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EREMOPHILANE-TYPE SESQUITERPENES FROM FRESH RHIZOMES OF *PETASITES JAPONICUS*

MOTOO TORI*, MAKIKO KAWAHARA and MASAKAZU SONO

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770, Japan

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Key Word Index—*Petasites japonicus*; Compositae, eremophilane-type sesquiterpene; epoxyeremophilanolide; eremopetasitenin; eremopetasinsulphoxide.

Abstract—Ten new eremophilane-type sesquiterpene lactones, eremopetasitenins A1, A2, B1, B2, C1, C2, C3, D1, D2, and D3, sulphoxide bearing lactone, eremopetasinsulphoxide, and two methoxyl derivatives, as well as seven previously known sesquiterpenes have been isolated from fresh rhizomes of *Petasites japonicus* and their structures determined by spectroscopic techniques. Epoxyeremophilanolides, eremopetasitenins A1, A2, B1, and B2, are proposed to be important biogenetic intermediates. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

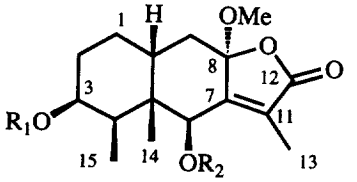
Petasites plants have been extensively investigated and many eremophilane-type sesquiterpene lactones have been reported [1–20]. In continuation of our investigation of biologically active substances, we collected *P. japonicus* in Tokushima prefecture and have studied the chemical constituents of the methanol extract of fresh rhizomes. Recently Kikuchi and co-workers [16–20] reported various types of terpenoids from the commercially available rhizomes of *P. japonicus*. We prefer to extract fresh materials, because labile compounds can be isolated by avoiding prolonged heating to dry the plant material. We now report the isolation and structure determination of ten new eremophilane-type sesquiterpene lactones, eremopetasitenins C1 (**1**), C2 (**2**), C3 (**3**), D1 (**4**), D2 (**5**), D3 (**6**), A1 (**7**), A2 (**8**), B1 (**9**), and B2 (**10**) [21], sulphoxide bearing lactone, eremopetasinsulphoxide (**11**), two methoxyl derivatives, **12** and **13**, which are presumably artefacts, and the previously known compounds, **14**–**20** [1, 2, 11, 15, 16].

RESULTS AND DISCUSSION

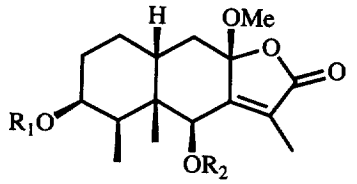
The ethyl acetate soluble part of the methanol extract of *P. japonicus* was separated by a combination of silica gel and Sephadex LH-20 CC as well as normal and reversed-phase HPLC. The ^{1}H NMR and IR spectra of eremopetasitenin C1 (**1**), $C_{21}H_{30}O_6$, showed the presence of a hydroxyl group [3500 cm^{-1}; δ_H 4.00 (*dt*)], a methoxyl group [δ_H 3.26 (*s*)], and an angelate ester [1730 cm^{-1}; δ_H 6.29 (1H, *qq*, J = 7.2 and 1.5 Hz, 2.08 (3H, *dq*, J = 7.3 and 1.5 Hz) and 1.99 (3H, *quint*, J = 1.5 Hz)], which was supported by the ^{13}C NMR spectrum (Tables 1 and 2). The IR spectrum also suggested the presence of a lactone moiety (1780 cm^{-1}). The ^{13}C NMR spectrum indicated the presence of six methyls, three methylenes, four methines and seven quaternary carbons (Table 2). Two of the oxygen atoms were assigned to an angelate, two to a lactone, one to a hydroxyl, and one to a methoxyl group. The degree of unsaturation of **1** is 7 and therefore this compound is tricyclic. The HMBC and HMQC spectra identified the eremophilane skeleton and the positions of its substituents are shown in Fig. 1. The stereochemistry was revealed by the NOESY spectrum (Fig. 2). The methyl group at C-5 had correlation peaks with the proton at C-10 and the methyl group at C-4. The proton at C-6 had an NOE with that of C-3, which means rings A and B were *cis*-fused. Moreover the methoxyl group at C-8 correlated with H-6, indicating the methoxyl group being α-oriented. The position of the angelate group was clarified by the correlation peak between H-6 and C-1″ in the HMBC spectrum. Therefore the gross non-steroidal conformation must be as shown in Fig. 2. The structure of eremopetasitenin C1 was established as depicted in structure **1**.

Eremopetasitenin C2 (**2**), $C_{25}H_{35}O_7S$ (by HRMS), had similar spectral data with **1**, although this had more peaks due to a 3-methylthiopropenoate moiety (Tables 1 and 2). The 2D-NMR data also indicated the eremophilane skeleton. The position of the angelate

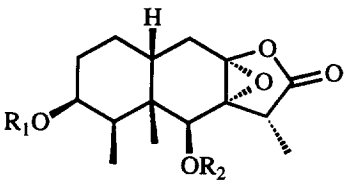
* Author to whom correspondence should be addressed.



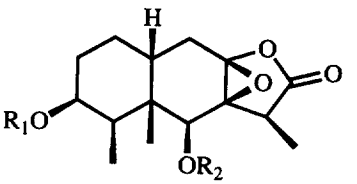
eremopetasitenin C1 (**1**): R_1=H, R_2=B
eremopetasitenin C2 (**2**): R_1=A, R_2=B
eremopetasitenin C3 (**3**): R_1=A, R_2=Me



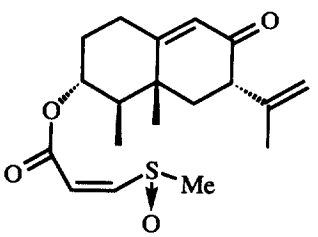
eremopetasitenin D1 (**4**): R_1=H, R_2=B
eremopetasitenin D2 (**5**): R_1=A, R_2=B
eremopetasitenin D3 (**6**): R_1=H, R_2=Me



