SESQUITERPENIC LACTONES OF *Inula aschersoniana* Janka var. *aschersoniana* SPECIES*

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Aerial parts of species *Inula aschersoniana* Janka var. *aschersoniana* have been shown to contain parthenolide (*I*), costunolide diepoxide (*III*) and the hitherto undescribed inusoniolide (*IV*) whose structure, including the absolute configuration, was determined using ^{1}H NMR and CD spectroscopy.

Within the framework of systematic investigation on sesquiterpenic lactones in species of the *Compositae* (*Asteraceae*) family we studied aerial parts of the species *Inula aschersoniana* Janka var. *aschersoniana* (family *Compositae*, tribe *Inuleae*) which has been already shown to contain parthenolide[1] (*I*) and 8β-hydroxy-11α,13-di-hydroeremanthin[2] (*II*). From this species we now isolated costunolide diepoxide (*III*), m.p. 163−164°C, of composition $C_{15}H_{20}O_4$. According to the ^{1}H NMR and IR spectra (see Experimental), the substance isolated was identical with the compound obtained earlier from species *Magnolia grandiflora* L. (family *Magnoliaceae*)[3]. This fact was proven by preparing costunolide diepoxide (*III*) from parthenolide (*I*) using the previously described procedure[3]. The synthetic diepoxide *III* was in all respects identical with the native compound.

Another substance, which we have isolated from the described material, was a hitherto undescribed non-crystalline sesquiterpenic lactone *IV* of composition $C_{15}H_{20}O_3$, $[\alpha]_D$ −20·3°, which we named inusoniolide. Its infrared spectrum indicated the presence of a γ-lactone grouping whose carbonyl is conjugated with an exomethylene double bond (1 766, 1 406 and 1 160 cm^{-1}). The spectrum further revealed a keto group in an aliphatic chain or in a six-membered ring (1 713 cm^{-1}), and a double bond (1 666 cm^{-1}). The mass spectrum exhibited a molecular peak at

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m/z 248 and the CD spectrum showed a Cotton effect at 245 nm $(\Delta\varepsilon\ -0\cdot5)$ and at 200 nm $(\Delta\varepsilon\ +9\cdot5)$. In the ^{1}H NMR spectrum (Table I) three proton signals proved the presence of a secondary methyl group $(\delta\ 1\cdot09$ d, $J = 7\cdot5$ Hz$)$ and a methyl ketone $(\delta\ 2\cdot18$ s$)$. In the downfield part of the spectrum we found characteristic signals of an exomethylene group $(\delta\ 6\cdot14$ d, $J = 3\cdot4$ Hz and $\delta\ 5\cdot46$ d, $J = 3\cdot1$ Hz$)$, a tri-substituted double bond proton $(\delta\ 5\cdot68$ m, $J = 2\cdot8$ and $1\cdot3$ $(2\times)$ Hz$)$ and with it interacting CH—O lactone ring proton $(\delta\ 4\cdot75$ m, $J = 10\cdot3$, $2\cdot8$ and $1\cdot8$ $(2\times)$ Hz$)$. The lactone ring thus must be closed in the position neighbouring with the double bond. The remaining ten protons in the molecule appeared as two non-analyzable multiplets in the regions $\delta\ 2\cdot27-2\cdot61$ (6 H) and $\delta\ 1\cdot64-1\cdot86$ (4 H). The ^{13}C NMR spectrum (Table I) confirmed the presence of the above-mentioned structural fragments $(CH_3—CH\mathord{<}, CH_3—C(=O)—, \mathord{>}C{=}CH—$ and an exomethylene-γ-lactone) and, moreover, proved four $–CH_2$-groups. The degree of unsaturation, following from the elemental formula, together with the found structural fragments and the ^{13}C NMR spectrum, show unequivocally a bicyclic molecule. All these data enabled us to assign the 4,5-seco-guaianolide (xanthanolide) structure IV to inusoniolide.

