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Secoorthosiphols A–C: three highly oxygenated secoisopimarane-type diterpenes from *Orthosiphon stamineus*

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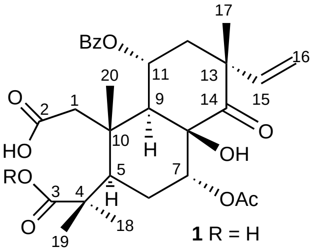
Abstract—Three new highly oxygenated 2,3-secoisopimarane-type diterpenes, named secoorthosiphols A (**1**), B (**2**) and C (**3**), have been isolated as extremely minor constituents from the aerial parts of the *Orthosiphon stamineus* from Okinawa. A unique unprecedented structural feature of such a ring A opened system in newly isolated compounds was encountered for the first time in isopimarane-type diterpenes. Secoorthosiphol C (**3**) represents the first example of a biogenetically unique and unconventional secoisopimarane-type diterpene having a cyano group. © 2002 Elsevier Science Ltd. All rights reserved.

Orthosiphon stamineus is an important traditional folk medicine extensively used in Southeast Asia.[1–3] In our search for cancer antiproliferative agents from natural sources, we have previously isolated a series of highly oxygenated isopimarane-type diterpenes and the diterpenes with a novel carbon framework named 'staminane' from *O. stamineus* of Vietnam and Myanmar.[4–7] However, there are no reports on the staminane-type diterpenes in *O. stamineus* from Okinawa.[8] Thus, we have investigated the constituents of *O. stamineus* cultivated in Okinawa and isolated three new highly oxygenated 2,3-secoisopimarane-type diterpenes named secoorthosiphols A–C (**1**–**3**) as extremely minor constituents (yields from methanolic extract: **1**, 0.00084%; **2**, 0.000061%; **3**, 0.00014%), together with three staminane-type and five isopimarane-type diterpenes. We herein report the isolation and structure elucidation of the new 2,3-secoisopimarane-type diterpenes **1**–**3** by spectroscopic and chemical means.

Secoorthosiphol A (**1**) was obtained as a colorless amorphous solid and showed $[\alpha]_D^{25}$ −155.6° (*c* 0.5, $CHCl_3$). Its molecular formula was determined by HR-FABMS to be $C_{29}H_{36}O_{10}$ [m/z 543.2272 $(M-H)^-$]. The IR spectrum of **1** showed the absorptions due to hydroxyl (3550 cm^{-1}), ester carbonyl (1710 cm^{-1}) and phenyl (1600, 1420 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) revealed signals due to four tertiary methyls (δ_H 1.15, 1.20, 1.11, 1.26), a vinyl (δ_H 5.92, 5.15, 4.96), two oxygen-substituted methine (δ_H 5.34, 5.53) and three aliphatic methylene groups (δ_H 2.63, 2.17; 2.08, 2.04; 2.54, 2.06), together with those of one acetyl and one benzoyl group. The ^{13}C NMR spectrum (Table 1) indicated the presence of a ketone, two acid carbonyl, two ester carbonyl and three oxygen-substituted carbons. The partial structures (bold line) obtained from the ^1H–^1H COSY and HMQC spectra were connected based on the long-range correlations observed in the HMBC spectrum (Fig. 1). Significant



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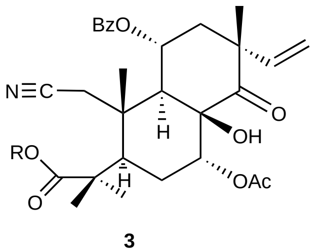
2 R = CH_3



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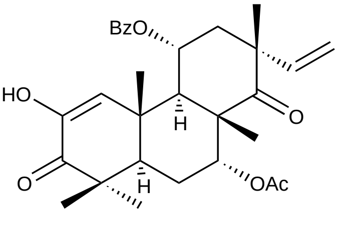