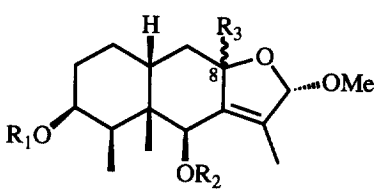
eremopetasitenin A1 (**7**): R_1=H, R_2=B
eremopetasitenin A2 (**8**): R_1=A, R_2=Me



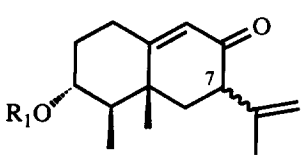
eremopetasitenin B1 (**9**): R_1=H, R_2=B
eremopetasitenin B2 (**10**): R_1=A, R_2=B



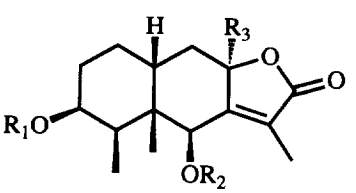
eremopetasinsulfoxide (**11**)



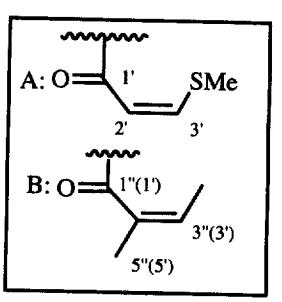
12: R_1=H, R_2=Me, R_3=α-OMe
13: R_1=H, R_2=B, R_3=β-OMe



14: 7αH, R_1=A
15: 7βH, R_1=A
16: 7αH, R_1=B
17: 7βH, R_1=B



18: R_1=H, R_2=Me, R_3=H
19: R_1=H, R_2=B, R_3=H
20: R_1=H, R_2=Me, R_3=OMe



moiety was shown by the correlation peak between H-6 and C-1″ and the 3-methylthiopropenoate moiety by that between H-3 and C-1′. The conformation and stereochemistry were shown by the NOESY spectrum (Fig. 2) to have a non-steroidal conformation. Thus the structure of eremopetasitenin C2 was established as depicted in structure **2**.

The spectral data of eremopetasitenin C3 (**3**), $C_{21}H_{30}O_6S$ (by HRMS), was very similar to that of **2**, although it lacked the angelate moiety. However, most of the spectral features were almost the same as demonstrated for **1** and **2**. The structure was determined by the 2D-NMR spectrum and the stereochemistry was shown by the NOESY spectrum to have a non-steroidal conformation. Therefore, the structure of eremopetasitenin C3 was determined as **3**.

Table 1. ^{1}H NMR data for eremopetasitenins **1–10** and petasinulphoxide (**11**)

