.7 Hz)



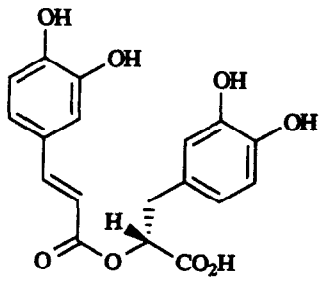
	R
1	Ac
2	H



	R^1	R^2
3	Ac	H
4	H	Ac



	R^1	R^2
5	Me	OMe
6	Me	H
7	H	H



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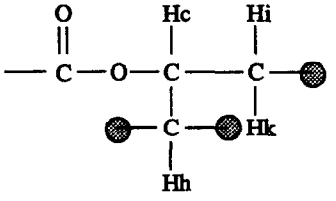
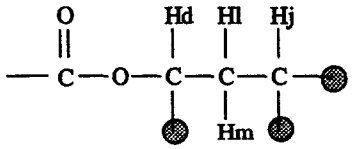
the location of the functional groups around rings B and C was elucidated. The location of the remaining functional group, an α,β-unsaturated carbonyl group, is restricted to ring A. The location of the carbonyl group and a proton on a double bond was elucidated to be at C-3 and C-1, respectively, from the facts that cross peaks for the signal [δ_C 196.2 (*s*)] from the proton signals due to H_a, 18-H_3 (δ1.14) and 19-H_3 (δ1.17) and those for the signal

Table 2. ^{13}C NMR data of orthosiphol D (**1**) and E (**2**) (measured in $CDCl_3$ at 100 MHz)

Carbon		1	2
1		142.3	126.3
2		142.2	143.7
3		196.2	199.9
4		44.5	43.4
5		44.8	45.2
6		22.9	22.7
7		70.5	70.7
8		75.2	75.2
9		45.9	46.5
10		40.4	39.7
11		69.2	69.1
12		39.0	39.3
13		48.0	48.1
14		208.4	208.7
15		140.0	140.7
16		115.8	115.0
17		26.7	26.6
18		21.5	21.8
19		25.9	25.9
20		19.7	20.6
OH			
OAc		20.1, 21.1 168.9, 169.2	21.0, 169.1
OBz	1′	133.5	133.5
	2′	128.6 (2C)	128.7 (2C)
	3′	129.8 (2C)	129.8 (2C)
	4′	129.8	130.1
	5′	165.7	165.8



Partial structure A



Partial structure B

[δ44.8 (C-5)] from the proton signal due to H_a were observed in the ^1H–^{13}C long range COSY ($J = 10$ Hz) spectrum. The substitution at C-2 by an acyloxy group was inferred from the chemical shift [δ142.3 (*d*)] of the signal assigned to C-1 [14]. The stereochemistry was

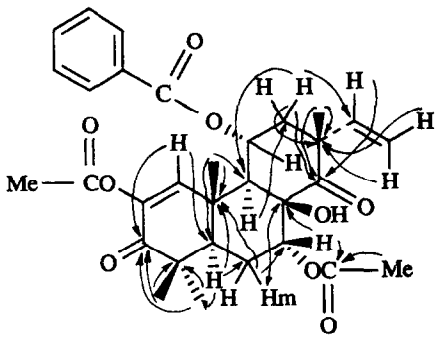


Fig. 1. Summary of the results of ^1H–^{13}C long range COSY spectrum ($J = 10$ Hz) for orthosiphol D (**1**).

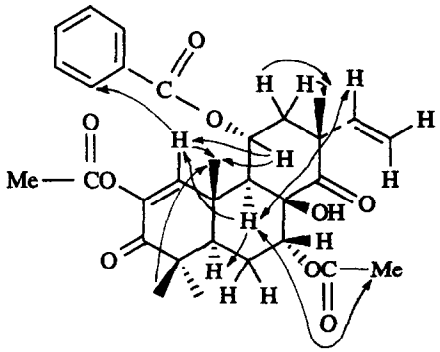


Fig. 2. Summary of the results of ^1H–NOESY spectrum for orthosiphol D (**1**).

determined from the results obtained by ^1H–NOESY experiment (Fig. 2). Thus, the structure of orthosiphol D was elucidated as mentioned except for the location of three acyloxyl groups, i.e. two acetoxyl and a benzoyloxyl group. An acetoxyl group was determined to be located on C-7 from the fact that H_d showed a cross peak with a carbonyl group of an acetoxyl group in the ^1H–^{13}C-long range COSY spectrum. The location of a benzoyloxyl group and another acetoxyl group were suggested to be at C-11 and C-2, respectively, based on the consideration of the co-existence of orthosiphol A (**3**) and B (**4**).

Orthosiphol E (**2**) $[\alpha]_D - 100°$ (MeOH) was also obtained as an amorphous powder. The molecular formula was determined as $C_{29}H_{34}O_8$ and is C_2H_2O less than that of orthosiphol D (**1**). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of orthosiphol E (**2**) showed the presence of a secondary acetoxyl group, a secondary benzoyloxyl group, two hydroxyl groups, an isolated carbonyl group, a vinyl group, an α,β-unsaturated carbonyl group and four tertiary methyl groups and are very similar to those of orthosiphol D (**1**). However, compound **2** had two hydroxyl groups and one acetoxyl group while compound **1** possessed one hydroxyl and two acetoxyl groups. The signal due to H-1 moved upfield by 0.35 ppm and the signals due to carbons of ring A changed (Table 2) in the ^1H and ^{13}C NMR spectra. These spectral data suggested that orthosiphol E has a structure which

corresponds to the 2-*O*-deacylated derivative of orthosiphol D (**1**). Acetylation of orthosiphol E (**2**) with a mixture of acetic anhydride and pyridine gave orthosiphol D (**1**). Based on the above findings, the structures of orthosiphols D and E were elucidated as **1** and **2**, respectively.

