STRUCTURE OF ISTANBULIN A AND ISTANBULIN B — TWO SESQUITERPENIC LACTONES FROM *Smyrnium olusatrum* L.*

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The structures of istanbulin A (*I*) and istanbulin B (*III*), including absolute configurations, have been derived on the basis of spectral methods, primarily a detailed analysis of ^{1}H NMR spectra and circular dichroism measurements. Both substances belong to the type of (4*R*,5*S*,10*R*)-eremophilan-8,12-olides.

Some time ago we published a paper[1] on the isolation of istanbulin A and istanbulin B from the roots of *Smyrnium olusatrum* L. (*Umbelliferae* family, *Smyrnieae* tribe). We also derived the basic structure for istanbulin A there. At that time we had too little istanbulin B for structural studies. A new detailed analysis of 200 MHz ^{1}H NMR spectra of both substances isolated (see the data in Table I) permitted us to determine the structures of both substances, including relative configurations.

The IR spectrum of istanbulin A (*I*) indicates the presence of an α,β-unsaturated γ-lactone grouping (1 758 cm^{-1}), a keto group (1 711 cm^{-1}) and a hydroxyl (3 380 and 3 565 cm^{-1}). The UV spectrum confirmed the presence of an α,β-unsaturated carbonyl group (λ_{max} 285, log ε 1·20). The mass spectrum had characteristic peaks at m/z 264 (M) and 246 (M − 18). The CD spectrum showed the presence of an α,β-unsaturated lactone grouping (220 nm, Δε −3.4; 243 nm, Δε +4·4) and an oxo group (289 nm, Δε −0·48). The ^{1}H NMR spectrum of istanbulin A indicates the presence of two tertiary methyls — one on an sp^2 carbon (δ 1·80, doublet, J = 1·6 Hz) and the other on an sp^3 carbon (δ 0·54, doublet, J = 0·5 Hz) — further one secondary methyl (δ 1·03, doublet, J = 6·7 Hz). No signals are present in the spectrum which could be assigned to the CH—O or olefinic protons. The signal at δ 4·05 belongs to the proton of the tertiary hydroxyl group. Using decoupling experiments we were able to assign all the hydrogen atoms and confirm the correctness of the basic structure[1] (Table I). However, the determination of the relative configuration on atoms $C_{(4)}$, $C_{(5)}$, $C_{(8)}$ and $C_{(10)}$ had still to be determined. The assumption that the methyl

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TABLE I

^{1}H NMR parameters of compounds *I*, *II* and *III* in deuteriochloroform

Compound	$H_{(2)}$, $H_{(2')}$	$H_{(3)}$ eq	$H_{(3')}$ ax	$H_{(4)}$ ax	$H_{(6)}$ eq	$H_{(6')}$ ax
I	2·45 m^a	1·96 m $\sum J_{3,2}$ = 9·5^b $J_{3,3'}$ = 13·8 $J_{3,4}$ = 3·8	1·63 m $\sum J_{3',2}$ = 18·6^b $J_{3',3}$ = 13·8 $J_{3',4}$ = 12·6	2·08 ddq $J_{4,3}$ = 3·8 $J_{4,3'}$ = 12·6 $J_{4,14}$ = 6·7	2·63 d $J_{6,6'}$ = 13·3	2·32 bd $J_{6',6}$ = 13·3 $J_{6',13}$ = 1·6 $J_{6',15}$ = 0·5
II^d	2·43 m^a (−0·02)	1·97 m $\sum J_{3,2}$ = 9·5^b $J_{3,3'}$ = 13·8 $J_{3,4}$ = 3·9 (0·01)	1·66 m $\sum J_{3',2}$ = 18·4^b $J_{3',3}$ = 13·8 $J_{3',4}$ = 12·4 (0·03)	2·13 ddq $J_{4,3}$ = 3·9 $J_{4,3'}$ = 12·4 $J_{4,14}$ = 6·7 (0·05)	2·74 d $J_{6,6'}$ = 13·4 (0·11)	2·22 bd $J_{6',6}$ = 13·4 $J_{6',13}$ = 1·5 $J_{6',15}$ = 0·5 (−0·10)
III	2·45 m^a	1·96 m $\sum J_{3,2}$ = 9·2^b $J_{3,3'}$ = 14·2 $J_{3,4}$ = 3·6	1·66 m $\sum J_{3',2}$ = 18·7^b $J_{3',3}$ = 14·2 $J_{3',4}$ = 12·9	2·02 ddq $J_{4,3}$ = 3·6 $J_{4,3'}$ = 12·9 $J_{4,14}$ = 6·6	2·76 d $J_{6,6'}$ = 13·6	2·12 bd $J_{6',6}$ = 13·6 $J_{6',13}$ = 1·7 $J_{6',15}$ = 0·9

