SESQUITERPENE LACTONES IN MONGOLIAN *Saussurea lipshitzii*

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Dedicated to the memory of Professor František Šorm.

Five known guaianolides were isolated from the areal part of *Saussurea lipshitzii* (*Asteraceae*) collected in South Gobi, Mongolia: cynaropicrin (*I*), janerin (*II*), chlorojanerin (*III*), 15-deschloro-15-acetoxychlorojanerin (*IV*), and 15-deschloro-15-hydroxychlorojanerin (*V*). The correlation between *I*—*V* and the β-configuration of the C-4/C-15 bond in *II*—*V* was shown by biomimetic chemical transformations, suggesting compound *I* as the possible common precursor.

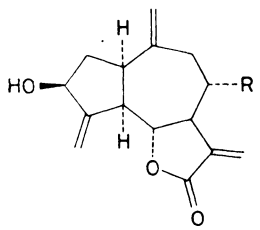
The large *Saussurea genus* (*Asteraceae*) is widely spread in Mongolia, where 42 species have been described until 1985 (ref.[1]). Only two of them — *S. salicifolia* and *S. involucrata* — have been investigated till now.[2] Their sesquiterpene lactones have already been known for 12 other *Saussurea* species from India, U.S.S.R., and Japan[3–13].

Most of the known *Saussurea* lactones are guaianolides with a C-10 exomethylene group and often bearing an oxygen function at C-3 or C-8. Specific for them are the functions connected with C-4: the 4,15-epoxide, the 4,5- or 4,15-double bond or a C-4 hydroxyl group. Reported are C-15 hydroxylated, acetoxylated and chlorinated guaianolides[5,7,11], too. Germacranolides, eudesmanolides and elemanolides have been found in *S. lappa*, *S. elongata* and *S. radix*[3,11,13]. Here we report the sesquiterpene lactones isolated from *S. lipshitzii* FILAT, collected in 1987 in South Gobi, Mongolia.

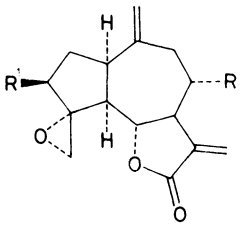
EXPERIMENTAL

Melting points were taken on a Kofler hot plate microscope and were not corrected. IR spectra were measured in KBr pellets or in layer; ^{1}H NMR (δ, J in Hz, TMS) spectra were measured in $CDCl_3$ (in $(CD_3)_2CO$ for *IV* and *VIII*) on a Bruker 400 MHz (compounds *I*—*III*) and 250 MHz (compounds *V*—*VIII*); CI MS (2-methylpropane) was measured on Jeol JMS-300. Merck's Silica 60 GF_{254} and benzene–acetone (4 : 1) mixture were used for TLC, sprayed with conc. H_2SO_4.

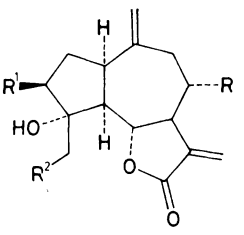
The overground part of the plants in full blossoming stage was collected in 1987 in South Gobi, dried in shadow (200 g) and extracted with 3 × 1 l ethanol at ambient temperature. After concentration i.v. and working up as usual, a crude lactone fraction (6·6 g) was isolated. A part of it (2·0 g) was separated on a Merck's Lobar (LiChroprep Si 60) column. Compounds *I*—*III* were additionally purified by PTLC of the less polar fraction of the column, while *IV* and *V* from the more polar ones. The lactones were identified by their ^{1}H NMR and m.p. data, compared with that in the references.