H	1*	2*	3*	4†	5*	6*	7†	8*	9*	10*	11*
1α	2.19 (*m*)	2.26 (*m*)	2.12 (*m*)	2.14 (*m*)	2.02 (*m*)	2.08 (*m*)	1.84 (*m*)	1.42 (*m*)	1.35 (*td*, 13.2, 4.1)	1.40 (*m*)	2.39 (*dquint*, 14, 2)
1β	1.70 (*m*)	1.58 (*m*)	1.54 (*m*)	1.28 (*m*)	1.32 (*m*)	1.25 (*m*)	1.49 (*m*)	1.70 (*m*)	1.56 (*m*)	1.40 (*m*)	2.51 (*td*, 14, 6)
2α	1.68 (*m*)	1.80 (*m*)	1.74 (*m*)	1.74 (*tt*, 14, 3)	1.73 (*m*)	1.71 (*tt*, 14, 3, 7)	1.76 (*m*)	1.75 (*m*)	1.78 (*m*)	1.90 (*m*)	1.55 (*m*)
2β	1.55 (*m*)	1.65 (*qd*, 12.1, 4.1)	1.68 (*qd*, 12.1, 4.4)	1.64 (*dtt*, 14, 3, 3)	1.72 (*m*)	1.60 (*m*)	1.53 (*m*)	1.68 (*m*)	1.56 (*m*)	1.65 (*m*)	2.21 (*m*)
3α	4.00 (*dt*, 11, 3)	5.02 (*dt*, 12.1, 4.1)	5.22 (*dt*, 12.1, 4.4)	3.84 *q*, 3)	5.07 (*q*, 2.9)	3.80 (*q*, 3.7)	4.19 (*dt*, 12, 4.5)	5.20 (*dt*, 12, 4.8)	4.25 (*dt*, 11.8, 4.2)	5.26 (*dt*, 16.2, 5.6)	—
3β	—	—	—	—	—	—	—	—	—	—	4.95 (*m*)
4α	2.06 (*m*)	2.32 (*m*)	2.48 (*qd*, 6.9, 4.4)	1.39 (*qd*, 7.3, 3)	1.58 (*m*)	1.28 (*qd*, 7.2, 3.7)	1.81 (*m*)	2.35 (*m*)	1.73 (*qd*, 7.4, 4.2)	1.93 (*m*)	1.64 (*m*)
6α	5.90 (*q*, 1.5)	5.90 (*q*, 1.4)	4.36 (*q*, 1.4)	5.68 (*s*)	5.68 (*s*)	3.94 (*s*)	5.76 (*s*)	3.87 (*s*)	5.09 (*br s*)	4.94 (*br s*)	1.91 (*t*, 14)
6β	—	—	—	—	—	—	—	—	—	—	2.04 (*dd*, 14, 4.6)
7β	—	—	—	—	—	—	—	—	—	—	3.12 (*dd*, 14, 4.6)
9α	2.18 (*m*)	2.18 (*d*, 12)	2.13 (*dd*, 14.0, 1.4)	2.00 (*m*)	1.97 (*t*, 13.7)	1.87 (*t*, 13.5)	2.24 (*d*, 15.2)	2.10 (*d*, 15.1)	2.02 (*br s*)	2.02 (*m*)	5.80 (*t*, 1.9)
9β	1.90 (*m*)	1.92 (*m*)	1.85 (*m*)	2.15 (*dd*, 13, 4)	2.16 (*dd*, 13.7, 4)	2.11 (*m*)	2.48 (*dd*, 15.2, 7.5)	2.35 (*m*)	2.61 (*dd*, 15.4, 6)	2.60 (*dd*, 15.6, 6)	
10β	1.85 (*m*)	1.89 (*m*)	1.82 (*m*)	2.28 (*dq*, 13, 4)	2.30 (*dq*, 13.7, 4)	2.16 (*m*)	1.94 (*m*)	1.85 (*m*)	1.88 (*m*)	1.92 (*m*)	—
11	—	—	—	—	—	—	2.98 (*q*, 7.3)	3.09 (*q*, 7.3)	2.95 (*q*, 7.1)	3.01 (*q*, 7.2)	—
12E	—	—	—	—	—	—	—	—	—	—	5.00 (*quint*, 1.6)
12Z	—	—	—	—	—	—	—	—	—	—	4.83 (*br s*)
13	1.83 (*d*, 1.5)	1.82 (*d*, 1.4)	2.01 (*d*, 1.4)	2.05 (*s*)	2.05 (*s*)	1.94 (*s*)	1.29 (*d*, 7.3)	1.44 (*d*, 7.3)	1.25 (*d*, 7.1)	1.26 (*d*, 7.2)	1.75 (*br s*)
14	0.98 (*s*)	0.97 (*s*)	0.81 (*s*)	1.32 (*s*)	1.28 (*s*)	1.34 (*s*)	1.03 (*s*)	0.83 (*s*)	1.09 (*s*)	1.10 (*s*)	1.24 (*s*)
15	0.97 (*d*, 7.3)	0.99 (*d*, 7.1)	0.97 (*d*, 7.1)	1.07 (*d*, 7.1)	0.95 (*d*, 7.1)	1.02 (*d*, 7.1)	0.95 (*d*, 7.1)	0.91 (*d*, 7.2)	0.90 (*d*, 7.4)	0.92 (*d*, 6.6)	0.96 (*d*, 6.8)
2′	—	5.80 (*d*, 10.2)	5.86 (*d*, 10.2)	—	5.84 (*d*, 10.2)	—	—	5.83 (*d*, 10.3)	—	5.79 (*d*, 10.2)	6.282 (*d*, 10.4)‡; 6.280 (*d*, 10.4)§
3′	—	7.01 (*d*, 10.2)	7.05 (*d*, 10.2)	—	7.08 (*d*, 10.2)	—	—	7.05 (*d*, 10.3)	—	7.02 (*d*, 10.2)	7.02 (*d*, 10.4)‡; 7.03 (*d*, 10.4)§
3″	6.29 (*qq*, 7.2, 1.5)	6.25 (*qq*, 7.2, 1.3)	—	6.20 (*qq*, 7.3, 1.4)	6.21, (*qq*, 7.3, 1.5)	—	6.30 (*q*, 7.3)	—	6.23 (*qq*, 7.3, 1.5)	6.18 (*qq*, 7.2, 1.2)	—
4″	2.08 (*dq*, 7.3, 1.5)	2.08 (*dq*, 7.2, 1.3)	—	2.06 (*dq*, 7.3, 1.4)	2.06 (*dq*, 7.3, 1.5)	—	2.11 (*dq*, 7.3, 1.5)	—	2.05 (*dq*, 7.3, 1.5)	2.06 (*dq*, 7.2, 1.2)	—
5″	1.99 (quint, 1.5)	2.00 (quint, 1.3)	—	1.93 (quint, 1.4)	1.93 (quint, 1.5)	—	1.99 (*s*)	—	1.95 (*dq*, 1.5, 1.5)	1.94 (*dq*, 1.2, 1.2)	—
OMe(6)	—	—	3.55 (*s*)	—	—	3.35 (*s*)	—	3.74 (*s*)	—	2.38 (*s*)	—
OMe(8)	3.26 (*s*)	3.30 (*s*)	3.19 (*s*)	2.98 (*s*)	2.98 (*s*)	3.19 (*s*)	—	—	—	—	—
SMe	—	2.38 (*s*)	2.41 (*s*)	—	2.41 (*s*)	—	—	2.40		—	2.854 (*s*)‡; 2.851 (*s*)§ (SOMe)

* 600 MHz.

† 400 MHz.

‡ Major isomer.

§ Minor isomer.

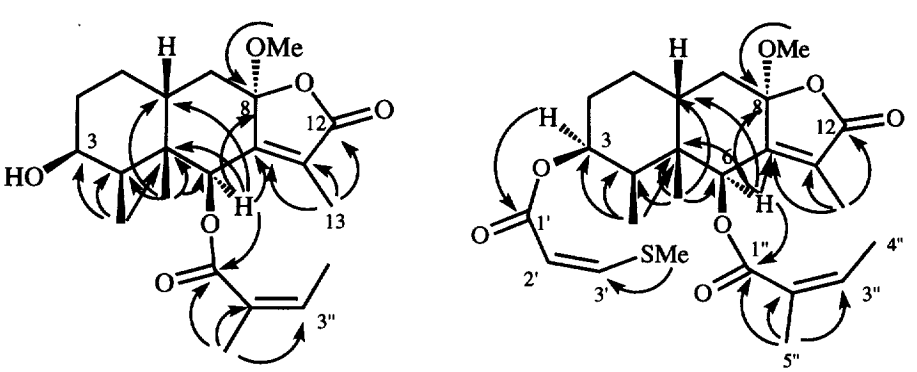
Table 2. ^{13}C NMR data for compounds **1**–**10** and petasinsulphoxide (**11**)

