Pergamon

PII: S0031-9422(98)00434-8

Phytochemistry Vol. 49, No. 8, pp. 2607–2610, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

HEPATOPROTECTIVE TRITERPENES FROM *SEDUM SARMENTOSUM*

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(*Received* 20 *April* 1998)

Key Word Index—*Sedum sarmentosum*; Crassulaceae; triterpene; sarmentolin; hepatoprotective activity.

Abstract—The known δ-amyrone, 3-epi-δ-amyrin, δ-amyrin as well as a new hydroperoxy triterpene, 18β-hydroperoxy-olean-12-en-3-one (named sarmentolin) were isolated as hepatoprotective agents from *Sedum sarmentosum*. Their structures were elucidated by spectral analysis and chemical reactions.

INTRODUCTION

Sedum sarmentosum Bunge has been used in folk medicine for the treatment of chronic viral hepatitis. Pharmaceutical granules of this plant have been used in clinics since 1971 [1]. In view of its reputation as an hepatoprotective drug, many investigators have studied its chemical constituents. Sarmentosin, a water-soluble cyanophoric glycoside, was considered as the active constituent of the drug [2, 3]. However, pharmaceutical granules that lack this compound but still show remarkable activity. This prompted us to extend our investigations on the plant. In previous papers, we reported the occurrence of the flavanoids [4] and sterols [5] in the plant. A detailed search for the hepatoprotective agents from this plant has led to the isolation of three known triterpenes and a novel hydroperoxy triterpene, 18β-hydroperoxy-olean-12(13)-en-3-one, named sarmentolin. In the present paper, we describe the isolation and structure elucidation of the active triterpenes from *S. sarmentosum* and their hepatoprotective activity.

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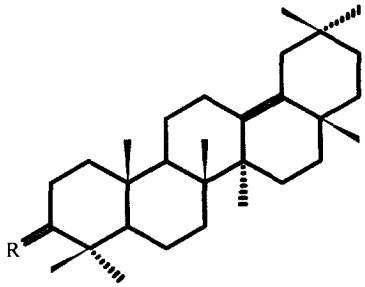
RESULTS AND DISCUSSION

The ethanol extract from *S. sarmentosum* was dissolved in ethanol–water (1:9) and extracted successively with petrol and ethyl acetate. Chromatography of the ethyl acetate extract gave four triterpenes: δ-amyrone (**1**, 1007 mg), 3-epi-δ-amyrin (**2**, 110 mg), δ-amyrin (**3**, 600 mg) and sarmentolin (**4**, 50 mg), respectively.

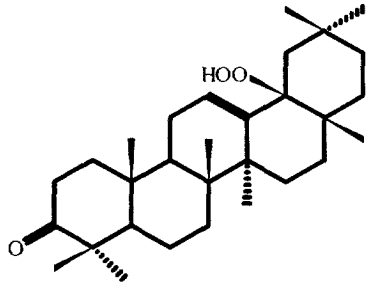
Sarmentolin (**4**) gave a $[M + H]^+$ peak at *m/z* 457.3699 on HRCIMS, corresponding to a molecular formula of $C_{30}H_{48}O_3$, which was also indicated by the ^{13}C NMR data. It gave a deep red color with Liebermann–Burchard reagent and the characteristic IR absorption bands (1384, 1353, 1326, 1295, 1264 cm^{-1}) of a pentacyclotriterpene [6]. The IR and ^{13}C NMR data show that **4** contained a carbonyl group [1695 cm^{-1}; δ_c 217.2 (*s*)], the positive Zimmermann colour reaction suggested it as a 3-keto group, and a hydroperoxy group [3423, 874, 816 cm^{-1}; δ_H 6.44 (1H, *s*) was lost on addition of D_2O and δ 87.2 (s, quaternary carbon bonded to oxygen function), EIMS *m/z* 438 $[M-H_2O]^+$] [7]. The ^1H NMR data also showed the presence of eight tertiary methyl groups (δ 0.85, 0.93, 1.00, 1.04, 1.05, 1.08, 1.13, 1.33) and a trisubstituted



