Sesquiterpene lactones. XXXVII. Germacranolides in the genus *Stizolophus* Cass.

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Abstract

Three germacranolides, hitherto unknown in this species, were isolated from the herb, *Stizolophus balsamita* (Lam.) Cass. ex Takht. They were identified as: 8 α (4'-hydroxy) - seneciolyloxy-9 α -hydroxyparthenolide, isospiciformin and 8 α ,9 α -dihydroxyparthenolide.

Key words: sesquiterpene lactones, *Stizolophus*, *Compositae*

INTRODUCTION

The genus *Stizolophus* Cass., numbering 6 species of plants occurring in the Caucasus and Central Asia, is considered by some botanists to be a section in the genus *Centaurea* L. Among these botanists is Wagenitz who, in *Flora Iranica* (1980) classified the plant under consideration here as *Centaurea balsamita* Lam. from the section *Stizolophus* (Cass.) DC. Knowledge of the chemistry of this plant therefore has a taxonomic aspect. To date, sesquiterpene lactones have been studied in two species of the genus *Stizolophus*. These were *S. balsamita* (Lam.) Cass. ex Takht (syn. *Centaurea balsamita* Lam.) and in *S. coronopifolius* (Lam.) Cass. (syn. *Centaurea coronopifolia* Lam.). The

following germacranolides were obtained from the former species: stizolin (I) (Mukhametzhanov et al. 1971), stizolicin (II) (Mukhametzhanov et al. 1970) and balsamin (III) (Rybalko et al. 1976); from the latter species in addition to stizolicin (II) (Mukhametzhanov et al. 1969), three more germacranolides described as: 1(10)-en-4 α ,5 β -epoxy-9 α -hydroxygermacranolide with 8 α -(4'-hydroxy) senecioate (IV), 1(10)-en-4 α ,5 β -epoxy-9 α -hydroxygermacranolide with 8 α -senecioate (V) and 1(10)-en-4 α ,5 β -epoxy-8 α -(4'-hydroxy)senecioate (VI) (Öksüz and Ayyildiz 1986)* were obtained from the latter species. Our study of the sesquiterpene lactones in the species *Stizolophus balsamita* led to the isolation of three germacranolides hitherto not described in this species. One of them is either identical with, or an enantiomer of, lactone IV (Fig. 1) first obtained from *S. coronopifolius* (Öksüz and Ayyildiz 1986), the next lactone VII is identical with, or an enantiomer of, the synthetically obtained lactone VII — a stereoisomer of spiciformin (VIII) (Shafizadeh and Bhadane 1973), named therefore, isospiciformin. The third lactone has not been described to date in literature, has structure IX and we propose to call it 8 α ,9 α -dihydroxyparthenolide (Fig. 1).

Fig. 1. I — stizolin, II — stizolicin, III — balsamin, IV — 8 α -(4'-hydroxy)senecioyloxy-9 α -hydroxygermacranolide, V — 4 α ,5 β -epoxy-8 α -senecioyloxy-9 α -hydroxygermacra-1(10),-11(13)-dien-6 α ,12-olide, VI — 4 α ,5 β -epoxy-8 α -(4'-hydroxy)senecioyloxygermacra-1(10),-11(13)-dien-6 α ,12-olide, VII — isospiciformin, VIII — spiciformin, IX — 8 α ,9 α -dihydroxyparthenolide

* We do not think that the names of lactones IV-VI given in the work by Öksüz and Ayyildiz are entirely correct. They should be: 4 α ,5 β -epoxy-8 α -(4'-hydroxy)senecioyloxy-9 α -hydroxygermacra-1(10),11(13)-dien-6 α ,12-olide (IV), 4 α ,5 β -epoxy-8 α -senecioyloxy-9 α -hydroxygermacra-1(10),11(13)-dien-6 α ,12-olide (V) and 4 α ,5 β -epoxy-8 α -(4'-hydroxy)senecioyloxygermacra-1(10),-11(13)-dien-6 α ,12-olide (VI).

