



STUDIES ON
A PISCICIDAL CONSTITUENT
OF HURA CREPITANS

by
Kanzo Sakata

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CONTENTS

I.	Introduction	
1.	Piscicidal Plants and their Active Constituents	1.
2.	Chemical Investigations on <u>Hura crepitans</u>	10.
II.	A Piscicidal Constituent of <u>Hura crepitans</u>	
1.	Isolation of Huratoxin	12.
	Experimental	14.
2.	Chemical Structure of Huratoxin	16.
2-1.	Characterization	16.
2-2.	Partial Structure	25.
2-3.	The Presence of an Orthoester	30.
2-4.	Skeleton and Structure	34.
	Experimental	38.
3.	Determination of the Absolute Stereo- structure of Huratoxin by X-ray Dif- fraction Analysis	55.
	Experimental	58.

4.	Some Interesting Aspects of Huratoxin and its Derivatives in their Reaction and Pmr Spectra	61.
	Experimental	65.
III.	Discussion on Biosynthesis of Macro- cyclic Diterpenes and Related Novel Compounds	68.
IV.	Piscicidal Activity of Huratoxin and of its Several Derivatives	74.
	Experimental	77.
V.	Summary	80.
	References	82.

I. Introduction

I-1. Piscicidal Plants and their Active Constituents^{1,2)}

There are various kinds of useful plants which have been employed for hundred years as medicines, insecticides, insect repellents, antiseptics, arrow poisons, fish poisons and so on. Up to date, extensive chemical studies have been carried out mainly on medicinal plants and insecticidal plants.

From medicinal plants a variety of cardiac glycosides and alkaloids were isolated and their structures were established. Chemical investigations on medicinal plants, however, were no so easy to reveal the actual active principles because of their complex remedial effects.

On the other hand, the bioassay method for the isolation of insecticidal constituents was standardized, and several compounds were isolated as the active constituents from the insecticidal plants. For example, rotenoids which were isolated as active principles of Derris species are the most famous natural pesticides. Subsequently mammein, quassin

and ryanodine were found to be active constituents of Mammea americana, Quassia amara and Tripterygium wilfordii, respectively. Further, many kinds of plants including the above mentioned are known to be poisonous for fish. More or less 300 species of plants are reported to have been used as fish poisons.^{3,4)} A list of piscicidal plants prepared by Dr. K.Kawazu^{1,2)} are shown in Table 1.

Though there are many kinds of piscicidal plants, a few of them have been investigated chemically and the active constituents revealed. On the other piscicidal plants, chemical investigations were carried out regardless of their piscicidal activity. The active constituents already isolated from piscicidal plants are as follows: rotenoids, coumarins, lignans, terpenoids, polyacetylenes, alkaloids, saponins, toxalbumins and so on. Here the author wishes to make a brief chemical review on the fish poisons hitherto isolated.

1) Rotenoids⁵⁾

Rotenone(48), deguelin(49) and elliptone(50) were isolated from Derris elliptica whose roots were

Table 1. Piscicidal Plants*

<u>Myricaceae</u>	<u>Myrica sapida</u>	bark
<u>Juglandaceae</u>	<u>Juglans mandshurica</u>	root, fruits
<u>Polygonaceae</u>	<u>Polygonum orientale</u>	leaves
<u>Menispermaceae</u>	<u>Anamirta paniculata</u>	fruits
	<u>Cocculus indicus</u>	root
	<u>Cissampelos pareira</u>	root
	<u>Stephania hernandifolia</u>	root
<u>Theaceae</u>	<u>Camelia drupifera</u>	
	<u>Ternstroemia</u> sp.	bark
	<u>Schima liukiensis</u>	bark
<u>Guttiferae</u>	<u>Calophyllum inophyllum</u>	leaves, fruits
	<u>C. muscigerum</u>	bark
<u>Capparidaceae</u>	<u>Gynandropsis gynandra</u>	seeds
<u>Pittosporaceae</u>	<u>Pittosporum ferrugineum</u>	leaves, fruits
<u>Leguminosae</u>	<u>Acacia catechu</u>	root
	<u>Albizzia acle</u>	bark
	<u>A. saponaria</u>	bark
	<u>A. procera</u>	bark
	<u>Entada phaseoloides</u>	fruits
	<u>Derris elliptica</u>	root
	<u>D. montana</u>	root
	<u>D. pubipetala</u>	root
	<u>D. scandens</u>	latex
	<u>D. robusta</u>	
	<u>Lonchocarpus sericeus</u>	
	<u>Milletia ichthyochtona</u>	seeds
	<u>M. sericea</u>	root
	<u>M. taiwaniana</u>	fruits
	<u>Pachyrrhizus erosus</u>	seeds
	<u>Pithecellobium ellipticum</u>	bark
	<u>Pongamia pinnata</u>	seeds, root
	<u>Robinia pseud-acasia</u>	
	<u>Tephrosia candida</u>	root, leaves
	<u>T. toxicaria</u>	bark
	<u>T. vogelii</u>	

[continued on next page]

Table 1. [continued from the preceding page]

<u>Erythroxylaceae</u>	<u>Erythroxylon cuneatum</u>	
<u>Euphorbiaceae</u>	<u>Alchornea parviflora</u>	whole plant
	<u>Cleistanthus collinus</u>	leaves
	<u>Excoecaria agallocha</u>	latex
	<u>E. cochinchinensis</u>	latex
	<u>Euphorbia antiquorum</u>	latex
	<u>E. neriifolia</u>	leaves
	<u>E. tirucalli</u>	latex
	<u>E. trigona</u>	latex
	<u>Hura crepitans</u>	latex
	<u>Jatropha curcas</u>	latex
	<u>Mallotus apelta</u>	seeds
	<u>M. philippinensis</u>	latex
	<u>Sapium indicum</u>	fruits
<u>Rutaceae</u>	<u>Acrornychia resinosa</u>	bark
	<u>A. laurifolia</u>	root
	<u>A. odorata</u>	bark
	<u>Zanthoxylum piperitum</u>	bark, fruits
<u>Meliaceae</u>	<u>Dysoxylon arborescens</u>	
	<u>Melia azedarach</u>	
	<u>Walsura piscidia</u>	bark
<u>Sapindaceae</u>	<u>Sapindus saponaria</u>	seeds, fruits
	<u>Serjania</u> sp.	
<u>Buxaceae</u>	<u>Buxus rolfiei</u>	fruits
<u>Tiliaceae</u>	<u>Grewia</u> sp.	
<u>Sterculiaceae</u>	<u>Pterospermum diversifolium</u>	root
<u>Thymelaeaceae</u>	<u>Wikstroemia ridleyi</u>	bark
<u>Flacourtiaceae</u>	<u>Casearia graveolens</u>	fruits
	<u>C. tomentosa</u>	fruits
<u>Lecythidaceae</u>	<u>Barringtonia</u> sp.	seeds, bark
<u>Umbelliferae</u>	<u>Hydrocotyle javanica</u>	leaves
<u>Primulaceae</u>	<u>Anagalis arvensis</u>	whole plant
<u>Myrsinaceae</u>	<u>Maesa cumingii</u>	leaves
	<u>M. pyrifolia</u>	bark
	<u>Aegiceras corniculatum</u>	bark

[continued on next page]

Table 1. [continued from the preceding page]

<u>Ebenaceae</u>	<u>Diospyros ebenaster</u>	immature fruits
	<u>D. lucida</u>	fruits
	<u>D. wallichii</u>	fruits
<u>Styracaceae</u>	<u>Styrax japonica</u>	fruits
<u>Apocynaceae</u>	<u>Thevetia peruviana</u>	wood
<u>Rubiaceae</u>	<u>Gardenia currani</u>	fruits
	<u>Randia dumetorum</u>	immature fruits
<u>Verbenaceae</u>	<u>Callicarpa candicans</u>	leaves
	<u>Vitex trifolia</u>	bark, fruits, leaves
<u>Acanthaceae</u>	<u>Jasticia hayatai</u> var. <u>decumbens</u>	whole plant
<u>Caprifoliaceae</u>	<u>Viburnum awabuki</u>	leaves
<u>Compositae</u>	<u>Ichthyothere terminalis</u>	leaves
	<u>Spilanthes acmella</u>	whole plant
<u>Dioscoreaceae</u>	<u>Dioscorea tokoro</u>	root
<u>Palmae</u>	<u>Rhapis cochinchinensis</u>	fruits

*Presented at the 6th conference on the chemistry of natural products, July 23, 1971, Chino, Nagano Pref.

employed as fish poisons, and tephrosin(51) and toxicarol(52) from Tephrosia species. These compounds also have insecticidal activity. Munduserone (53)⁶⁾ and pachyrrizone(54)⁷⁾ are active constituents of Mundulea sericea and Pachyrrhizus erosus, respectively.

