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ISTANBULIN D AND ISTANBULIN E, TWO NEW SESQUITERPENE LACTONES FROM *SMYRNIUM CRETICUM*

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Key Word Index—*Smyrnium creticum*; Umbelliferae; sesquiterpene lactones; eremophilenolide; istanbulin D; istanbulin E.

Abstract—Two new sesquiterpene lactones, istanbulin D and istanbulin E, were isolated from the benzene and chloroform concentrates of *Smyrnium creticum*. The latter concentrate also yielded sitosterol and sitosteryl 3-β-D-glucoside. The structures of istanbulin D and E were assigned on the evidence of spectral methods.

INTRODUCTION

In a continuation of a phytochemical investigation of the genus *Smyrnium* (Umbelliferae) [1–6] we report the isolation and structure determination of two new sesquiterpene lactones of the eremophilenolide type. The benzene concentrate of the aerial parts of *Smyrnium creticum* yielded a new sesquiterpene lactone, istanbulin D, and the chloroform concentrate yielded sitosterol and sitosteryl 3-β-D-glucoside together with another new compound istanbulin E.

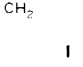
RESULTS AND DISCUSSION

Istanbulin D was identified as 1, 8-dihydroxy-4, 15, 7, 11-eremophiladien-8, 12-olide (**1**) and istanbulin E as 1-oxo-8-hydroxy-3, 7, 9-eremophilatrien-8, 12-olide (**2**) on the basis of UV, IR and ^{1}H NMR evidence and, in the case of the former compound, the ^{13}C NMR spectrum.

The UV spectrum of istanbulin D was similar to those of istanbulin A, B and C [2, 6], the λ_{max} at 221 nm being characteristic for an α, β-unsaturated γ-lactone. The IR (KBr) spectrum indicated the presence of one or more hydroxyl groups (3400 cm^{-1}), an α,β-unsaturated γ-lactone (1740 cm^{-1}), unsaturation (1690 cm^{-1}) and an exocyclic methylene (1645, 890 cm^{-1}). In the ^{1}H NMR spectrum two broad one-proton singlets at δ 4.65 and 4.85 confirmed the presence of an exocyclic methylene. The presence of two signals at 7.1 (*br. s*) and 4.6 (*br. s*), which disappeared by D_2O exchange, indicated two hydroxyl groups, while the lack of a lactonic proton signal at 4.8 suggested that one of the hydroxyl groups could be at C-8 similar to that of istanbulin A. Methyl singlets were at 0.85 (C-5) and 1.70 (C-11). The chemical shift of the C-5 methyl protons is considered direct evidence for the configuration at C-10; in *cis*-annelated systems the chemical shift of the C-5 methyl protons is of the value 0.9–1.2, while in *trans*-annelated systems it is 0.5–0.9 (in $CDCl_3$) [7, 8]. The C-5 methyl protons of istanbulin D appeared at 0.85 with an upfield solvent effect of DMSO-d_6 [9], therefore the configuration at C-10 is considered to be *cis*. The stereochemistries at C-1 and C-8 were not determined at this stage. In the mass spectrum, the molecular ion peak at m/z 264 confirmed the molecular formula $C_{15}H_{20}O_4$ and the base peak at m/z 246 $[M - H_2O]^+$ indicated the presence of a secondary alcohol group.

Noise decoupled and off-resonance ^{13}C NMR spectra, when compared to those of istanbulin C (**3**), confirmed the proposed structure. The replacement of the C-1 carbonyl signal by a doublet at 77.89 indicated a secondary hydroxyl group. When β- and γ-effects of this group were studied (Table 1) it was found that the secondary hydroxyl group could only be at the C-1 position. Other possible positions for this group could be at C-2, C-3, C-6 and C-9 which in each case would cause chemical shifts other than those given in Table 1. Due to the β-effect of this group, the C-2 triplet appeared at 48.88 instead of 39.49. The hydroxyl group also was responsible for the upfield shift of C-5 (γ-effect) to 42.38 compared with 49.14 in **3**. The doublet of **3** at 70.05 (C-8) was replaced in istanbulin D by a singlet at 105.01 thus clearly indicating the presence of a hydroxyl group in that position. This was responsible for the upfield shift of C-6 which appeared at 31.53 (*t*) instead of 37.12 (*t*) (γ-effect).

Istanbulin E was obtained only in a very small amount. Nevertheless, its structure was deduced



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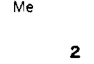

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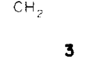

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Table 1. Comparison of ^{13}C NMR spectral data for istanbulin C and istanbulin D

	Istanbulin D (**1**)	Istanbulin C (**3**)
C-1	77.89 (*d*)	211.18 (*s*)
C-2	48.88 (*t*)	39.45 (*t*)
C-3	24.69 (*t*)	24.75 (*t*)
C-4	148.78 (*s*)	144.21 (*s*)
C-5	42.38 (*s*)	49.14 (*s*)
C-6	31.52 (*t*)	37.12 (*t*)
C-7	162.05 (*s*)	160.49 (*s*)
C-8	105.01 (*s*)	70.05 (*d*)
C-9	34.38 (*t*)	34.13 (*t*)
C-10	49.99 (*d*)	47.97 (*d*)
C-11	121.66 (*s*)	120.81 (*s*)
C-12	172.80 (*s*)	174.25 (*s*)
C-13	11.56 (*q*)	16.76 (*q*)
C-14	108.32 (*t*)	110.63 (*t*)
C-15	8.89 (*q*)	8.28 (*q*)

