Studies on a Piscicidal Constituent of *Hura crepitans*

Part I. Isolation and Characterization of Huratoxin and its Piscicidal Activity[†]

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Huratoxin, $[\alpha]_D^{28} + 55.1$ ($c = 2.69$, CHCl_3), a piscicidal constituent, was isolated from the sap of *Hura crepitans*. It was elucidated to have the formula $\text{C}_{34}\text{H}_{48}\text{O}_8$ and contain a 1,3-trideca-dienyl, an isopropenyl, a secondary methyl, an α -methylcyclopentenone, an epoxide and three hydroxyl (primary, secondary and tertiary) groups. Its piscicidal activity was evaluated to be about 10 times that of rotenone.

Hura crepitans L. (Euphorbiaceae) is a tree originally found in the American tropics, which has many thorns on its trunk and is a good store of milky sap. It has long been recognized as a dangerous plant. Ingestion of two or three seeds produces severe emesis and purging. Contact with the plant produces severe skin irritation¹⁾ in some individuals. The milky sap was known to be a fish poison,²⁾ sometimes in America, an arrow poison.

Studies on the drastic substances of this plant had not yet given any clear-cut result until a haemoagglutinating constituent, crepitine, was

isolated from this sap by Richet,³⁾ which was characterized by Jaffé and Seidl⁴⁾ to be a kind of glycolipoprotein (LD_{50} in mice: 187 mg/kg, agglutination of rabbit blood: 30 $\mu\text{g}/\text{ml}$).

We isolated a piscicidal constituent from the sap of *Hura crepitans*, collected in Bolivia. The isolation procedure was guided by piscicidal test. The sap was continuously extracted first with ether and then with methanol. The piscicidal activity was found in both of the ether and methanol extracts, but not in the residue.

The ether extracts were chromatographed on silicic acid-Celite by stepwise elution with benzene containing an increasing ratio of ethyl acetate. The activity was found in the fraction eluted with 22.5% ethyl acetate in benzene. This fraction was further purified by chromatography on Florisil and by preparative thin-layer chromatography. A piscicidal constituent was obtained in a yield of *ca.* 0.008% of the sap used, and was named "huratoxin."

[†] This report was presented at the 14th Symposium on the Chemistry of Natural Products in Fukuoka, Oct. 29, 1970, by K. Sakata, K. Kawazu and T. Mitsui, *Symposium Papers*, 227 (1970).

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1) J. M. Kingsbury, "Poisonous Plants of the United States and Canada," Prentice-Hall, Englewood Cliffs, New Jersey, 1964, p. 190.

2) I. H. Burkill, "A Dictionary of the Economic Products of the Malay Peninsula," Vol. I, University Press, Oxford, England, 1935, p. 1223~1224.

3) C. Richet, *Compt. rend. soc. biol.*, **66**, 763 (1903) [*C.A.*, **4**, 595³ (1910)]; *ibid.*, **68**, 252 (1905) [*C.A.*, **4**, 1987³ (1910)].

4) W. G. Jaffé and D. S. Seidl, *Experientia*, **25**, 891 (1969).

The active constituent in methanol extracts was proved to be identical with the active substance isolated from the ether extracts.

The piscicidal activity of huratoxin was evaluated, by using killie-fish (*Himadaka* in Japanese, *Oryzias latipes*) as test fish, and the experimental data are shown in Table I. The 24- and 48-hr median tolerance limits estimated by straight line graphical interpolation are listed in Table II, together with those of rotenone,

TABLE I. PISCICIDAL ACTIVITY OF HURATOXIN TO KILLIE-FISH

Conc. of Huratoxin ppm (μ M)	Number of test fish added originally	Number of test fish surviving after	
		24 hr	48 hr
0.0029 (0.0050)	5	0	0
0.0022 (0.0037)	5	1	0
0.0016 (0.0027)	5	1	1
0.0012 (0.0020)	5	3	2
0.0009 (0.0015)	5	4	5
control	5	5	5

TABLE II. THE MEDIAN TOLERANCE LIMITS OF HURATOXIN, CALLICARPONE, ROTENONE AND SODIUM PENTACHLOROPHENOXIDE

	Median tolerance limit			
	24 hr		48 hr	
	ppm	μ M	ppm	μ M
Huratoxin	0.0014	0.0024	0.0011	0.0019
Callicarpone	0.042	0.13	0.024	0.072
Rotenone	0.013	0.033	0.012	0.030
Sodium penta-chlorophenoxide	0.25	0.87	0.24	0.83

callicarpone⁵⁾ and sodium pentachlorophenoxide for comparison. Huratoxin is about 10 times as toxic as rotenone to killie-fish.

