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DITERPENOIDS FROM *SALVIA SPLENDENS*

DA-PENG HU, KAZUYOSHI KAWAZOE and YOSHIHISA TAKAISHI*

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770, Japan

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Key Word Index—*Salvia splendens*; Labiatae; diterpene; clerodan; splenolide.

Abstract—The methanol extract of aerial parts of *Salvia splendens* afforded three new diterpenes: 15,16-epoxy-1β-hydroxy-*trans*-cleroda-2,13(16),14-trieno-12,17; 19,18-diolide: 15,16-epoxy-11β-acetoxy-*trans*-cleroda-2,13(16),14-trieno-12,17: 19,18-diolide and 1β,11β-diacetoxy-15-hydroxy-*trans*-cleroda-2,13-dieno-12,17; 15,16; 19,18-triolide named splenolide A, B and C. Their structures were established on the basis of spectroscopic studies. © 1997 Elsevier Science Ltd

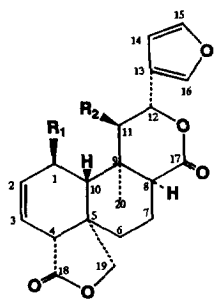
INTRODUCTION

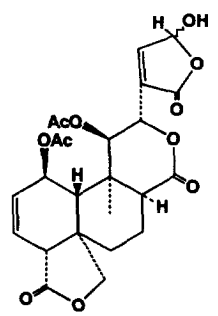
In the course of our search for bioactive metabolites from plants [1–5], we have been interested in the genus *Salvia* plants and started a study of the chemical components of *Salvia splendens*. *Salvia* species are widespread in tropical and temperate zones and a number have found local use as medicinal and culinary herbs. *Salvia splendens* is a Brazilian species which is now widely used for ornamental purpose. There are several reports [6–9] on the chemical investigation of *S. splendens*. In this paper, we report the isolation and the structure elucidation of three new diterpenoids, splenolide A (**1**), B (**2**) and C (**3**), and the known compounds **4** and **5**.

RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl acetate-soluble fraction from the methanol extracts of aerial parts of *Salvia splendens* yielded compounds **1**–**5**. Splenolide A (**1**) was assigned the molecular formula $C_{20}H_{22}O_6$ by HREI-mass spectrometry and its IR spectrum showed lactones (1765 and 1698 cm^{-1}) and hydroxy (3423 cm^{-1}) band absorptions. The ^{13}C NMR spectrum exhibited 20 carbon resonances including one methyl, four methylenes, ten methines and five quaternary carbons. The ^1H and ^{13}C NMR spectral data also showed the presence of one disubstituted double bond [δ_H 5.60 (1H, *dd*, J = 10.3, 1.5 Hz), 5.93 (1H, *dd*, J = 10.3, 1.5 Hz)], two lactones [δ_C 173.2, 176.2], one furan ring [δ_H 6.41, 7.40, 7.45 (each 1H, *brs*), δ_C 108.5 (*d*), 125.5 (*s*), 139.6 (*d*), 143.7 (*d*)], two methines attached to an oxygen function [δ_H 4.36 (1H, *d*, J = 9.8 Hz), δ_C 65.3; δ_H 5.75 (1H, *dd*, J = 12.2, 3.4 Hz), δ_C 72.6] and one methylene attached to an oxygen function [δ_H 4.20, 4.35 (each 1H, AB*q*, J = 9.1 Hz)]. The spectral data of **1** were very similar to those of salviarin (**4**) which was isolated from the same plant [6]. Comparison of the ^{13}C NMR spectral data of compounds **1** and **4** showed almost the same chemical shifts except for C-1, 2 and 10. This fact suggested that in the structure of **1**, the hydroxy methine replaced the methylene at C-1 found in **4**.