EXPERIMENTAL

General procedures. ^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz. TMS as int. standard. EIMS: 70 eV; CC: silica gel 60 (0.040–0.063 mm, Merck); TLC and prep. TLC: silica gel 60 F_{254} plates (0.25 and 0.5 mm in thickness, respectively.

Plant material. The plant material used was cultivated in Okinawa prefecture, Japan.

Isolation procedures. The methanolic extract obtained from the aerial parts of *Orthosiphon stamineus* BENTH. (1.1 kg) was concd *in vacuo*. The residue (81 g) was dissolved in 90% MeOH (330 ml) and the soln was partitioned between *n*-hexane (300 ml × 3). The 90% MeOH layer was concd *in vacuo*. The residue was suspended in H_2O (300 ml) and the suspension was extracted with EtOAc (300 ml × 3). After being washed with H_2O (100 ml × 3), the EtOAc extract was dried and evapd *in vacuo* to give a residue (17.73 g) which was chromatographed over silica gel (600 g) with $CHCl_3$–MeOH as eluant with increasing MeOH content. $CHCl_3$ (1 l), $CHCl_3$–MeOH (49:1) (3 l), $CHCl_3$–MeOH (19:1) (2 l), $CHCl_3$–MeOH (93:7) (2 l), $CHCl_3$–MeOH (9:1) (2 l), $CHCl_3$–MeOH 17:3 (2.5 l), $CHCl_3$–MeOH 4:1 (2.5 l), $CHCl_3$–MeOH 7:3 (2.5 l) and MeOH (2 l) were passed successively collecting 200 ml fractions (Column I).

Fr. nos 16–32 gave a residue (5.885 g) which was chromatographed over silica gel (80 g) with $CHCl_3$–MeOH (49:2) collecting 100 ml fractions (Column II). The residue (285 mg) from fr. no. 2 was further purified by silica gel (20 g) chromatography using Et_2O as eluant to give scutellarein tetramethyl ether (**6**) (16.3 mg) [6]. Fr. nos 3–5 gave a residue (4.227 g) which was chromatographed over silica gel (200 g) with Et_2O as eluant collecting 15 ml fractions. Fr. nos 25–30 gave a residue (295 mg) which was separated by prep. TLC (solvent: *n*-hexane–Et_2O 1:1, developed 3 ×) to give orthosiphol D (**1**) (57.9 mg) and orthosiphol E (**2**) (58.3 mg) as amorphous powders. Fr. nos 32–34 gave a residue (435 mg), a portion (100 mg) of which was purified by prep. TLC (solvent: *n*-hexane–Et_2O 1:1, developed 2 ×; solvent: *n*-hexane–isopropanol 17:3, developed 3 ×) to give orthosiphol A (**3**) (34.4 mg) [11]. Fr. nos 44–54 gave a residue (249 mg), an aliquot (40 mg) of which was purified by prep. TLC (*n*-hexane–Et_2O 3:7 developed 3 × and then developed with Et_2O) to give orthosiphol B (**4**) (20 mg) [11]. Fr. nos 64–75 gave a residue (31 mg) which was purified by prep. TLC (Et_2O, developed 2 ×) to give salvigenin (**7**) (13.2 mg) [12]. The Me_2CO washings of the column gave a residue (155 mg), an aliquot of the residue (106 mg) was purified by prep. TLC (benzene–EtOAc 4:1, developed 3 ×) to give scutellarein tetramethyl ether (**6**) (56.1 mg) [6] sinensetin (**5**) (42.2 mg) [8].

The MeOH washings of column I gave a residue (2.122 g) which was purified by a CC over Toyo pearl (solvent EtOH–H_2O 3:2) collecting 6 ml fractions. Fr. nos 24–30 gave a residue (1.20 g), an aliquot (350 mg) of which was further purified by a chromatography over Lobar RP-8 with MeOH–H_2O (1:1) as an eluant collecting 7 ml fractions. Fr. nos 9–11 gave rosmarinic acid (**8**) (271 mg) [13]. Among the isolated compounds, known compounds were identified by direct comparison or by comparisons of the spectral data with those reported. The physical properties of the new compounds are as follows.

Orthosiphol D (**1**). Amorphous powder, $[\alpha]_D^{21.6}$ −96° (MeOH; *c* 0.21); UV λ_{max}^{MeOH} (log ε): 231 (4.29); IR $\nu_{max}^{CHCl_3}$: 3575, 1720, 1610, 1460, 1380, 1280, 1240–1210, 1115, 1045, 1030 and 915 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); EIMS *m/z* 552.2345 $[M]^+$. $C_{31}H_{36}O_9$ requires: 552.2359.

Orthosiphol E (**2**). Amorphous powder, $[\alpha]_D^{21.6}$ −99.7° (MeOH; *c* 0.47); UV λ_{max}^{MeOH} (log ε): 231 (4.15) and 271 (3.55); IR $\nu_{max}^{CHCl_3}$: 3575, 3450, 1720, 1610, 1460, 1380, 1280, 1240–1210, 1115, 1050, 1030, 915 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); EIMS *m/z* 510.2249 $[M]^+$. $C_{29}H_{34}O_8$ requires: 510.2254.

Acetylation of orthosiphol E (**2**). Orthosiphol E (**2**) (7.4 mg) was dissolved in a mixt. of pyridine (0.2 ml) and Ac_2O and the mixt. was left overnight at room temp. After addition of excess MeOH, the mixt. was concd *in vacuo*. The residue was purified by prep. TLC (*n*-hexane–Et_2O 1:1, developed 3 ×) to give orthosiphol D (**1**) (3.3 mg). This compound was completely identical with natural orthosiphol D in all respects.

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