2) Coumarins

Erosnin(55)⁸⁾ and pachyrrizin(56)⁷⁾ were isolated together with rotenone from the seeds of

Pachyrrhizus erosus(Leguminosae) whose leaves and seeds were used as fish poisons. Mammein(57),⁹⁾ is contained in Mammea americana and also shows insecticidal activity. Kawazu et al.¹⁰⁾ isolated (+)-inophyllolide(58) and its derivatives(59 and 60) as active constituents of the leaves of Calophyllum inophyllum(Guttiferae). On the other hand, Polonsky¹¹⁾ et al. isolated (+)-inophyllolide and calophyllolide(61) possessing anticoagulant activity from the seed of the same plant.

3) Lignanes

Cleistanthus collinus(Euphorbiaceae)^{12,13)} has been reported to contain diphyllin(62), collinusin(63) and cleistanthin(64) as piscicidal constituents. Diphyllin is also present in Justicia procumbens.¹⁴⁾ Methyl ether of diphyllin, justicidin A(65),¹⁵⁾ was isolated together with justicidin B(66)¹⁵⁾ as the active constituents of a Formosan piscicidal plant, Justicia hayatai var. decumbens. Ohta et al. reported that they isolated justicidin C(67), D(68), E(69) and F(70) with piscicidal activity as well as justicidine A, B and diphyllin

from J. procumbens.^{16,17)}

4) Terpenoids

Picrotoxinin(71) and picrotin(72) which are classed in the realm of sesquiterpenes of abnormal structure are the piscicidal principles of Anamirta paniculata(Menispermaceae). Picrotoxinin and its related compounds are also found in various species as toxic principles.¹⁸⁾ Callicarpone(73),¹⁹⁾ a constituent of Callicarpa candicans(Verbenaceae) which is used as a fish poison in Palau, is the first example of diterpene possessing piscicidal activity. Subsequently, a diterpene-acid, maingayic acid(74),²⁰⁾ was isolated as the piscicidal constituent of Callicarpa maingayi.

5) Polyacetylenes

Ichthyothere terminalis(Compositae) was known to be used as a fish poison by the natives of the Lower Amazon Basin. Polyacetylenic compounds, ichthyothereol(75) and its acetate(76), which are poisonous to mammals as well, are active constituents of the leaves of this plant.²¹⁾

6) Alkaloids

From Cissampelos pareira (Menispermaceae),^{1,2)} alkaloids of biscoclaurine type such as bebeerine, sepeerine, cissampareine and 4''-O-methylcurine were isolated as piscicidal principles. Isotrilobine, (±)-tetrandrine, fangchinoline, and isochondrodendrine were revealed to be active constituents of Stephania hernandiifolia (Menispermaceae).^{1,2)} Though many kinds of alkaloids are isolated from piscicidal plants, few of them have been confirmed to be causative principles.

7) Saponins

Saponins, which usually have anticoagulant activity, are widely distributed in the realm of plants, being found in more than 60 species. Many kinds of piscicidal plants have been reported to contain saponins causative of their biological activity, and some sapogenins were isolated and identified from Albizzia procera (Leguminosae), Barringtonia acutangula (Lecythidaceae) and Aegiceras corniculatum (Myrsinaceae).^{1,2)}

Though about 300 species are reported to have been used as fish poisons, only a few of them have been chemically investigated. However, the piscicidal constituents already elucidated are various kinds of compounds as stated above.

Many of the piscicidal plants are also useful for folk remedies. Recently several antitumor substances have been isolated from higher plants, some of which are piscicidal plants.

It is interesting and important to investigate chemical constituents of the piscicidal plants and to reveal the active constituents of them. It is expected to find out novel compounds with any biological activity.

I-2. Chemical Investigations on Hura crepitans

Hura crepitans(Euphorbiaceae) is a tree originally found in the American tropics, which has many thorns on its trunk and a good store of milky sap. It is occasionally planted in the extreme southern United States, Hawaii and South East Asia. It has been recognized as a dangerous plant.²²⁾ Ingestion of two or three seeds produces severe emesis and purging. Contact with the plant produces severe skin irritation in some individuals. The milky sap was known to be a fish poison,^{3,4)} and sometimes was used as an arrow poison in America. In Java it was also considered to be effective for the treatment of leprosy.³⁾

A few chemical investigations have been carried out on this plant. Jaffé et al.²³⁾ reported the isolation of hurain, a new plant protease, from the sap, barks and roots. During the course of chemotaxonomical studies on the Euphorbiaceae, were identified 17 known tetracyclic triterpenoids²⁴⁾ in the sap of H. crepitans. Insecticidal activity²⁵⁾ of this plant has also been reported. However, no

definite informations had been furnished on the drastic substances in this plant until Richet²⁶⁾ isolated from the sap a haemoagglutinating constituent, crepitine, which was characterized by Jaffé et al.²⁷⁾ to be a kind of glycolipoprotein(LD₅₀ in mice: 187mg/kg, agglutination of rabbit blood: 30µg/ml).

Under such circumstances, the author started the isolation of a piscicidal principle from the sap of this plant. The sap was collected in Bolivia. Ultimately the active constituent was isolated in pure state and named "huratoxin". Huratoxin is about 10 times as toxic as rotenone which is a representative of the known fish poisons. According to the procedure stated in the following chapter, its structure was revealed to be a novel orthoester consisting of a diterpene-hexaol and a 2,4-tetradecadienoic acid. Hitherto only five orthoesters have ever been isolated as natural substances: tetrodotoxin,^{28,29)} bersaldigenin-1,3,5-orthoacetate,³⁰⁾ daphnetoxin,³¹⁾ mezerein^{32,33)} and huratoxin.

II. A Piscicidal Constituent of Hura crepitans

II-1. Isolation of Huratoxin^{34,35)}

The milky sap of Hura crepitans was collected in Bolivia. The isolation procedure (Fig. 1) of huratoxin from the sap was guided by the piscicidal activity against the killie-fish(Oryzias latipes). 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 chromatographed on Florisil and the active fraction was purified 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". The active constituent in the methanol extracts was proved to be identical with the active substance isolated from

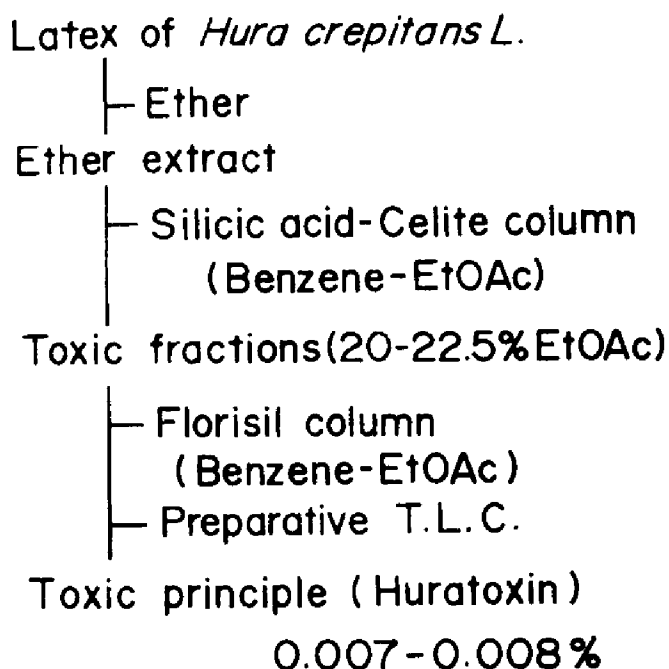


Fig. 1.

the ether extracts.

