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CALLICARPONE, A FISH-KILLING COMPONENT OF CALLICARPA CANDICANS

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The leaf of Callicarpa candicans (Burm. f.) Hochr. (1) has long been used for stupefying fishes by the natives of Palau and Philippine Islands.

We isolated an active principle, pale yellow needles, m.p. 111-112°, $[\alpha]_D^{23°}$ -188°(c 1.0, chloroform), from the dried leaves (1.5% yield) by ether extraction followed by treatments with acetone and n-hexane. It exhibited ten times stronger toxicity against loach fish (Misgurnus anguillicaudatus (Cantor)) than rotenone.

The formula $C_{20}H_{28}O_4$ (M.W. 332*) was assigned to the active principle, named callicarpone, for which we propose the structure I on the basis of the following evidences.

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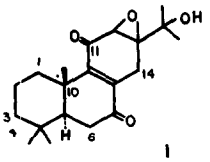
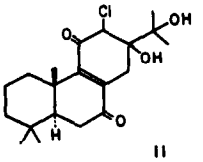
* The molecular weight was determined by the mass spectrum. The molecular ion peak did not appear, but the highest mass peak was observed at m/e 314 ($M-H_2O$).

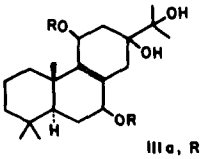
It was shown spectroscopically* that callicarpone (I) had five tertiary methyl groups (3H singlets at 0.90, 0.92, 1.18, 1.29 and 1.39 p.p.m.), a hydroxyl group ($\nu_{max}^{CCl_4}$ 3575 cm^{-1}, 1H singlet at 1.92 p.p.m. which disappeared on shaking with deuterium oxide) that should be tertiary since on treatment with acetic anhydride - sodium acetate it was readily dehydrated, ethylenic group ($\nu_{max}^{CCl_4}$ 1640 cm^{-1}, shoulder) which was tetra-substituted because of no olefinic proton signals in the NMR spectrum and more than one carbonyl group ($\nu_{max}^{CCl_4}$ 1700-1675 cm^{-1} br.). A 1H doublet at 3.71 p.p.m. (J=1.2 c.p.s.**), characteristic of a proton attached to carbon bearing oxygenated substituent, coupled with the finding that no alkoxyl and no other hydroxyl groups than the tertiary hydroxyl were involved suggested the presence of an epoxide ring. In addition the NMR spectrum showed a pair of 1H doublets at 2.43 and 3.41 p.p.m. (J=19.5 c.p.s.**), suggestive of methylene protons deshielded by carbonyl group, the more deshielded of which was further coupled through long range (J=1.2 c.p.s.**) to the proton (3.71 p.p.m.) attached to the epoxide ring.



I



II



IIIa, R=H
b, R=Ac

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* Unless otherwise stated, the nuclear magnetic resonance (NMR) spectra were taken in deuteriochloroform solution with tetramethylsilane as internal standard, and the infrared (IR) spectra were taken in nujol mull.

** These couplings were verified by spin decoupling.

Callicarpone (I), treated with pyridine hydrochloride or hydrochloric acid in dioxane gave a chlorohydrin (II), $C_{20}H_{29}ClO_4$, m.p. 213-214°, $[\alpha]_D^{23°}$ -37.6°(c 1.0, chloroform), λ_{max}^{EtOH} 265.5 mμ (ε 9.28 x 10^3), which showed IR absorption bands (chloroform) at 3617, 3535 (hydroxyl), 1707 and 1679 cm^{-1} (carbonyl) and NMR signals at 4.15 (1H doublet, J=0.8 c.p.s.; H-C-Cl), at 0.93, 0.94, 1.38 (3H singlet each) and 1.43 (6H singlet)(five H_3C-C-) and at around 3.3 and 2.2 p.p.m. (two OH).

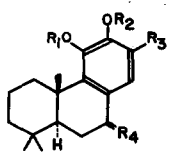
Lithium aluminum hydride reduction of I gave a tetraol (IIIa), $C_{20}H_{34}O_4$, λ_{max}^{EtOH} 210 mμ (ε 4.01 x 10^3), showing four hydroxyl proton signals and two 1H signals at 4.38 (triplet, J=8.5 c.p.s.) and 4.91 p.p.m. (multiplet) (H-C-OH) in pyridine, which on lead tetraacetate oxidation afforded acetone. Under normal acetylation conditions IIIa formed a diacetate (IIIb), which showed two 1H signals at 5.29 (triplet, J=8.5 c.p.s.) and 5.87 (multiplet) (H-C-OAc) and two hydroxyl proton signals at 2.5 and 3.0 p.p.m. This indicated that IIIa consisted of two secondary and two tertiary hydroxyl groups making 1,2-glycol.

The formation of II and IIIa confirmed that two ketone groups forming ene-1,4-dione system (λ_{max}^{EtOH} 266.5 mμ, ε 6.68 x 10^3) were present and the epoxide ring was attached to α-hydroxy isopropyl group in I. It was therefore concluded that callicarpone (I) was a tricarbocyclic diterpene.