I, R = *a*



II, R = *a* ; R^1 = OH
VI, R = *b* ; R^1 = OAc
IX, R = *c* ; R^1 = OH



III, R = *a* ; R^1 = OH ; R^2 = Cl
IV, R = *a* ; R^1 = OH ; R^2 = OAc
V, R = *a* ; R^1 = R^2 = OH
VII, R = *b* ; R^1 = R^2 = OAc
VIII, R = *a* ; R^1 = OH ; R^2 = I

In formulae *I*–*IX*: *a* = O—CO—  , *b* = O—CO—  , *c* = O—CO— 

Compound *I*: cynaropicrin, yield 50 mg, viscous oil, R_F 0·55; compound *II*: janerin, yield 25 mg, viscous oil, R_F 0·48; compound *III*: chlorojanerin, yield 15 mg, m.p. 151—153°C, R_F 0·63; compound *IV*: 15-deschloro-15-acetoxychlorojanerin, yield 15 mg, m.p. 143—145°C ($CHCl_3$, colourless oil according to ref.[7]), R_F 0·38; compound *V*: 15-deschloro-15-hydroxychlorojanerin, yield 10 mg, viscous oil, R_F 0·25; compound *VI*: janerin diacetate, from 5 mg of *II*, 0·2 ml Ac_2O and 0·2 ml pyridine, 1 h at ambient temperature, purified by PTLC, viscous oil, CI MS: 447 (M^+, 1%); compound *VII*: 15-deschloro-15-hydroxychlorojanerin triacetate, *a*) from 5 mg of *IV*, *b*) from 5 mg of *V*, as above, viscous oil, CI MS: 507 (M^+, 1%). ^{1}H NMR (250 MHz, $CDCl_3$): 2·09 s, 3 H (OAc); 2·11, 6 H (2 OAc); 3·18 dddd, 1 H (H-7, J = 3·0, 3·5, 9·2, 9·2); 3·50 ddd, 1 H (H-1, J = 8·0, 8·0, 10·5); 4·31 d, 1 H (H-15, J = 12·0); 4·42 dd, 1 H (H-6, J = 9·2, 11·0); 4·62 d, 1 H (H-15, J = 12); 4·84 s, 2 H (H-17); 4·96 s, 1 H (H-14); 5·10 m, 2 H (H-3 and H-8); 5·17 s, 1 H (H-14); 5·67 d, 1 H (H-13, J = 3·0); 5·98 br s, 1 H (H-16); 6·26 d, 1 H (H-13, J = 3·5); 6·46 s, 1 H (H-16). Compound *VIII*: 15-iodojanerin, from 10 mg of *II*, 80 mg NaI in 1 ml water and 80 mg CH_3COONa in 1 ml CH_3COOH stirred 1 h at ambient temperature. The mixture, diluted with water, extracted with ether, washed to neutral and dried over Na_2SO_4, yielded 8 mg colourless crystals, m.p. 200—203°C. CI MS: 490 (M^+, 1%). ^{1}H NMR (250 MHz, CD_3COCD_3): 2·74 dd, 1 H (H-9, J = 5·0, 15·0); 3·20 dddd, 1 H (H-7, J = 3·0, 3·5, 9·4, 9·4); 3·63 d, 1 H (H-15, J = 10·5); 3·71 ddd, 1 H (H-1, J = 8·0, 8·0, 10·0); 4·0 d, 1 H (H-15, J = 10·5); 4·10 br d, 1 H (H-3, J = 6·0); 4·35 br s, 2 H (H-17); 4·80 br s, 1 H (H-14); 4·96 dd, 1 H (H-6, J = 9·4, 11·0); 5·11 br s, 1 H (H-14); 5·22 m, 1 H (H-8); 5·60 d, 1 H (H-13, J = 3·0); 5·98 br s, 1 H (H-16); 6·02 d, 1 H (H-13, J = 3·6); 6·27 br s, 1 H (H-16).

Janerin diacetate (*VI*) from cynaropicrin (*I*): 10 mg of *I* were acetylated as above. The product, purified by PTLC, was dissolved in 0·5 ml $CHCl_3$ and 5 mg *m*-CPBA in 0·5 ml $CHCl_3$ were added (see ref.[18]). After 20 h at 0°C the solvent was removed i.v. and the residue purified by PTLC. The product was identical with *VI*, prepared by acetylation of *II*.

15-Deschloro-15-hydroxychlorojanerin (*VI*) from janerin (*II*): a solution of 5 mg of *II* in 0·2 ml dioxane and 10 mg *p*-TSA in 0·5 ml water was stirred 15 min at 90°C. The product, purified by PTLC, was identical with *V*.