Compound	$C_{(8)}$—R ax	$H_{(9)}$ eq	$H_{(9')}$ ax	$H_{(10)}$ ax	$C_{(4)}$—CH_3 eq	$C_{(5)}$—CH_3 ax	$CH_{(11)}$—CH_3
I	4·05 bs	2·39 dd $J_{9,9'}$ = 14·3 $J_{9,10}$ = 3·4	1·75 dd $J_{9',9}$ = 14·3 $J_{9',10}$ = 12·8	2·82 bdd $J_{10,9}$ = 3·4 $J_{10,9'}$ = 12·8	1·03 d $J_{14,4}$ = 6·7	0·54 d $J_{15,6'}$ = 0·5	1·80 d $J_{13,6'}$ = 1·6
II^d	8·32 bs	2·73 m^c (0·34)	1·77 dd $J_{9',9}$ = 14·3 $J_{9',10}$ = 12·3 (0·02)	2·73 m^c (−0·09)	1·04 d $J_{14,4}$ = 6·7 (0·01)	0·59 d $J_{15,6'}$ = 0·5 (0·05)	1·87 d $J_{13,6'}$ = 1·5 (0·07)
III	4·64 bdd $J_{8,9}$ = 6·7 $J_{8,9'}$ = 11·3 $J_{8,13}$ = 1·7	2·47 m^c	1·46 ddd $J_{9',8}$ = 11·3 $J_{9',9}$ = 13·8 $J_{9',10}$ = 13·1	2·47 m^c	1·04 d $J_{14,4}$ = 6·6	0·55 d $J_{15,6'}$ = 0·9	1·82 t $J_{13,6'}$ = 1·7 $J_{13,8}$ = 1·7

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[a] Overlap of strongly coupled hydrogens $H_{(2)}$ and $H_{(2')}$; [b] only the sums of the corresponding J can be determined from the multiplets $H_{(3)}$ or $H_{(3')}$; [c] the overlap of the signals of $H_{(9)}$ and $H_{(10)}$ does not permit determination of $J_{9,10}$; [d] TAI-induced acylation shifts $\Delta\delta$ (differences of δ_H in *II versus I*) are given in brackets.