Although huratoxin was not crystallizable, it showed a single spot on the thin-layer chromatogram of silica gel G with various developing solvent systems. It was visualized by characteristic reddish brown coloration on spraying 4% ammonium metavanadate in 50% sulfuric acid or detected by spraying 0.5% KMnO_4 . But it could not be visualized by vaniline-conc. sulfuric acid. Huratoxin is easily soluble in any usual organic solvents, but not in water. It

was proved to consist of carbon, hydrogen and oxygen atoms by the qualitative and elemental analysis.

EXPERIMENTAL

Isolation of huratoxin

The milky sap of Hura crepitans was collected in Bolivia. The isolation procedure was guided by the killie-fish bioassay.¹⁹⁾ 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, 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 a silicic acid(150 g)-Celite 545(300 g). Each eluant(1 liter) consisting of benzene and ethyl acetate, and the ethyl acetate concentration was increased stepwise(0, 10, 15, 20, 22.5 and 30%). The piscicidal activity was found in the fraction eluted with 22.5% ethyl acetate in benzene. This 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 chromatogram using various developing solvent systems(benzene-ethyl acetate(1:1)(Rf 0.5), chloroform-ethyl acetate (1:1)(Rf 0.5), benzene-ether(1:2)(Rf 0.5), benzene-acetone(5:1)(Rf 0.6)), but tailing on aluminum oxide G. Visualization of the spot was effected by spraying 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, $\lambda_{\max}$ 231nm(ϵ 28,000), sh. 240nm(ϵ 21,000); ir(CCl_4), 3500(OH), 1700(C=O), 1680, 1638(C=C) cm^{-1} ; pmr(100 MHz), 0.89(3H, perturbed t.), 1.18(3H, d., $\underline{J}=6$), 1.27(s., polymethylene), 1.80 (6H, broad s.), 2.94(H_P , d., $\underline{J}=2.5$), 3.46(H_O , s.), 3.74(OH), 3.84(H_L , H_M , broad s.), 3.84(H_N , m.), 3.85 (OH), 4.10(OH, d., $\underline{J}=3$), 4.27(H_K , d., $\underline{J}=3$, $+\text{D}_2\text{O} \rightarrow$ s.), 4.45(H_J , d., $\underline{J}=2.5$), 4.92(H_G , m.), 5.04(H_F , m.), 5.6-

6.4(3H), 6.77(1H, d.d., $\underline{J}$ =15, 8.5), 7.64(H_A , m.).

II-2. Chemical Structure of Huratoxin^{34,35,36)}

II-2-1. Characterization

Huratoxin(1) showed* ir absorption bands (Fig.2) at 3500(OH), 1700(C=O), 1680 and 1638(C=C) cm^{-1} , and uv absorption maximum at 231nm(ϵ 28,000) and shoulder at 240nm(ϵ 21,000). Its pmr spectrum** (Fig. 3) 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, H_F , H_G , m.) and other five

* Unless otherwise stated, the uv absorption spectra were taken in ethanol and the pmr spectra in deuteriochloroform at 60 MHz. Chemical shifts were expressed as δ values(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.

** In order to grasp the pmr spectral data, each protons which are important for the structure argument of 1 were named H_A , H_F , H_G , as depicted in Fig. 3.

olefinic protons. The disappearance of signals equivalent to three protons by addition of D_2O showed that three hydroxyl groups exist in huratoxin. It is noticeable that huratoxin showed carbonyl band at 1700cm^{-1} but no prominent band near 1250cm^{-1} characteristic for ester function in its ir spectrum. This suggests 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 0.95ppm 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(Fig. 4), the two-proton broad singlet(3.84, H_L , H_M) observed in that of 1 changed into a AB quartet(3.62 and 4.68) with a large coupling constant($J=12\text{Hz}$), and the one-proton singlet(H_K) in 1 was shifted downfield by 1.3ppm to 5.54ppm. This observation indicates the presence of

a primary and a secondary hydroxyl group depicted by (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 addition of D_2O pointed to the presence of a tertiary hydroxyl group in 1.

Hexahydrohuratoxin(2) also provided its diacetate(4), m.p. 62-62.5°C, needles, $[\alpha]_D^{17} +68.7^\circ$ ($c=1.79$, $CHCl_3$), on acetylation with acetic anhydride and pyridine. It was deacetylated to 2 with ammonia in methanol like diacetylhuratoxin(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 exposing in the air. This fact, together with their uv absorption maximum(231nm, ϵ 28,000), suggests the presence 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 had disappeared as well as the terminal methylene proton signals. This shows these olefinic

protons are attributed to the diene, which is deduced to be disubstituted. In the pmr spectrum of 2 the singlet at 1.27ppm 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 disubstituted 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 mixing melting point of its 2,4-dinitrophenylhydrazone.

From the latter fraction was obtained a ketoenal(5). The pmr spectrum of 5(Fig. 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., $J=5$, 2.5Hz). The aldehydic proton is the X part of an ABX type splitting pattern with two olefinic protons at 6.66 and 6.81ppm. This pmr and uv (212nm, sh., ϵ 13,000 and $\lambda_{\max}$ 238nm, ϵ 7500) spectra revealed the presence of an α,β -unsaturated aldehyde group in 5. The coupling constant ($J_{AB}=15\text{Hz}$) 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 $C_{29}H_{30}O_{12}$. 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, $C_{34}H_{48}O_8$, 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 aldehydic proton nor olefinic protons (Fig. 6), but a broadened one-proton multiplet at 5.2ppm which was turned to singlet by 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 . This 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 7.61ppm. The ir and uv spectra of 1 suggested the absence of benzene or furan ring. The spin-decoupling experiments(Fig. 7) disclosed that the one-proton multiplet at 7.61ppm was related not only to the one-proton multiplet at 3.84ppm with a coupling constant of 2.0Hz but also to the broad singlet at 1.80ppm assigned to a vinylic methyl group. This fact and the other spectroscopic characteristics of 2($\lambda_{\max}$ 240nm(ϵ 7200), $\nu_{\max}$ 1700, 1635 cm^{-1}) which are in good agreement with those of α -methyl- α,β -unsaturated cyclopentenone compounds illustrated in Fig. 8 are suggestive of the presence of α -methyl- α,β -unsaturated cyclopentenone 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 to yield octahydrohuratoxin, which gave triacetyl octahydrohuratoxin(7) by acetylation with acetic anhydride and pyridine. It showed no uv absorption maximum over 210nm(ϵ 1400). In its pmr

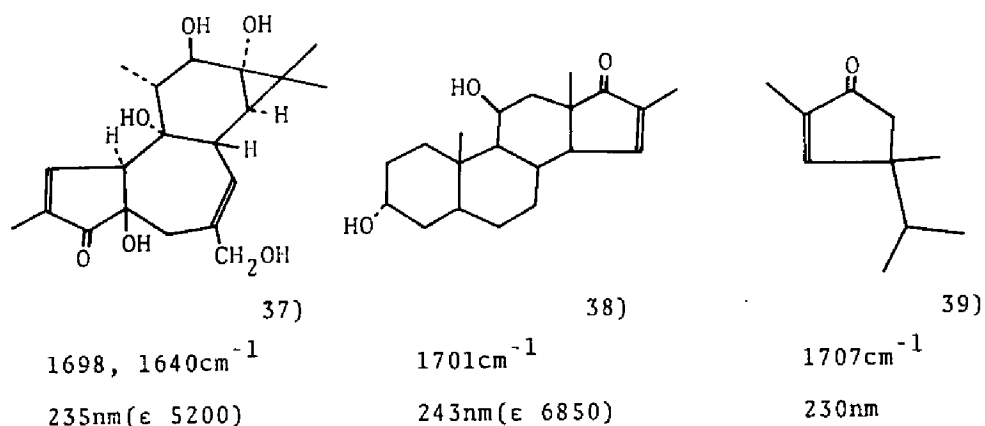


Fig. 8.

