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STRUCTURE AND STEREOCHEMISTRY OF HURATOXIN, A PISCICIDAL CONSTITUENT OF HURA CREPITANS

Kanzo Sakata*, Kazuyoshi Kawazu** and Tetsuo Mitsui
Department of Food Science and Technology,
Kyoto University, Kyoto, Japan.

Norio Masaki
Faculty of Pharmaceutical Science,
Kyoto University, Kyoto, Japan.

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Hura crepitans (Euphorbiaceae) has long been recognized as a dangerous plant and the sap as a fish poison in South America.[1] We isolated a piscicidal constituent and named it "huratoxin"[2] which was about 10 times as toxic as rotenone. We wish to present a preliminary report on the structure elucidation of huratoxin.

The ether extraction of the sap collected in Bolivia, followed by chromatographic purification guided by the piscicidal tests against the killie-fish (Oryzias laptipes), yielded huratoxin(I), (ca. 0.008% of the sap), glassy resin, $[\alpha]_D^{28°}$ +55.1° (c 2.69, $CHCl_3$); λ_{max}^{EtOH} 231nm(ε 28000), sh. 240nm(ε 21000); $\nu_{max}^{CCl_4}$ 3500 (OH), 1700(C=O), 1680, 1638(C=C)cm^{-1}; δ($CDCl_3$) 0.92(3H, perturbed t.), 1.18(3H, d., J=6Hz), 1.27(ca. 10H, broad s.), 1.80(6H, broad s.), 2.94(1H, d., J=2.5Hz), 3.46(1H, s.), 3.84(2H, broad s.), 3.84(1H, m.), 4.23(1H, s.), 4.45(1H, d., J=2.5 Hz), 4.92(1H, m.), 5.04(1H, m.), 5.6-6.4(3H), 6.77(1H, d.d., J=15, 8.5Hz), 7.61 ppm(1H, m.).

Chemical reactions together with spectral data clarified that huratoxin(I) has the formula $C_{34}H_{48}O_8$ and contains a 1,3-tridecadienyl, an isopropenyl, a secondary methyl, an α-methylcyclopentenone, an epoxide and three hydroxyl(pri-

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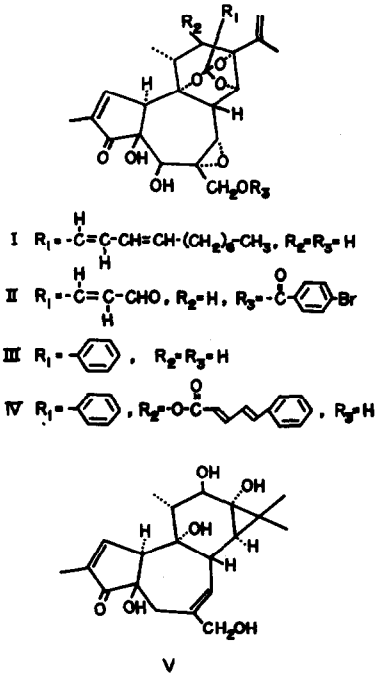
* Present address: Pesticides Synthesis Lab. 3, The Institute of Physical and Chemical Research, Wako, Saitama, Japan.

** Present address: Department of Agricultural Chemistry, Okayama University, Okayama, Japan.



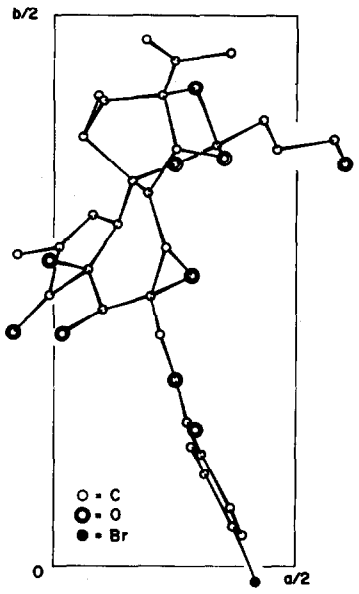


Fig. 1. The molecule of p-bromobenzoylcrepital viewed along the c axis.

mary, secondary and tertiary) groups.[2] Huratoxin(I) was elucidated to be a tetradeca-2,4-dienoic acid orthoester of a diterpene-hexaol by acid hydrolysis of I and formation of an orthoacetate of the diterpene-hexaol chlorohydrin.