groups are *cis*-oriented as in all so far known furoeremophilanes and eremophilenolides[2] reduces the number of possible configurational types from 16 to 8, *i.e.* 4 pairs of enantiomers in which the hydrogen atom on $C_{(10)}$ and the hydroxyl on $C_{(8)}$ can be oriented *cis* or *trans* with respect to both methyl groups, *i.e. trans*, *trans*; *trans*, *cis*; *cis*, *trans*, and *cis*, *cis*. In the spectrum of istanbulin A the couplings of $H_{(4)}$ with methylene hydrogens $H_{(3)}$ and $H_{(3')}$ have J values 12·6 and 3·8 Hz, and of $H_{(10)}$ with methylene hydrogens $H_{(9)}$ and $H_{(9')}$ the values 12·8 and 3·4 Hz. This indicates clearly a rigid structure with dihedral angles of hydrogens close to 180° and 60° in both fragments. From an inspection of models* it follows that this situation requires the ring A to assume the $_1C^4$ conformation with an equatorial $C_{(4)}$-methyl and an axial $C_{(5)}$-methyl, while the conformational requirements on the ring B depend on the configurational type. In the case of the configuration *trans*, *trans*, the chair $_9C^6$ is required for the ring B, while for the type *trans*, *cis*, the boat conformation $B^{10,7}$, in the case of configuration *cis*, *cis* the chair $^9C_6$ and finally for the configuration *cis*, *trans* the boat of the type $B_{10,7}$. The signal of the angular $C_{(5)}$-methyl occurs at a considerably high field (δ 0·54), which is in agreement with the axial position and the $_1C^4$ conformation in which it comes into the shielding zone of the carbonyl group. The methyl signal is split by long-range interaction with one of the methylene hydrogens on $C_{(6)}$ (0·5 Hz). This interaction is typical of *trans*-annellated rings with a *trans*-1,2-diaxial arrangement of the angular methyl and the interacting hydrogen, and it was observed in a series of *trans*-furoeremophilanes[3], steroids[4] and triterpenes[5]. In *cis* annellated systems it may occur only in the case of the so-called non-steroidal conformation of the ring A (equatorial $C_{(5)}$-methyl and axial $C_{(4)}$-methyl), which, however, is excluded in the case of istanbulin A by the values of the coupling constants for hydrogens in the positions $C_{(4)}$, $C_{(3)}$ and $C_{(10)}$, $C_{(9)}$. Hence, the rings A and B are *trans*-annellated and the hydrogen on $C_{(10)}$ should be axial and assume *trans*-configuration with respect to the angular $C_{(5)}$--methyl group. Thus the types *cis, cis*, and *cis*, *trans* may be excluded. A detailed analysis shows that the type *trans*, *cis* is also improbable because at the given conformation $B^{10,7}$ of the ring B the hydrogens on $C_{(6)}$ assume dihedral angles of about 0° and 120° with respect to the angular methyl group. The type *trans*, *trans* remains as the sole one which fulfils all the requirements. In it the hydrogens on $C_{(3)}$, $C_{(6)}$ and $C_{(9)}$ may be assigned configurationally on the basis of the discussed interactions. The assignment is in agreement with the expected differences in chemical shifts because the signals of hydrogens in axial positions – $H_{(3')}$, $H_{(6')}$ and $H_{(9')}$ – appear at a higher field than their equatorial counterparts. The *trans*-configuration of the $C_{(8)}$-hydroxyl is also supported by the homoallylic coupling between the $C_{(11)}$-methyl and the hydrogens in the position $C_{(6)}$. This interaction is a non-zero one (1·6 Hz)

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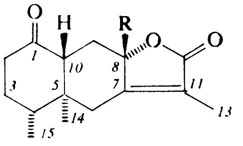
* In the following text only one enantiomeric series with α-configuration of the methyl groups on $C_{(4)}$ and $C_{(5)}$ is considered.

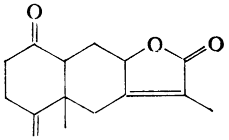
only for hydrogen $H_{(6')}$ to which we have assigned an axial position, while for $H_{(6)}$ it is equal to zero. This is in agreement with the dihedral angles of hydrogens $H_{(6')}$ and $H_{(6)}$ with respect to the plane of the double bond $C_{(7)}=C_{(11)}$, determined from the model for *trans*, *trans* type to be about 100° or 20°, respectively, and the known dependence $^5J_{\text{homoallyl}}$[6]. The acylation shifts ($\Delta\delta$) observed in the spectrum after the *in situ* reaction of istanbulin A with trichloroacetyl isocyanate[7,8] (see Table 1) are in agreement with the *trans* configuration of the $C_{(8)}$-hydroxyl. For $H_{(9)}$ the acylation shift is $\Delta\delta = 0{\cdot}34$ ppm, while for $H_{(9')}$ it is only $\Delta\delta = 0{\cdot}02$ ppm, which corresponds to the *gauche* or *trans* arrangement of OH with respect to the equatorial $H_{(9)}$ or axial $H_{(9')}$, respectively. In addition to this practically the same upfield acylation shift of $\Delta\delta = -0{\cdot}10$ ppm was observed for the axial hydrogens $H_{(10)}$ and $H_{(6')}$ which are in a similar 1,3-diaxial arrangement with the $C_{(8)}$—OH group, which is in agreement with the known change of the van der Waals effect after the acylation of the OH group. So it may be concluded that according to the ^{1}H NMR data the structural formula *I* corresponds to istanbulin A, and formula *II* to the product of its TAI acylation.

The absolute configuration of istanbulin A has been deduced from its CD spectrum which contains a maximum at 289 nm with $\Delta\varepsilon$ $-0{\cdot}48$, corresponding to $n \rightarrow \pi^*$ transition of the isolated carbonyl group. According to the octant rule and in connection with the presently derived relative configuration and conformation of the substance investigated the absolute configuration expressed by formula *I* could be proposed, *i.e.* (4*R*,5*S*,8*S*,10*R*)-1-oxo-8-hydroxyeremophil-7(11)-en-8,12-olide.