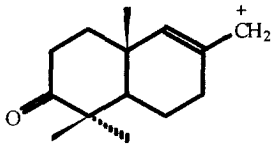
1 R=O

2 R=H, OH

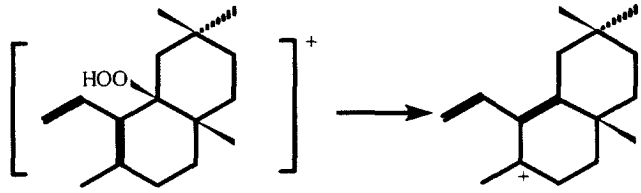
3 R=OH, H



4



m/z 205



m/z 250 m/z 217

Scheme 1.

double bond, indicating the presence of an olean 12(13)-ene system [8,9]. Further evidence was obtained from the diagnostically important EIMS fragment ions (*m/z* 205 and 217) (Scheme 1) associated with an RDA fragmentation in ring-C [10].

The hydroperoxy group in **4** was assigned at C-18 by HMBC correlation. Both protons of the C-17 methyl group and the C-12 olefinic proton correlated to the carbon signal at δ 87.2 (*s*). All proton and carbon signals were assigned by DEPT, ^1H–^1H COSY, ^{13}C–^1H COSY, HMQC and HMBC (Table 1).

The stereochemistry at C-18 was deduced from the effect of the hydroperoxy group on the 12–13 olefinic bond in **4**. This effect was revealed by the ^{13}C NMR data [11,12]. If the D/E ring-junction is *cis*, as usually present in an olean-12(13)-ene type triterpene, the 18-hydroperoxy group is equatorial and quite close to the π electron system of the 12–13 olefinic bond. Because of the steric influence due to the polar character of the hydroperoxy, C-12 was deshielded by 4.0 ppm and C-13 shielded by 5.4 ppm compared to the C-12 and C-13 chemical shift in β-amyrin [13]. However, if the D/E ring is *trans*, the 18-hydroperoxy assumes α-axial configuration and is far removed from 12–13 olefinic bond and leaves them unaffected. Therefore, the stereochemistry at C-18 was unequivocally established as 18β-hydroperoxy in **4**.

Triterpenes bearing a hydroperoxy moiety are rarely distributed in nature. So far only two compounds have been reported [7,14]. Sarmentolin **4** is the third triterpenoid hydroperoxide isolated from plants.

Identification of three known triterpenes isolated from *S. sarmentosum* was achieved by spectroscopic methods (Mp, IR, ^1H NMR, ^{13}C NMR and EIMS) as well as chemical reactions. Their structures were elucidated as δ-amyrone (**1**) [15,16], 3-epi-δ-amyrin (**2**) [17] and δ-amyrin (**3**) [18]. The C13–C18 double bond of δ-amyrone (**1**) could not be catalytically hydrogenated using PtO_2 as a catalyst, but the 3-keto group of **1** could be reduced to give **2** and **3**. On oxidation of **2** and **3** with Jones' reagent, both of them yield δ-amyrone (**1**).

It was found that δ-amyrone (**1**) markedly lowered the level of serum glutamic–pyruvic transaminase of mice treated with 0.1 ml/kg tetrachloromethane in olive oil [19]. Further research into the hepatoprotective activity of **2**–**4** on liver injury mice is being carried out.

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data of compound **4** and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data of compounds **1–3** (δ values from TMS; J in Hz)

