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TOXICANTS FROM MANGROVE PLANTS, V. ISOLATION OF THE PISCICIDE, 2-HYDROXY-5-METHOXY-3-UNDECYL-1,4 BENZOQUINONE (5-*O*-METHYLEMBELIN) FROM *AEGICERAS CORNICULATUM*[1]

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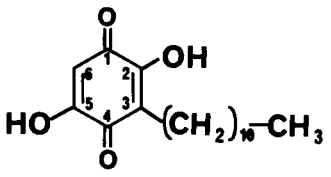
ABSTRACT.—Extracts of the twigs and stems of the mangrove plant *Aegiceras corniculatum* demonstrated toxicity to fish (*Tilapia nilotica*). 5-*O*-Methylembelin was isolated and was shown to be toxic to fish at a concentration of 1 ppm within a period of 75 min. The structure of 5-*O*-methylembelin was determined by a study of spectroscopic properties and comparison with an authentic synthetic sample.

Approximately one-fourth of the world's tropical coastline is dominated by mangroves, an association of trees including *Aegiceras* spp. They are found in the tidal mudflats where the water is brackish, which is usually where streams and rivers join the sea. Mangrove species are easily distinguished by their intricate clumps of roots that grow extensively and support the upper plant parts a meter or two above the water. In the Philippines, more than 140,000 hectares of mangrove swamps are in existence. Ecologically, mangroves are important in maintaining and building the soil, as a reservoir in the tertiary assimilation of wastes, and in the global cycle of certain gases. They also serve as a natural habitat for wildlife, birds, and shellfish.

Aegiceras corniculatum Blanco (syn. *Aegiceras majus*) is a shrub or small tree that is commonly known in the Philippines as "saging-saging" and belongs to the family Aegiceritaceae (1,2). The presence of saponins and diterpenes in *A. corniculatum* was reported by a number of investigators (3–6). Examination of an orange pigment by Hensens and Lewis (7) yielded the hydroquinones embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) [**1**] and rapanone (3,5-dihydroxy-3-tridecyl-1,4-benzoquinone) [**2**]. There was no biological activity reported for these components. An ethno-



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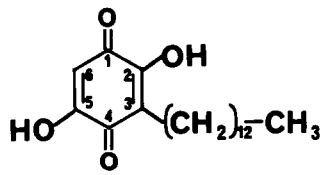

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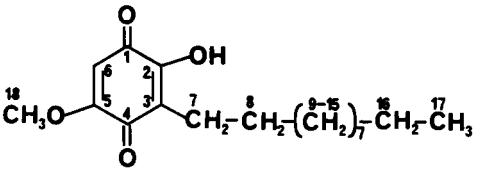
[1]For Part IV, see D.H. Miles, A.A. de la Cruz, A.M. Ly, D.S. Lho, E.D. Gomez, J.A. Weeks, and J. Atwood, "Toxicants from Mangrove Plants," ACS Symposium Series No. 330, American Chemical Society, Chicago, 1985, pp. 491–495.

botanical survey of mangrove vegetation (8) in Southeast Asia revealed that the leaves and bark of *A. corniculatum* contained toxic chemicals. Juvenile fish (*Tilapia nilotica*) were killed instantly when exposed to extracts of this species (9).

This paper reports the presence of the piscicide 2-hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone (5-*O*-methylembelin) [**3**] in *A. corniculatum*. This is the first report of 5-*O*-methylembelin from nature. Initial studies on *A. corniculatum* (9) indicated that the highest level of toxicity to fish resided in the aqueous fraction from the 95% EtOH extract of the roots and the petroleum ether extract of the stems/twigs. Priority was given to examination of the stems and twigs since they were more readily available.



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5-*O*-Methylembelin [**3**] was isolated as orange crystals (mp 95–96°) by utilization of preparative tlc with the same solvent system. Low resolution ms gave a molecular ion of $[M]^+$ 308 ($C_{18}H_{28}O_4$). The fragmentation pattern obtained was characteristic of a parent 1,4-benzoquinone. The fragment ion at *m/z* 280 could result from the loss of one molecule of carbon monoxide. Similarly, fragment ions at *m/z* 93, 83, 69, and 55 have been known to occur in various 1,4-benzoquinone derivatives. The base peak at *m/z* 168 could be derived by the loss of a decene side chain. The uv spectrum gave a broad band at λ max 285 nm, which is consistent with the absorption of a 1,4-benzoquinone substituted with hydroxy or methoxy. The ir spectrum gave absorptions for hydroxyl (3340 cm^{-1}), alkene (1640 cm^{-1}), quinoid carbonyl (1580 cm^{-1}), and methoxy (1300 cm^{-1}) functionalities. The low carbonyl absorption at 1580 cm^{-1} indicated substitution of the quinoid ring.

