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EREMOPHILENOLIDES AS OXIDATIVE ARTIFACTS OF FURANOEREMOPHILANES

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In previous studies with the roots of *Smyrnium olusatrum* (1), *Symrnium connatum* (2), and *Symrnium creticum* (3), we have only isolated eremophilenolides (istanbulin A, B, C, D, and E) using $CHCl_3$ as extraction and elution solvent, which is now known to increase the instability of furanosesquiterpenes (4, 5). Recently, we studied the fruits of *S. olusatrum* (4) and *Smyrnium cordifolium* (6) using a mixture of petroleum ether and Et_2O for extraction and elution. We obtained a furanogermacrane (glechomafuran), a furanoeremophilane (6), and eremophilenolides (7). Because the facile oxidation of furanosesquiterpenes to corresponding lactones is well known (8, 9), and in order to establish the presence or total lack of eremophilenolides as native compounds in the plant, we decided to reinvestigate the roots of *S. olusatrum*.

Fresh and dried root materials were extracted with a mixture of petroleum ether and Et_2O, and another batch of the dried root material of the same collection was extracted with $CHCl_3$. The elutions of the columns for the first two extracts were completed rather quickly; for the $CHCl_3$ extract it was much slower. We observed that the fresh roots contain only minor amounts of eremophilenolides (istanbulin A and B) while dried roots yielded four times as much of the same compounds; on the other hand, the chloroform extract yielded about 50 times as much istanbulin A and B. This observation led us to the following conclusion: eremophilenolides are present in the fresh plant in minor quantities, while drying of the plant material increases their amounts to some extent. However, solvent effects (*e.g.*, $CHCl_3$), as well as air and light exposure, cause a fast oxidation of furanosesquiterpenes to corresponding lactones, a process that accounts for most of the lactones reported previously from this plant. We suspect that many other reports of large amounts of sesquiterpene lactones from plants that produce furanosesquiterpenes may require reinvestigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded on the following instruments: uv, Varian Techtron 635; ir, Perkin-Elmer 577; ^{1}H-nmr, NT-200 MHz; ms, DuPont 21-491. Adsorbants for column chromatography and tlc were from E. Merck.

The roots of *S. olusatrum* L. (Umbelliferae) were collected from Istanbul in June 1982 when the fruits were ripe.

EXTRACTION AND ISOLATION OF THE COMPOUNDS.—Two different extraction and isolation procedures were used for fresh (500 g) and dried (200 g) root materials of *S. olusatrum*. In the first method, the plant material was macerated with petroleum ether (boiling point, 40-70°)-Et_2O (1:1) overnight, filtered, and evaporated to dryness *in vacuo* at room temperature. The residues were chromatographed on silica gel columns (2×30 cm), elution of the columns was started with petroleum ether, a gradient of Et_2O was added up to 100%, which was followed by the addition of MeOH up to 100%, and was separately completed within 3 h. The fractions thus obtained were compared with authentic samples of istanbulin A and B, and those which contained these compounds were cleaned on preparative tlc plates and identified by spectral methods. In this procedure, fresh roots yielded 3 mg of istanbulin A and 5 mg of istanbulin B, while dried roots yielded 10 mg of the former and 20 mg of the latter compounds.

In the second method, dried plant material was extracted with $CHCl_3$ in a Soxhlet; the extract was evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column (4×50 cm), a slow elution of the column was performed with $CHCl_3$, a gradient of EtOAc was added up to 100%, and the elution was completed within 2 wk. Istanbulin B (200 mg) was obtained from $CHCl_3$-EtOAc (1:1) fractions, and

istanbulin A (110 mg) was obtained from fractions of the same solvents (1:2). In addition to istanbulin A and B, glechomafuran, β-sitosterol, β-lanosterol, and α-amyrine were isolated from dry and fresh roots. All compounds were identified by uv, ir, ^{1}H-nmr, and ms spectral data, as well as by authentic sample comparisons.

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