C	4	4	1	2	3
1	1.92 (1H, *m*); 1.42 (1H, *m*)	39.3	39.3	33.6	38.8
2	2.52 (1H, *m*); 2.38 (1H, *m*)	34.1	34.1	25.5	27.4
3		217.2	218.2	76.3	79.0
4		47.2	47.2	37.6	38.8
5	1.33 (1H, *t*, *J* = 6.7 Hz)	55.3	54.8	49.2	55.4
6	1.49 (2H, *br d*, *J* = 6.7 Hz)	19.7	19.8	18.5	18.5
7	1.57 (1H, *m*); 1.39 (1H, *m*)	32.2	26.5	34.8	34.9
8		40.1	40.9	41.4	41.0
9	1.69 (1H, *t*, *J* = 9 Hz)	46.1	50.0	50.7	50.7
10		36.7	36.9	37.5	37.3
11	2.03 (2H, *dd*, *J* = 9, 3.7 Hz)	23.8	22.2	21.7	21.8
12	5.63 (1H, *d*, *J* = 3.7 Hz)	125.7	26.5	26.6	26.5
13		139.6	134.1	134.6	134.3
14		42.7	44.7	44.9	44.7
15	1.90 (1H, *m*); 1.04 (1H, *m*)	25.5	25.0	25.2	25.0
16	2.01 (1H, *m*); 0.93 (1H, *m*)	30.7	36.5	36.8	36.7
17		37.3	34.5	34.7	34.6
18		87.2	133.6	133.1	133.1
19	1.98 (1H, *br s*); 1.15 (1H, *br s*)	36.6	39.6	39.5	39.4
20		30.9	33.3	33.4	33.3
21	1.49 (1H, *m*); 1.24 (1H, *m*)	33.8	35.3	35.6	35.4
22	1.88 (1H, *m*); 0.93 (1H, *m*)	31.5	38.7	38.7	38.7
23	1.08 (3H, *s*)	26.7	21.0	22.3	17.7
24	1.04 (3H, *s*)	21.4	26.9	28.3	28.1
25	1.05 (3H, *s*)	15.3	16.3	16.2	15.5
26	1.00 (3H, *s*)	16.7	17.6	17.7	16.4
27	1.33 (3H, *s*)	24.5	21.2	21.6	21.4
28	0.85 (3H, *s*)	20.1	24.1	24.2	24.1
29	0.93 (3H, *s*)	35.1	23.8	23.9	23.8
30	1.12 (3H, *s*)	28.3	32.3	32.4	32.4
-OOH	6.44 (1H, *s*)				

EXPERIMENTAL

General

M.p.'s: uncorr; ^{1}H NMR and ^{13}C NMR: 400 and 100 MHz (TMS as internal standard in $CDCl_3$), respectively. 2D NMR spectra (^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMQC and HMBC): 400 MHz using standard pulse sequences; optical rotations: $CHCl_3$; MS: 70 eV, direct inlet system: CC: silica gel 60 (200–300 mesh); TLC: precoated silica gel (Merck silica gel 60 F-254). Detection of compounds by spraying with 7.5% H_2SO_4 EtOH soln followed by heating at 105°C for 3 min.

Plant material

The fresh herb (*S. sarmentosum* Bunge) was collected from Ying county, Zhejiang Province, China, in August 1993. A voucher specimen (No. 930824) is available for inspection at the Herbarium of China Pharmaceutical University, Nanjing, China.

Extraction and isolation of compounds

The fresh whole herb materials (70 kg) were extracted successively with 90 and 80% EtOH. Removal of solvent gave a syrup. The syrup was dissolved in 10% EtOH and partitioned with petrol, EtOAc and *n*-BuOH, respectively. Chromatography of the EtOAc extract on silica gel (10 × 110 cm) using a petrol EtOAc gradient gave 180 fractions (1000 ml each) which were collected and combined according to their TLC behavior. Fractions 11–17 gave δ-amyrone (**1**, 1007 mg), fractions 20–22 gave 3-epi-δ-amyrin (**2**, 110 mg), fractions 39–44 gave δ-amyrin (**3**, 600 mg), fractions 49–53 were recrystallized from EtOH–$CHCl_3$ sarmentolin to give (**4**, 50 mg).

δ-Amyrone (**1**)

Needles, m.p. 202–203°C $[\alpha]_D^{28}$ +2.2° ($CHCl_3$, *c* 0.11); IR ν_{max}^{KBr} cm^{-1}: 2960, 2860, 1706, 1383, 1361, 1349, 1294, 1263; ^{1}H NMR ($CDCl_3$): δ 0.68, 0.88, 1.00, 1.02, 1.08, 1.16 (each 3H, *s*), 0.92 (6H, s); ^{13}C NMR ($CDCl_3$): Table 1; MS *m/z* (rel. int.): 424.3755 $[M]^+$ ($C_{30}H_{48}O$ requires 424.3707) (23.4), 409 (13.8), 205 (100), 189 (20.5), 109 (60.3), 95 (42.9).