Although huratoxin was not crystallizable, it was proved to be homogeneous by thin-layer chromatography on silica gel G in various de-

veloping solvent systems. It was visualized by characteristic reddish brown when sprayed with the reagent composed of ammonium metavanadate and 50% sulfuric acid. Huratoxin was proved to consist of carbon, hydrogen and oxygen by the qualitative and elemental analysis.

Huratoxin (*1*) showed* IR $\nu_{\max}$ cm^{-1} : 3500 (OH), 1700 (C=O), 1680 and 1638 (C=C), and UV $\lambda_{\max}$ nm (ϵ): 231 (28,000), and 240 (21,000) as a shoulder. Its PMR spectrum (Fig. 1) exhibited signals ascribed to *n*-alkyl group (0.92, 3H, perturbed t. and 1.27, ca. 10H, broad s.), two methyl groups on double bonds (1.80, 6H, broad s.), terminal methylene (4.92 and 5.04, 1H each, m.) and other five olefinic protons. The disappearance of signals equivalent to three protons by the addition of D₂O showed three hydroxyl groups exist in huratoxin. It is noticeable that huratoxin showed carbonyl band at 1700 cm^{-1} but no prominent band near 1250 cm^{-1} characteristic for ester function in its IR spectrum. This suggests that no ester function is present in *1*.

Catalytic hydrogenation of *1* with platinum catalyst in ethanol gave hexahydrohuratoxin (*2*). The PMR spectrum of *2*, in which a new doublet ($J=7\text{Hz}$) equivalent to six protons were observed at 0.95 ppm instead of the signals characteristic for the terminal methylene and the one vinylic methyl indicated the presence of an isopropenyl group in *1*.

On acetylation with acetic anhydride and pyridine *1* gave diacetylhuratoxin (*3*), which was reconverted to *1* by treatment with ammonia in methanol. In the PMR spectrum of *3*, the two-proton broad singlet (3.84) observed in that of *1* changed into an AB quartet (3.62 and 4.68) with a large coupling constant ($J=12\text{Hz}$), and the one-proton singlet in *1* was

* Unless otherwise stated, the UV absorption spectra were taken in ethanol and the PMR spectra in deuteriochloroform at 60 MHz. Chemical shifts were expressed in ppm from tetramethylsilane as internal standard and coupling constant in Hz. Singlet, doublet, double doublet, triplet and multiplet are abbreviated to s., d., d.d., t. and m., respectively.

5) K. Kawazu, M. Inaba and T. Mitsui, *Agr. Biol. Chem.*, **31**, 494, 498 (1967).

FIG. 1. Proton Magnetic Resonance Spectrum of Huratoxin (CDCl_3 , 60MHz).

shifted downfield by 1.3 ppm to 5.54 ppm. This observation indicates the presence of a primary and a secondary hydroxyl group as depicted (a) and (b). The existence of (b) was confirmed by taking the PMR spectrum of *1* in d_6 -DMSO. One hydroxyl proton signal of *3* which was confirmed by the addition of D_2O pointed to the presence of a tertiary hydroxyl group in *1*.

Hexahydrohuratoxin (*2*) also provided its diacetate (*4*), mp $62\sim 62.5^\circ\text{C}$, needles, $[\alpha]_D^{17} + 68.7^\circ$ ($c=1.79$, CHCl_3), by acetylation with acetic anhydride and pyridine. It was deacetylated to *2* with ammonia in methanol like diacetyl-huratoxin (*3*). Comparison of the PMR spectra of *2* and *4* also supported the presence of a primary and a secondary hydroxyl group.