C	1*	2*	3*	4*	5*	6*	7†	8*	9*	10*	11*
1	27.0	26.8	26.9	20.6	21.1	20.6	27.5	27.3	28.2‡	29.0‡	30.4
2	28.8	25.2	25.5	27.7	24.7	27.8	29.3	26.2	28.6	25.4	31.3
3	68.2	71.2	71.3	71.5	72.9	71.7	68.0	71.2	68.3	71.3	75.4
4	38.6	35.7	35.3	32.9	32.3	33.0	39.0	35.5	39.7‡	36.6‡	47.0
5	46.0	46.1	47.3	42.2	42.2	43.0	41.3	42.4	41.0‡	41.0‡	40.0
6	71.1	71.0	79.7	70.9	70.7	79.6	67.0	76.6	68.2	68.0	41.6
7	155.0	155.2	157.1	150.7	150.3	153.5	65.4	67.0	63.6‡	63.7‡	50.3
8	106.8	106.9	106.8	106.9	106.7	107.6	86.9	86.9	87.8	87.6	198.3
9	37.7	38.0	37.5	38.2	38.1	37.8	25.0	25.0	26.7‡	26.7‡	124.9
10	35.1	35.1	35.0	35.1	34.8	34.3	33.9	33.4	35.7‡	35.5‡	163.9
11	162.2	126.5	126.5	130.4	130.8	129.3	40.7	40.3	43.0	43.0	143.1
12	170.9	170.9	171.4	170.7	170.5	170.8	175.9	176.7	175.8	176.0	114.6
13	8.1	8.0	8.4	9.0	9.0	8.8	10.9	11.5	11.2	11.2	20.1
14	20.0	19.8	18.7	19.0	18.6	18.5	20.4	19.3	19.6	19.3	17.1
15	7.6	8.8	8.8	12.8	12.3	12.9	6.8	7.7	6.4‡	8.1‡	10.4
1′	—	165.6	166.1	—	166.0	—	—	165.9	—	165.5	165.6
2′	—	113.4	113.5	—	112.9	—	—	113.3	—	113.2	124.23§; 124.16¶
3′	—	151.6	151.7	—	152.5	—	—	151.7	—	151.7	159.7§; 159.9¶
1″	166.6	166.6	—	167.1	167.0	—	166.5	—	167.7	167.2	—
2″	162.4	126.7	—	127.0	126.9	—	126.4	—	126.8	127.0	—
3″	142.0	141.1	—	140.8	140.9	—	141.8	—	140.8	140.1	—
4″	16.1	16.1	—	15.9	15.9	—	16.1	—	16.0	15.9	—
5″	20.7	20.7	—	20.6	20.5	—	20.6	—	20.5	20.5	—
6-OMe	—	—	59.8	—	—	59.2	—	—	—	—	—
8-OMe	50.4	50.5	50.0	50.5	50.5	51.0	—	—	—	—	—
SMe	—	19.3	19.3	—	19.3	—	—	19.2	—	19.3	—
SOMe	—	—	—	—	—	—	—	—	—	—	40.6§; 40.7¶

* 150 MHz.
† 100 MHz.
‡ Broad peak.
§ Major isomer.
¶ Minor isomer.

The ^1H NMR spectrum of eremopetasitenin D1 (**4**), $C_{21}H_{31}O_6$ (by HRMS), was quite similar to that of **1**, although the chemical shift of the methoxyl group appeared at a much higher field than that of **1**. However, the 2D-NMR spectrum revealed that this compound also had the eremophilane skeleton and the angelate moiety attached to the C-6 position. The NOESY spectrum showed that the conformation of the molecule is steroidal contrary to compounds **1**–**3** (Fig. 2), which was supported by the coupling pattern of H-3α (quartet with $J = 3$ Hz). Consequently the methoxyl group at C-8 position must be β, which was the same direction as the angelate at C-6. Thus the structure of eremopetasitenin D1 was established as **4**.

Eremopetasitenin D2 (**5**), $C_{25}H_{33}O_7S$ (by HRMS),



eremopetasitenin C1 (1) **eremopetasitenin C2 (2)**

Fig. 1. HMBS Correlations detected for eremopetasitenins C1 (**1**) and C2 (**2**).

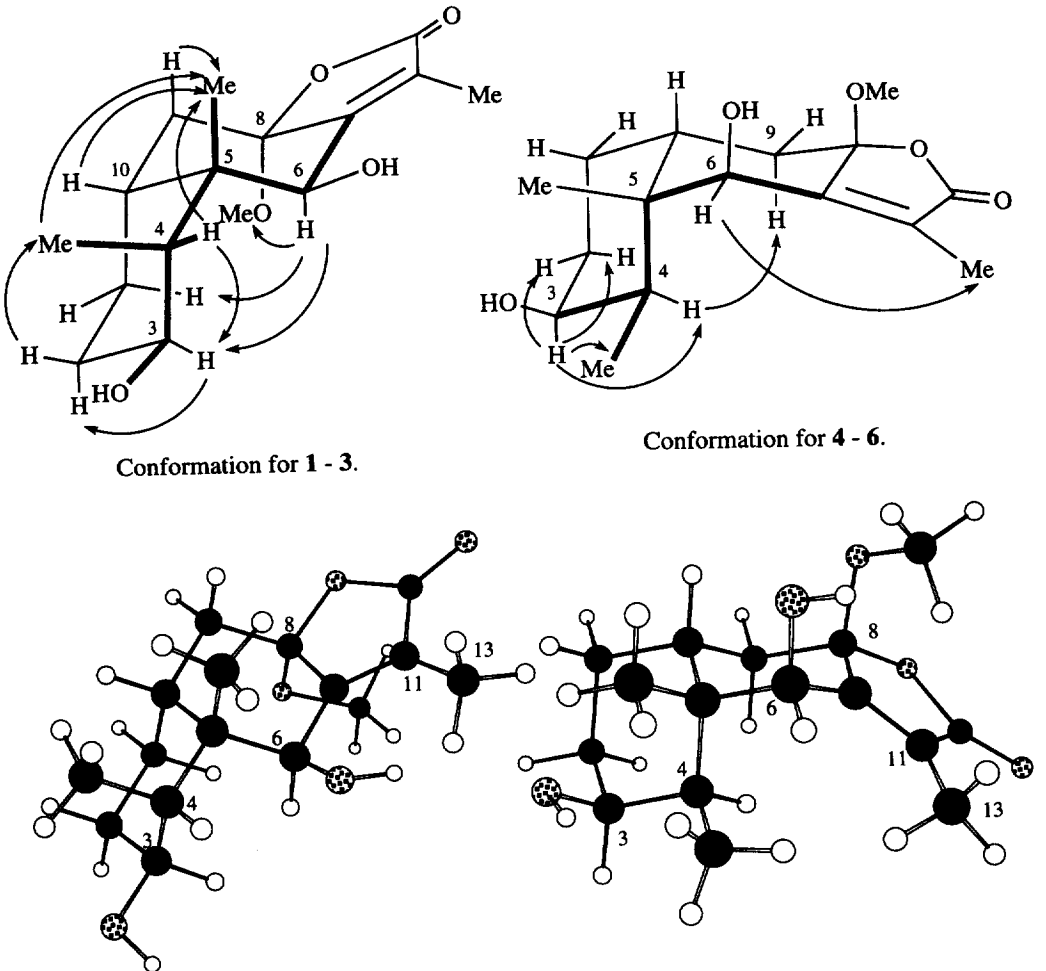


Fig. 2. Conformations and NOE's detected for compounds **1–3** and **4–6** (substituents at the C-3 and C-6 positions were denoted as OH for clarity).

