Sesquiterpene lactones from *Crepis setosa* Hall.

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Abstract

The roots of *Crepis setosa* Hall. afforded four sesquiterpene lactones, among them three glycosides, all derived from 11 β ,13-dihydrozaluzanin C and not previously reported from this species.

Key words: *Crepis setosa*, *Compositae*, sesquiterpene lactones, chemotaxonomy

INTRODUCTION

Earlier studies of some members of the genus *Crepis* L. (*Compositae*, tribe *Lactuceae*) yielded several new structurally related guaianolides as well as their acyloxy and 3- β -D-glucosyloxy derivatives (Kisiel 1983a, 1983b, 1984, Kisiel and Kohlmünzer 1987, 1989). The present paper describes sesquiterpene lactone constituents of *Crepis setosa* Hall. which have been isolated for the first time from the plant.

RESULTS AND DISCUSSION

The ethanol extract of the roots of the plant on repeated silica gel column chromatography afforded four derivatives of 11 β ,13-dihydrozaluzanin C, from the fractions eluted with a mixture of chloroform and methanol (9:1).

The least polar lactone was purified by acetylation and chromatography. The ^1H NMR data of the acetate (Ib) closely resembled those of the aglycone of ixerin F (Ia) (Kisiel and Kohlmünzer 1987) except for the H-9 and H-3 signals which were shifted downfield due to acetylation of two secondary

hydroxyl groups at C-9 and C-3. Two acetyl signals were observed at δ 2.14 and δ 2.18 (Table 1). Therefore, the parent compound was identified as 9 α -hydroxy-11 β ,13-dihydrozaluzanin C (I) (Fig. 1.) which was recently reported from *Lactuca laciniata* Makino (Nishimura et al. 1986b).

Table 1

^1H NMR spectral data of compounds I, Ib, II and III (300 MHz, δ -values)

H	I	Ib	II	III
H-1	3.33 ddd br	3.13 ddd br	2.84 ddd br	2.84 q br
H-2	2.38 dt	2.49 dt	2.33 dt	2.24 dt
H-2'	1.75 dt	1.80 dt	1.75 dt	1.66 dt
H-3	4.58 dd br	5.59 dd br	4.53 dd br	4.46 t br
H-5	2.86 t br	2.88 t br	2.79 t br	2.77 dd br
H-6	3.96 t	3.97 t	4.02 t	4.00 t
H-7			1.90 m	2.03 ddd
H-8	2.29 m	2.29 m	2.12 dddd	3.62 dddd
H-8'	1.53 m	1.47 m	1.34 m	
H-9	4.63 t	5.55 t	2.53 dt	2.68 dd
H-9'			2.02 ddd	2.14 dd
H-11	2.23 dq	2.32 dq	2.23 dq	2.56 dq
H-13	1.24 d	1.24 d	1.23 d	1.36 d
H-14		5.18 s	4.97 s br	5.02 s
H-14'	5.09 s	5.10 s	4.93 s br	4.95 s
H-15	5.37 t	5.42 t	5.39 t	5.26 t br
H-15'	5.29 t	5.29 t	5.30 t	5.24 t br
OAc		2.18 s		
		2.14 s		

J (Hz): Compounds I and Ib: 1, 2 = 8; 1, 2' = 7; 1, 5 = 5; 6 = 6; 7 = 9.5; 2, 2' = 14; 2, 3 = 8; 2', 3 = 7; 3, 15 = 5; 15 = 2; 7, 8 = 3; 7, 8' = 7; 11 = 11; 8, 9 = 8'; 9 = 3; 8, 8' = 13; 11, 13 = 7. Compound II: 1, 2 = 8; 1, 2' = 7; 1, 5 = 5; 6 = 6; 7 = 9.6; 2, 2' = 14; 2, 3 = 8; 2', 3 = 7; 3, 15 = 5; 15 = 2; 7, 8 = 8; 9 = 8; 9' = 5; 7, 8' = 10; 7, 11 = 11; 8', 9' = 9; 8, 8' = 9; 9' = 12; 11, 13 = 7. Compound III: 1, 2 = 1; 2' = 1; 5 = 7.8; 2, 2' = 13; 2, 3 = 2'; 3 = 7.8; 5, 6 = 6; 7 = 7; 8 = 10; 7, 11 = 11; 8, 9 = 5; 8, 9' = 8.6; 9, 9' = 13; 11, 13 = 7.

Fig. 1. The structures of sesquiterpene lactones isolated from *Crepis setosa* Hall. and their chemical transformation products

The more polar lactone was shown to be 11 β ,13-dihydrozaluzanin C-3- β -D-glucoside (IIa) on the basis of its chemical transformations. Hydrolysis with β -glucosidase revealed glucose as the sugar and an aglycone II whose R_f value was almost the same as that of zaluzanin C. However, in its ^1H NMR spectrum the exomethylene proton signals were replaced by a double quartet at δ 2.23 for H-11 and a doublet at δ 1.23 for H-13, indicating the presence of a 11,13-dihydroderivative II. The α -orientation of the methyl group was deduced from the chemical shift of H-13 and the couplings of H-11. Finally, the structure of the glycoside IIa was confirmed by direct comparison with an authentic sample prepared by sodium borohydride reduction of vernoflexuoside (3- β -D-glucoside of zaluzanin C) (Kisiel 1975). The glycoside IIa was also isolated from *Lactuca laciniata* Makino (Nishimura et al. 1986b).