To confirm the structure of **1**, we measured the 2D NMR spectrum. From the ^1H–^1H COSY spectrum, the partial structures, I: >CH—CH(OH)—CH=CH—CH<, II: —CH_2—CH_2—CH< and III: —O—CH—CH_2— were established. The proton signal at δ_H 2.03 (1H, *d*, J = 9.8 Hz, H-10) was correlated with the carbon signals at δ_C 24.1 (C-20), 35.9 (C-9), 42.6 (C-5), 65.3 (C-1) and 71.1 (C-19), the proton signal at δ_H 5.60 (H-3) with the carbon signals at δ_C 42.6 (C-5), 52.4 (C-4) and 65.3 (C-1) in the ^{13}C–^1H long range correlation spectrum. These facts clearly indicated that the partial structure I could be put in





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1: R1=OH, R2=H
2: R1=H, R2=OAc
4: R1=H, R2=H
5: R1=OAc, R2=OAc

* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data for compounds **1–5**

C	1	2	3	4	5
1	65.3	19.5	66.5	18.9	66.8
2	135.5	129.3	128.8	128.8	128.7
3	120.1	120.6	122.3	121.2	122.1
4	52.4	52.2	51.3	52.1	51.2
5	42.6	41.6	42.4	41.4	42.4
6	32.2	32.1	31.5	32.4	31.7
7	18.9	23.8	19.5	21.9	29.0
8	50.8	49.1	49.7	49.0	50.2
9	35.9	39.0	39.9	35.1	39.9
10	44.2	37.9	41.4	38.2	41.4
11	43.8	76.4	76.0	40.8	76.0
12	72.6	71.5	71.1	70.5	71.2
13	125.5	121.6	134.5	124.7	121.5
14	108.5	108.6	148.6	108.3	108.6
15	143.7	144.0	97.2	143.8	144.3
16	139.6	141.4	168.7	139.6	141.8
17	176.2	175.4	169.2	175.4	169.2
18	173.2	169.5	174.2	171.4	174.1
19	71.1	70.0	70.6	70.0	70.4
20	24.1	19.0	20.2	23.7	19.0
OAc	—	169.0	170.8	—	170.1
	—	20.7	19.2	—	21.8
	—	—	170.8	—	169.8
	—	—	21.8	—	19.9

ring-A and the hydroxy group was located on C-1. Other ^1H and ^{13}C NMR signals were assigned in the same manner (Table 1). The relative stereochemistry of the hydroxy group on C-1 was determined to be β from the coupling constant ($J = 9.8$ Hz) of H-1 with H-10 (β-ax). Thus, the structure of splenolide A (**1**) was formulated as shown.

Splenolide B (**2**) contained a furan ring (δ_C 108.6, 121.6, 141.4, 144.0), a γ-lactone (δ_C 169.5), a δ-lactone (δ_C 175.4), and a disubstituted double bond (δ_C 120.6, 129.3) moieties like salviarin (**4**). Compound **2** also contained an acetyl ester moiety as evidenced by the proton signal at δ_H 1.99 (3H, *s*) and the carbon signals at δ_C 20.7 and 169.0. The FAB-HR-mass spectrum of **2** showed the peak due to $[M+H]^+$ at *m/z* 401.1611 which agreed with a molecular formula for **1** as $C_{22}H_{24}O_7$. The ^1H and ^{13}C NMR spectral data of **2** were very similar to those of **4** except for the proton signals of H-11 [**2**: δ_H 5.20 (*d*, $J = 10.7$ Hz), **4**: δ_H 1.72 (*dd*, $J = 15.0$, 5.0 Hz), 2.24 (*dd*, $J = 15.0$, 12.0 Hz)] and H-12 [**2**: δ_H 5.28 (*brd*, $J = 10.7$ Hz), **4**: δ_H 5.36 (*dd*, $J = 15.0$, 12.0 Hz)] and acetyl methyl in compound **2**, and the carbon signals of C-9, C-11 and C-13 (Table 1). From these facts, the structure of **2** was established as 11-acetoxysalviarin. The relative stereochemistry of the acetoxy group on C-11 was determined to be β from the NOESY spectrum. In the NOESY spectrum of **2**, the proton signal at δ_H 5.20 (H-11) was correlated with the proton signals at δ_H 0.98 (H_3-20) and 2.68 (H-8), the proton signal at δ_H 5.28 (H-12) with the proton signal at δ_H 2.21 (H-10). From these facts the structure of splenolide B was determined as shown (**2**).