Huratoxin (*1*) and its diacetate (*3*) are unstable and decomposed on exposure to air. This fact, together with their UV absorption maximum (231 nm, ϵ 28,000), suggests the existence of a conjugated diene in *1*. Di- or tri-substituted diene could be worked out for this diene by application of the Woodward rule

to this maximum. In the PMR spectrum of hexahydrohuratoxin (*2*) the four olefinic proton signals as well as the terminal methylene proton signals had disappeared. This shows that these olefinic protons are attributed to the diene, which is deduced to be di-substituted. In the PMR spectrum of *2* the singlet at 1.27 ppm attributed to polymethylene is remarkably sharpened as compared with that of *1*. On the basis of the foregoing the *n*-alkyl group is anticipated to be connected with the di-substituted diene.

The ethyl acetate solution of *3* was ozonized with two molar equivalents of ozone. The resulting ozonide was reductively degraded by catalytic hydrogenation with platinum catalyst. A volatile aldehyde was captured as its 2,4-dinitrophenylhydrazone and identified as formaldehyde. The remaining reaction mixture was concentrated and chromatographed on silicic acid-Celite. From the first eluate a straight chain aldehyde was obtained and identified as *n*-decanal by its mass spectrum and measurement of mixed melting pointed of its

2,4-dinitrophenylhydrazone.

From the latter fraction was obtained a ketoenal (5). The PMR Spectrum of 5 is similar to that of 3 except the following aspects: the signals ascribed to *n*-alkyl and isopropenyl group were not observed, but an additional acetyl group (2.30, 3H, s.) and an aldehydic proton (9.75, 1H, d.d., $J=5, 2.5\text{Hz}$). The aldehydic proton is the X part of an ABX type splitting pattern with two olefinic protons at 6.66 and 6.81 ppm. This PMR and UV (λ_{max} nm (ϵ): 212 (shoulder 13,000), 238 (7500)) spectra revealed the presence of an α,β -unsaturated aldehyde group in 5. The coupling constant ($J_{\text{AB}}=15\text{Hz}$) of the ABX type splitting pattern showed the *trans*-configuration of the double bond. The isopropenyl group was oxidized by ozone to the aforesaid formaldehyde and the additional acetyl group, and the diene to *n*-decanal and the α,β -unsaturated aldehyde group. In this partial ozonolysis were unequivocally oxidized only the one double bond of the diene and the terminal methylene. This indicates that the partial structure (c) and an isopropenyl group exist in 1.

As huratoxin (1) was not crystallizing and easily air-oxidized, it was not expected to provide a reliable elemental analytical data. Moreover no molecular ion peak was observed in the mass spectra of 1 and all of the other derivatives. The ketoenal (5), however, showed its parent peak at m/e 546. By high resolution mass spectrometry the formula of 5 was determined to be $\text{C}_{29}\text{H}_{30}\text{O}_{12}$. On the basis of the fact that the diacetylhuratoxin (3) was unambiguously degraded to the ketoenal (5), *n*-decanal and formaldehyde on the above mentioned ozonolysis, the molecular formula, $\text{C}_{34}\text{H}_{48}\text{O}_8$, was assigned to huratoxin (1). The elemental analysis of diacetyl hexahydrohuratoxin (4) also supported the molecular formula of 1.

An aldehyde hydrate (6), as well as 5, was obtained by ozonolysis with three molar equivalents of ozone. Though 6 gave a positive silver mirror reaction, it showed neither al-

dehydic proton nor olefinic protons, but a broadened one-proton multiplet at 5.2 ppm which was turned to singlet by the addition of D_2O in its PMR measurement. The aldehyde hydrate (6) was shown in its PMR spectrum to have two more hydroxyl groups than 5. The mass spectrum of 6 exhibited the highest mass peak at m/e 520 with generation of water vapor. The difference in mass units between the highest mass peaks of 5 and 6 is 26 which corresponds to C_2H_2 . These evidence clarified that 5 was further oxidized with ozone at the double bond of the α,β -unsaturated aldehyde to 6 which had a hydrated aldehyde group.