had the similar spectral data with those of **2**. The substituents and their positions were the same as those of **2**, judging from the 2D-NMR spectra. However, the configuration of the methoxyl group was opposite to that of **2**, revealed by the NOESY spectrum. Thus compound **5** adopts a steroidal conformation (Fig. 2), while **2** is in a non-steroidal conformation. Therefore, the structure of eremopetasitenin D2 was established as **5**.

Eremopetasitenin D3 (**6**), $C_{17}H_{27}O_5$ (by HRMS), had two methoxyl groups at the C-6 and C-8 positions, determined by the HMBC spectrum. Since the NOESY spectrum indicated the NOE between H-6 and H-13, H-4α and H-9α, this also must have a steroidal conformation. This was supported by the coupling pattern of the H-3α (quartet with $J = 3.7$ Hz), which had NOE peaks with H-2α, H2β, H4α, and H-15. Thus the structure was determined as **6**.

The molecular formula of eremopetasitenin A1 (**7**) [21] was determined as $C_{20}H_{29}O_6$ by the HRMS. The degree of unsaturation of **7** was seven and thus this compound must be tetracyclic (Table 3). The eremophilanolide skeleton was shown by the HSQC and HMBC spectra as well as the IR spectrum (1800 cm^{-1}). Therefore, five of the six oxygen atoms were assigned to the lactone, the angelate and the methoxyl group. The last oxygen, and thus the presence of the fourth ring, was suggested by the ^{13}C NMR chemical shifts of C-7 and C-8 to be an epoxide ring (δ_C 65.4 and 86.9) [22, 23]. Thus this compound must be one of the biosynthetic intermediates in the series of the eremophilanolides. Bohlmann *et al.* [9] isolated an epoxide which had very similar spectral data. However, they did not determine the stereochemistry of the epoxide ring. The NOESY spectrum of **7** clearly indicated that both the epoxide ring and the methyl group at C-13 were α-oriented, while dimethyl groups and the angelate moiety and the hydroxyl groups were β-oriented as shown in Fig. 3. Therefore, the structure of eremopetasitenin A1 was established as **7**.

Eremopetasitenin A2 (**8**), $C_{20}H_{29}O_6S$ (by HRMS) [21], had a 3-methylthiopentanoate moiety at C-3 position instead of the hydroxyl group as in the case of **7**. Furthermore the methoxyl group was substituted at the C-6 position as revealed by the HMBC spectrum. This also had an epoxide ring at the C-7 and C-

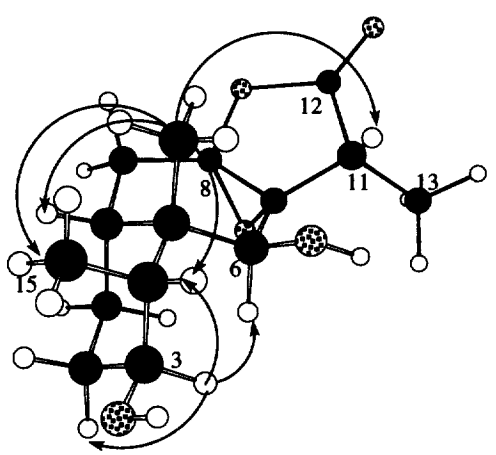
Table 3. ^{13}C NMR data for compounds **12–17**

C	12*	13*	14*	15*	16†	17†
1	27.2	21.0	30.5	30.6	30.6	30.5
2	28.9	27.7	33.8	31.6	33.7	31.5
3	68.9	72.1	73.5	73.0	73.4	72.8
4	38.0	33.0	43.4	47.3	43.3	47.1
5	45.5	41.0	40.0	39.9	40.0	39.8
6	79.7	71.1	37.5	41.6	37.6	41.5
7	133.6	138.2	50.3	50.3	50.3	50.1
8	112.0	112.7	199.0	198.4	199.0	198.1
9	39.2	39.6	123.7	124.5	123.7	124.4
10	35.6	35.9	168.2	166.8	167.6	167.3
11	131.5	130.0	143.4	143.3	143.3	143.1
12	109.3	109.4	113.8	114.3	113.9	114.2
13	9.8	10.0	20.7	20.0	20.7	19.9
14	19.0	19.0	21.0	17.1	21.1	17.0
15	7.6	12.7	11.6	10.4	11.7	10.4
1′	—	—	166.3	166.2	168.1	166.5
2′	—	—	112.8	112.8	127.9	127.7
3′	—	—	152.6	152.5	138.1	137.9
4′	—	—	—	—	15.8	15.6
5′	—	—	—	—	20.6	20.4
1″	—	167.3	—	—	—	—
2″	—	127.6	—	—	—	—
3″	—	139.6	—	—	—	—
4″	—	15.9	—	—	—	—
5″	—	20.7	—	—	—	—
6-OMe	59.1	—	—	—	—	—
8-OMe	49.2	49.4	—	—	—	—
12-OMe	56.1	55.3	—	—	—	—
SMe	—	—	19.3	19.2	—	—

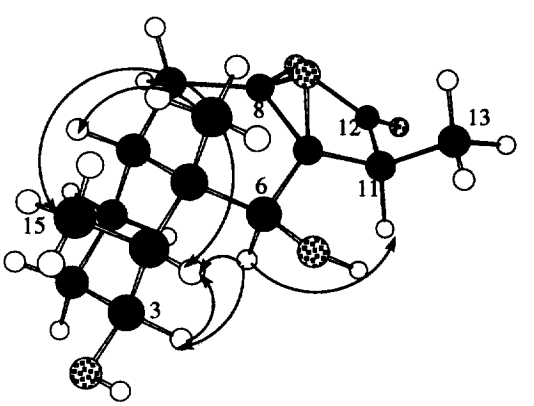
* 150 MHz.
† 100 MHz.