Janerin (*I*) from chlorojanerin (*III*): to 10 mg of *III*, dissolved in 0·2 ml EtOH, 2·0 ml a saturated solution of $NaHCO_3$ in EtOH–water (1 : 1) was added and left overnight. The product purified by PTLC was identical with *I*.

RESULTS AND DISCUSSION

The pure individual α-methylene-γ-lactones *I*–*V* (IR: $\tilde{\nu}$ 1 750 cm^{-1}, ^1H NMR: two vinyl doublets at δ 5·20–6·20 ppm) were isolated from the crude lactone fraction by consecutive column and PTL chromatography on silica. For all compounds was common the loss of 102 mass units ($C_4H_6O_3$ from M^+ CI MS mode) and the ^1H NMR signals at δ 5·96 (s, H), 6·35 (s, H) and 4·39 (s, 2 H), (the latter two at δ 6·25 and 4·34 for *IV*), typical for 4-hydroxymethacrylic acid. The other ^1H NMR signals evidenced a distinct structural resemblance between the sesquiterpenic skeletons of compounds *I*–*V*. Exhaustive ^1H NMR decoupling experiments established them as *trans*-6,12-guaianolides with a β-hydroxyl at C-3, an exomethylene at C-10 and an α-4-hydroxymethacrylic residue at C-8. The differences between *I*–*V* consisted of the substituents at C-4 and C-15. The compcund *I* with a C-4 exomethylene was identified as cynaropicrin[14], the 4,15-epoxide *II* – as janerin[15,16]. Compounds *III*, *IV* and *V* bear an α-hydroxyl group at C-4, but different substituents at C-15: a chlorine atom in *III* (chlorojanerin[17]), an acetoxy group in *IV* (15-deschloro-15-acetoxychlorojanerin[7]), and a hydroxyl group in *V* (15-deschloro-15-hydroxychlorojanerin[7]). The ^1H NMR data of compounds *II*–*V* corresponded well with these in the references, while *I* was identical with an authentic sample of cynaropicrin.

The literature references showed some discrepancy concerning the stereochemistry of the 4,15-epoxide in janerin (*II*). Gonzales[15] suggested a β-epoxide, accepted later by others[7,16], though with some reservations. In the meantime Stevens[18] epoxidized cynaropicrin diacetate to janerin diacetate *VI*, and assumed an α-epoxide in *II*. This stereochemistry was extended by analogy to repin (*IX*), and confirmed further by its X-ray analysis[19]. Nevertheless, Singh et al.[7] admitted a β-epoxide in *II*, i.e. an αC-4/15 bond, but in contrary they accepted a βC-4/15 bond in lactones related to *II*, however having an open epoxide ring.

The simultaneous presence of the lactones *I*–*V* in *S. lipshitzii*, which is valid for *S. candicans*[7], too, suggested links to a common precursor. This was supported by the following biomimetic transformations. The epoxide *II*, janerin, was converted to the tetrol *V*. Its triacetate *VII* was identical with the triacetate prepared by acetyla-

tion of the natural monoacetate *IV*. Acetylation of *I*, followed by epoxidation with *m*-CPBA[18] yielded the epoxidiacetate *VI*, identical with the janerin diacetate prepared from *II*. Treatment of *II* with NaI in acetic acid yielded the iodide *VIII*. Attempts for converting the iodide *VIII* to the acetate *IV* by treatment with CH_3COONa in dry acetone[20] or with CH_3COOAg in acetic acid[21] produced only an epoxide, identical in all respects with the natural janerin *II*. Hence, the compounds *II* – *VIII* have the same βC-4/15 configuration. This biomimetic transformations make very likely that the C-4 exomethylene lactone *I*, cynaropicrin, could be a common precursor of its C-4/C-15 oxygenated analogues.

It was reported recently, that repin inhibits in vitro the growth of chicken embryo neurons[19] and this was connected with the neurotic disease Equine Nigropalidal Encephalomalacia observed at horses, using food contaminated with *Acroptilion repens* (*Centaurea repens*), which contains repin. So far as one of the lactones in the Mongolian *Saussurea lipshitzii* is janerin, a C-8α-acyl analogue of repin, a similar effect of this plant on horses could be expected. This could be of importance for Mongolia with her very large horse stock.

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