It was inferred from the above mentioned structural characteristics the diterpene part of I was very similar to phorbol(V)[3] isolated from Croton tiglium (Euphorbiaceae). Although we could work out the possible structure I(apart from the stereochemistry) which well accounted for the all chemical and spectral data, the chemical evidence alone was not conclusive. It was expected to establish the structure for huratoxin by a X-ray diffraction analysis of its derivative containing a heavy atom. Huratoxin(I) was converted to a number of derivatives containing a bromine atom, but all attempts to obtain a well crystallizable derivative had failed until p-bromobenzoylcrepital was produced as follows.

Huratoxin(I) was treated with p-bromobenzoylchloride in pyridine to afford p-bromobenzoylhuratoxin, which was ozonized with one molar equivalent of ozone in ethyl acetate at -70°C. The ozonide was cleaved with triphenylphosphine and

chromatographed to yield, together with n-decanal, p-bromobenzoylcrepital(II), which was found to crystallize in colorless plates, m.p. 254-256°, Dm 1.442 $g \cdot cm^{-3}$, from ethyl acetate-n-hexane(4:1). These single crystals are orthorhombic, space group $P2_12_12_1$, with four formula units of $C_{31}H_{31}O_{10}Br$ in a unit cell of dimensions a=12.83, b=29.64, c=7.60 Å. 1704 independent reflections from equiinclination Weissenberg photographs taken along the a and c axes with CuKα radiation were measured.

The structure was solved by the heavy atom method and refined by the least squares methods to an R-factor of 18.2%. The X-ray analysis of the crystal of this derivative unambiguously established that it has the absolute stereostructure shown in II. The perspective view of the molecule along the c axis is shown in Fig. 1, where all the atoms in the asymmetric unit of the crystal, excluding hydrogen, are given.

Now, composed of crepital and n-decanal, huratoxin can be represented by the formula I. The coupling constant(J_{AB}=15Hz) of the ABX type splitting pattern of the α,β-unsaturated aldehyde group of II showed the trans-configuration of the double bond which was consistent with the result of the X-ray analysis.

Huratoxin, although unique in an orthoester form, is reminiscent of phorbol esters[4] isolated as tumor promoting substances from Croton tiglium(Euphorbiaceae). Recently from Euphorbia species was reported the isolation of the diterpenes (6,20-epoxy-lathyrol[5], 12-desoxy-phorbol[6], 16-hydroxy-12-desoxy-phorbol[7] and ingenol[8]) having structures very similar to phorbol. Moreover, quite recently daphnetoxin(III)[9] and mezerein(IV)[10] were isolated from Daphne mezereum(Thymelaeaceae) and to our surprise the diterpene part of huratoxin proved to be actually identical with that of daphnetoxin. The occurrence of the same diterpene alcohol as orthoesters in Euphorbiaceae and Thymelaeaceae species may give additional support to the possibility[9,10] of a close relationship between the two families.

The exceedingly strong toxicity of huratoxin may well be explained by this structure where the highly oxygenated diterpene moiety is bonded through orthoester function to the long aliphatic chain. The fact that this toxicity vanished on acetylation of its hydroxyl groups suggests the highly oxygenated moiety acts as an active site of the molecule. An abrupt drop(1/10000) in toxicity

caused by removal of the side chain of C_{10} suggests the long aliphatic chain also plays a significant role, particularly in making the molecule penetrate easily into the cell. The participation of the orthoester function in the toxicity seems to be worthy of further studies.

Full details of the chemical investigation will be published in Agr. Biol. Chem. and the X-ray work in Acta Cryst. B.

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