8 positions (Table 2) [22, 23]. The stereochemistry was inferred by the NOESY spectrum and found to have similar configurations to those of **7** (Fig. 3). Therefore, the structure of eremopetasitenin A2 was established as **8**.

The ^1H NMR spectrum of eremopetasitenin B1 (**9**), $C_{20}H_{29}O_6$ (by HRMS) [21], was very similar to that of **7**. The substituents and their positions were the same as those of **7** as revealed by the 2D-NMR spectra. However, the NOESY spectrum of **9** indicated the



eremopetasitenins A1 (**7**) and A2 (**8**).



eremopetasitenins B1 (**9**) and B2 (**10**)

Fig. 3. Conformations and NOE's detected for compounds **7–10** (substituents at the C-3 and C-6 positions were denoted for OH for clarity).

NOE between H-6α and H-3α; H-6α and H-4α; H-6α and H-11α; H-14 and H-15; H-14 and H-10β, which suggest that rings A and B were *cis* to each other and the epoxide ring and the methyl group at C-13, as well as the angelate moiety were all β-oriented (Fig. 3). Thus the structure of eremopetasitenin B1 was established as **9**.

The penultimate product was named eremopetasitenin B2 (**10**), $C_{24}H_{33}O_7S$ (by HRMS) [21], and its ^1H NMR spectrum was similar to **2**, **5**, and **9**. It had both the angelate and 3-methylthiopropenoate moiety judging from ^1H and ^{13}C NMR data (Tables 1 and 2). The positions of these moieties were revealed by the HMBC spectrum (Fig. 4) and the stereochemistry was determined by the NOESY spectrum (Fig. 3). Thus the structure was established as **10**.

The last new compound was eremopetasinsulphoxide (**11**), $C_{19}H_{27}O_4S$ (by HRMS), which had a sulphoxide moiety that is relatively rare in nature. Unfortunately, despite repeated HPLC, this material was a mixture of two diastereoisomers (*ca* 2:1) due to the sulphoxide moiety (Tables 1 and 2). The ^1H NMR chemical shift at δ_H 2.85 due to the methyl group was lower than that of methylthio group [24] and higher than that of methyl sulphone [25]. The ^{13}C NMR chemical shift δ_C 40.6 was also lower than that of methylthio group (Table 2) [24]. Thus we concluded that **11** had a sulphoxide side chain at the C-3 position as revealed by the HMBC spectrum, although it had a ketone instead of the lactone grouping. The stereochemistry was the same as that of *S*-petasin (**15**). Therefore, the structure of eremopetasinsulphoxide was determined as **11**.

Compounds **12** and **13** were presumably artefacts. Compound **12** had three methoxyl groups, while compound **13** had two methoxyl groups. Neither had a lactone (Table 3). If they were derived from the endoperoxide by direct reduction, orientations of the methoxyl groups at C-8 and C-12, which was revealed by the NOESY spectrum, should be the same. Therefore, we suspect that these are artefacts, although the details are not clear at this stage. Compounds **14**–**20** were all previously reported [1, 2, 11, 15, 16] and their structures were assigned by comparison with the reported data or by independent 2D-NMR works.

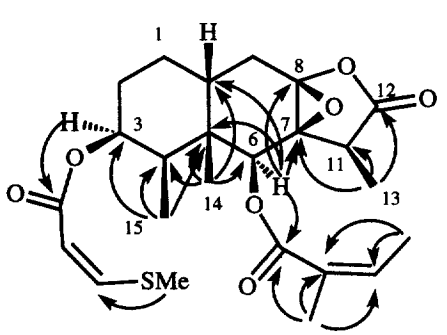


Fig. 4. HMBS Correlations detected for eremopetasitenin B2 (**10**).

A plausible biosynthetic pathway is shown in Fig. 5. Oxidation of furan provides an endoperoxide, which rearranges into a diepoxide, the route of which was previously discussed in the case of a diterpene [26]. Further opening of one of the epoxides affords epoxylactones. This type of compound must be a biosynthetic intermediate and few compounds of this type have been isolated previously. This is probably due to the fact that we used the fresh material for investigation, which was very important for detection of such labile compounds.

EXPERIMENTAL

General. ^1H NMR: 400 or 600 MHz, ^{13}C NMR: 100 MHz (in $CDCl_3$ soln, TMS as int. standard). CC: Silica gel 60 (70–230 mesh, Merck) and Sephadex LH-20 (Pharmacia). TLC: silica gel 60 F_{254} plates (Merck).

Isolation. The rhizomes of *P. japonicus* were collected in Tokushima Prefecture in June, 1995. The voucher specimen was deposited in the herbarium of Tokushima Bunri University. The half-dried (overnight) rhizomes (860 g) were extracted with MeOH to afford a residue (66 g). The extract was partitioned with EtOAc (16 g), which was subjected to silica gel CC (hexane–EtOAc, gradient) to give 7 frs. Fr. 4 (1.5 g) was further sepd by silica gel CC (hexane–EtOAc, gradient) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) followed by HPLC (hexane–EtOAc, 9:1) to afford eremopetasitenins A2 (**8**) (11.7 mg), B2 (**10**) (4.1 mg), **16** (13 mg), and **17** (181 mg), Fr. 5 (5.2 g) was separated by silica gel CC (hexane–EtOAc, gradient) to afford 9 frs (A–I). Fr. D (582 mg) was purified by silica gel CC (hexane–EtOAc, gradient) and HPLC (hexane–EtOAc, 4:1) to give eremopetasitenins C2 (**2**) (28.8 mg) and D2 (**5**) (19.6 mg). Fr. E (949 mg) was similarly sepd to afford eremopetasitenins A1 (**7**) (14 mg), B1 (**9**) (4 mg), C3 (**3**) (2.2 mg), **14** (157 mg), and **15** (219 mg). Fr. H (207 mg) was further sepd by HPLC (hexane–EtOAc, 7:3) to afford eremopetasitenin D1 (**4**) (8 mg). Fr. 6 (1.8 g) was sepd by silica gel CC (hexane–EtOAc, gradient) followed by HPLC (CH_2Cl_2–EtOAc, 4:1) to afford eremopetasitenins C1 (**1**) (14 mg), D3 (**6**) (5.2 mg), **12** (2.5 mg), **13** (3.9 mg), **19** (9.8 mg), and **20** (65 mg). Fr. 7 (5.4 g) was similarly separated to afford **11** (3.4 mg) and **18** (21 mg).