Hydrogenation of δ-amyrone (**1**)

A pure sample of **1** (10 mg) was dissolved in EtOH (5 ml) and hydrogenated with PtO_2 at atm. pres. for 24 h with stirring. The soln was filtered and after removal of the solvent under reduced pressure the residue was chromatographed by CC [6 g silica gel 60, 200–300 mesh, eluting with petrol EtOAc (1:49)] to give 3-epi-δ-amyrin (**2**) and δ-amyrin (**3**) (1:1, total 7.6 mg).

3-Epi-δ-amyrin (**2**)

Needles, m.p. 209–210°C $[\alpha]_D^{28}$ −47.5° ($CHCl_3$; *c* 0.22); IR ν_{max}^{KBr} cm^{-1}: 3385, 3045, 2950, 2865, 1636,

1472, 1465, 1372, 1280, 1043, 976; ^{1}H NMR ($CDCl_3$): δ 0.68, 0.81, 0.91, 0.93, 0.99, 1.15 (each 3H, *s*), 0.84 (6H, *s*), 3.4 (1H, *br*, *s*); ^{13}C NMR ($CDCl_3$): Table 1; MS *m/z* (rel. int.) 426[M]$^+$ ($C_{30}H_{50}O$) (15.2), 411 (4.4), 218 (43.4), 205 (53.6), 204 (33.9), 190 (45.9), 135 (34.9), 109 (100), 95 (83.5).

*Oxidation of 3-epi-δ-amyrin (**2**)*

To a soln of **2** (20 mg) in Me_2CO (7 ml), was added 7 drops of Jones' reagent within 10 min. The mixture was kept at 0°C for 2 h with stirring and then it was diluted with H_2O and the product extracted with $CHCl_3$. The $CHCl_3$ extract was washed, dried and evapd to give a ketone (15.8 mg). The spectrum of the ketone was identical to that of δ-amyrone.

*δ-Amyrin (**3**)*

Needles, m.p. 214–215.5°C; $[\alpha]_D^{28}$ −52.4° ($CHCl_3$; *c* 0.20); IR ν_{max}^{KBr} cm^{-1}: 3411, 2960, 2920, 2860, 1382, 1361, 1342, 1294, 1244; ^{1}H NMR ($CDCl_3$): δ 0.68, 0.75, 0.91, 0.97, 1.00, 1.14 (each 3H, *s*), 0.83 (6H, *s*), 3.21 (1H, *m*); ^{13}C NMR ($CDCl_3$): Table 1; EIMS *m/z* (rel. int.) 426.3896 [M]$^+$ ($C_{30}H_{50}O$ requires 426.3864) (26.1), 411 (9.8), 408 (24.2), 229 (12.1), 218 (51.9), 205 (95.4), 189 (84.0), 109 (100), 95 (76.7), 69 (48.2), 55 (48).

*Oxidation of δ-amyrin (**3**)*

A pure sample of **3** (20 mg) was oxidized with Jones' reagent, processing as in Section 3.7, to give δ-amyrone (16 mg).

*Sarmentolin (**4**)*

Needles, m.p. 210–211°C; $[\alpha]_D^{28}$ +83.6° ($CHCl_3$, *c* 0.11); IR ν_{max}^{KBr} cm^{-1}: 3423, 3047, 2948, 2855, 1695, 1655, 1461, 1384, 1264, 924, 874, 845, 816; ^{1}H NMR ($CDCl_3$): Table 1; ^{13}C NMR ($CDCl_3$): Table 1; CIMS *m/z* (rel. int.) 457.3621 [M + H]$^+$ ($C_{30}H_{48}O_3H$ requires 457.3605) (19.2), 439 [M–H_2O + H]$^+$ (70.2), 423 (86.9), 307 (100); EIMS *m/z* (rel. int.) 438 [M–H_2O]$^+$ (4.4), 423 (16.1), 356 (4.8), 224 (24.6), 217 (6.2), 205 (17.2), 145 (11.7), 109 (13.7), 105 (18.7), 95 (25.9), 70 (100).

Acknowledgements—We wish to thank Dr Xianguo Zhao (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China) for botanical identification of *S. sarmentosum*, Dr Yanlong Guo (China Pharmaceutical University) for the measurements of MS and Ms Ping Wang (Shanghai Institute of Meteria Medica, Shanghai, China) for performing the 2D NMR spectra.

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