The PMR spectrum of huratoxin (1) exhibited a characteristic one-proton multiplet at 7.61 ppm. The IR and UV spectra of 1 suggested the absence of benzene or furan ring. The spindecoupling experiments disclosed that the one-proton multiplet at 7.61 ppm was related not only to the one-proton multiplet at 3.84 ppm with a coupling constant of 2.0Hz, but also to the broad singlet at 1.80 ppm assigned to a vinylic methyl group. This fact and the other spectroscopic characteristics of 2 (λ_{max} nm (ϵ): 240 (7200), ν_{max} cm^{-1} 1700, 1635) which are in good agreement with those of α -methylcyclopentenone compounds illustrated in Fig. 2 are suggestive of the presence of α -methylcyclopentenone structure. This partial structure (d) was confirmed as follows:

Huratoxin (1) absorbed four molar equivalents of hydrogen over a large amount of platinum catalyst in ethanol, yielding octahydrohuratoxin which gave triacetyl octahydrohuratoxin (7) by acetylation with acetic anhydride and pyridine. It showed no UV absorption maximum over 210 nm (ϵ 1400). In its PMR spectrum the characteristic one-proton multiplet (7.61) observed in 1 had shifted to the ordinary olefinic region (5.71), and appeared an additional acetoxyl methyl (2.02) and a proton (5.55) attached to the carbon atom bearing this acetoxyl group. Moreover the vinylic methyl suffered a high field shifts by 0.2 ppm. Its

FIG. 2.

IR spectrum showed the absence of the strong absorption bands due to the enone structure (1700, 1635 cm^{-1}). On lithium and liquid ammonia reduction diacetyl hexahydrohuratoxin (4) afforded a cyclopentanone (8) which showed no absorption maximum over 210 nm (ϵ 1000) and a carbonyl absorption band at 1755 cm^{-1} .

FIG. 3.

Diacetyl hexahydrohuratoxin (4) reacted with hydrogen bromide in acetic acid to yield its bromohydrin, which could be reconverted to

4 by being heated in aqueous ethanol. This fact suggests the presence of 1,2-oxide ring and this easy reversibility is worth noting. Similarly, triacetyl octahydrohuratoxin (7) gave the corresponding bromohydrin (9), the PMR spectrum of which exhibited a one-proton doublet at 4.97 ppm ($J=10\text{Hz}$), instead of one-proton broad singlet at 3.36 ppm. The partial structure (e) was inferred to exist in 1.

The PMR spectra of almost all derivatives of huratoxin exhibited a three-proton doublet ($J=6\sim 7\text{Hz}$) at about 1.0 ppm. This indicates the presence of a secondary methyl group in 1.

On the basis of the foregoing, it is argued that 1 should have all the functional groups illustrated in Fig. 3.

EXPERIMENTAL

Isolation of huratoxin. The milky sap of *Hura crepitans* was collected in the southern part of Bolivia. The isolation procedure was guided by the killie-fish bioassay.⁵⁾ A test solution was prepared by adding an acetone solution (0.5 ml) of material to be tested into aerated water (150 ml).

The sap (10 kg) was continuously extracted with ether in a continuous extractor, and the extract was concentrated to a brown residue weighing 165 g. A part (4 g) of this residue was adsorbed on silicic acid (20 g) and chromatographed on silicic acid (150 g)-Celite 545 (300 g). Each eluant (1 liter) consisted of benzene and ethyl acetate, and the ethyl acetate concentration was increased stepwise (0, 10, 15, 20, 22.5, 30%). The piscicidal activity was found in the fraction eluted with 22.5% ethyl acetate in benzene. This

6) E. Hecker, H. Bartsch, H. Bresch, M. Gschwendt, E. Härle, G. Kreibich, H. Kubinyi, H. U. Schairer, Ch. Szczepanski and H. W. Thielmann, *Tetrahedron Letters*, 3165 (1967); L. Crombie, M. L. Games and D. J. Pointer, *J. Chem. Soc. (C)*, **1968**, 1347.

7) D. Taub, R. D. Hoffsommer, H. L. Slates, C. H. Kuo and N. L. Wendler, *J. Am. Chem. Soc.*, **82**, 4012 (1960).