The mixture of the most polar lactones was found to consist of two isomeric glycosides. The major component of the mixture could be obtained pure and be identified by direct comparison with an authentic sample of ixerin F (Ia) isolated from *Crepis biennis* L. (Kisiel and Kohlmünzer 1987) and reported earlier from *Ixeris* (Asada et al. 1984) and *Picris* (Nishimura et al. 1986a) species.

Since the attempted chromatographic separation of the minor component was without success, the mixture was hydrolysed with β -glucosidase. The hydrolysis yielded glucose and a mixture of two aglycones which were separated by repeated column chromatography. One of them gave an ^1H NMR spectrum superimposable on that of 9 α -hydroxy-11 β ,13-dihydrozaluzanin C (I). The ^1H NMR chemical shifts of the other combined with the mass spectral fragments of its diacetate indicated that the compound was an 8 α -hydroxy derivative (III) of II. The α orientation of the hydroxyl group at C-8 was deduced from both the chemical shift and the coupling constants of the H-8 signal. For comparison, ^1H NMR spectra of guaianolides with the β -oriented hydroxyl groups (Kisiel 1983a, 1983b, 1984) at C-8 showed H-6 and H-8 chemical shifts at lower field and had a completely different pattern of coupling constants involving H-7, H-8 and H-9. From those results, however, the position of attachment of the sugar moiety could not be deduced. The structure IIIa for the glycoside seemed to be likely as all the other β -glycosides isolated from *Crepis* species had the glucose moieties at C-3 (Kisiel 1984, Kisiel and Kohlmünzer 1987, 1989).

The chemistry of *Crepis setosa* Hall. is similar to that of other members of the genus, the isolated compounds are all derived from 11 β , 13-dihydrozaluzanin C, three of them (I, IIa, IIIa) found in *Crepis* species for the first time.

EXPERIMENTAL

Mass spectra were recorded by direct inlet at 15 eV ionisation. ^1H NMR spectra were measured in CDCl_3 at 300 MHz with TMS as an internal standard. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography. TLC was performed on Merck silica gel 60 plates. Sesquiterpene

lactone glycosides isolated previously in this laboratory and their chemical transformation products were used as authentic samples for comparison (co-TLC, colour reactions, IR, MS) with known compounds. The roots of *Crepis setosa* Hall. were collected in August 1984 from flowering cultivated plants at the garden of medicinal plants of the Institute of Pharmacology, Polish Academy of Sciences, in Kraków.

EXTRACTION AND ISOLATION

Dried and ground plant material (157 g) was extracted with ethanol at room temperature with stirring. After filtration, the solvent was removed under vacuum to yield 10 g of crude syrup. The syrup was pre-adsorbed on silica gel and fractionated by column chromatography (silica gel, benzene-ethyl acetate mixtures followed by chloroform-methanol mixtures). Seventy fractions (100 ml each) were collected and monitored by TLC. Chloroform-methanol (9:1) mixtures (fractions 24-63) eluted successively sesquiterpene lactones.

Fraction 26 contained impure I which showed the same R_f value and colour reactions on TLC as the aglycone of Ia. Its acetyl derivative (acetic anhydride-pyridine, room temperature, overnight) could be purified satisfactorily on a silica gel column eluted with benzene-ethyl acetate (95:5) to give 4 mg of noncrystalline Ib whose the previously unreported ^1H NMR spectrum is listed in Table 1.

Fractions 34-36 contained amorphous glycoside IIa (9 mg) which on hydrolysis with β -glucosidase in the manner described earlier (Kisiel 1984) gave glucose identified by paper chromatography and noncrystalline aglycone II whose ^1H NMR spectrum is listed in Table 1. The physical properties of IIa were identical to those of the NaBH_4 reduction product of vernoflexuoside (Kisiel 1975), authentic sample.

Fractions 42-63 (432 mg) contained more polar glycosides of the same R_f values. The pure major component of the glycosides was obtained from the later part of the fractions and identified as Ia by direct comparison with an authentic sample of ixerin F. The identification was confirmed by hydrolysis with β -glucosidase which afforded glucose as a sugar moiety and an aglycone I (for ^1H NMR spectrum see Table 1). The earlier part of the fractions contained a mixture of two isomeric glycosides. All attempts to separate them by chromatography failed. So the mixture of both compounds was hydrolysed with β -glucosidase and the aglycones were chromatographed on a silica gel column eluted with chloroform-methanol (98:2) to give further amounts of I and III as a semicrystalline gum: IR (chloroform): 3600, 1765 cm^{-1} ; ^1H NMR: see Table 1; MS of diacetate, m/z (rel. int. %): 348 (M^+) (0.3), 306 ($\text{M}-42^+$) (13.7), 288 ($\text{M}-60^+$) (1.0), 246 ($\text{M}-60-42^+$) (9.9), 228 ($\text{M}-2 \times 60^+$) (11.7), 175 (29.8), 150 (50.7), 122 (18.2), 43 (100).

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Laktony seskwiterpenowe Crepis setosa Hall.

Streszczenie

W etanolowym wyciągu z korzeni pępowy szczeciniastej (*Crepis setosa* Hall., *Compositae*) stwierdzono obecność czterech laktonów seskwiterpenowych typu gwajanu: 9 α -hydroxy-11 β , 13-dihydrozaluzaniny C (I) i glikozydu tego związku — ikseryny F (Ia) oraz glikozydów 11 β ,13-dihydrozaluzaniny C (IIa) i 8 α -hydroxy-11 β ,13-dihydrozaluzaniny C (IIIa). Związki te wyodrębniono po raz pierwszy z badanego gatunku, a ich identyfikację przeprowadzono metodami chemicznymi i spektralnymi.