According to the IR spectrum istanbulin B (*III*) contains an α,β-unsaturated γ-lactone group (1 750 cm^{-1}), an oxo group (1 715 cm^{-1}) and a double bond (1 670 cm^{-1}). The UV spectrum confirmed the presence of an α,β-unsaturated system ($\lambda_{\max}$ 223, log ε 4·11) and a keto group ($\lambda_{\max}$ 283, log ε 1·53). The mass spectrum displayed characteristic peaks of *m*/*z* 248 (M), 137 and 57. The CD spectrum confirmed the presence of an α,β-unsaturated system (220 nm, $\Delta\varepsilon$ $-1{\cdot}69$; 238 nm, $\Delta\varepsilon$ $+5{\cdot}08$) and a keto group (290 nm, $\Delta\varepsilon$ $-0{\cdot}17$). The ^{1}H NMR spectrum of istanbulin B has similar characteristic features as istanbulin A – two tertiary methyl groups (sp^2-CH_3; δ 1·80 triplet, $J = 1{\cdot}7$ and 1·7 Hz; sp^3-CH_3: δ 0·55 doublet, $J = 0{\cdot}9$ Hz) and one secondary methyl (δ 1·04, doublet, $J = 6{\cdot}6$ Hz). Instead of the hydroxyl group signal a broad doublet of doublets at δ 4·64 appears in the spectrum. Together with further spectral data this led to the assumption of the same skeleton as in istanbulin A, but with a hydrogen on $C_{(8)}$ instead of a hydroxyl, *i.e.* to the structure *III*. The chemical shifts of corresponding hydrogens of both substances are very similar (the differences are lower than 0·1 ppm, with the exception of the hydrogens on $C_{(6)}$ and $C_{(9)}$, which are affected by the structural change on $C_{(8)}$). The comparable coupling constants also do not differ by more than 0·5 Hz. All this shows that the two substances do not differ even from the point of view of relative configurations. For the configuration on $C_{(4)}$, $C_{(5)}$ and $C_{(10)}$ the same arguments may be applied as in the case of compound *I*. The configuration on $C_{(8)}$ follows from the values of the couplings $J_{8,9} = 6{\cdot}7$ and $J_{8,9'} = 11{\cdot}3$ Hz which are explicable only for $H_{(8)}$ with a *trans* configuration with respect to the $C_{(5)}$-methyl. The atom $H_{(8)}$ then assumes an axial position and makes with the plane of the double bond $C_{(7)}{=}C_{(11)}$ a similar angle as $H_{(6)}$. In agreement with this the $C_{(11)}$-methyl gives a triplet with the same homoallylic couplings $J_{13,6'} \approx J_{13,8} \approx 1{\cdot}7$ Hz. According to the ^1H NMR data istanbulin B must thus have the structure *III*.



I, R = OH
II, R = OCONHCOCCl$_3$
III, R = H



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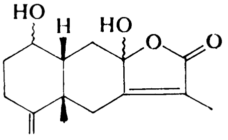

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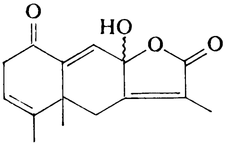

VI

We derived the absolute configuration of istanbulin B from its CD spectrum which is very similar to the spectrum of istanbulin A (*I*). On the basis of similar arguments the absolute configuration expressed by formula *III* follows for istanbulin B.

Istanbulin A (*I*) and B (*III*) are the first eremophilanolides which were identified in the species of the *Umbelliferae* family[1]. Istanbulin C (*IV*) from *Smyrnium connatum* Boiss, et Kotschy[9], istanbulin D (*V*) and istanbulin E (*VI*) from *Smyrnium apiifolium* Willd. (= *S. creticum* Mill.)[10] have been described later. According to the stereostructure istanbulin A (*I*) and B (*III*) differ from the eremophilanolides described so far (isolated from the species of the tribe *Senecioneae* of the *Compositae* family[2]) mainly by the *trans* annellation of the six-membered homo-cycles and further — from the point of view of absolute configuration — by the opposite orientation of the methyl groups on $C_{(4)}$ and $C_{(5)}$. If disregarding the stereostructure of the three-carbon side chain on $C_{(7)}$, istanbulin A (*I*) and B (*III*) may be reckoned among eremophilanolides of the nootkatane type[2,11]. Hence, the lactones *I* and *III* represent the first members of the so far undescribed stereostructural type of (4*R*,5*S*,10*R*)-eremophilan-8,12-olides.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The IR spectra were measured on a Zeiss UR 20 (Jena) spectrophotometer, in chloroform. The mass spectra were measured on an AEI MS 902 instrument, and the UV spectra on a Beckman UV, model DB spectrophotometer, in methanol. Circular dichroism was determined on a Roussel Jouan Dichrographe CD 185 in methanol and optical rotation on a objective polarimeter Perkin Elmer, in methanol. The ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, in deuteriochloroform as solvent, using tetramethylsilane as interrnal reference.

