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JUSTICIDIN A AND B, THE FISH-KILLING COMPONENTS OF *JUSTICIA HAYATAI* VAR. *DECUMBENS*

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Justicidin A and B are the active principles of *Justicia Hayatai* var. *decumbens* which has been used as a fish-killing drug for many hundred years among the natives of the Pescadores (Pung Fu islands) of Taiwan.

We have isolated them into the crystalline mixture from dried plant (yield 0.1 %) by the ethanol extraction and subsequent purification through alumina column chromatography. Repeated thin layer chromatography on silica gel could only separate the mixture into almost equal amount of justicidin A and B.

Justicidin A and B exhibited strong toxicity against *Oryzias latipes*, TLm 0.049 and 0.028 ppm after 24 hours respectively (1), which were comparable to that of rotenone and ten times stronger than that of pentachlorophenol.

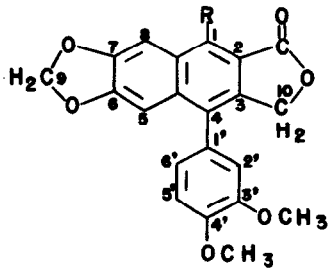
Justicidin A (I), $C_{22}H_{18}O_7$, mp 263°, no optical rotation, was a

highly unsaturated colourless compound having blue fluorescence under the ultraviolet irradiation. It contained three aromatic methoxyls (the n.m.r. spectrum), and one methylenedioxy group (chromotropic acid test (2) and the infrared spectrum, ν^{KBr} 927 cm^{-1} (3)). The α,β-unsaturated five membered lactone suggested from the infrared spectrum, ν^{KBr} 1765 cm^{-1}, was confirmed through transforming of 1 into the water-soluble carboxylate compound in the boiling conc. alkali, and again by regenerating the original one after acidification of the solution. The ultraviolet spectrum, $\lambda_{max}^{CHCl_3}$ 265, 295, 315, and 355 mμ (log ε 4.35, 4.13 4.13 and 3.33), was superimposable with that of diphyllin (11), clearly indicating that their chromophores must be same.

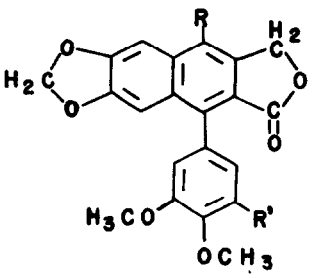
The n.m.r. spectrum (4) of 1 could allow us to define the disposition of the substituents upon a phenylnaphthalene skelton unambiguously as the proposed structure, that is, n.m.r. ($CDCl_3$) C_1-OCH_3 δ 4.09 (3 H, s), C_5-H 7.54 (1 H, s), C_8-H 7.05 (1 H, s), C_9-CH_2 6.10 (2 H, q, J=1), C_{10}-CH_2 5.52 (2 H, s), $C_{2'}$-H 6.80 (1 H, s), $C_{3'}$-OCH_3 3.80 (3 H, s), $C_{4'}$-OCH_3 4.03 (3 H, s), $C_{5'}$-H 6.90 (1 H, d, J=8), $C_{6'}$-H 6.75 (1 H, d. J=8). The two doublet signals of AB type (they are well differenciated from other signals in the mixed solvent of $CDCl_3$ and dimethylsulfoxide) could be attributed to the ortho-coupling of $C_{5'}$ and $C_{6'}$ protons, leaving only the possibility that two methoxyls should be located at $C_{3'}$ and $C_{4'}$ positions.

Finally justicidin A was compared with methyl ether of diphyllin (5) and it was concluded that they were identical on the basis of complete coincidence of their infrared spectra and of no depression of the mixed melting point. Further support upon C_4-methoxyl was obtained from the infrared absorption of diphyllin's lactone, ν^{nujol} 1705 cm^{-1}, to have

shifted to ν_{max}^{nujol} 1770 cm^{-1} after methylation (6). Since the positions of $C_{3'}$, $C_{4'}$-dimethoxyls of diphyllin had been assigned by Murakami et al (6) only from biogenetic considerations, we could have added further evidence upon diphyllin's structure also.



I, R=OCH_3
II, R=OH
III, R=H



IV, R=OH R'=OCH_3
V, R=H R'=OCH_3

Justicidin B (III), $C_{21}H_{16}O_6$, M.W. 364 (Mass spectrum), mp 240°, no optical rotation, was easily supposed from it's molecular formula to be the compound of one less methoxyl than I. The ultraviolet spectrum, $\lambda_{max}^{CHCl_3}$ 260, 295, 310, and 350 mμ (log ε 4.52, 4.13, 4.13 and 3.41), suggested that the chromophore must be same with that of I. III had a new singlet proton at δ 7.18 in place of a methoxyl at δ 4.09 in the n.m.r. spectrum, in addition to protons which were appeared similarly in that of I. The AB type of two protons, δ 6.80 and 7.00 (J=8), was also observed, indicating that two methoxyls in III, δ 3.80 and 4.03, must be located at $C_{3'}$ and $C_{4'}$ positions. Thus the structure of justicidin B could be reasonably represented as III.

Naturally occuring products having a 4-arylnaphthalene skelton are very rare, and only two, dehydropodophyllotoxin (IV) (7) and diphyllin (II), have been reported besides of our justicidins.

Justicin, $C_{16}H_{12}O_5$, mp 216°, and isojusticin, $C_{16}H_{12}O_5$, mp 252°, which have been isolated from Justicia procumbens L. var leucanta Honda by T. Tsukamoto (8), seems us to be the mixture of justicidin A and B. It is quite interesting to have found that dehydroanhydropicropodophyllin (V) (9) did not exhibit any appreciable fish-killing properties in spite of it's close structural similarity with justicidins.

Footnotes and References

(1) We wish to thank Dr. T.Tamura and Mr. K.Kimura of this faculty for the fish toxicity measurement.

(2) F.Feigle and L.Heinberger, Microchim. Acta., 806 (1955)

(3) C.K.Briggs, P.F.Highet, W.C.Wildman, J. Amer. Chem. Soc., 78 2899 (1956)

(4) N.m.r. spectra were measured at 60 Mc.; shifts are expressed as values (p.p.m.) from tetramethylsilane as internal standard; coupling constauts (J) are expressed in c.p.s.

(5) The sample of diphyllin methyl ether was kindly supplied by Dr. T.Murakami, Tokyo College of Science.

(6) T, Murakami, A. Matsushima, J. Pharm. Soc. Japan, 81, 1596 (1961)

(7) H.Kofod, C. Jørgensen, Acta. Chem. Scand., 8, 1294 (1954)

(8) T.Tsukamoto and Y.Kishimoto, J.Pharm. Soc, Japan, 75, 1565 (1955)

(9) We wish to thank Dr. A.Schrecker, National Institute of Health, U.S.A. for the gift of a sample of dehydroanhydropicropodophyllin.