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TOXICANTS FROM MANGROVE PLANTS, VI. HERITONIN, A NEW PISCICIDE FROM THE MANGROVE PLANT *HERITIERA LITTORALIS*[1]

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ABSTRACT.—The mangrove plant *Heritiera littoralis* has been utilized by natives of the Philippines as a fish poison. The petroleum ether extract of this plant has shown toxicity to fish (*Tilapia nilotica*). A new piscicide, which was assigned the name heritonin [**1**], has been identified as one of the compounds with ichthyotoxicity. The structure was elucidated by spectroscopic methods.

Mangrove plants provide a source of compounds having physiological activity. *Heritiera littoralis* Dryand (Sterculiaceae) is a mangrove plant native to the Philippines and other tropical countries (1). The observation that local fisherman used this plant to kill fish prompted this continuing chemoecological study of mangrove toxins in the Philippines. Initial studies of *H. littoralis* indicated that the petroleum ether extract of the roots showed toxicity to fish (*Tilapia nilotica*) within 20 min at a concentration of 100 ppm (2).

In this paper, we report the isolation and structure determination of the compound heritonin [**1**] from *H. littoralis*. Heritonin [**1**] was toxic to *T. nilotica* with 10% mortality in 12 h at a concentration of 100 ppm.

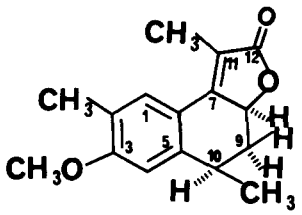
RESULTS AND DISCUSSION

Pure heritonin [**1**] was recrystallized from C_6H_6-Et_2O (1:1) as colorless long needles (mp 115–116°) from a fraction obtained by chromatography (80% C_6H_6/Et_2O) on Si gel of the petroleum-ether-soluble fraction. Fractions 1–4 were chromatographed on Si gel and eluted with 100% C_6H_6. The C_6H_6 fractions were purified by preparative tlc [$CHCl_3$-C_6H_6-MeOH (6:3:1)]. A molecular formula of $C_{16}H_{18}O_3$ was established by hrms (*m/z* $[M]^+$ 258.124, calcd 258.126) and indicated eight degrees of unsaturation. Aromaticity in the molecule was indicated by the fact that the molecular ion at *m/z* 258 was also the base peak. Furthermore, fragmentations at *m/z* 229 $[M-CO]^+$ and *m/z* 228 $[M-CHO]^+$ were typical of a phenol.

The presence of an α,β unsaturated-γ-lactone was determined by the uv (cyclohexane) absorption at 226 nm and by an ir (KBr) band at 1750 cm^{-1}. The aromatic nature of heritonin [**1**] was confirmed by the ^1H nmr ($CDCl_3$, 200 MHz) spectrum, which showed signals at δ 6.84 (s, 1H) and 7.41 (s, 1H) for two isolated protons on an aromatic ring, and also by the uv spectrum, which showed absorptions at 220, 284, and 304 nm.

The ^1H-nmr spectrum also showed evidence of three nonequivalent methyl resonances at δ 1.46 (d, 3H, $J=7$ Hz), 2.13 (s, 3H), and 2.25 (s, 3H). Two of

[1]For Part V, see E.D. Gomez, O. de la Cruz-Giron, A.A. de la Cruz, B.S. Joshi, V. Chittawong, and D.H. Miles, "Toxicants from Mangrove Plant, V: Isolation of the Principle 2-Hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone (5-*O*-methylembelin) from *Aegiceras corniculatum*." *J. Nat. Prod.*, **52**, 649 (1989).



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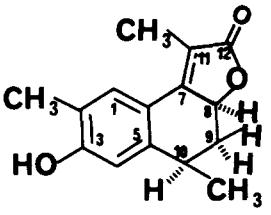

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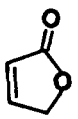

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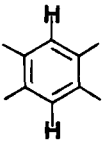