The presence of hydroxy and methoxy groups in compound **3** was further supported by a one-proton singlet at 3.50 ppm and a three-proton singlet at 3.85 ppm in the ^{1}H-nmr spectrum. The ^{1}H-nmr spectrum also contained signals at 0.90 ppm (t, 3H) for a methyl group, 1.30 ppm (m, 18H) for a $-(CH_2)_9-$ moiety, and 5.82 ppm (s, 1H) for a quinoid proton. The ^{13}C nmr was also consistent with the assignment of compound **3** as 5-*O*-methylembelin. The tentative assignments are given in Table 1. The signals at 180.0 and 181.0 ppm could be assigned to carbonyl groups at C-1 and C-4. No specific assignment was made.

TABLE 1. ^{13}C-nmr Spectrum of 5-*O*-Methylembelin [**3**].

Carbon	ppm	Carbon	ppm
C-17	13.4	C-6	101.0
C-16	21.2	C-3	118.0
C-9,10,11,12 13,14,15	28.0	C-2	150.0
C-8	27.0	C-5	160.0
C-7	31.0	C-1 or C-4	180.0
C-18	55.0	C-4 or C-1	181.0

The oxygen-bearing carbons at C-2 and C-5 were consistent with signals at 150.0 and 160.0 ppm. The signals at 101.0 and 118.0 ppm can be attributed to the C-6 and C-3 carbons of the quinone ring, while the absorption at 55.0 ppm can be assigned to the methoxy carbon (C-18). The signal at 31.0 ppm is consistent with an allylic carbon (C-7), and the signal at 13.4 ppm is consistent with a terminal methyl group (C-17) on a long-chain alkane. A methylene group (C-16) adjacent to a terminal methyl group is consistent with the signal at 21.2 ppm. This leaves the very large signals at 28.0 ppm to be assigned to carbons 9–15 with one remaining signal, which is assigned to C-8.

The final proof of the structure was obtained by comparison with a sample that had been synthesized from embelin by Joshi and Kamat (10). Compound **3** was shown to be identical with synthetic 5-*O*-methylembelin by mmp and comparison of the tlc and gc with an authentic sample.

The compound 5-*O*-methylembelin [**3**] demonstrated toxicity to fish (*Tilapia nilotica*) at a concentration of 1 ppm within a period of 75 min. Also the growth of the fungus *Pythium ultimum* was inhibited by the presence of 5-*O*-methylembelin (zone of inhibition = 8 mm diameter for a solution of 1 mg of compound **3** in 0.1 ml of $CHCl_3$).

EXPERIMENTAL

ISOLATION OF 5-*O*-METHYLEMBELIN [**3**].—Samples of the twigs and stems of *A. corniculatum* were collected at the Mangrove Forest Reserves of the Mangrove Research Center in Pagbilao, Quezon Province, Philippines. Voucher specimens are maintained in the herbarium. The plant material was air-dried and then ground in a Wiley mill. The stems and twigs (700 g) were successively extracted in a Soxhlet apparatus with petroleum ether, Me_2CO, and MeOH. The petroleum ether extract was selected for further examination due to its toxicity to fish in the "quick screening test (9)." Removal of the petroleum ether in vacuo produced 1.1% yield of a yellow-brown residue. The solid material was subjected to attempts at recrystallization by treatment with petroleum ether and then MeOH. A yellow solid (Fraction A) precipitated. The brown filtrate (Fraction B) obtained from the crystallization of fraction A was subjected to preparative tlc with Si gel 60–200 mesh and 10% MeOH in $CHCl_3$. The component with R_f value of 0.55 was isolated and gave compound **3** as orange crystals, mp 95–96°; ir (pellet) 3340, 1640, 1580, 1370, 1300, 1190, 1000 cm^{-1}; uv (95% EtOH) 285 nm (log $\epsilon = 2.60$); ^1H nmr ($CDCl_3$) 0.90 (t, 3H), 1.30 (broad singlet, 18H), 2.40 (t, 2H), 3.85 (s, 3H), 3.50 (s, 1H), and 5.82 (s, 1H); ^{13}C nmr ($CDCl_3$) 13.4, 21.2, 28.0, 27.0, 31.0, 55.0, 101.0, 118.0, 150.0, 160.0, 180.0, 181.0 ppm; ms *m/z* $[M]^+$ 308, with other fragments at *m/z* 93, 83, 69, 55. Compound **3** was identical by mmp, tlc, and gc comparison with an authentic sample of 5-*O*-methylembelin [**3**].

ACKNOWLEDGMENTS

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