Eremopetasitenin C1 (**1**). $[\alpha]_D^{22}$ −21.1° (*c* 0.94, $CHCl_3$); CIHRMS (CH_4) *m/z* 347.1846 $[M-31]^+$ $C_{20}H_{27}O_5$ requires 347.1859; CIMS (CH_4) *m/z* 379 $[M+1]^+$, 347, 329, 307, 279 (base), 247, 229, 203, 157, 101, and 83; ν_{max} cm^{-1}: 3500, 1780, 1730, 1660; $[\theta]$ 253 nm −1330 ($CHCl_3$); ^1H and ^{13}C NMR see Tables 1 and 2.

Eremopetasitenin C2 (**2**). $[\alpha]_D^{24}$ −62.5° (*c* 0.32, EtOH); CIHRMS (CH_4) *m/z* 479.2089 $[M+1]^+$ $C_{25}H_{35}O_7S$ requires 479.2104; CIMS (CH_4) *m/z* 479 $[M+1]^+$, 447, 407, 379, 361, 347, 329, 307, 289, 261, 229 (base), 217, 161, 119, and 101; ν_{max} cm^{-1}: 1800, 1760, 1720, 1700, 1650; ^1H and ^{13}C NMR see Tables 1 and 2.

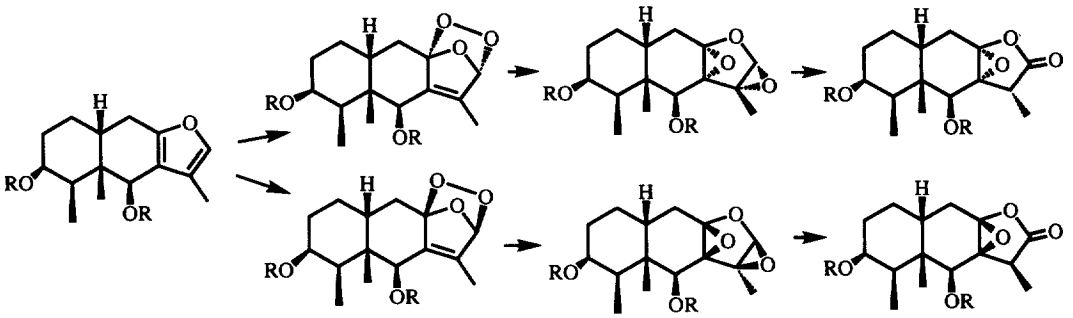


Fig. 5. Possible biosynthetic pathways to eremopetasitenins A and B.

*Eremopetasitenin C*3 (**3**). $[\alpha]_D^{20}$ −142.9° (*c* 0.32, $CHCl_3$); CIHRMS (CH_4) *m/z* 410.1773 $[M]^+$ $C_{21}H_{30}O_6S$ requires 410.1763; MS *m/z* 410 $[M]^+$, 379, 349, 293 (base), 229, 170, 119, and 101; ν_{max} cm^{-1}: 1780, 1700, 1580; ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin D*1 (**4**). $[\alpha]_D^{24}$ +116.5° (*c* 0.74, $CHCl_3$); CIHRMS (CH_4) *m/z* 379.2115 $[M+1]^+$ $C_{21}H_{31}O_6$ requires 379.2121; CIMS (CH_4) *m/z* 379 $[M+1]^+$, 361, 347, 319, 307, 279, 261, 247 (base), 231, 203, 175 and 149; ν_{max} cm^{-1}: 1760, 1715, 1640; [θ] 251 nm −1940 ($CHCl_3$); ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin D*2 (**5**). $[\alpha]_D^{24}$ +64.8° (*c* 0.88, EtOH); CIHRMS *m/z* 478.2010 $[M]^+$ $C_{25}H_{33}O_7S$ requires 478.2026; CIMS (CH_4) *m/z* 507 $[M+29]^+$, 478 $[M]^+$, 447, 407, 379, 329, 289, 261, 247, 229, 217, 203, 147, 119, 101 (base), 91 and 83; ν_{max} cm^{-1}: 1770, 1700, 1570; ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin D*3 (**6**). $[\alpha]_D^{22}$ +97.8° (*c* 6.03, $CHCl_3$); CIHRMS (CH_4) *m/z* 311.1861 $[M+1]^+$ $C_{17}H_{27}O_5$ requires 311.1858; CIMS (CH_4) *m/z* 311 $[M+1]^+$, 293, 279 (base), 261, 247, 229, 185, 170, and 154; ν_{max} cm^{-1}: 1780; [θ] 247 nm +1580 ($CHCl_3$); ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin A*1 (**7**). $[\alpha]_D^{24}$ −23.2° (*c* 1.40, $CHCl_3$); CIHRMS (CH_4) *m/z* 365.1955 $[M+1]^+$ $C_{20}H_{29}O_6$ requires 365.1964; CIMS *m/z* 365 $[M+1]^+$, 347, 283, 265, 247, 237, 219, 191, 165, 121, 101, 83 (base), 55; ν_{max} cm^{-1}: 3400, 1800, 1720; ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin A*2 (**8**). $[\alpha]_D^{24}$ −27.8° (*c* 1.19, $CHCl_3$); CIHRMS *m/z* 397.1668 $[M+1]^+$ $C_{20}H_{29}O_6S$ requires 397.1685; CIMS *m/z* 397 $[M+1]^+$, 379, 331, 307, 279 (base), 261, 247, 229, 155, 123, 101; ν_{max} cm^{-1}: 1800, ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin B*1 (**9**). $[\alpha]_D^{20}$ −55.6° (*c* 0.63, $CHCl_3$); CIHRMS *m/z* 365.1971 $[M+1]^+C_{20}H_{29}O_6$ requires 365.1964; CIMS *m/z* 365 $[M+1]^+$, 265, 247, 237, 209, 191, 149, 123, 101, 83 (base); ν_{max} cm^{-1}: 3400, 1807, 1707; [θ] 293 nm +243 ($CHCl_3$); ^1H and ^{13}C NMR see Tables 1 and 2.

