ISOLATION OF THE CEMBRANOLIDE DITERPENES DIHYDROSINULARIN AND 11-*EPI*-SINULARIOLIDE FROM THE MARINE MOLLUSK *PLANAXIS SULCATUS*

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Marine mollusks have been identified as a rich source of novel compounds (1-3). The diversity of the chemical nature of these compounds can be partially attributed to the dietary sources (4,5) inasmuch as mollusks are known to concentrate selectively compounds present in their diet. Although cembranolide diterpenes have been isolated in ever-growing numbers from a variety of marine as well as terrestrial sources (6-8), there is only one report about their presence in a marine mollusk (9). In this communication, we report the isolation of dihydrosinularin and 11-*epi*-sinulariolide from the mollusk *Planaxis sulcatus* Born. Dihydrosinularin and 11-*epi*-sinulariolide acetate have been isolated previously from soft corals (6,10-12) which, together with gorgonians, are the richest sources of marine cembranoids (6,10-12). It should be pointed out that this is the first report on the occurrence of the parent 11-*epi*-sinulariolide in a marine invertebrate. It is expected these diterpenes are of dietary origin although the possibility of biotransformation of a precusor by the mollusk cannot be ruled out at this time. Both dihydrosinularin and 11-*epi*-sinulariolide have been reported to have marginal antineoplastic activity against P-388 lymphocytic leukemia (12).

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus. Spectra were recorded on the following instruments: ir, Perkin-Elmer model 283; nmr, Nicolet NT 300; hplc, Waters Associates Model LC 1200 equipped with a 10 μ silica gel cartridge in a Radial Compression Module (RCM 100) and a model 401 differential refractometer.

EXTRACTION AND ISOLATION.—*P. sulcatus* (Phylum: Mollusca; Family Planaxidae) was collected at Heron Island on the Great Barrier Reef of Australia. The iPrOH extract of the mollusk after concentration followed by lyophilization gave a residue, which was subjected to a series of partitioning between hexanes, CCl_4, $CHCl_3$ versus increasing concentrations of H_2O in MeOH (12). The residue from the $CHCl_3$ fraction upon chromatography on Sephadex LH-20 [MeOH-CH_2Cl_2 (9:1 v/v) as eluting solvent] followed by hplc on silica gel (1% MeOH in CH_2Cl_2) gave two crystalline compounds.

Dihydrosinularin.—Mp 110-112°, $[\alpha]_D = -44$ (lit. mp 112°, $[\alpha]_D = -45°$). We confirmed the structure by direct comparison with an authentic sample using accepted techniques (mixed mp, ir, ^1H nmr, ms).

11-Epi-*sinularoilide.*—Crystallized from MeOH, mp 175-177°; ^1H nmr (300 MHz, $CDCl_3$) δ 1.20 (3H, s, 20-Me), 1.34 (3H, s, 18-Me), 1.58 (3H, s, 19-Me), 2.93 (1H, dd, J=8.2,2.8 Hz, 3-H), 4.08 (1H, m, 11-H), 5.14 (1H, bd, J=2 Hz, 7-H), 5.42, and 6.20 (1H each, s, 17α-H, 17β-H); ^{13}C nmr 206.0 (C15), 144.3 (C16), 134.7 (C8), 126.6 (C17), 124.1 (C7), 86.9 (C11), 68.7 (C12), 63.8 (C3), 60.4 (C4), 38.1 (C5), 35.8 (C1), 35.1 (C9), 33.0 (C13), 31.6 (C6), 31.3 (C14), 26.9 (C2), 24.9 (C10), 22.8 (C20), 15.9 (C18), 15.19 (C19). Acetate: mp 163-165°. The mp, ^1H nmr, ^{13}C nmr, COSY, and CSCM, of the acetate were indistinguishable from that of an authentic sample[1] (6).

Full details of the isolation and identifications and COSY and CSCM spectra are availble upon request from the senior author.

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[1]Gary E. Martin, University of Houston, 1984, unpublished data.

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MAJOR FLAVONOIDS OF *TEPHROSIA NUBICA*

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Tephrosia Pres. (Leguminosae-Papilionoideae) is a large tropical and subtropical genus estimated to contain about three hundred species (1). *Tephrosia* has been used medicinally and as a fish poison (2). Chemical studies on a number of species have revealed the presence of rotenoids (3,4) and a range of isoflavones (5-8), flavanones/chalcones (9-13), flavonols (14-16), and flavones; prominent among the flavones is a group of 5,7-oxygenated (17-19) and 7-oxygenated (20-24) compounds characterized by the occurrence of a C-8 prenyl unit and prenylated flavan (25).

We undertook the chemical investigation of *Tephrosia nubica* (Boiss) Baker.

EXPERIMENTAL

PLANT MATERIAL.—*T. nubica* was collected from Gabal Elba at the boundry between Egypt and Sudan and authenticated by Dr. Lofty Boulos, Professor of Taxonomy at the National Research, Cairo, Egypt.

EXTRACTION AND ISOLATION OF THE FLAVONOIDS.—Air-dried and powdered herb material of *T. nubica* (150 g) was defatted in a continuous extraction apparatus with petroleum ether. The defatted powder was then exhaustively extracted with MeOH in a Soxhlet apparatus. The alcoholic extract was concentrated under reduced pressure, and the resulting gum was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$ residue (1.8 g) was subjected to flash column chromatography (54 g of silica gel), eluting with C_6H_6, C_6H_6-MeOH (99:1), (98:2), (97:3), (96:4), and (95:5). Fractions (50 ml) were collected by utilizing the distinctive fluorescence of the components as shown by tlc [$CHCl_3$-MeOH (19:1) and C_6H_6-EtOAc (8:3) as solvent systems]. The least polar compounds obtained from the above column were further purified by silica gel ptlc using the Chromatotron with $CHCl_3$-MeOH (99:1) and (98:2) as solvent systems. Complete purification of the compounds was achieved by semipreparative hplc (10×250 mm silica gel column, 5μ Supelco, hexane-EtOH, 4:1) which gave 10 mg of semiglabrin (21), 5 mg of pseudosemiglabrin (23), 30 mg of apollinine (23), and 50 mg of laneolatin (20). The structures of all compounds were determined by spectral analysis (ir, ms, and ^1H nmr) as well as comparison with published data.

Full details of the isolation and identification of the compounds are available on request from BBJ.