MATERIAL AND METHODS

Stizolophus balsamita was cultured in the garden of the Chair of Medicinal Plants in Poznań (Poland). The "lactone fraction" was obtained using 465 g of dried and crushed herb, according to the method of Drożdż and Piotrowski (1973). The preliminary chromatographic check of the extract was done on silica gel. The most suitable solvent systems were found to be a 3:1 (v/v) mixture of chloroform and acetone and a 1:1 (v/v) mixture of chloroform and ethyl acetate. The chromatograms were sprayed with sulfuric acid and heated to a temperature of 100°C for 3 min. Seven to ten spots of compounds staining green, red or grey were obtained.

The "lactone fraction" (3.1 g) was applied to a column (100 cm long and 2 cm in diameter) packed with 180 g of silica gel. The compounds were eluted first with a mixture of chloroform-ethyl acetate 4:1 (v/v), then with chloroform containing increasing amounts of ethyl acetate (chloroform: ethyl acetate at 3:1, 1:1, 1:2 and 1:4 were used). The compounds so obtained were identified using the following spectral methods: ^1H NMR, MS, IR, CD.

Isospiciformin (VII). A compound needing further purification dominated in fraction 6. Its dry residue (0.31 g) was rechromatographed on small particle silica gel (18 g) using an 18 $\times$ 1.5 cm column and ethyl ether. A chromatographically uniform substance (17.8 mg) was obtained. ^1H NMR (200 MHz; CDCl_3): H-1: 5.37 bt ($J_{1,2} \sim 8.3$; $J_{1,14} = 1.2$); H-5: 2.66 d ($J_{5,6} = 9.0$); H-6: 3.44 dd ($J_{6,5} = 9.0$; $J_{6,7} = 9.6$); H-7: 3.00 m ($J_{7,6} = 9.6$; $J_{7,8} = 5.4$; $J_{7,13} = 3.0$; $J_{7,13'} = 2.6$); H-8: 4.02 ddd ($J_{8,7} = 5.4$; $J_{8,9} = 2.4$; $J_{8,9'} = 10.8$); H-9: 2.80 vbd ($J_{9,9'} = 13.0$; $J_{9,8} = 2.4$); H-13: 6.48 dd ($J_{13,13'} = 1.3$; $J_{13,7} = 3.0$); H-13': 6.29 dd ($J_{13',13} = 1.3$; $J_{13',7} = 62.6$); H-14: 1.83 d ($J_{14,1} = 1.2$); H-15: 1.28 s.

Mass: 246, 228, 207, 204, 188.

IR (cm^{-1} , CHCl_3): 3440 (hydroxyl), 1761 (γ -lactone), 1652 (double bond).

CD (nm, $\Delta\epsilon$): 252, -2.0; 228, ± 0 ; +16.4 (last reading).

8 α ,9 α -dihydroxyparthenolide (IX). The solvents were distilled off from fraction 12 (chromatographically uniform), the remainder was dissolved in chloroform and ethyl ether was added. After cooling, a substance crystallized which was then filtered off and washed with ethyl ether. Crystals (33.6 mg) with a melting point of 218-220°C, were obtained.

^1H NMR (200 MHz; CDCl_3): H-1: 5.67 bdd ($J_{1,2} = 12.8$; $J_{1,2'} = 5.0$); H-5: 2.69 d ($J_{5,6} = 8.0$); H-6: 3.96 dd ($J_{6,5} = 8.0$; $J_{6,7} = 6.7$); H-7: 3.61 m; H-8: 3.92 um; H-9: 4.38 bs; H-13: 6.50 dd ($J_{13,13'} = 1.0$; $J_{13,7} = 3.4$); H-13': 6.06 dd ($J_{13',13} = 1.0$; $J_{13',7} = 3.0$); H-14: 1.75 bs; H-15: 1.29 s.