TABLE I
^{1}H and ^{13}C NMR data of inusoniolide (IV) in CDCl$_3$

	^{1}H NMR		^{13}C NMR	
Proton	δ	$(J$ in Hz$)$	Carbon	δ
H-2			C-1	145·89 s
H-2′	2·27−2·61 m		C-2	33·01 t
H-3			C-3	41·56 t
H-3′			C-4	207·69 s
H-5	5·68 m $(J(5, 2) \approx J(5, 2') \approx J(5, 10) \approx 1\cdot3;$		C-5	123·63 d
	$J(5, 6) = 2\cdot8)$		C-6	81·31 d
H-6	4·75 m $(J(6, 5) = 2\cdot8; J(6, 7) = 10\cdot3;$		C-7	47·65 d
	$J(6, 2) \approx J(6, 10) \approx 1\cdot8)$		C-8	23·82 t
H-7	2·27−2·61 m		C-9	30·73 t
H-8			C-10	37·40 d
H-8′	1·64−1·86 m		C-11	139·88 s
H-9			C-12	170·19 s
H-9′			C-13	118·65 t
H-10	2·27−2·61 m		C-14	15·64 q
H-13	6·16 d $(J(13, 7) = 3\cdot4)$		C-15	29·90 q
H-13′	5·46 d $(J(13', 7) = 3\cdot1)$			
H-14	1·09 d $(J(14, 10) = 7\cdot5)$			
H-15	2·18 s			
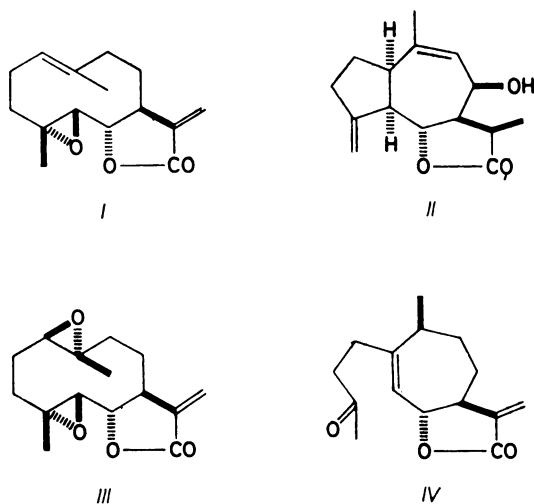
The *trans*-annelation of the lactone ring follows from the allylic coupling constants $J(7, 13) = 3.4$ Hz and $J(7, 13') = 3.1$ Hz, the vicinal coupling constant $J(6, 7) = 10.3$ Hz and from the use of the so-called lactone rule[4]. In the hitherto described xanthanolides with γ-lactone ring closed at C(6) (for a review see refs[5,6]) the lactone ring is invariably *cis*-annelated and the found values of the coupling constants $(J(6, 7) = 9-10$ Hz and $J(7, 13) \approx J(7, 13') = 2.5-3$ Hz) do not differ substantially from those found by us. However, there is a marked difference between the chemical shifts of the H-6 proton signals. Whereas in the spectra of the described *cis*-xanthan--6,12-olides the H-6 proton signal appears at about δ 5·50, in the spectrum of our compound *IV* this signal is located at δ 4·75. The upfield shift of the H-6 proton signal thus reflects the change in the annelation of the lactone ring from *cis* to *trans* and may be used as an important argument in favour of the *trans*-fusion in the lactone *IV*. Similar characteristic differences in the chemical shifts of the H-6 proton signal were observed by us for *cis*- and *trans*-2-oxo-1(10),3-dien-6,12-olides[7] and we also discussed the reasons of this phenomenon. We have no direct evidence on the configuration of the methyl group at the C(10) carbon atom in compound *IV* and the tentatively suggested β-configuration is based on analogy with the structurally closest known compounds – ivalbatin[8] (*V*) and parthemollin[9,10] (*VI*) – in which the β-configuration of the C(10) methyl has been proven by X-ray diffraction. We also used comparison of the ^{13}C NMR data of these compounds (or their acetates) for assignment of some signals in the ^{13}C NMR spectrum of inusoniolide (*IV*). In this connection, it is worth notice that comparison of ^{1}H NMR spectra of acetyl-ivalbatin[8] (*VII*) and fruticosin[12] (in which the position of the acetate group was not

V, R¹= H ; R²= OH
VI, R¹= OH ; R²= H
VII, R¹= H ; R²= OAc

VIII

IX
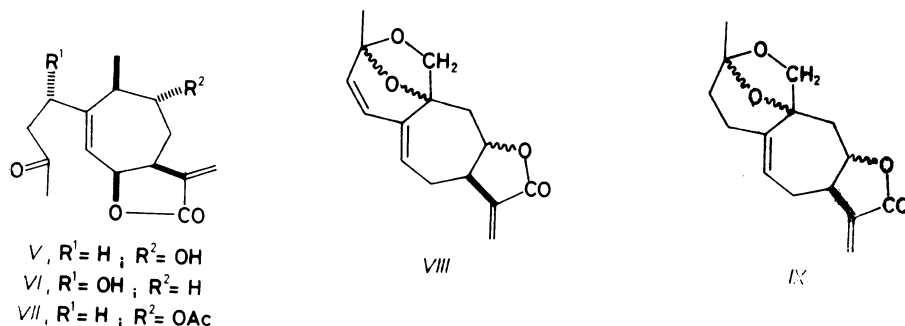
determined) shows unequivocally that both these compounds are structurally identical.

The absolute configuration at C(7) in inusoniolide (*IV*) was derived from its CD spectrum. According to the Geissman's rule[11], the Cotton effect of the exomethylene--γ-lactone grouping at 245 nm (Δε −0·5), together with the *trans*-annelation of the γ-lactone ring closed at C(6) (as indicated by the ¹H NMR spectrum), corresponds to the S-configuration at the C(7) carbon atom.