Isolation of Istanbulin A and Istanbulin B

Dry, ground roots (1·5 kg) of *S. olusatrum* L. (*Umbelliferae* family, tribe *Smyrnieae*), collected in the surroundings of Istanbul (Turkey) in 1967 (a voucher sample is deposited in the herbarium of the Farmaceutical Faculty of the University of Istanbul under the No ISTE 11013) were extracted with methanol at room temperature. The ethanolic solution was concentrated in a vacuum to a small volume and the syrupy residue was extracted with a mixture of methanol, n-hexane and water (6 : 25 : 2). The aqueous layer was separated and the substantial part of the solvent evaporated in a vacuum. The residue was extracted with chloroform and the chloroform solution was evaporated. The residue (5·0 g) was chromatographed on a silica gel column (5 × 60 cm) using a mixture of chloroform and ethyl acetate (1 : 1) for elution. From the first fractions istanbulin B was isolated, m.p. 167°C, $[\alpha]_D^{20}$ +120·6 and the composition $C_{15}H_{20}O_3$. IR spectrum (in cm^{-1}): 1 750 (α,β-unsaturated lactone), 1 715 (keto group), 1 670 (double bond). UV spectrum (nm, log ε): 223, 4·11; 283, 1·53. Mass spectrum (*m/z*): 248 (M), 137, 57. CD spectrum (nm, Δε): 220, −1·69; 238, +5·07; 290, −0·17. For $C_{15}H_{20}O_3$ (248·3) calculated: 72·55% C, 8·12% H; found: 72·82% C, 8·25% H. In further fractions istanbulin A was eluted, m.p. 246°C, $[\alpha]_D^{20}$ +81·5 and the composition $C_{15}H_{20}O_4$. IR spectrum (in cm^{-1}): 3 380, 3 565 (hydroxyl), 1 711 (ketone), 1 758 (α,β-unsaturated lactone). UV spectrum (nm, log ε): 211, 4·16; 285·, 1·20. Mass spectrum (*m/z*): 264 (M), 246 (M−17). CD spectrum (nm, Δε): 220, −3·4; 243, +4·4; 289, −0·5. For $C_{15}H_{20}O_4$ (264·3) calculated: 68·16% C, 7·63% H, 0·38% H act.; found: 68·23% C, 7·70% H, 0·54% H act.

Elemental analyses were carried out in the analytical laboratory of our Institute, under the direction of Dr J. Horáček, by Mrs V. Rusová. The IR, UV *and* CD *spectra were measured and interpreted by Dr S. Vašíčková. The mass spectra were measured and interpreted by Dr L. Dolejš. Optical rotation was measured by Mrs Z. Ledvinová. We thank them all.*

REFERENCES

1. Ulubelen A., Öksüz S., Samek Z., Holub M.: Tetrahedron Lett. *1971*, 4455.
2. Pinder A. R.: Fortschr. Chem. Org. Naturst. *34*, 114 (1977).
3. Jizba J., Samek Z., Novotný L., Najdenova E., Boeva A.: This Journal *43*, 1113 (1978).
4. Bhacca N. S., Williams D. H.: *Applications of* NMR *Spectroscopy in Organic Chemistry*, p. 118. Holden-Day, San Francisco 1964.
5. Buděšínský M.: Unpublished results.
6. Barfield M., Sternhell S.: J. Amer. Chem. Soc. *94*, 1905 (1972).
7. Goodlett V. W.: Anal. Chem. *37*, 431 (1965).
8. Samek Z., Buděšínský M.: This Journal *44*, 558 (1979).

9. Ulubelen A., Ates N., Nishida T.: Phytochemistry *18*, 338 (1979).
10. Ulubelen A., Abdolmaleky H.: Phytochemistry *21*, 2128 (1982).
11. Mac Leod W. D. jr: Tetrahedron Lett. *1965*, 4779.

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