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 shift by 0.2ppm. Its 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 210nm(ϵ 1000) and a carbonyl absorption band at 1755 cm^{-1} . All the facts mentioned above show the presence of an α -methyl- α,β -unsaturated cyclopente-

none in 1.

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 notice. Similarly, triacetyl octahydrohuratoxin(7) gave the corresponding bromohydrin(9), the pmr spectrum of which exhibited a one-proton doublet at 4.97ppm($J=10\text{Hz}$), instead of one-proton

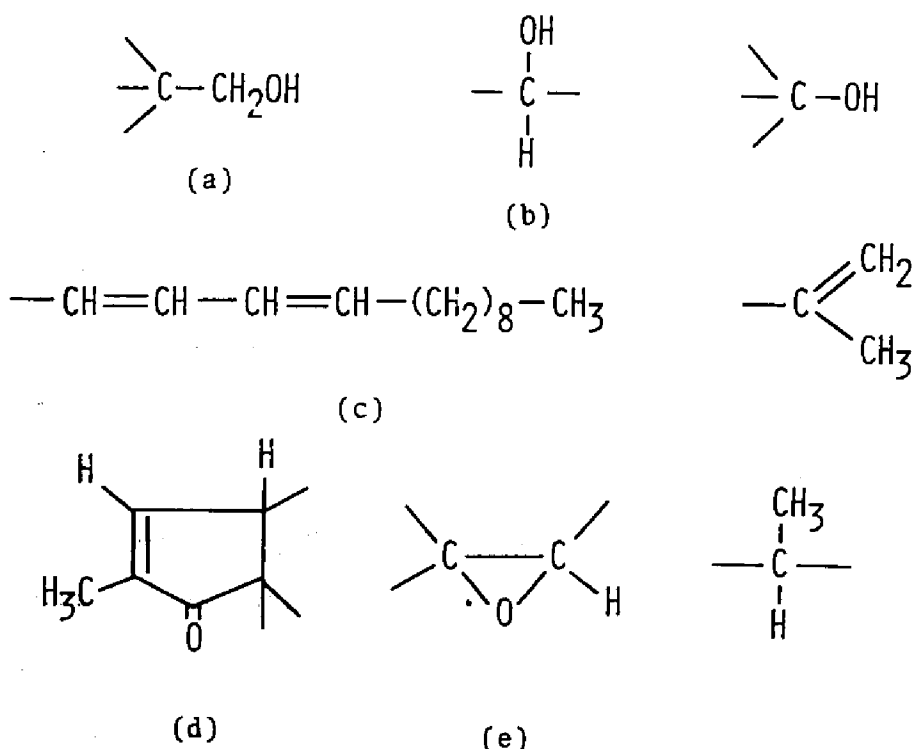


Fig. 9.

broad singlet at 3.36ppm. The partial structure (e) was inferred to exist in 1.

The pmr spectra of almost all derivatives of huratoxin exhibited a three-proton doublet ($J=6-7\text{Hz}$) at about 1.0ppm. 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. 9.

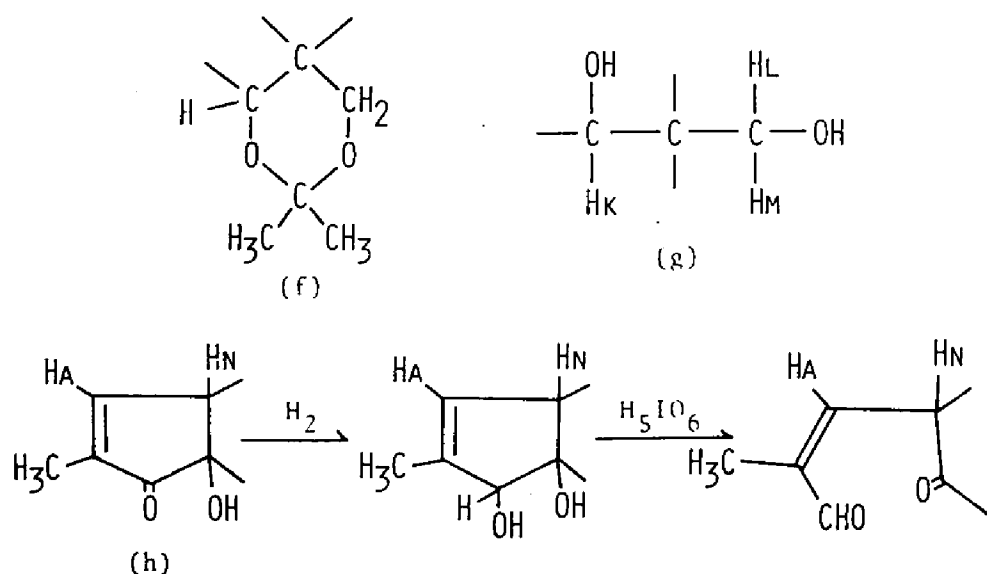
II-2-2. Partial Structure

Hexahydrohuratoxin(2) gave an acetonide(10) when it was treated with acetone in the presence of a catalytic amount of p-toluenesulphonic acid. The pmr spectrum(100 MHz) of 10 showed a six-proton singlet at 1.46ppm attributed to two quarternary acetonide methyl groups as well as one hydroxyl proton signal at 3.37ppm. The acetonide(10) afforded no acetate on the usual acetylation with acetic anhydride and pyridine. This indicates the isopropylidene group was introduced to the primary and the secondary hydroxyl groups as depicted in the

partial structure (f). Accordingly the partial structure (g) could be worked out for 1.

Octahydrohuratoxin, in which the α,β -unsaturated ketone was reduced to the corresponding allylic alcohol, reacted with one molar equivalent of periodic acid in dry ether⁴⁰⁾ to give an aldehyde-ketone(11). Its uv($\lambda_{\max}$ 239nm, ϵ 10,000), ir($\nu_{C=O}$ 1710, 1688 and $\nu_{C=C}$ 1640 cm^{-1}) and pmr(10.13, 1H, broad s., 6.46, H_A , m., 1.82, 3H, m.) spectra indicate the presence of a moiety containing an α -methyl- α,β -unsaturated aldehyde and a ketone in 11. This is well explained in terms of the partial structure (h).

Hexahydrohuratoxin(2) gave a compound(12) on



oxidation with one molar equivalent of ethereal periodic acid,⁴⁰⁾ whereas its diacetate did not react with periodic acid. This fact is suggestive of the presence of a 1,2-diol in 1. The 1,2-diol was inferred to consist of the secondary and the tertiary hydroxyl groups.

In the pmr spectrum(Fig. 10) the compound(12) exhibited no aldehydic proton, though it gave a positive silver mirror reaction. A vinyl methyl appeared at 2.06ppm as a doublet($J=1.2\text{Hz}$) and a characteristic proton, H_A , at 7.61ppm as a quartet($J=1.2\text{Hz}$). The proton, H_N , to which H_A was coupled with a coupling constant of 1.5Hz in 2 had disappeared. Spin-decoupling experiments(Fig. 10) clarified that H_A was coupled to the vinyl methyl only. A one-proton doublet at 4.31ppm was changed to a singlet by addition of D_2O , and was, therefore, assigned to a proton(H_K) on the hydroxyl-carrying carbon atom.