8) W. E. Doering, M. R. Willcott, III and M. Jones, Jr., *J. Am. Chem. Soc.*, **84**, 1224 (1962).

fraction was further chromatographed on Florisil 70 times as much as the sample, and eluted stepwise with benzene containing an increasing ratio (5% step) of ethyl acetate. Then huratoxin could be obtained in nearly pure state. If further purification was needed, it could be purified by silicic acid column or preparative thin-layer chromatography (benzene-ethyl acetate, 1:1). Huratoxin (1) showed a single spot on silica gel G(E. Merck, 0.25 mm thick) thin-layer chromatography using various developing solvent systems [benzene-ethyl acetate (1:1) (*R_f* 0.5), chloroform-ethyl acetate (1:1) (*R_f* 0.5), benzene-ether (1:2) (*R_f* 0.5), benzene-acetone (5:1) (*R_f* 0.6)], but tailing on aluminum oxide G. Visualization of the spot was effected by spraying with a solution of ammonium metavanadate (2.0 g) in 50% sulfuric acid (50 ml) (characteristic reddish brown spot) or 5% potassium permanganate in water. Huratoxin (1): glassy resin, $UV\lambda_{max}nm(\epsilon)$: 231 (28,000), shoulder 240 (21,000); IR $\nu_{max}(CCl_4)cm^{-1}$: 3500 (OH), 1700 (C=O), 1680, 1638 (C=C); PMR, see Fig. 1.

Piscicidal activity. *Oryzias latipes* (killie-fish, *hime-daka* in Japanese) averaging 350 mg in weight and 3~3.5 cm in length were used as the test fish, which were not fed for two days before they were used in a test. As the test containers 200 ml beakers served, in which 150 ml of water was kept. The water must be aerated by means of an air-pump for a few hours. An acetone solution (0.5 ml) of a test compound of a known concentration was added with vigorous stirring. Five test fish were introduced to the test solution. The solution prepared by adding only 0.5 ml of acetone served as control. Fish having died during the test were immediately removed from the solution and those which have been surviving in each test container were observed and recorded exactly 24 hr and 48 hr after their introduction. The experimental data are listed in Table I. The median tolerance limits were estimated by a straight-line graphical interpolation using survival percentages at two successive concentration of the test series which were lethal to more than half and to less than half of the test fish. The experimental data for toxicity of callicarpone, rotenone and sodium pentachlorophenoxide to the killie-fish are listed for comparison with that of huratoxin in Table II. But the test condition is different, that is, the test solution in the case of callicarpone *etc.* are composed of 10 liter of water and an acetone solution (1 ml) of a test compound of a known concentration (in the case of sodium pentachlorophenoxide, methanol solution was used).

Hexahydrohuratoxin (2). A mixture of PtO_2 (10 mg) and ethanol (12 ml) was prehydrogenated until no further uptake of hydrogen occurred. A solution of huratoxin (73.0 mg) in ethanol (2 ml) was added and the hydrogenation was continued at atmospheric pressure and room temperature until 3 molar equivalents of hydrogen was absorbed (40 min). The catalyst was filtered off and the filtrate was evaporated to yield a colorless resinous matter (71 mg). It was chromatographed on a silicic acid-Celite column (benzene-ethyl acetate) to yield 2(68 mg): $UV\lambda_{max}nm(\epsilon)$: 242 (7200); IR $\nu_{max}(CCl_4)cm^{-1}$: 3550 (OH), 1700 (C=O), 1635 (C=C); PMR δ 0.92 (3H, perturbed t.), 0.92 (6H, d., *J*=7Hz), 1.14 (3H, d., *J*=6Hz), 1.27 (s., polymethylene), 1.80 (3H, m.), 2.80 (1H, d., *J*=2.5Hz), 3.48 (1H, s.), 3.74 (1H, m.), 3.80 (2H, broad s.), 4.24 (1H, s.), 4.36 (1H, d., *J*=2.5Hz), 7.60 (1H, m.).