from the spectral data. The IR (KBr) spectrum showed one or more hydroxyl groups (3400 cm^{-1}), an α, β-unsaturated γ-lactone (1765 cm^{-1}) and a ketone (1740 cm^{-1}) as well as unsaturation (1690 cm^{-1}). The ^1H NMR spectrum taken in $CDCl_3$ indicated the presence of three methyl groups at δ 1.22 (*s*, H-5), 1.84 (*d*, $J = 2$ Hz, H-4) and 1.90 (*d*, $J = 2$ Hz, H-11), again showing the configuration of C-10 to be *cis* [7, 8]. A multiplet centered at 5.65 represented the vinylic hydrogen on C-3 coupled to the hydrogens on C-2, and a doublet ($J = 2$ Hz) at 5.34 indicated the presence of a second vinylic hydrogen. The lack of extended conjugation indicated by the UV spectrum (λ_{max} 221 nm) clearly showed that the second double bond could only be at C-9. The lack of a lactonic proton indicated that the hydroxyl group should be at C-8. In the mass spectrum, the molecular ion peak at *m/z* 260 indicated the molecular formula $C_{15}H_{16}O_4$, the fragmentation pattern was similar to those of other istanbulins.

EXPERIMENTAL

The aerial parts of *Smyrnium creticum* Mill. (Umbelliferae) were collected from the European section of Turkey (Tekirdağ) in May 1978 and identified by Prof. Dr. A. Baytop. A voucher was deposited in the Herbarium of the Faculty of Pharmacy (Istanbul) ISTE 43818. The powdered plant material (1.6 kg) was extracted in a Soxhlet with C_6H_6, $CHCl_3$ and EtOH. The C_6H_6 concentrate (98 g) was extracted with 60% aq. EtOH and the aq. EtOH was evaporated to dryness *in vacuo*. The residue (20 g) was fractionated on a Si gel (Merck) column (4 × 55 cm). The elution was initiated with $CHCl_3$ and the polarity of the solvent was increased by addition of EtOH. Istanbulin D was obtained from fractions 72–78 ($CHCl_3$–EtOH, 1:1) as a mixture which was then separated on a second Si gel column and crystallized from EtOH, the yield was 20 mg, mp 204–206°. (Found: C, 68.32; H, 7.53. $C_{15}H_{20}O_4$ requires: C, 68.18; H, 7.57%.) UV λ_{max}^{EtOH} nm: 221 (log ϵ 4.10). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200, 2960, 2860, 1740, 1690, 1645, 1440, 1330, 1275, 1240, 1130, 1080, 1020, 940, 890, 680. ^1H NMR (90 MHz, DMSO-d_6, TMS): δ 0.85 (3H, *s*), 1.7 (3H, *s*), 4.6 (1H, *br. s*, disappeared with D_2O), 4.65 (1H, *br. s*), 4.85 (1H, *br. s*), 7.1 (1H, *br. s*, disappeared with D_2O). MS *m/z* (rel. int.): 264 $[M]^+$ (10), 249 $[M-CH_3]^+$ (5), 246 $[M-H_2O]^+$ (100), 236 $[M-CO]^+$ (20), 231 $[M-CH_3-H_2O]^+$ (20), 228 $[M-2\times H_2O]^+$ (40), 218 $[M-CO-H_2O]^+$ (30), 213 $[M-CH_3-2\times H_2O]^+$ (70). ^{13}C NMR (DMSO-d_6, TMS): δ 172.8 (*s*), 162.05 (*s*), 148.78 (*s*), 121.66 (*s*), 108.32 (*t*), 105.01 (*s*), 77.89 (*d*), 49.99 (*d*), 48.88 (*t*), 42.38 (*s*), 34.38 (*t*), 31.52 (*t*), 24.69 (*t*), 11.56 (*q*), 8.89 (*q*).

Istanbulin E was obtained from the $CHCl_3$ extract (77.5 g) of the plant. Fractions 44–46 ($CHCl_3$–EtOH, 9:1) yielded 3 mg of istanbulin E, when the extract was fractionated on a Si gel column (4 × 50 cm). After EtOH crystn mp 246–248°. (Found: C, 69.28; H, 6.15. $C_{15}H_{16}O_4$ requires C, 69.22; H, 6.15%.) UV λ_{max}^{EtOH} nm: 221 (log ϵ 4.15). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1765, 1740, 1690, 1435, 1280, 1170, 1085, 1050, 980. ^1H NMR (200 MHz, $CDCl_3$, TMS): δ 1.22 (3H, *s*), 1.84 (3H, *d*, $J = 2$ Hz), 1.90 (3H, *d*, $J = 2$ Hz), 2.6 (1H, *dd*), 2.8 (1H, *dd*), 3.8 (2H, *br. s*), 5.34 (1H, *d*, $J = 2$ Hz), 5.65 (1H, *m*). MS *m/z* (rel. int.) 260 $[M]^+$ (50), 245 $[M-CH_3]^+$ (4), 242 $[M-H_2O]^+$ (6); 232 $[M-CO]^+$ (18), 217 $[M-CH_3-CO]^+$ (75).

Subsequent fractions from the same column yielded sitosterol and sitosteryl 3-β-D-glucoside, these were identified by TLC and IR comparisons with standards as well as by mmp's. In the case of the sitosteryl 3-β-D-glucoside hydrolysis (glucose and sitosterol) and acetylation were also performed.

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