Mass: 280 (M), 262 (M-18), 244 (M-18-18), 233, 216, 215, 137
 IR (cm^{-1} , CHCl_3): 3500, 3430 (hydroxyl), 1742 (γ -lactone), 1646 (double bond).
 CD (nm, $\Delta\epsilon$): 255, +0.6; 242, ± 0 ; 210, -3.2 (last reading).

8 α -(4'-hydroxy)seneciolyoxy-9 α -hydroxyparthenolide (IV). The thirteenth fraction began to crystallize in the elutant after it had been distilled off to a volume of about 5 cm^3 . The crystals were filtered off and rinsed with chloroform. A compound with a melting point of 180-182°C (13.1 mg) was obtained.

^1H NMR (200 MHz; CDCl_3): H-1: 5.66 bdd ($J_{1,2}$ 12; $J_{1,2}$ 3.5); H-5: 2.62 d ($J_{5,6}$ = 9.4); H-6: 4.35 dd ($J_{6,5}$ = 9.4; $J_{6,7}$ = 6.5); H-7: 3.83 m ($J_{7,6}$ = 6.5; $J_{7,8}$ = 3.8; $J_{7,13}$ = 3.2); H-8: 4.57 bd ($J_{8,7}$ = 3.8; $J_{8,9}$ = 0.7); H-9: 4.38 bs; H-13: 6.28 dd ($J_{13,13'}$ = 0.8; $J_{13,7}$ = 3.6); H-13': 5.67 dd ($J_{13,13'}$ = 0.8; $J_{13,7}$ = 3.2); H-14: 1.80 t ($J_{14,1}$ $J_{14,2}$ 1.3); H-15: 1.27 s; Sen-4-OH: H-2'': 6.03 q (J = 1.5); H-4'': 4.16 bs; H-1'': 2.02 d (J = 1.5).

Mass: 378 (M), 262 (M-116), 244 (M-116-18), 233, 215, 179, 99 ($\text{C}_5\text{H}_7\text{O}_2$), 71 ($\text{C}_4\text{H}_7\text{O}$).

IR (cm^{-1} , CHCl_3): 3425 (hydroxyl), 1745, 1760 sh (γ -lactone), 1712 (α , β -unsaturated ester), 1665 (double bond) CD (nm, $\Delta\epsilon$): 252, -1.0; 240, ± 0 ; 210, -5.9 (last reading).

SUMMARY OF RESULTS

Three germacranolides were isolated from *Stizolophus balsamita*. They are IV, VII and IX which, except for isospiciformin (VII), are characterized by a 4 α ,5 β -epoxide, a trans double bond between C-1 and C-10, a trans γ -lactone ring on C-6 and C-7 coupled with an exomethyl group, and a free or estrified hydroxyl group in position C-8. This type of germacranolide is found in both *S. balsamita* and *S. coronopifolius*. Only does isospiciformin stand out from among the discussed germacranolides by the position of its γ -lactone ring in C-8. Most of the isolated germacranolides from both species of the genus *Stizolophus* have a free C-9 hydroxyl group. Substituents in this position in sesquiterpene lactones are found only very rarely.

The data obtained in this study are not sufficiently conclusive to warrant a judgement stating unequivocally if *Stizolophus* is to be recognized as an independent genus or a section in the genus *Centaurea*. Further phytochemical studies of other species in *Stizolophus* may give an answer to this question.

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Laktony seskwiterpenowe. XXXVII. Germakranolidy w rodzaju Stizolophus Cass.

Streszczenie

Wyizolowano i zidentyfikowano z ziela *Stizolophus balsamita* (Lam.) Cass. ex Takht. trzy nieznanne u tego gatunku germakranolidy. Są to 8 α (4'-hydroksy)senecioyloksy-9 α -hydroksypartenolid, izospiciformina i 8 α ,9 α -dihydroksypartenolid.