Diacetylhuratoxin (3). Acetylation of huratoxin (268 mg) with acetic anhydride and pyridine in a usual manner furnished 3(260 mg) after chromatography on silicic acid-Celite 545 (1:1) (benzene-ethyl acetate); $UV\lambda_{max}nm(\epsilon)$: 231 (28,000); IR $\nu_{max}(CCl_4)cm^{-1}$: 3580 (OH), 1760, 1746, 1710 (C=O), 1671, 1632 (C=C); PMR δ 0.92 (3H, perturbed t.), 1.15 (3H, d., *J*=6Hz), 1.27 (s., polymethylene), 1.77 (3H, broad s.), 1.81 (3H, m.), 2.03, 2.20 (3H each, s.), 3.02 (1H, d., *J*=2.5Hz), 3.09 (1H, s., OH), 3.38 (1H, s.), 3.62 (1H, d., *J*=12Hz), 3.95 (1H, m.), 4.43 (1H, d., *J*=2.5Hz), 4.68 (1H, d., *J*=12Hz), 4.92 (1H, m.), 5.04 (1H, m.), 5.54 (1H, s.), 5.6~6.7 (4H), 7.52 (1H, m.).

Diacetylhexahydrohuratoxin (4). Hexahydrohuratoxin (2) (20 mg) was acetylated with acetic anhydride (0.5 ml) in pyridine (0.5 ml) at room temperature overnight. The reaction mixture was poured onto ice-water and extracted with ether. The ether layer was washed with dil. HCl, dil. Na_2CO_3 and water saturated with NaCl, dried over anhydrous sodium sulfate, and then the solvent was evaporated. The residue was purified with preparative thin-layer chromatography (benzene-ethyl acetate, 2:1) to yield 4(20 mg). It was crystallized from 85% ethanol: mp 62~62.5°C (needles); $[\alpha]_D^{17} + 68.7^\circ$ ($c=1.79$, $CHCl_3$); $UV\lambda_{max}nm(\epsilon)$: 240 (9500); IR $\nu_{max}(CCl_4)cm^{-1}$: 3500 (OH), 1760, 1727, 1703 (C=O), 1632 (C=C), 1240 (-O-C=O); PMR δ 0.92 (3H, perturbed t.), 0.93 (6H, d., *J*=6Hz), 1.14 (3H, d., *J*=6Hz), 1.27 (s., polymethylene), 1.77 (3H, m.), 2.05, 2.20 (3H each, s.), 2.88 (1H, d., *J*=2.5Hz), 3.05 (OH), 3.36 (1H, s.), 3.60 (1H, d., *J*=12Hz), 3.88 (1H, m.), 4.32 (1H, d., *J*=2.5Hz), 4.69 (1H, d.,

$J=12\text{Hz}$), 5.52 (1H, s.), 7.49 (1H, m.). Found: C, 67.2; H, 8.8. Calcd. for $\text{C}_{38}\text{H}_{58}\text{O}_{10}$: C, 67.63; H, 8.86%.