The isolation of parthenolide (*I*) and costunolide diepoxide (*III*) agrees with the common occurence of sesquiterpenic germacrane lactones in the tribe *Inuleae* and particularly in the genus *Inula*[5,6]. Xanthanolides (4,5-secoguaianolides) also occur in this taxa, although they are not so numerous as e.g. germacranolides or eudesmanolides[5,6]. However, the described xanthanolides in both taxa mentioned have the γ-lactone ring closed on C(8), mostly with the β-configuration of the C(8)—O bond. Thus, inusoniolide is the first representative of xanthanolides from *Inula* genus and *Inuleae* tribe* and the first xanthanolide with *trans*-annelation of the γ-lactone ring to C(6).

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The IR spectra were measured in chloroform on a Perkin–Elmer PE 580 spectrophotometer. The ¹H and ¹³C NMR spectra (200 MHz or 50·3 MHz, respectively) were measured on a Varian XL-200 instrument in deuterochloroform with tetramethylsilane (for ¹H) and deuterochloroform (for ¹³C; δ(CDCl₃) = 77·0) as interral reference. Chemical shifts and coupling constants of protons were obtained by first order analysis from the expanded spectra (2 Hz/cm) using exponential multiplication with gaussian apodization function for resolution enhancement. The multiplicity

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* For some species of the *Inuleae* tribe, e.g. *Geigeria africana* (p. 372), *G. aspera* (p. 373) etc., griesenin (*VIII*) and its dihydro derivative *IX* are reported[6] as xanthanolides with the γ-lactone ring closed at C(6). However, the structure of these xanthanolides clearly shows that the γ-lactone ring is closed at C(8).

of signals in ^{13}C NMR spectra was determined using the "attached proton test" pulse sequence[13]. The mass spectra were measured on an AEI MS 902 spectrometer. Optical rotation was determined on an objective polarimeter Perkin–Elmer 141 and CD spectra were measured on a Roussel––Jouan CD 185 dichrographe.

Isolation of sesquiterpenic lactones. Dry aerial parts of the species *Inula aschersoniana* JANKA var. schersoniana STOY., STEF. et KITAN (*Compositae*) (voucher RL 286/76 is deposited in the herbarium of Zakład Roślin Leczniczych, Akademia Medyczna, Poznań, Poland) (3 kg) were worked up in the described manner[14] and the obtained so-called lactone fraction (6 g) was chromatographed on a column of Kieselgel 60 (Merck, 500 g) in light petroleum–chloroform (1 : 1) and then with chloroform. The first fractions afforded non-crystalline inusoniolide (*IV*, 2·5 g); $[\alpha]_D$ −20·3° (*c* 0·37, pyridine). IR spectrum (cm^{-1}): 1 766, 1 406, 1 160 (methylene-γ--lactone); 1 713, 1 252, 1 265 (aliphatic ketone); 1 666 (double bond). Mass spectrum, *m/z*: 248 (M), 230 (M-18), 190 (M-58). CD spectrum (nm, $\Delta\varepsilon$): 245, −0·5; 200, +9·5 (last reading). For the ^{1}H and ^{13}C NMR data see Table I. For $C_{15}H_{20}O_3$ (248·3) calculated: 72·53% C, 8·12% H; found: 72·25% C, 7·94% H.

Further fractions were subjected to preparative thin-layer chromatography on Silufol in chloroform and afforded parthenolide (*I*), $C_{15}H_{20}O_3$, m.p. 109—110°C; $[\alpha]_D$ +65° (*c* 0·1, methanol), which showed no depression of mixture melting point with an authentic specimen.

Still further fractions, on column chromatography on silica gel followed by preparative thin--layer chromatography on Silufol, afforded diepoxycostunolide (*III*, 150 mg), $C_{15}H_{20}O_4$, m.p. 163—164°C; $[\alpha]_D$ −30° (*c* 0·1, methanol); no m.p. depression on admixture with costunolide diepoxide, prepared from parthenolide by a described procedure[3]. IR spectrum (cm^{-1}): 1 770 (C=O, γ-lactone); 1 663, 1 621 (C=C). Mass spectrum, *m/z*: 264 (M), 246 (M-18), 235 (M − CHO), 217 (M − CHO − 18). ^{1}H NMR spectrum: 1·35 d, 3 H (H-14, *J*(14,9) = 9·6); 1·40 s, 3 H (H-15); 2·74 m, 1 H (H-7, *J*(7, 6) = 9·2; *J*(7, 8) = 7·2; *J*(7, 8′) = 1·7; *J*(7, 13) = 3·6; *J*(7, 13′) = 3·3); 2·86 dd, 1 H (H-1, *J*(1, 2) = 9·9; *J*(1, 2′) = 1·7); 2·91 d, 1 H (H-5, *J*(5,6) = 8·6); 3·95 t, 1 H (H-6, *J*(6, 5) = 8·6; *J*(6, 7) = 9·2); 5·63 d, 1 H (H-13′, *J*(13′, 7) = 3·3); 6·35 d, 1 H (H-13, *J*(13, 7) = 3·6).

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