On the usual acetylation with acetic anhydride and pyridine, 5 gave a diacetate(13, $\nu_{C=O}$ 1762, 1752 and 1728, $\nu_{C=C}$ 1619 cm^{-1}) which also exhibited no aldehydic proton in its pmr spectrum. The protons,

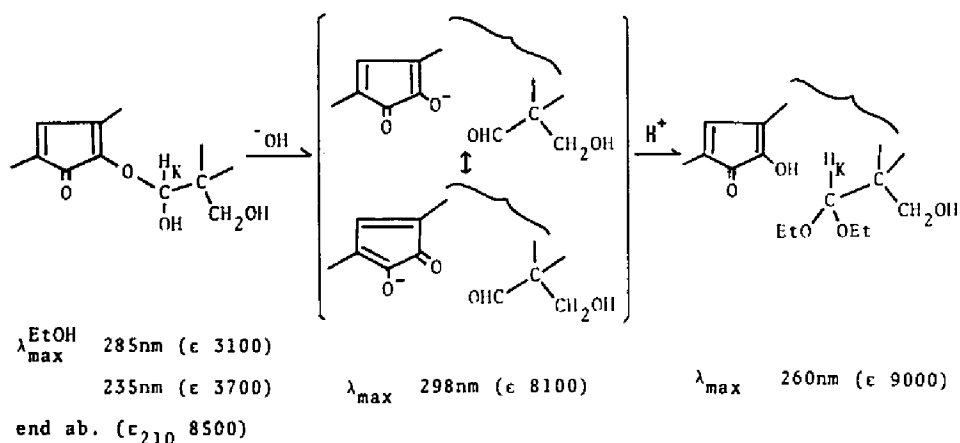
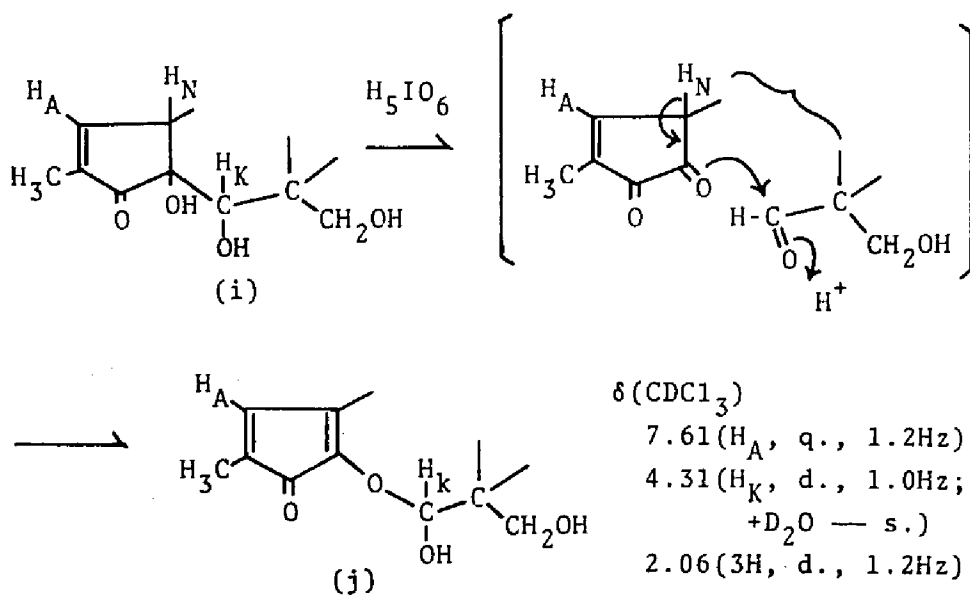


Fig. 11.

H_L , H_M and H_K were deshielded by 0.22, 0.48 and 1.22ppm, respectively, on this acetylation.

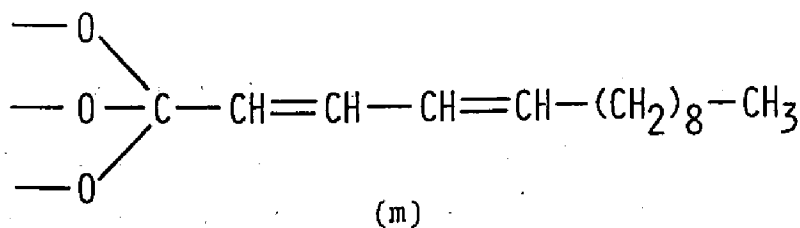
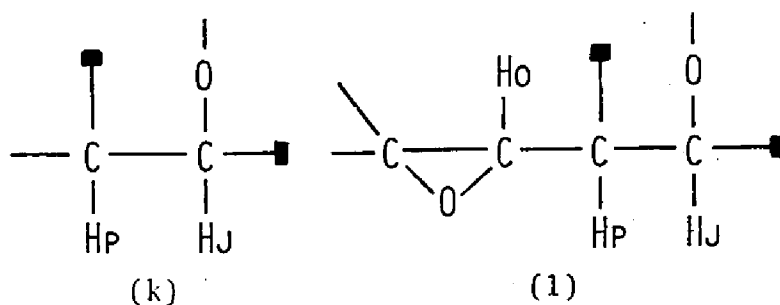
These observations suggested the presence of a hemiacetal structure shown by (j) in 12. The



behavior of 12 in its uv spectra shown in Fig.11. also supports the partial structure (j). Therefore, the presence of the partial structure (i) in 1 is apparent.

A pair of doublets, H_P and H_J , ($J=2.5\text{Hz}$) is observed at about 2.9 and 4.2ppm, respectively, in the pmr spectra of derivatives. The chemical shifts suggest the presence of the partial structure (k).

Diacetyl hexahydrohuratoxin(4) reacted with hydrogen bromide in acetic acid to yield its bromohydrin(14) which was easily reconverted to 4. In the pmr spectrum of 14 the signal ascribed to H_O



appeared as doublet($J=10\text{Hz}$). Spin-decoupling experiments(Fig. 12) clarified that H_p is coupled to H_J and H_O ($J_{pJ}=2.5$, $J_{pO}=10\text{Hz}$) and that H_O is coupled to H_p only($J_{Op}=10\text{Hz}$). From the fact that the bromohydrin(14) could not be acetylated, the hydroxyl group resulting from the cleavage of the epoxide was inferred to be tertiary. Then the partial structure (k) was connected with (e), and expanded to (l).

II-2-3. The Presence of an Orthoester

Hexahydrohuratoxin(2) was heated under reflux in aqueous ethanol containing hydrogen chloride to give a diterpene-polyol(15) and ethyl myristate which was identified with the authentic sample by its mass spectrum. In huratoxin(1) a straight chain of 14 carbon atoms, therefore, was proved to be connected with the diterpene moiety through ether linkages, and the partial formula (c) is extended to (m).

The polyol(15), inferred to be a chlorohydrin by its positive Beilstein test, gave a diacetate(16), triacetate A(17) and triacetate B(18) on acetylation

with acetic anhydride and pyridine. The pmr spectra of 15, 16, 17 and 18 exhibited the presence of all characteristic protons($H_A \sim H_P$), and 15 was accordingly deduced to have suffered no significant rearrangement in its carbon skeleton on the acid hydrolysis. Comparison of the pmr spectrum of 16 with those of 2 and 4 showed that the primary and the secondary hydroxyl groups originally present in 1 were acetylated in 16.

The pmr spectrum(Fig. 13) of triacetate A(17) showed a down field shift of H_J by 1.49ppm compared with that of the diacetate(16). It is obvious that the hydroxyl group on the H_J -bearing carbon atom was also acetylated in 17. The spectrum exhibited the signal assigned to H_O as a doublet($J=4.0\text{Hz}$) which was changed to a singlet by addition of D_2O . This revealed that H_O is attached to the carbon atom bearing the hydroxyl group resulting from the epoxide. Table 2 illustrates that the hydroxyl group on the H_O -bearing carbon atom of 18 was acetylated, and the hydroxyl group on the H_J -bearing carbon atom was free.