Ozonolysis of diacetylthuratoxin (3). A solution of diacetylthuratoxin (3) (200 mg) in ethyl acetate (10 ml) was cooled to -70°C with dry ice and ethanol, and treated with an ethyl acetate solution of 2 molar equivalents of ozone. Prehydrogenated platinum catalyst (50 mg) was added into the reaction mixture and hydrogenation was carried out at room temperature until the reaction mixture showed negativity against potassium iodide-starch test. Nitrogen gas was passed through the reaction mixture and volatile aldehyde was trapped as its 2,4-dinitrophenylhydrazone. It was identified as formaldehyde by its IR spectrum and measurement of mixed melting point with an authentic sample. The remaining reaction mixture was evaporated and chromatographed on silicic acid (15 g)-Celite 545 (30 g) by stepwise elution with benzene containing an increasing ratio of ethyl acetate. The first eluate was further chromatographed on silicic acid to yield *n*-decanal (25 mg). Identity with an authentic sample was established by its mass and PMR spectra and melting point of its 2,4-dinitrophenylhydrazone. The latter eluate (30% ethyl acetate in benzene) was purified by preparative thin-layer chromatography (benzene-ethyl acetate, 1:1) to yield a ketoenal (5) (102 mg): UV λ_{max} nm(ϵ): 212 (sh. 13,000), 238 (7500); IR ν_{max} (CHCl_3) cm^{-1} : 3570 (OH), 1760~1738, 1705 ($\text{C}=\text{O}$), 1635 ($\text{C}=\text{C}$); PMR δ 1.15 (3H, d., $J=6.5\text{Hz}$), 1.78 (3H, d.d., $J=2.3, 1.0\text{Hz}$), 2.02, 2.20, 2.30 (3H each, s.), 3.10 (1H, d., $J=2.5\text{Hz}$), 3.20 (OH), 3.41 (1H, s.), 3.66 (1H, d., $J=12\text{Hz}$), 3.97 (1H, m.), 4.66 (1H, d., $J=12\text{Hz}$), 4.86 (1H, d., $J=2.5\text{Hz}$), 5.56 (1H, s.), 6.66 (1H, d.d., $J=15, 5.0\text{Hz}$), 6.81 (1H, d.d., $J=15, 2.5\text{Hz}$), 7.47 (1H, m.), 9.57 (1H, d.d., $J=5.0, 2.5\text{Hz}$); high-resolution mass spectrum M^+ 546.178 (Calcd. 546.173 for $\text{C}_{27}\text{H}_{30}\text{O}_{12}$); MS m/e (relative intensity) M^+ 546(0.3), 503(0.7), 443(0.2), 426(0.2), 413(0.1), 403(0.3), 343(1.0), 301(1.5), 283(3.8), 255(3.2), 243(1.5), 229(2.5), 83(18), 69(9.0), 60(11), 55(20), 43(100), 28(29).

When three molar equivalents of ozone was introduced into the ethyl acetate solution of diacetylthuratoxin (3), an aldehyde hydrate (6) was obtained as well as the ketoenal (5): UV λ_{max} nm(ϵ): 240.5 (7000); IR ν_{max} (CHCl_3) cm^{-1} : 3570 (OH), 1760~1740, 1708 ($\text{C}=\text{O}$), 1632 ($\text{C}=\text{C}$); PMR δ 1.18 (3H, d., $J=6.5\text{Hz}$), 1.78 (3H, m.), 2.03, 2.20, 2.32 (3H each, s.), 3.10 (1H, m.), 3.22 (OH), 3.40 (1H, s.), 3.66 (1H, d., $J=12\text{Hz}$),

3.99 (1H, m.), 4.66 (1H, d., $J=12\text{Hz}$), 4.83 (1H, m.), 5.2 (1H, broad m., $+\text{D}_2\text{O}\rightarrow\text{s.}$), 5.56 (1H, s.), 7.53 (1H, m.); MS m/e : M^+ 18 520(0.4), 477(0.5), 460(0.4), 417(1.0), 400(1.6), 387(0.7), 371(0.7), 357(0.8), 346(1.5), 343(1.6), 337(1.6), 301(2.5), 283(5.1), 261(11), 255(7.4), 243(3.7), 229(3.7), 165(3.7), 151(3.8), 123(5.5), 95(5.0), 71(5.0), 69(7.5), 55(7.5), 43(100), 28(8.0).

Triacetyl octahydrohuratoxin (7). A solution of huratoxin (1) (90 mg) in ethanol (2 ml) was added to a prehydrogenated mixture of PtO_2 (30 mg) in ethanol (20 ml) was hydrogenated at atmospheric pressure and room temperature until 4 molar equivalents of hydrogen was consumed (2 hr). The catalyst was filtered off and the ethanol solution was evaporated to yield a colorless residue (89 mg). It was chromatographed on a silicic acid-Celite column (benzene-ethyl acetate) to yield octahydrohuratoxin (71 mg), which gave triacetyl octahydrohuratoxin (7) (73 mg) by acetylation with acetic anhydride-pyridine at room temperature; UV $\lambda_{\text{end ab.}}$ (ϵ_{210} 1400); IR ν_{max} (CCl_4) cm^{-1} : 3590(OH), 1750 ($\text{C}=\text{O}$, broad), 1228 ($-\text{O}-\text{C}=\text{O}$); PMR δ 0.90 (3H, perturbed t.), 0.92 (6H, d., $J=6\text{Hz}$), 1.18 (3H, d., $J=6\text{Hz}$), 1.27 (s., polymethylene), 1.60 (3H, m.), 2.02, 2.04, 2.14 (3H each, s.), 2.84 (1H, s., OH), 2.86 (1H, d., $J=2.5\text{Hz}$), 3.36 (1H, s.), 3.50 (1H, d., $J=12\text{Hz}$), 4.30 (1H, d., $J=2.5\text{Hz}$), 4.56 (1H, d., $J=12\text{Hz}$), 5.56 (1H, s.), 5.64 (1H, m.), 5.71 (1H, m.).