*Eremopetasitenin B*2 (**10**). $[\alpha]_D^{25}$ −66.9° (*c* 0.32, $CHCl_3$); CIHRMS *m/z* 465.1953 $[M+1]^+$ $C_{24}H_{33}O_7S$ requires 465.1947; CIMS *m/z* 465 $[M+1]^+$, 447, 419, 391, 365, 347, 309, 265, 247 (base), 231, 219, 203, 191: ν_{max} cm^{-1}: 1820, 1725, 1705; ^1H and ^{13}C NMR see Tables 1 and 2.

Eremopetasinsulphoxide (**11**). $[\alpha]_D^{24}$ +17.0° (*c* 0.30, EtOH); CIHRMS *m/z* 351.1639 $[M+1]^+$ $C_{19}H_{27}O_4S$ requires 351.1630; CIMS *m/z* 351 $[M+1]^+$, 333, 287, 245, 231, 217 (base), 201, 189, 148, 135, 105; ν_{max} cm^{-1}: 1715, 1670; [θ] 323 nm +1235 (MeOH); ^1H and ^{13}C NMR see Tables 1 and 2.

Compound **12**. $[\alpha]_D^{20}$ −39.0° (*c* 0.21, $CHCl_3$); CIHRMS *m/z* 327.2104 $[M+1]^+C_{18}H_{31}O_5$ requires 327.2172; CIMS *m/z* 395 $[M+1]^+$, 393, 377, 363, 345, 331, 309, 295, 281, 263, 231 (base), 213, 159, 101, 83; ν_{max} cm^{-1}: 3550, 1770, 1725, 1660; ^{13}C NMR see Table 3.

Compound **13**. $[\alpha]_D^{20}$ +0.62° (*c* 0.16, $CHCl_3$); CIHRMS *m/z* 395.2419 $[M+1]^+$ $C_{22}H_{35}O_6$ requires 395.2404; CIMS *m/z* 327 $[M+1]^+$, 309, 295 (base), 277, 245, 231, 213, 187, 138; ν_{max} cm^{-1}: 3450, 1805, 1740; ^{13}C NMR see Table 3.

Neo-S-petasin **14** [11]. $[\alpha]_D^{20}$ −6.47° (*c* 0.35, $CHCl_3$); CIHRMS *m/z* 335.1672 $[M+1]^+$ $C_{19}H_{27}O_3S$ requires 335.1681; CIMS *m/z* 335 $[M+1]^+$, 277, 245, 231, 217 (base), 201, 175, 161, 148, 133; ν_{max} cm^{-1}: 1700, 1680, 1640, 1580; ^{13}C NMR see Table 3.

S-*petasin* **15** [11]. $[\alpha]_D^{24}$ +57.7° (*c* 1.5, $CHCl_3$); CIHRMS *m/z* 335.1669 $[M+1]^+$ $C_{19}H_{27}O_3S$ requires 335.1681; CIMS *m/z* 335 $[M+1]^+$, 279, 245, 231, 217 (base), 201, 175, 161, 148, 137; ν_{max} cm^{-1}: 1700, 1690, 1640, 1580; ^{13}C NMR see Table 3.

Neopetasol angelate **16** [1, 2]. $[\alpha]_D^{22}$ −87.6° (*c* 1.35, $CHCl_3$); CIHRMS *m/z* 317.2117 $[M+1]^+$ $C_{20}H_{29}O_3$ requires 317.2117; CIMS *m/z* 317 $[M+1]^+$ (base), 245, 217, 201, 189, 161, 148, 135; ν_{max} cm^{-1}: 1720, 1690, 1640; [θ] 322 nm+1404 (EtOH); ^{13}C NMR see Table 3.

Petasin **17** [1, 2]. $[\alpha]_D^{24}$ +39.6° (*c* 1.08, $CHCl_3$); MS *m/z* 316 $[M]^+$, 233, 216, 201, 188, 161, 148 (base), 133, 105; ν_{max} cm^{-1}: 1720, 1680, 1640; ^{13}C NMR see Table 3.

Compound **18** [16]. $[\alpha]_D^{20}$ −13.3° (*c* 0.60, $CHCl_3$); CIHRMS *m/z* 281.1728 $[M+]^+$ $C_{16}H_{25}O_4$ requires 281.1753; MS *m/z* 281 $[M+1]^+$ (base), 263, 249, 231, 203, 140, 121, 133, 105; ν_{max} cm^{-1}: 1750, 1680.

Compound **19** [15]. $[\alpha]_D^{22}$ −130.5° (*c* 1.25, $CHCl_3$);

CIHRMS m/z 349.2022 $[M+1]^+$ $C_{20}H_{29}O_5$ requires 349.2015; MS m/z 349 $[M+1]^+$, 330, 248, 231, 121, 83 (base); ν_{max} cm^{-1}: 1760, 1730.

Compound **20** [16]. $[\alpha]_D^{22}$ $-168.2°$ (c 0.68, $CHCl_3$); CIHRMS m/z 311.1869 $[M+1]^+$ $C_{17}H_{27}O_5$ requires 311.1859; MS m/z 311 $[M+1]^+$, 278, 261, 247, 170 (base), 154, 123; ν_{max} cm^{-1}: 1770.

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