On the basis of the foregoing, it was inferred that huratoxin(1) is an orthoester, and H_J should be

Table 2. Significant Signals in the PMR Spectra of Huratoxin(1), Hexahydrohuratoxin(2), Diacetyl hexahydrohuratoxin(4), Polyol diacetate(16), Polyol triacetate A(17), and Polyol triacetate B(18).

	H _J	H _K	H _L	H _M	H _O	H _P
<u>1</u>	4.45(d., <u>J</u> =2.5)	4.23	3.84	3.84	3.46	2.94(d., <u>J</u> =2.5)
<u>2</u>	4.36(d., <u>J</u> =2.5)	4.24	3.80	3.80	3.48	2.80(d., <u>J</u> =2.5)
<u>4</u>	4.32(d., <u>K</u> =2.5)	5.52	4.69(d., <u>J</u> =12)	3.60(d., <u>J</u> =12)	3.36	2.88(d., <u>J</u> =2.5)
<u>16</u>	3.88	5.17	4.58(d., <u>J</u> =12)	4.14(d., <u>J</u> =12)	4.38	3.35(d., <u>J</u> =2.0)
<u>17</u>	5.37	5.13	4.70(d., <u>J</u> =12)	3.96(d., <u>J</u> =12)	4.26	3.55(d., <u>J</u> =2.5)
<u>18</u>	4.08	5.21	4.49(d., <u>J</u> =12)	3.99(d., <u>J</u> =12)	5.45	3.45(d., <u>J</u> =1.5)

attached to one of the three carbon atoms which contribute to the orthoester linkage of 1. If this assumption is correct, triacetate A(17) in which the hydroxyl group on the H_J-bearing carbon atom is acetylated will easily produce an orthoacetate.

Triacetate A(17) crystallized in prisms from ethyl acetate-n-hexane, but its melting point was not sharp(193-208°C). Thin-layer chromatography showed that 17 had been converted to another substance after melting. The mass spectrum of 17 exhibited the highest mass peak at m/e 542, which is 18 mass unit smaller than the mass number, 560, expected for the molecular ion peak of 17. This is also suggestive of the easy preparation of the

orthoacetate from 17.

Triacetate A (17) was heated for a few minutes under nitrogen gas to yield the orthoacetate(19). Its pmr spectrum(Fig. 14) showed a three-proton singlet at 1.63ppm instead of one of the three acetyl methyl signals, which were present in the pmr spectrum of 17. The chemical shift(1.63ppm) of this methyl is well consistent with those of methyls of orthoacetates: bersaldigenin-1,3,5-orthoacetate(1.59ppm)³⁰⁾ which was recently isolated as one of a few naturally occurring orthoesters, cevagenin C-orthoacetate diacetate(1.58 ppm)⁴¹⁾ and 16-dehydrosabine D-orthoacetate diacetate (1.58ppm).⁴¹⁾ In the orthoacetate(19) two hydroxyl groups were still free, and accordingly the polyol(15) was revealed to be a heptaol, that is, three hydroxyl groups originally present in 1, three resulting from the hydrolysis of the orthoester linkage and one resulting from the cleavage of the epoxide with hydrogen chloride.

The mass spectrum of 19 exhibited the same highest mass peak(m/e 542) and the same fragmentation pattern as the spectrum obtained from triacetate A(17) This shows that 17 was changed to 19 before electron

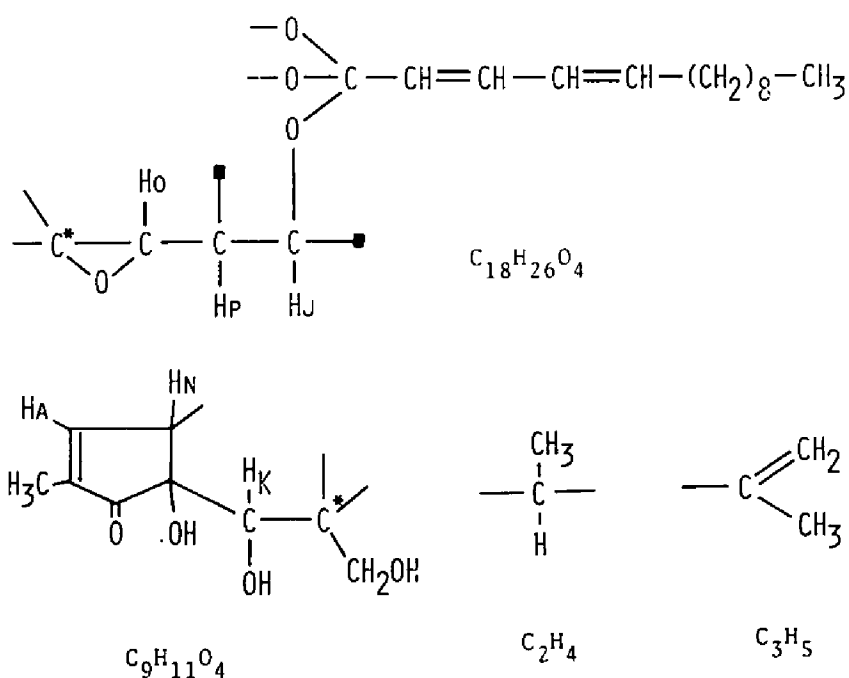
bombardment in a mass spectrometer.

The unidentified three of the eight oxygen atoms of 1 were thus accounted for as an orthoester function. On the basis of the foregoing, huratoxin (1) was proved to be a 2,4-tetradecadienoic acid orthoester of a diterpene-hexaol.

II-2-4. Skeleton and Structure

Huratoxin(1) is now represented by the partial structures in Fig. 15. The unidentified atoms of 1 are C_2H_2 or C_3H_2 . The elucidated partial structures, in conjunction with the molecular formula, $C_{34}H_{48}O_8$, demand that the diterpene part of 1 is tricarbocyclic. These structural characteristics stated above remind us of phorbol(26),³⁷⁾ isolated from Croton tiglium, a plant of the same family as Hura crepitans.

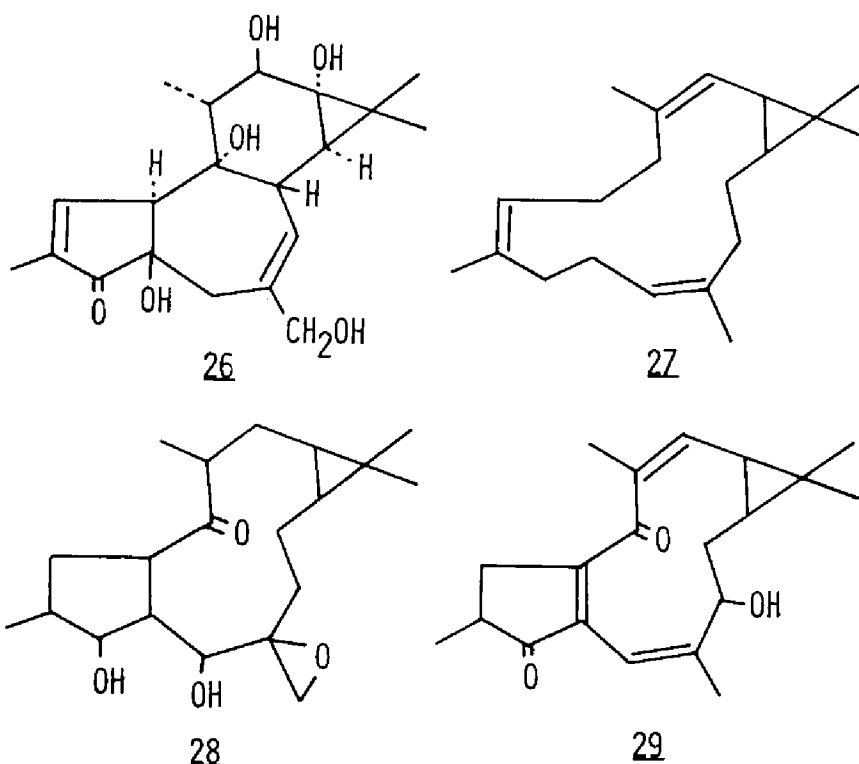
The skeleton of 1, therefore, is expected to be very similar to that of phorbol. Phorbol is not probably biosynthesized from geranylgeranyl pyrophosphate in the same sequence as common bi-, tri- or tetracyclic diterpenes, and is assumed to be formed by recyclisation of such a macrocyclic



C_2H_2 or C_3H_2 were unidentified.

