HETEROCYCLES, Vol. 65, No. 4, 2005, pp. 887 - 891
Received, 17th January, 2005, Accepted, 10th February, 2005, Published online, 10th February, 2005

EREMOPETASITENIN A_3 AND 8-OXOEREMOPHIL-6-EN-12-OIC ACID FROM *PETASITES JAPONICUS* SSP. *GIGANTEUS*

Motoo Tori,* Moeko Kume, Katsuyuki Nakashima, Masakazu Sono, and Masami Tanaka

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan. e-mail:tori@ph.bunri-u.ac.jp

Abstract – Seven eremophilane-type sesquiterpenes have been isolated from *Petasites japonicus* ssp. *giganteus* collected in Hokkaido, and two of them have been found to be new. Eremopetasitenin A_3 was a lactone containing an epoxide at the C-7 and C-8 positions, whose structure was established mainly based on spectroscopic evidence including 2D NMR spectra. The other one was an eremophilane-type carboxylic acid.

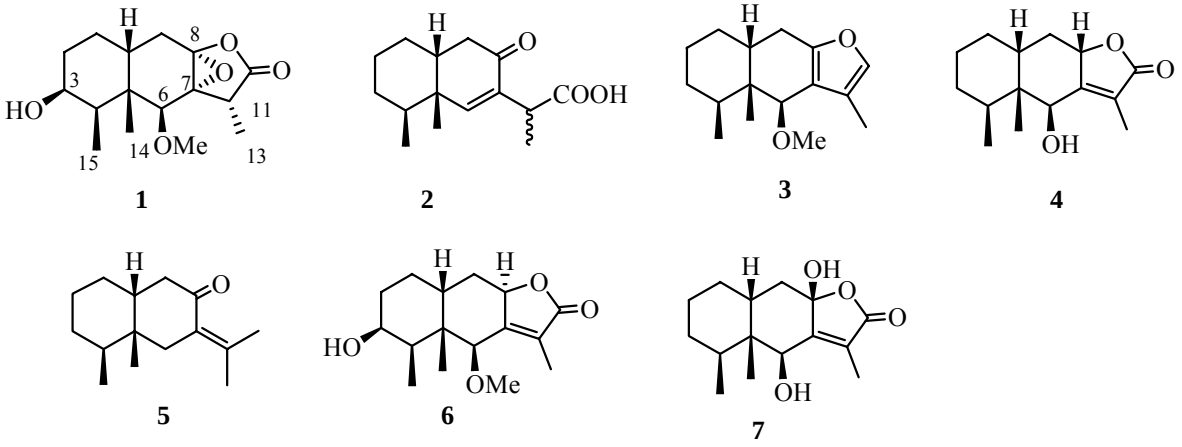
INTRODUCTION

We have been studying the chemical constituents from plants belonging to Compositae, especially *Petasites*,[1,2] *Farfugium*,[3] *Solidago*,[4] *Eupatorium*,[5-7] *Aster*,[8] *Ligularia*,[9] and so on. Quite recently several reports on isolation of eremophilane-type sesquiterpenes from *Petasites japonicus* ssp. *giganteus* Kitam.[10] and *Ligularia virgaurea* ssp. *oligocephala*[11,12] appeared and these reports prompted us to publish our results. In 1997, we found several new eremophilane-type sesquiterpenes from the rhizomes of *Petasites japonicus*[1,2] which are presumably biosynthetic intermediates. Because these lactones are not so stable, they have rarely been isolated so far. We have also found similar compounds in *Farfugium japonicum*.[3] Because *P. japonicus* ssp. *giganteus*[10] growing in the Hokkaido area, is a close species to *P. japonicus*, we believe this plant also produces such type of lactones as a biosynthetic intermediate. We have now reinvestigated the chemical constituents of *P. japonicus* ssp. *giganteus* and isolated a new lactone as well as a carboxylic acid.

RESULTS AND DISCUSSION

The methanol extract of rhizomes of this plant was subjected to silica gel column chromatography followed by HPLC (see EXPERIMENTAL) to isolate seven compounds, among which compounds (**1**) and (**2**) were new.

