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THE PISCICIDAL CONSTITUENTS OF *CALOPHYLLUM INOPHYLLUM* LINN.

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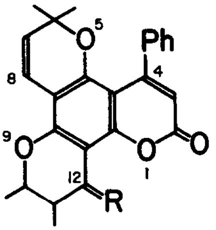
Calophyllum inophyllum Linn. (*Guttiferae*) is a tree commonly found in Southeast Asia. The seed, bark and leaf of the tree are reputed to be a fish poison.

The chemical constituents of the nuts were investigated (1) and three 4-phenylcoumarin derivatives, calophyllolide, (±)-inophyllolide and calophyllic acid were isolated and their structures established by Polonsky (2). From the leaves four triterpenes of the friedelin group were isolated by Govindachari *et al* (3).

We here wish to report the isolation and the identification of the piscicidal constituents of the leaves. The isolation of the constituents was guided by the killie-fish bioassay (4). The ether extracts obtained from the dried leaves (1.4 kg) were chromatographed on a silicic acid - celite column and eluted stepwise with benzene containing an increasing ratio of ethyl acetate. The piscicidal activity was found in the fractions eluted with 11% ethyl acetate. Further chromatography of the fractions on Florisil, followed by preparative thin layer chromatography or recrystallizations, afforded compounds I (112 mg), II (19 mg) and III (631 mg) as the active constituents.

Compounds I and II showed piscicidal activity four times stronger than III, but one-fifth as strong as pentachlorophenol.



I , II : R=O
III : R=H, OH

Compound I, $C_{25}H_{22}O_5$, m.p. 188-191°, $[\alpha]_D^{20°}$ + 13° (*c* 1.1, chloroform) showed UV absorption maxima (in ethanol) at 240 (shoulder)(ϵ 2.1x10^4), 257(sh.)(2.9x10^4), 266(3.1x10^4) and 302 mμ (2.5x10^4) and IR absorption bands (nujol mull) at 1727(lactone), 1688(aryl ketone), 767 and 703 cm^{-1}(monosubstituted benzene). Its mass spectrum showed the molecular ion peak at *m/e* 402 and prominent peaks at *m/e* 387(M-15), 331(M-15-56) and 303(M-15-56-28).

These spectral data which agree closely with those for (±)-inophyllolide, m.p. 186-188°, reported by Polonsky (5,6) indicate that I is (+)-inophyllolide. This is supported by a complete agreement of the NMR spectra in carbon tetrachloride of I and (±)-inophyllolide.

It may be noteworthy that inophyllolide has been isolated in an optically active form from the leaves, while it was obtained in optically inactive form from the nuts of the same plant.

Compound II, $C_{25}H_{22}O_5$, an isomer of I, m.p. 149-151°, $[\alpha]_D^{20°}$ +70°(*c* 1.2, chloroform) showed the same characteristic bands in the IR spectrum as I. Its UV and mass spectra were exactly similar to those of I. The NMR spectrum of II (TABLE I) differs from that of I only in the chemical shifts of the methyl protons and the protons at C-10 and C-11 and the splitting pattern of the latter. These observations enable us to conclude both compounds are the 10, 11-*cis* and *trans* isomers. Comparison of the coupling constants, $J_{10,11}$ (11.5 cps in I and 3.7 cps in II) reveals that II is the *cis* isomer while I is the *trans*. It is of some biogenetic interest that both of the *cis* and *trans* isomers have been isolated from the same plant.

TABLE I NMR Data ($CDCl_3$, 60 Mc).

Compounds	I	II	III
C-6 $(CH_3)_2$	0.95, s 0.98, s	0.97, s	0.94, s
C-11 CH_3	1.24, d, *J*=7.2	1.18, d, *J*=7.2	1.17, d, *J*=7.2
C-10 CH_3	1.56, d, *J*=6.6	1.44, d, *J*=6.8	1.43, d, *J*=7.0
C-11 H	2.59, m, *J*=7.2, 11.5	2.67, m, *J*=7.2, 3.7	2.27, m
C-10 H	4.32, m, *J*=6.6, 11.5	4.73, m, *J*=6.8, 3.7	4.43, m, *J*=7.0, 3.3
C-7 H	5.42, d, *J*=10	5.42, d, *J*=10	5.36, d, *J*=10
C-8 H	6.56, d, *J*=10	6.56, d, *J*=10	6.55, d, *J*=10
C-3 H	6.04, s	6.05, s	5.96, s
C-12 H	-----	-----	5.17, d, *J*=5.4
Phenyl H_5	7.3	7.3	7.3

Chemical shifts from TMS are expressed in ppm and coupling constants in cps.
Symbols s, d and m stand for singlet, doublet and multiplet, respectively.

Compound III, $C_{25}H_{24}O_5$, m.p. 200-202°, $[\alpha]_D^{20°}$ +43°(*c* 1.8, acetone), λ_{max}^{EtOH} (ε) 235(1.5x10^4), 280(sh.)(1.4x10^4), 286(1.5x10^4) and 337 mμ(8.9x10^3) showed IR bands at 3430(hydroxyl), 1717 (α,β-unsaturated δ-lactone), 767 and 703 cm^{-1}(monosubstituted benzene). The mass spectrum showed the intense peaks due to the ions of M-H_2O(*m/e* 386) and M-CH_3-H_2O(*m/e* 371) as well as the characteristic peaks appearing in that of I or II. Its NMR spectrum showed signals at 5.17(1H, doublet, *J*=5.4 cps, $\underline{H}-\overset{|}{\underset{|}{C}}-OH$) and at 3.32 ppm(1H, broad, hydroxyl proton) besides all the signals observed in that of II. These data suggest that III is a dihydro derivative in which the C-12 ketone of II is replaced by a secondary alcohol group. This was confirmed by the oxidation with chromic acid - pyridine which gave a ketone, m.p. 147-149°, $[\alpha]_D^{20°}$ +77° (*c* 1.1, chloroform), identical with II in respects of the mixed melting point, optical rotation and UV, IR and NMR spectra. Compound III is the first natural 4-phenylcoumarin possessing a 2,3-dimethylchromanol ring.

Studies of the absolute configuration of the dimethylchromanol ring are in progress.

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