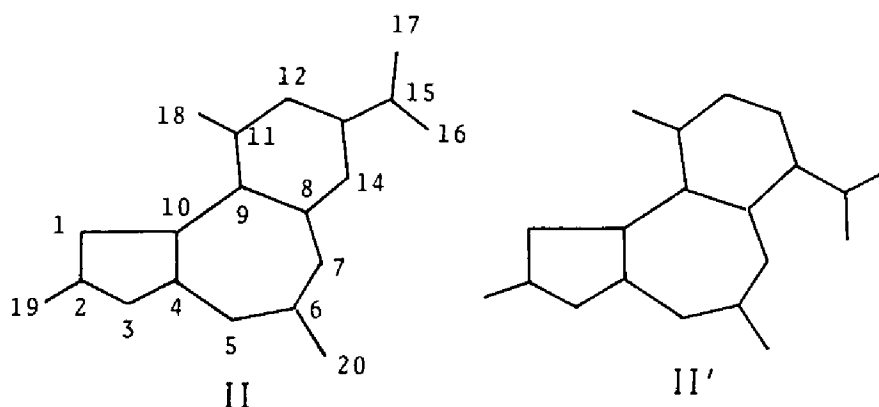
(The carbon atoms with an asterisk may be identical.)

Fig. 15. Partial Structures of Huratoxin diterpene as casbene(27)⁴²⁾ which has recently been reported as a cyclisation product of geranylgeranyl pyrophosphate with enzyme preparation of castor bean (Rcinus communis, Euphorbiaceae)(cf. Chapter III.). Recently, macrocyclic diterpenes(6,20-epoxy-lathyrol (28)^{43,44)} 7-hydroxy-lathyrol,⁴⁵⁾ bertiyadionol(29)⁴⁶⁾ and jatrophone⁴⁷⁾) were isolated from Euphorbia, Bertya and Jatropha species(Euphorbiaceae), respec-



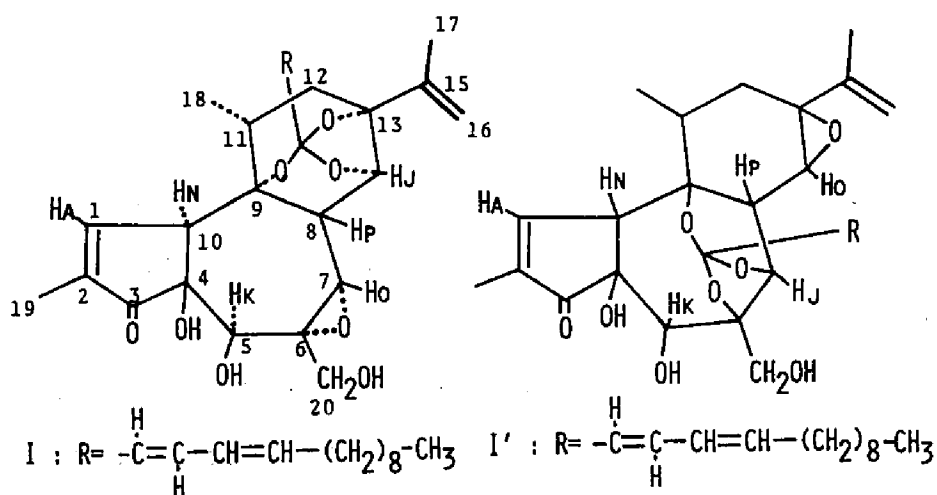
tively. This supports the assumption that the diterpenes such as phorbol(26) are formed by re-cyclisation of macrocyclic diterpenes. On this assumption possible skeletons are formulated for 1, but the positions of the pendant groups already elucidated rule out the other skeletons than II and II'.

When the structure of 1 is formulated on the basis of these skeletons, it becomes very important to decide the position of H_p . From the partial



structures of 1, H_p must be settled down at C-8, and the skeleton II' is ruled out. Consequently, the possible structures are limited to I and I'.

In the pmr spectrum of bromohydrin(14), which could be easily reconverted to diacetyl hexahydrohuratoxin(4), H_N was shifted upfield by 0.75ppm



compared with that of 4, while H_L and H_M suffered a down field shift by 0.24 and 1.15ppm, respectively. This remarkable effect on the chemical shift caused by the simple cleavage of the epoxide can not be explained by the formula I'. This remarkable shift of H_L , H_M , and H_N would be more easily comprehended in the formula I. The proton, H_N , having been subjected to the anisotropic effect of the epoxide, was shifted upfield on the cleavage of the epoxide ring, and H_L and H_M were subjected to the anisotropic effect of the hydroxyl group generated by the epoxide cleavage. Then the formula I' is ruled out and the structure of huratoxin(1) is, therefore, represented by the formula I apart from the stereochemistry.

EXPERIMENTAL

Unless otherwise noted, uv spectra were measured in ethanol and pmr spectra in deuteriochloroform solution. The pmr spectra were obtained on Varian A-60 and HA-100D nmr spectrometers. Double-resonance experiments were performed with the latter instrument. Chemical shifts are expressed as δ (ppm) from tetra-

methylsilane as internal standard. The mass, ir and uv spectra were recorded on a Hitachi RMU-6D mass spectrometer, a Shimadzu recording infrared spectrometer AR-275 and a Hitachi Perkin-Elmer EPS-3T spectrophotometer, respectively.

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(1) (73.0 mg) in ethanol (2 ml) was added and the hydrogenation was continued at atmospheric pressure and at room temperature until 3 molar equivalents of hydrogen was consumed (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, λ_{max} 242 nm (ϵ 7200); ir (CCl_4), 3550 (OH), 1700 (C=O), 1635 (C=C) cm^{-1} ; pmr, 0.92 (3H, perturbed t.), 0.92 (6H, d., $\underline{J}=7$), 1.14 (3H, d., $\underline{J}=6$), 1.27 (s., polymethylene), 1.80 (3H, m.), 2.80 (H_p , d., $\underline{J}=2.5$), 3.48 (H_O , s.), 3.74 (H_N , m.), 3.80 (2H, broad s.), 4.24 (H_K , s.), 4.36 (H_J , d., $\underline{J}=2.5$), 7.60 (H_A , m.).

Diacetylhuratoxin(3)

Acetylation of huratoxin(1)(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, $\lambda_{\max}$ 231nm(ϵ 28,000); ir(CCl_4), 3580(OH), 1760, 1746, 1710(C=O), 1671, 1632(C=C) cm^{-1} ; pmr, 0.92(3H, perturbed t.), 1.15(3H, d., $\underline{J}=6$), 1.27(s., polymethylene), 1.77(3H, broad s.), 1.81(3H, m.), 2.03, 2.20(3H each, s.), 3.02(H_P , d., $\underline{J}=2.5$), 3.09(1H, s., OH), 3.38(H_O , s.), 3.62(H_M , d., $\underline{J}=12$), 3.95(H_N , m.), 4.43(H_J , d., $\underline{J}=2.5$), 4.68(H_L , d., $\underline{J}=12$), 4.92(H_G , m.), 5.04(H_F , m.), 5.54(H_K , s.), 5.6-6.7(4H), 7.52(H_A , m.).