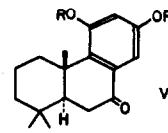
Treatment of I with sodium carbonate in methanol gave a diphenol A (IVa), $C_{20}H_{28}O_4$, (75% yield), which exhibited intense green color with ferric chloride in methanol, turning to wine-red on addition of sodium carbonate, and a di-

phenol B (Va), $C_{17}H_{22}O_3$, (10-15% yield), no color change with ferric chloride in methanol, but purple in the chloroform solution.

The NMR spectrum of IVa showed proton signals (five methyls) at 0.95, 0.97, 1.38 (3H each) and 1.68 p.p.m. (6H) which were observed also in the NMR spectrum of I, suggesting the same carbon skeleton as I was kept. The IR absorption bands at 1780 br. (phenol acetate) and 1697 cm^{-1} (aryl ketone) and the NMR signals at 2.23, 2.33 (3H singlets, acetate methyls) and 7.98 p.p.m. (1H singlet, benzene ring proton) of its acetate (IVb) enabled us to conclude that IVa was o-diphenolic diterpene ketone whose carbonyl was located in benzylic position. In the NMR spectrum of IVb instead of two downfield methyl proton signals (1.35-1.70 p.p.m.), a 3H singlet at 2.05 ($C\underline{H}_3-\overset{|}{C}=C$) and a couple of 1H multiplets at 5.08 and 5.22 p.p.m. ($C\underline{H}_2=\overset{|}{C}$) appeared. These three signals were not observed in the NMR spectrum of its dihydro-acetate (IVc), but two 3H doublets at 1.20 and 1.24 (J=7 c.p.s.) and a 1H septuplet centered at 2.94 p.p.m. (J=7 c.p.s.), attributable to isopropyl group attached to benzene ring. From these data it was apparent that α-hydroxy isopropyl group was present in IVa and consequently in I, which was dehydrated to isopropenyl group on acetylation of IVa. The structure of IVa was established by converting it to IVd, $C_{23}H_{32}O_4$, m.p. 167-169°, $[\alpha]_D^{23°}$ +43°(c 1.0, methanol) and to IVe, $C_{22}H_{34}O_2$, m.p. 89.5-91°, $[\alpha]_D^{23°}$ +100°(c 0.5, ethanol), which were identified with cryptojaponol acetate (2) and 11-methoxy ferruginol methyl ether (3), respectively by melting point, optical rotation, IR and NMR spectra.



IVa, $R_1 = R_2 = H$, $R_3 = $  , $R_4 = O$
b, $R_1 = R_2 = Ac$, $R_3 = $  , $R_4 = O$
c, $R_1 = R_2 = Ac$, $R_3 = $  , $R_4 = O$
d, $R_1 = Ac$, $R_2 = Me$, $R_3 = $, $R_4 = O$
e, $R_1 = R_2 = Me$, $R_3 = $  , $R_4 = H_2$



Va, R = H
b, R = Ac

The IR absorption bands of Va at 3230-3120 br. (hydroxyl) and 1668 cm^{-1} (conjugated carbonyl) and those of its acetate (Vb) at 1780-1765 br. (phenol acetate) and 1695 cm^{-1} (aryl ketone), in conjugation with the NMR signals at 2.24 and 2.30 (two acetate methyls), 6.99 and 7.71 p.p.m. (a pair of 1H doublets, J = 3 c.p.s., meta-coupling ring protons) revealed that Va was a m-diphenol ketone. The loss of two downfield 3H singlets (attributable to $(CH_3)_2\overset{1}{C}$-OH) indicated that acetone was lost on the reaction.

The placement of the epoxide linkage in 12, 13-position in I explained reasonably these findings as follows.
In case of a base attacking the hydrogen attached to the epoxide I was rearranged to IVa, while I furnished Va with loss of acetone in case of a base attacking the tertiary hydroxyl hydrogen.

The studies on stereochemistry of the epoxide ring are now in progress.

We wish to thank Prof. S. Takei for his constant encouragements, Prof. E. Wenkert for his supply of copies of the IR and NMR spectra of 11-methoxy ferruginol methyl ether, and Prof. T. Kondo for the authentic sample of cryptojaponol acetate. We are indebted to Dr. T. Shingu for the NMR spectra, Prof. M. Nakajima and Mr. T. Sakata for the IR spectra, Mr. A. Kato for the mass spectra and Dr. M. Inaba for collection of the plant.

References

1. This plant had been named Callicarpa cana Linn. [Car A Linne, Mantissa Plantarum, Generum Ed. VI & Specierum Ed. II, p. 198, Holmiae (1771)] before Dr.H.N.Moldenke made the name synonym.[H. N. Moldenke, A Resume of the Verbenaceae, Avicenniaceae, Stilbaceae, Symphoremaceae, Eriocaulaceae of the World as to Valid Taxa, Geographic Distribution and Synonymy. Yonkers, N.Y., U.S.A.(1959)]

2. T. Kondo, M. Suda and M. Teshima, J. Pharm. Soc. Japan, 82, 1252 (1962).

3. C. H. Brieskorn, A. Fuchs, J. B-son Bredenberg, J. D. McChesney and E. Wenkert, J. Org. Chem., 29, 2293 (1964).