Lithium-liquid ammonia reduction of diacetyl hexahydrohuratoxin (4). Liquid ammonia (ca. 10 ml) was trapped by introducing ammonia gas into a vessel cooled to -70°C with dry ice and ethanol, and lithium metal was thrown into it. The lithium metal dissolved and the solution became bluish. Dry ether solution of diacetyl hexahydrohuratoxin (4) (160 mg) was added dropwise. When the bluish color had disappeared, more lithium metal was added until the solution turned faint blue. Ammonium chloride (ca. 100 mg) was added and the reaction mixture was kept at room temperature to disperse ammonia. Then, large amounts (200 ml) of ether was added and the insoluble matter was washed thoroughly with ether and filtered off. The ether layer was washed with water, dried over anhydrous sodium sulfate, and concentrated to yield resinous matter (150 mg). It was acetylated with acetic anhydride-pyridine, and chromatographed on a silicic acid (8 g)-Celite 545 (8 g) column by stepwise elution with benzene containing an increasing ratio of ether. The fraction eluted with 15% ether in benzene afforded a cyclopentanone (8) (20 mg); UV $\lambda_{\text{end ab.}}$ (ϵ_{210} 1000);

IR $\nu_{\max}$ (CCl₄) cm⁻¹: 3580 (OH), 1760, 1755, 1746 (C=O), 1240, 1120, 1045; PMR δ 0.90 (3H, perturbed t.), 0.92 (6H, d., $J=6$ Hz), 1.27 (s., polymethylene), 2.17, 2.19 (3H each, s.), 2.92 (1H, d., $J=2$ Hz), 3.08 (1H, s., OH), 3.31 (1H, s.), 3.50 (1H, m.), 3.54 (1H, d., $J=12$ Hz), 4.28 (1H, d., $J=2$ Hz), 4.62 (1H, d., $J=12$ Hz), 5.39 (1H, s.).

Bromohydrin (9) of triacetyl octahydrohuratoxin (7). To a solution of 7 (40 mg) in acetic acid (1.2 ml) was added dropwise a solution of 47% hydrobromic acid (0.7 ml) in acetic acid (0.7 ml). The reaction mixture was kept at 10~15°C for 1 hr and evaporated *in vacuo* under 30°C. The residue was chromatographed on silicic acid (2 g)-Celite 545 (2 g) by stepwise elution with *n*-hexane containing an increasing ratio of ether. From the fraction eluted with 40% ether in *n*-hexane was obtained a bromohydrin (9) (27 mg); positive to

Beilstein test; UV $\lambda_{\text{end ab.}}$ (ϵ_{210} 3500); IR $\nu_{\max}$ (CCl₄) cm⁻¹: 3590 (OH), 1765~1735 (C=O), 1630 (C=C, broad), 1225 (—O—C=O), 1075, 1030, 895; PMR δ 0.92 (3H, m.), 0.94 (6H, d., $J=6$ Hz), 1.27 (s., polymethylene), 1.60 (3H, m.), 2.03, 2.10, 2.17 (3H each, s.), 2.97, 3.50 (1H each, s., OH), 4.50 (1H, d., $J=12$ Hz), 4.78 (1H, d., $J=2.5$ Hz), 4.97 (1H, d., $J=10$ Hz), 5.07 (1H, d., $J=12$ Hz), 5.22 (1H, s.), 5.57 (1H, m.), 5.70 (1H, m.).

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