Splenolide C (**3**), $C_{24}H_{26}O_{11}$ showed a carbonyl band at 1766, 1757 and 1735 cm^{-1} and a hydroxy band at 3432 cm^{-1}. The ^1H NMR spectrum revealed the presence of one methyl [δ_H 1.07 (*s*)], two acetyl methyls [δ_H 2.03 and 2.20], one methylene attached to an oxygen function [δ_H 4.15, 4.35 (each 1H, *ABq*, $J = 13.2$ Hz)] and seven methines from δ_H 5.21 to 7.24. The ^{13}C NMR spectrum of **3** exhibited 24 carbon resonances including five carbonyl ester carbon signals at δ_C 168.7, 169.2, 170.8 × 2, 174.2, four double bond carbon signals at δ_C 122.3, 128.8, 134.5 and 148.6. The ^{13}C NMR spectral data of **3** was very similar to that of splendidin (**5**) except for a furan ring in compound **5**. From the ^1H–^1H COSY and ^{13}C–^1H COSY spectra, the partial structures, I: >CH—CH—CH=CH—CH<, II: >CH—CH_2—CH_2— and III: >CH—CH< were established. In the ^{13}C–^1H long range correlation spectrum, the proton signal at δ_H 2.87 (H-4) was correlated with the carbon signals at δ_C 122.3 (C-3), 128.8 (C-2) and 174.2 (C-18), the proton signal at δ_H 1.07 (H_3-20) with the carbon signals at δ_C 39.9 (C-9), 41.4 (C-10), 49.7 (C-8) and 76.0 (C-11), the proton signal at δ_H 2.80 (H-8) with the carbon signals at δ_C 41.4 (C-10) and 169.2 (C-17). From these correlations, the partial structures I, II and III could be put in rings -B and -C, respectively, and the assignments of ^{13}C and ^1H NMR data were established as shown in Table 1 and experiments except for C-13, C-14, C-15 and C-16. Remaining signals in the NMR spectral data are the proton signal at δ_H 6.11 (1H, *br s*) and 7.24 (1H, *s*) and carbon signals at δ_C 97.2 (*d*), 134.5 (*s*), 148.6 (*d*) and 168.7 (*s*). In the ^{13}C–^1H long range correlation spectrum, the proton signal at δ_H 6.11 was correlated with the carbon signals at δ_C 134.5, and the proton signal at δ_H 7.24 with the carbon signals at δ_C 97.2 and 168.7. From these results, the structure of the side chain in compound **3** could be one of two possible structures, IV: 13-ene-16-hydroxy-16, 15-olide or V: 13-ene-15-hydroxy-15, 16-olide. In the NOESY spectrum, the proton signal at δ_H 6.11 was correlated with the proton signal at δ_H 7.24. These facts clearly indicated that the structure of the side chain in compound **3** was partial structure V.

The relative stereochemistry was determined as shown below. In the NOESY spectrum, the methyl proton signal at δ_H 1.07 (H_3-20) was correlated with the proton signals at δ_H 5.51 (H-1), 4.35 (H-19), 2.80 (H-8) and 5.21 (H-11), the proton signals at δ_H 5.61 (H-12) with the acetyl methyl proton signal at δ_H 2.20 (C-11 OAc), the proton signals at δ_H 2.45 with the acetyl methyl proton signal at δ_H 2.03 (C-1 OAc). From these correlations, the C-1 acetoxy and C-11 acetoxy groups could be assigned to β, and the other relative stereochemistry also determined as shown. The coupling constants of H-1 ($J = 9.3$ Hz) and H-11 ($J = 10.3$ Hz) also supported the above conclusion.

Thus, the structure of splenolide C (**3**) was determined as shown.

Compounds **4** and **5** were identified from spectral data comparison to be salviarin [6] and splendidin [7]. In the course of our structure elucidation, we knew that the ^{13}C NMR assignment of splendidin [7] is not correct. Thus, we confirmed the ^{13}C NMR assignments of compound **5** by using 2D NMR spectra, results are shown in Table 1.