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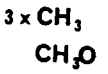

c

the resonances were singlets, which was proof of their attachment to quaternary carbons. The third methyl group with double multiplicity could be assigned to a methine carbon. The ^{1}H-nmr spectrum also gave signals for a methylene proton at δ 2.63 (m, 2H, $J = 5$ Hz), a benzylic proton at δ 3.10 (q, 1H), a proton on a carbon-bearing oxygen at δ 4.89 (dt, 1H, $J = 3$ Hz) and a methoxy proton at δ 3.90 (s, 3H). The data therefore indicated that the molecule contained the following partial structures: partial structures **a** and **b** accounted for seven degrees of unsaturation, which suggested that an additional ring must be present in order to complete the unsaturation number of eight suggested by the molecule. Incorporation of partial structures **a** and **b** into the ring and consideration of the isoprene rule led to the assignment of basic skeleton of heritonin [**1**]. Further justification for this assignment was the fact that this structure contained the cadinane framework (3,4). The ir spectrum was identical with that for heritol [**2**] except for the absence of an hydroxy group band and the addition of a methoxy group (5). The mass spectrum fragmentation pattern of heritonin resembled that of heritol. The presence of the same peaks at *m/z* 229, 215, 128, 115, and 77 was particularly diagnostic of a similarity of structure between heritonin and heritol. Further justification for the structure of heritonin was the ^{1}H-nmr spectrum, which was identical upon comparison with that of heritol except for the absence of an hydroxy group and the addition of a methoxy group as shown in Table 1. Therefore, the structure can be assigned as shown for heritonin [**1**].

TABLE 1. ^{1}H-nmr Chemical Shifts (δ) of Heritonin [**1**] and Heritol [**2**] (200 MHz, $CDCl_3$).

Proton	Heritonin	Heritol
H-1	6.84 s	6.85 s
H-4	7.41 s	7.42 s
H-8	4.89 dd	4.90 dd
H-9	2.63 m	2.62 m
H-10	3.10 m	3.10 m
H-13	2.13 s	2.18 s
H-14	2.25 s	2.30 s
H-15	3.90 s (OMe)	5.22 s (OH)
H-16	1.46 d	1.42 d

There is a need for biodegradable agrochemicals that could be compatible with the environment. Toxic compounds such as heritonin [**1**] have potential as natural pesticides. Heritonin [**1**] is interesting because it possesses toxic properties and is a new structure of the cadinane sesquiterpene class with an unusual oxygenation pattern and aromatic ring. The toxicity of heritonin [**1**] to fish and related organisms is also currently under examination for the purpose of

evaluating its potential for agrochemical utilization.

EXPERIMENTAL

PLANT MATERIAL.—*H. littoralis* was collected in the Mangrove Forest Reserve in Pagbilao, Quezoa Province, The Philippines, May 1983. A voucher specimen has been deposited in the herbarium of the Marine Science Center, University of the Philippines.

ISOLATION OF HERITONIN [**1**].—The air-dried and ground roots (21 kg) were extracted with hexane in a Soxhlet apparatus for 16 h, and the solvent was evaporated in vacuo to yield 98.2 g of crude hexane extract. The fraction showed toxicity to fish (100% mortality within 20 min at a concentration of 100 ppm).

The crude hexane extract was chromatographed on a Si gel column (600 g), which was prepared in hexane. The polarity of the solvent (hexane) was increased in 10% increments by mixing with Et_2O until pure Et_2O was added. Twelve 250-ml fractions, which were eluted with C_6H_{14}-Et_2O (8:2), were collected from the Si gel column. Fractions 1–4 were combined after examination on analytical tlc [Et_2O-C_6H_{14} (80:20)]. The solvent of the combined fractions 1–4 was evaporated in vacuo to give 2.98 g of residue which was rechromatographed on a Si gel column (40 g) using C_6H_6 and C_6H_6-$CHCl_3$ (90:10→0:100) as eluents and monitored by tlc [Et_2O-C_6H_{14} (80:20)]. Five 125-ml subfractions eluted with C_6H_6 were collected and combined, and the solvent was concentrated to a small volume which, on standing for a few hours, gave crude crystalline material. These crystals were purified by preparative tlc [$CHCl_3$-C_6H_6-MeOH (6:3:1)]. Three clearly defined bands were located by uv light and separately eluted with C_6H_{14}-Et_2O (1:1). The eluted band 3 afforded 20 mg of colorless very long needle-shaped crystals: mp 115–116°; ir (KBr) 2945, 1750, 1660, 1600, 750 cm^{-1}; uv (cyclohexane) λ max 220, 226, 284, 304 nm.

Other significant peaks: hrms *m/z* (composition %) 258 (100), 243 (30), 229 (13), 215 (18), 199 (25), 187 (16), 172 (13), 128 (16), 77 (8); ^1H nmr ($CDCl_3$) δ 1.46 (d, 3H, $J = 7$ Hz), 2.13 (s, 3H), 2.25 (s, 3H), 2.63 (m, 2H), 3.10 (m, 1H), 3.90 (s, 3H), 4.89 (dd, 1H, $J = 3$ Hz), 6.84 (s, 1H), 7.41 (s, 1H).

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