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Table 1. ^1H and ^{13}C NMR data for compounds **1**–**3** in $CDCl_3$ (*J* values in parentheses)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.63 d (20.3)	39.7	2.32 d (20.3)	39.5	2.57 d (18.2)	
	2.17 d (20.3)		2.22 d (20.3)		2.28 d (18.2)	29.3
2		176.8		171.1		
3		185.6		179.1		117.8
4		44.8		45.3		181.3
5	3.08 dd (12.7, 3.1)	40.6	3.05 dd (15.2, 2.7)	41.0	2.58 dd (15.2, 2.6)	46.5
6	2.08 m	22.8	2.07 m	23.4	2.16 td (15.2, 2.6)	42.7
	2.04 m		1.98 dt (15.2, 2.7)		1.97 dt (15.2, 2.6)	24.5
7	5.34 br s	71.2	5.30 t (2.7)	71.0	5.31 br s	
8		75.0		75.0		70.2
9	3.58 d (7.1)	42.5	3.78 d (7.3)	41.4	3.11 d (7.3)	74.6
10		44.2		44.2		44.3
11	5.53 t (7.1)	69.3	5.60 dd (7.3)	69.3	5.64 t (7.3)	44.2
12	2.54 dd (15.4, 7.1)	39.4	2.53 dd (15.6, 7.3)	39.0	2.58 dd (18.1, 7.3)	68.7
	2.06 d (15.4)		2.09 m		2.14 d (18.1)	39.4
13		47.8		47.8		
14		209.2		209.3		47.8
15	5.92 dd (17.6, 10.8)	140.7	5.95 dd (17.6, 10.8)	140.4	6.01 dd (17.8, 10.7)	208.6
16	5.15 d (10.8)	114.4	5.15 d (10.8)	114.5	5.22 d (10.7)	140.0
	4.96 d (17.6)		4.98 d (17.6)		4.99 d (17.8)	115.2
17	1.15 s	25.6	1.18 s	25.5	1.19 s	
18[a]	1.20 s	28.8	1.18 s	27.9	1.29 s	25.7
19[a]	1.11 s	21.9	1.20 s	23.5	1.35 s	25.3
20	1.26 s	20.9	1.20 s	19.9	1.30 s	23.2
7-OAc						18.7
CO<u>CH</u>$_3$	2.07 s	20.7	2.09 s	20.9	2.06 s	
<u>CO</u>CH$_3$		169.6		169.3		20.8
11-OBz						169.4
1′		130.3		131.2		
2′,6′	7.80 d (8.1)	129.4	8.03 dd (7.6, 1.5)	129.5	8.20 d (7.5)	129.4
3′,5′	7.34 t (8.1)	128.3	7.46 t (7.6)	128.5	7.50 t (7.5)	130.2
4′	7.50 t (8.1)	132.9	7.58 tt (7.6, 1.5)	133.2	7.61 t (7.5)	128.6
CO		165.5		165.5		133.7
3-O<u>C</u>H$_3$			3.57 s	52.0		165.8

[a] Assignments were based on the ^{13}C NMR chemical shift values of isopimarane-type diterpenes obtained from *O. stamineus*. The C-18 resonates always at lower field than C-19.[4–7]

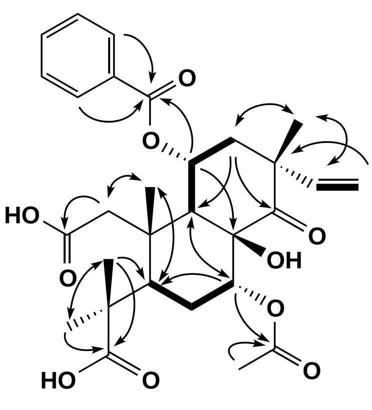

Figure 1. Partial structures (bold line) and key HMBC correlations (arrows) in **1**.

correlations were observed between the ester carbonyl carbon at δ_C 169.6 (7-OCO) and the protons at δ_H 2.07 (7-OCOCH$_3$) and δ_H 5.34 (H-7) and between the ester carbonyl carbon at δ_C 165.5 (11-OCO) and the phenyl protons at δ_H 7.80 (H-2′,6′) and δ_H 5.53 (H-11), allowing the locations of the benzoyl and acetoxyl groups to be at C-11 and C-7, respectively. Thus, the structure of secoorthosiphol A was concluded to be represented by the structure **1**.

The ring A dicarboxylic nature of **1** was further confirmed by treatment of **1** with diazomethane to give the dimethyl ester: δ_H 3.53, 2.96; δ_C 51.8, 50.0; FABMS m/z 571 $(M-H)^-$.