EXPERIMENTAL

^1H NMR: 270 and 400 MHz with TMS as int. stand; ^{13}C NMR: 100.2 MHz; CC: silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and Toyopearl HW-40 (Tosho); HPLC GPC (H-2002, Shodex).

Plant material. Aerial parts of *Salvia splendens* were collected in January 1994 at Shiwahori-cho, Hiroshima prefecture, Japan.

Extraction and isolation. The dried aerial parts (7.13 kg) of *Salvia splendens* were extracted with MeOH (15 × 3) at 60°. The MeOH extracts were concd *in vacuo* to give a residue (300 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concd to give a residue (148 g), which was chromatographed on silica gel. The column was eluted with solvents of increasing polarity [hexane–EtOAc (3:1, 2:1, 1:1, 1:2), EtOAc, EtOAc–MeOH (19:1, 9:1), MeOH] to give 36 frs. Fr. 6 (3.3 g) was chromatographed on silica gel ($CHCl_3$–MeOH, 97:3) to give 13 frs (fr. 6.1–6.13). Fr. 6.5 (1.19 g) was chromatographed on Toyopearl HW-40 ($CHCl_3$–MeOH, 4:1), silica gel Et_2O–MeOH, 99:1) and crystallized from Et_2O to give **1** (17 mg). Fr. 5 (6.5 g) was chromatographed on silica gel (CH_2Cl_2–Me_2CO, 19:1) to give 13 frs (fr. 5.1–5.13). Fr. 5.2 (202 mg) was chromatographed by using HPLC (GPC, $CHCl_3$) to give **3** (30 mg) and **5** (66 mg). Fr. 5.1 (1.41 g) was chromatographed on silica gel (CH_2Cl_2–Me_2CO, 24:1) to give 6 frs (fr. 5.1.1–5.1.6), fr. 5.1.1 (80 mg) and fr. 5.1.2 (200 mg) were chromatographed by using HPLC (GPC, $CHCl_3$) to give **4** (30 mg) and **2** (99 mg), respectively.

Splenolide A (**1**). Needles, mp 207–208°, $[\alpha]_D^{25}$ −135.3° ($CHCl_3$ *c* 0.77), IR ν_{max}^{KBr} cm^{-1}: 3423, 1765, 1698, 1023, 877. ^1H NMR ($CDCl_3$–CD_3OD, 1:1): δ 1.13 (3H, *s*), 1.35 (1H, *dd*, J = 14.2, 14.2 Hz, H-6), 1.79 (1H, *br d*, J = 14.2 Hz, H-6), 1.66 (1H, *dd*, J = 13.7, 13.2 Hz, H-11), 1.93 (1H, *dddd*, J = 14.2, 14.2, 4.4, 4.4 Hz, H-7), 2.03 (1H, *d*, J = 9.8 Hz, H-10), 2.44 (1H, *br s*, H-8), 2.45 (1H, *br d*, J = 14.2 Hz, H-7), 2.82 (1H, *d*, J = 1.5 Hz, H-4), 3.12 (1H, *dd*, J = 12.2, 2.4 Hz, H-11), 4.20, 4.35 (each 1H, AB*q*, J = 9.1 Hz, H_2-19), 4.36 (1H, *d*, J = 9.8 Hz, H-1), 5.60 (1H, *dd*, J = 10.3, 1.5 Hz, H-3), 5.75 (1H, *dd*, J = 12.2, 3.4 Hz, H-12), 5.93 (1H, *dd*, J = 10.3, 1.5 Hz, H-2), 6.41 (1H, *br s*, H-14), 7.40 (1H, *br s*, H-15), 7.45 (1H, *br s*, H-16). ^{13}C NMR ($CDCl_3$–CD_3OD, 1:1): Table 1. EI-MS *m/z* (rel. int.): 358 $[M]^+$ (36), 340 (100), 253 (22), 230 (18), 202 (75), 143 (54), 94 (64), 91 (43). HR-MS *m/z* 358.1439 $[M]^+$ $C_{20}H_{22}O_6$ required 358.1416.