Compound (**1**), named eremopetasitenin A_3, showed a quasi-molecular ion peak at *m/z* 297 (CIMS), whose exact mass was measured as $C_{16}H_{25}O_5$ in HRCIMS. The characteristic IR absorption band at 1800 cm^{-1} indicated the presence of a γ-lactone as experienced in the case of eremopetasitenins.[1,2] There are two *sec*-methyl and one *tert*-methyl groups in the ^1H NMR spectrum. The ^{13}C NMR spectrum showed the presence of a carbonyl group (δ 176.0) as well as four carbons bearing an oxygen function, two of which were quaternary. From the degree of unsaturation, this compound must be tetra-cyclic. A careful analysis of the 2D NMR spectra indicated the eremophilane skeleton having a γ-lactone with an epoxide at the C-7 and C-8 positions. The methoxy group must be at the C-6 position, because HMBC correlation between the methoxy protons and the carbon at the C-6 was observed. The hydroxy group was determined to be located at the C-3 position, because HMBC correlation between the *sec*-methyl group (δ 0.71, H-15) and the carbon at δ 68.0 (C-3) was observed. The NOESY spectrum as shown in Figure 1 showed correlations between H-3 and H-6, H-3 and H-4, H-10 and H-15, H-10 and H-14, and H-14 and H-11, respectively. Therefore, rings A and B must be *cis*-fused and the lactone ring should be on the same side of both methyl groups at the C-4 and C-5 positions, indicating the epoxide ring and the methyl group at the C-11 position are both in the α-side. As the conformation of this molecule must be as shown in Figure 1 based on the NOESY spectrum, both the hydroxy group at the C-3 and the methoxy group at the C-6 position should be β.



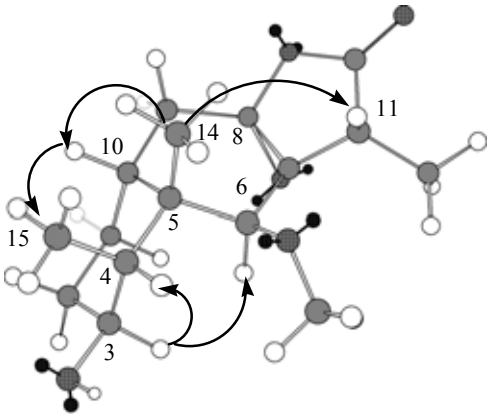


Figure 1 The selected NOEs detected in compound (**1**).

Compound (**2**) showed a quasi-molecular ion peak at *m/z* 251 (molecular formula $C_{15}H_{23}O_3$ from HRCIMS). The IR spectrum indicated the presence of a carboxylic acid, which was supported by the ^{13}C NMR signal at δ 180.0. The other carbonyl absorption at δ 199.8 must be attributed to a carbonyl moiety. A careful analysis of the 2D NMR spectra resulted in the structure depicted in the figure. The stereostructure was also established by the analysis of the NOESY spectrum. Because an NOE between H-10 and H-14 was observed, rings A and B should be *cis*-fused. Furthermore, an NOE between H-4α and H-9α indicated that both methyl groups at the C-4 and C-5 positions had *cis* stereochemistry. Therefore, the structure of compound (**2**) was determined as depicted in the figure. The stereochemistry at the C-11 position was not clarified.

The other components were all known compounds and were identified by comparison with the authentic samples or the published data. It is very interesting to note that we could not isolate bakkenolide[13] from the rhizomes of this plant. Petasalbin methyl ether (**3**)[14] was the most abundant and the other components were just minor in the rhizomes collected for this study. This phenomenon is presumably due to the variation of the harvested season and the place of collection.[9,15]

EXPERIMENTAL

GENERAL

The specific rotations and the CD spectra were taken on a JASCO DIP-1000 and J-725 polarimeter, respectively. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were taken on a Varian Unity 600 (600 MHz and 150 MHz, respectively) and a JEOL ECP 400 (400 MHz and 100 MHz, respectively) spectrometer. MS spectra including high-resolution mass spectra were recorded on a JEOL JMS-700 MStation. Chemcopak Nucleosil 50-5 (4.8×250 mm) was used for HPLC (JASCO pump system). Silica gel 60 (70-230 mesh, Fuji Sylisia) was used for column chromatography and silica gel 60 F_{254} plates (Merck) were used for TLC.