The relative stereochemistry of **1** was determined by coupling constants and ROESY experiment (Fig. 2). The large coupling constants for H-5 ($J_{5,6ax}=12.7$), H-9 and H-11 ($J_{9,11}=7.1$) indicated them to be axial, while the small *J* values for H-7 ($J_{6eq,7}=2.4$ Hz, $J_{6ax,7}=0$ Hz) indicated its equatorial nature. In the ROESY spectrum, significant correlations were observed between H$_3$-20 and H-11, between H-5 and H-9 and between H-11 and H$_3$-17 as depicted in Fig. 2. These data suggested that rings B and C should have chair conformation.

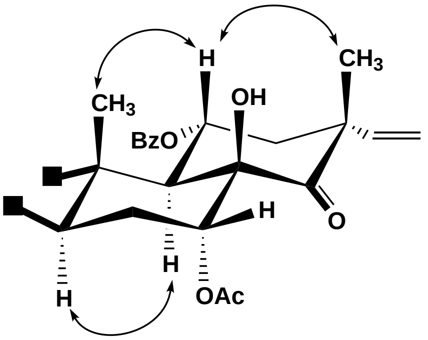

Figure 2. Key ROESY correlations in **1**.

The ^{1}H and ^{13}C NMR spectra of secoorthosiphol B[9] (**2**) were almost superimposable to those of **1** (Table 1), except for the presence of one additional methoxyl signal (δ_H 3.75, δ_C 52.0). This was consistent with the molecular formula $C_{30}H_{38}O_{10}$ determined from HR-FABMS [m/z 557.2385 $(M-H)^-$]. The location of the methoxy group was determined to be at the position 3 based on the significant HMBC correlations between the ester carbonyl carbon at δ_C 179.0 (C-3) and the methoxyl protons at δ_H 3.57 (-OCH_3) and two tertiary methyl protons (δ_H 1.18, 1.20) assigned for H_3-18 and H_3-19. In conclusion, **2** was determined as 3-*O*-methylate of secoorthosiphol A (**1**).

Secoorthosiphol C (**3**)[10] was obtained as a colorless amorphous solid and displayed $[\alpha]^{25}_{D}$ −99.6° (c 0.05, $CHCl_3$). The quasimolecular ion $(M-H)^-$ at m/z 524.2281 in the HR-FABMS indicated the presence of one nitrogen atom in **3** and is consistent with the molecular formula $C_{29}H_{36}NO_8$. The IR spectrum of **3** showed a characteristic sharp absorption for the cyano group (2250 cm^{-1}) together with the absorptions for hydroxyl, ester carbonyl and phenyl groups. The ^{1}H NMR spectrum of **3** was identical to that of **1**. Comparison of the ^{13}C NMR spectrum showed that, except for the upfield shift of the signal for the carboxyl group (δ_C 176.8, C-2) of **1** to δ_C 117.8 in **3**, the remaining carbon signals were also identical. In the HMBC experiment, this carbon was correlated with the protons at δ_H 2.57 and 2.28 (H_2-1), suggesting the location of the cyano group in the position 2. Thus, secoorthosiphol C was determined to have the structure formula **3**.

To the best of our knowledge, secoorthosiphols A–C (**1**–**3**) are the first examples of 2,3-secoisopimarane-type diterpenes, which may be produced through an oxidative cleavage of the C_2–C_3 bond of orthosiphol E (**4**),[11] isolated from the same extract. Secoorthosiphol C (**3**), on the other hand, is the first example of a biogenetically unique isopimarane-type diterpene having a cyano group. Further work on the antiproliferative activities of **1**–**3** against several carcinoma cell lines are in progress and will be reported in due course.

Acknowledgements

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10. Secoorthosiphol C (**3**): Colorless amorphous solid, $[\alpha]^{25}_{D}$ −99.6° (c 0.05, $CHCl_3$). IR ν_{max} 3550, 2250, 1710, 1600, 1420, 1370, 1280, 1110, 1050 cm^{-1}. HR-FABMS 524.2281 [calcd for $C_{29}H_{38}NO_8$ $(M-H)^-$, 524.2284].
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