Splenolide B (**2**). Needles, mp. 272–274°, $[\alpha]_D^{25}$ −135.5° ($CHCl_3$ *c* 1.09), IR ν_{max}^{KBr} cm^{-1}: 1752, 1231, 1046, 875. ^1H NMR ($CDCl_3$): δ 0.98 (3H, *s*, H_3-20), 1.34 (1H, *br t*, J = 13.8, Hα-6), 1.89 (1H, *br d*, J = 14.2 Hz, Hβ-6), 1.95 (1H, *dddd*, J = 12.2, 12.2, 4.4, 4.4 Hz, H-1), 1.99 (3H, *s*, OAc), 2.08 (1H, *m*, H-7), 2.21 (1H, *dd*, J = 12.2, 4.9 Hz, H-10), 2.45 (1H, *ddd*, J = 12.2, 4.9, 2.3 Hz, H-1), 2.59 (1H, *ddd*, J = 18.6, 4.9, 4.9 Hz, H-7), 2.68 (1H, *br s*, H-8), 2.80 (1H, *br s*, H-4), 4.21 (2H, *s*, H_2-19), 5.20 (1H, *d*, J = 10.7 Hz, H-11), 5.28 (1H, *d*, J = 10.7 Hz, H-12), 5.63 (1H, *br d*, J = 9.8 Hz, H-3), 6.01 (1H, *m*, H-2), 6.40 (1H, *d*, J = 1.5 Hz, H-14), 7.40 (1H, *d*, J = 1.5 Hz, H-15), 7.45 (1H, *s*, H-16). ^{13}C NMR ($CDCl_3$): Table 1. FAB-MS *m/z* (rel. int.): 401 $[M+H]^+$ (1), 340 $[M-CH_3COOH]^+$ (100), 322 (16), 278 (19), 216 (10), 203 (14), 189 (49), 176 (23), 133 (74), 129 (15), 105 (19), 91 (40), 81 (19), 43 (66). FAB-HR-MS *m/z* 401.1595 $[M+H]^+$ $C_{22}H_{25}O_7$ required 401.1600.

Splenolide C (**3**). Amorphous powder, $[\alpha]_D^{25}$ −145.2° ($CHCl_3$ *c* 1.60), IR ν_{max}^{KBr} cm^{-1}: 3432, 1766, 1376, 1242, 1043. ^1H NMR ($CDCl_3$): δ 1.07 (3H, *s*, H_3-20), 1.43 (1H, *br t*, J = 12.7 Hz, H-6), 1.86 (1H, *br d*, J = 14.7 Hz, H-6), 1.99 (1H, *m*, H-7), 2.03, (3H, *s*, C-1, OAc), 2.20 (3H, *s*, C-11, OAc), 2.45 (1H, *d*, J = 9.3 Hz, H-10), 2.47 (1H, *m*, H-7), 2.80 (1H, *br s*, H-8), 2.87 (1H, *br s*, H-4), 4.15, 4.35 (each 1H, AB*q*, J = 13.2 Hz, H_2-19), 5.21 (1H, *d*, J = 10.3, H-11), 5.51 (1H, *br d*, J = 9.3 Hz, H-1), 5.61 (1H, *d*, J = 10.3 Hz, H-12), 5.71 (1H, *br d*, J = 9.8 Hz, H-3), 5.89 (1H, *br d*, J = 9.8 Hz, H-2), 6.11 (1H, *br s*, H-15), 7.24 (1H, *br s*, H-14). ^{13}C NMR ($CDCl_3$): Table 1. EI-MS *m/z* (rel. int.): 490 $[M]^+$ (1), 430 $[M-CH_3COOH]^+$ (8), 388 (17), 370 (75) $[M-CH_3COOH\times 2]^+$ (18), 356 (16), 340 (9), 279 (8), 202 (20), 167 (12), 149 (27), 143 (23), 91 (24), 43 (100). HR-MS *m/z* 490.1509 $[M]^+$ $C_{24}H_{26}O_{11}$ required 490.1475.

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