PLANT MATERIAL

The rhizomes of *P. japonicum* ssp. *giganteus* (14 kg) were collected in Obihiro, in June 2003. A voucher specimen (TBU-MT-200301) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

EXTRACTION AND ISOLATION

Partially dried rhizomes (14 kg) were cut into pieces and extracted with MeOH (*ca*. 40 L) for *ca.* a month at rt to give a crude extract (528 g). A part of this MeOH extract (96 g) was subjected to silica gel column chromatography (elution with hexane-EtOAc, in gradient) to afford 3 fractions. The 1st fraction (511 mg) was further separated by silica gel column chromatography (elution with hexane-EtOAc, in gradient) to afford petasalbin methyl ether (**3**, 408 mg). The 2nd fraction (936 mg) was further separated by silica gel column chromatography (elution with hexane-EtOAc, in gradient) to give 8 fractions and each fraction was purified by HPLC (Nucleosil 50-5, 4.8×250 mm, elution with hexane-EtOAc in different concentration) to give **1** (3.4 mg), **2** (4 mg), **4** (3.8 mg),[16] fukinone (**5**, 13 mg),[17] **6** (1.1 mg),[18] and **7** (8.3 mg).[19]

Eremopetasitenin A_3 (**1**): Colorless amorphous. $[\alpha]_D^{20}$ -5.1° (*c* 0.44; EtOH); ν_{max} (FT) cm^{-1}: 3440, 1800; MS (Cl) *m/z* 297 $[M+H]^+$ (base), 279, 247; HRMS (CI) Obs. 297.1693 $[M+H]^+$ (calcd for $C_{16}H_{25}O_5$ 297.1702); ^{13}C NMR (150 MHz, C_6D_6) δ 6.7 (C-15), 11.8 (C-13), 19.4 (C-14), 25.2 (C-9), 27.4 (C-1), 29.9 (C-2), 33.3 (C-10), 38.7 (C-4), 40.4 (C-11), 42.2 (C-5), 61.6 (O-Me), 66.8 (C-7), 68.0 (C-3), 76.8 (C-6), 87.0 (C-8), 176.0 (C-12); ^1H NMR (600 MHz, C_6D_6) δ 0.51 (3H, s, H-14), 0.71 (3H, d, *J*=7.4 Hz, H-15), 0.85 (1H, dq, *J*=13.7, 4.2 Hz, H-1β), 1.05 (1H, m, H-10), 1.10 (1H, qd, *J*=13.7, 4.4 Hz, H-2β), 1.30 (3H, d, *J*=7.1 Hz, H-13), 1.36 (1H, m, H-2α), 1.42 (1H, qd, *J*=13.7, 4.1 Hz, H-1α), 1.64 (1H, br d, *J*=15.1 Hz, H-9α), 1.89 (1H, dd, *J*=15.1, 7.7 Hz, H-9β), 1.92 (1H, m, H-4α), 2.79 (1H, q, *J*=7.1 Hz, H-11β), 2.99 (3H, s, OMe), 3.35 (1H, s, H-6α), 3.55 (1H, dt, *J*=13.7, 4.4 Hz, H-3α); CD (*c* 6.5×10^{-4} M, EtOH) $[\theta]_{205\ nm}$ -3700, $[\theta]_{238\ nm}$ +2050.

8-Oxoeremophil-6-en-12-oic acid (**2**): Colorless amorphous. $[\alpha]_D^{16}$ +17.7° (*c* 1.06; EtOH); ν_{max} (FT) cm^{-1}: 3500~2500, 1710, 1680; MS (CI) *m/z* 251 $[M+H]^+$ (base), 233, 58; HRMS (CI) Obs. 251.1644 $[M+H]^+$ (calcd for $C_{15}H_{23}O_3$ 251.1647); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9 (C-13), 16.1 (C-15), 20.4 (C-2), 20.7 (C-14), 27.0 (C-1), 30.1 (C-3), 36.0 (C-4), 38.5 (C-11), 39.0 (C-10), 39.4 (C-9), 39.5 (C-5), 136.0 (C-7), 157.6 (C-6), 180.0 (C-12), 199.8 (C-8); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, d, *J*=6.6 Hz, H-15), 1.13 (3H, s, H-14), 1.30 (3H, d, *J*=7.1 Hz, H-13), 1.32 (1H, m, H-3), 1.38 (1H, m, H-1), 1.46 (2H, m, H-2, H-3), 1.54 (1H, m, H-2), 1.73 (1H, m, H-1), 1.81 (1H, m, H-4), 2.05 (1H, m, H-10), 2.32 (1H, dd, *J*=17.2, 4.4 Hz, H-9β), 2.70 (1H, dd, *J*=17.2, 12.1 Hz, H-9α), 3.64 (1H, q, *J*=7.1 Hz, H-11), 6.70 (1H, s, H-6); CD (*c* 1.3×10^{-4} M, EtOH) $[\theta]_{243\ nm}$ +8600, $[\theta]_{344\ nm}$ +800.

ACKNOWLEDGEMENTS

We thank Mrs. Tomoko Omatsu, Obihiro Hokkaido, for help in collecting the plant and identification.

Miss Yasuko Okamoto, of this University, is also thanked for measurement of MS spectra.

REFERENCES

1. M. Tori, M. Kawahara, and M. Sono, *Tetrahedron Lett.*, 1997, **38**, 1965.
2. M. Tori, M. Kawahara, and M. Sono, *Phytochemistry*, 1998, **47**, 401.
3. M. Tori, Y. Shiotani, M. Tanaka, K. Nakashima, and M. Sono, *Tetrahedron Lett.*, 2000, **41**, 1797.
4. M. Tori, A. Katto, and M. Sono, *Phytochemistry*, 1999, **52**, 487.
5. M. Tori, Y. Takeichi, H. Kuga, K. Nakashima, and M. Sono, *Heterocycles*, 2000, **52**, 1075.
6. M. Tori, Y. Ohara, K. Nakashima, and M. Sono, *J. Nat. Prod.*, 2000, **64**, 1048.
7. M. Tori, Y. Takeichi, H. Kuga, K. Nakashima, and M. Sono, *Chem. Pharm. Bull*., 2002, **50**, 1250.
8. M. Tori, J. Murata, K. Nakashima, and M. Sono, *Spectroscopy*, 2001, **15**, 119.
9. R. Hanai, X. Gong, M. Tori, S. Kondo, K. Otose, Y. Okamoto, T. Nishihama, A. Murota, Y. Shen, S. Wu, and C. Kuroda, *Bull. Chem. Soc. Jpn*., 2005, **78**, in press.
10. Y. Goto, Y. Kojima, T. Nakayama, and M. Terazawa, *Phytochemistry*, 2001, **57**, 109.
11. Q. –X Wu, Y. –P. Shi, and L. Yang, *Org. Lett*., 2004, **6**, 2313.
12. Q. –X. Wu, Y. –P. Shi, and L. Yang, *Planta Med*., 2004, **70**, 479.
13. N. Abe and R. Onoda, *Tetrahedron Lett.*, 1968, 369 and K. Shirahata, T. Kato, Y. Kitahara, and N. Abe, *Tetrahedron*, 1969, **25**, 3179.
14. K. Naya, M. Nakagawa, M. Hayashi, K. Tsuji, and M. Naito, *Tetrahedron Lett*., 1971, 2961.
15. M. Tori, Y. Takeichi, H. Kuga, K. Nakashima, and M. Sono, *Heterocycles*, 2000, **52**, 1075 and M. Tori, Y. Takeichi, H. Kuga, K. Nakashima, and M. Sono, *Chem. Pharm. Bull.*, 2002, **50**, 1250.
16. Y. Moriyama and T. Takahashi, *Bull. Chem. Soc. Jpn*., 1976, **49**, 3196.
17. K. Naya, I. Takagi, Y. Kawaguchi, and Y. Asada, *Tetrahedron*, 1968, **24**, 5871.
18. Y. Yaoita and M. Kikuchi, *Chem. Pharm. Bull*., 1994, **42**, 1944.
19. Y. Moriyama and T. Takahashi, *Chem. Pharm. Bull*., 1976, **24**, 360.