Diacetyl hexahydrohuratoxin(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 evaporated to dryness. 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: m.p. 62-62.5°C(needles); $[\alpha]_D^{17} +68.7^\circ$ ($c=1.79$, CHCl_3); uv, λ_{max} 240nm(ϵ 9500); ir(CCl_4), 3500(OH), 1760, 1727, 1703(C=O), 1632(C=C), 1240(-O-C=O) cm^{-1} ; pmr, 0.92(3H, perturbed t.), 0.93(6H, d., $J=6$), 1.14(3H, d., $J=6$), 1.27(s., polymethylene), 1.77(3H, m.), 2.05, 2.20(3H each s.), 2.88(H_P , d., $J=2.5$), 3.05(OH), 3.36(H_O , s.), 3.60(H_M , d., $J=12$), 3.88(H_N , m.), 4.32(H_J , d., $J=2.5$), 4.69(H_L , d., $J=12$), 5.52(H_K , s.), 7.49(H_A , 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 diacetylhuratoxin(3)

A solution of diacetylhuratoxin(3)(200 mg) in ethyl acetate(10 ml) was chilled 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-dinitro-phenylhydrazone. 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} 212nm(sh. ϵ 13,000), 238nm(ϵ 7500); ir(CHCl_3), 3570(OH), 1760-1738, 1705(C=O) cm^{-1} , 1635(C=C) cm^{-1} ; pmr, 1.15(3H, d., $\underline{J}$ =6.5), 1.78(3H, d.d., $\underline{J}$ =2.3, 1.0), 2.02, 2.20, 2.30(3H each, s.), 3.10(H_P , d., $\underline{J}$ =2.5), 3.20(OH), 3.41(H_O , s.), 3.66(H_M , d., $\underline{J}$ =12), 3.97(H_N , m.), 4.66(H_L , d., $\underline{J}$ =12), 4.86(H_J , d., $\underline{J}$ =2.5), 5.56 (H_K , s.), 6.66(1H, d.d., $\underline{J}$ =5.0, 15), 6.81(1H, d.d., $\underline{J}$ =15, 2.5), 7.47(H_A , m.), 9.57(1H, d.d., $\underline{J}$ =5.0, 2.5);

high-resolution mass spectrum, M^+ 546.178 (Calcd. 546.173 for $C_{27}H_{30}O_{12}$); mass spectrum, m/e (relative intensity) 546(M^+) (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} 240.5nm (ϵ 7000); ir($CHCl_3$), 3570(OH), 1760-1740, 1708(C=O), 1632(C=C) cm^{-1} ; pmr, 1.18(3H, d., $J=6.5$), 1.78(3H, m.), 2.03, 2.20, 2.32(3H each s.), 3.10(H_P , m.), 3.22(OH), 3.40(H_O , s.), 3.66(H_M , d., $J=12$), 3.99(H_N , m.), 4.66(H_L , d., $J=12$), 4.83(H_J , m.), 5.2(1H, broad m., $+D_2O \rightarrow$ s.), 5.56(H_K , s.), 7.53(H_A , m.); mass spectrum, m/e 520($M^+ - 18$) (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, λ_{end} ab. (ϵ_{210} 1400); ir(CCl_4), 3590(OH), 1750(C=O, broad), 1228(-O-C=O) cm^{-1} ; pmr, 0.90(3H, perturbed t.), 0.92(6H, d., $\underline{J}=6$), 1.18(3H, d., $\underline{J}=6$), 1.27(s., polymethylene), 1.60(3H, m.), 2.02, 2.04, 2.14(3H, each s.), 2.84(1H, s., OH), 2.86(H_P , d., $\underline{J}=2.5$), 3.36(H_O , s.), 3.50(H_M , d., $\underline{J}=12$), 4.30(H_J , d., $\underline{J}=2.5$), 4.56(H_L , d., $\underline{J}=12$), 5.56(H_K , s.), 5.64(1H, m.), 5.71(1H, m.).

Lithium-liquid ammonia reduction of diacetyl hexahydrofuratoxin(4)

Liquid ammonia(ca. 10 ml) was trapped by introducing ammonia gas into a vessel chilled 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 hexahydrofuratoxin(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(CCl_4), 3580(OH), 1760, 1755, 1746($\text{C}=\text{O}$), 1240, 1120, 1045 cm^{-1} ; pmr, 0.90(3H, perturbed t.), 0.92(6H, d., $\underline{J}=6$), 1.27(s., polymethylene), 2.17, 2.19(3H each, s.), 2.92(H_P , d., $\underline{J}=2$), 3.08(1H, s., OH), 3.31(H_O , s.), 3.50(H_N , m.), 3.54(H_M , d., $\underline{J}=12$), 4.28(H_J , d., $\underline{J}=2$), 4.62(H_L , d., $\underline{J}=12$), 5.39(H_K , 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(CCl_4), 3590(OH), 1765-1735($\text{C}=\text{O}$), 1630($\text{C}=\text{C}$, broad), 1225($-\text{O}-\text{C}=\text{O}$), 1075, 1030, 895 cm^{-1} ; pmr, 0.92(3H, m.), 0.94(6H, d., $\underline{J}=6$), 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(H_M , d., $\underline{J}=12$), 4.78(H_J , d., $\underline{J}=$

2.5), 4.97(H_O , d., $\underline{J}=10$), 5.07(H_L , d., $\underline{J}=12$), 5.22(H_K , s.), 5.57(1H, m.), 5.70(1H, m.).

Acetonide(10) of hexahydrohuratoxin

To a solution of hexahydrohuratoxin(2)(40 mg) in absolute acetone(10 ml) was added a catalytic amount of *p*-toluenesulfonic acid, and the reaction mixture was left at room temperature overnight. After addition of 5% aqueous Na_2CO_3 (1 ml), the reaction mixture was extracted with ether. The ether layer was washed with water saturated with NaCl, dried and concentrated. The residue was purified by preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate, 5:1) to yield an acetonide(10)(36 mg); $[\alpha]_D^{22} +55.6^\circ$ ($c=1.93$, $CHCl_3$), uv, λ_{max} 241nm(ϵ 8100), ir(CCl_4), 3541(OH), 1716(C=O), 1638(C=C), 1230, 1116, 1083 cm^{-1} ; pmr(100 MHz), 0.89(3H, perturbed t.), 0.92(6H, d., $\underline{J}=7$), 1.10(3H, d., $\underline{J}=7$), 1.27(s., polymethylene), 1.46(6H, s.), 1.78(3H, d.d., $\underline{J}=3$, 1.5), 2.78(H_P , d., $\underline{J}=2.5$), 3.18(H_O , s.), 3.37(OH), 3.60(H_M , d., $\underline{J}=13$), 3.74(H_N , m.), 4.02(H_L , d., $\underline{J}=13$), 4.22(H_K , s.), 4.31(H_J , d., $\underline{J}=2.5$), 7.45(H_A , m.).

Hemiacetal(12)

To a solution of hexahydrohuratotoxin(2) (76 mg) in dry ether(12 ml) was added 1.5 molar equivalents of ethereal periodic acid.⁴⁰⁾ After 2 hr at room temperature, the reaction mixture was concentrated and purified by preparative thin-layer chromatography (silica gel GF₂₅₄, benzene-ethyl acetate, 2:1) to yield a hemiacetal(12) (7.2 mg) as well as the starting material(50 mg); uv, end ab.(ϵ_{210} 8500), $\lambda_{\max}$ 235nm(ϵ 3700), 285nm(ϵ 3100); $\lambda_{\max}$ did not change in the acidic condition, $\lambda_{\max}^{\text{NaOH-EtOH(pH 9)}}$, 298nm(ϵ 8100), $\lambda_{\max}$ 260nm(ϵ 9000) when the alkaline solution was acidified to pH 4; ir(CCl₄), 3460(OH, broad), 1725(C=O), 1620(C=C), 1065, 1035, 920cm⁻¹; pmr(100 MHz), 0.88(3H, perturbed t.), 0.91(6H, d., $\underline{J}$ =6.5), 1.04(3H, d., $\underline{J}$ =7), 1.27(s., polymethylene), 2.06(3H, d., $\underline{J}$ =1.2), 3.50(H_P, d., $\underline{J}$ =2.5), 3.50(H_M, d., $\underline{J}$ =13), 3.58(OH), 4.13(H_L, d., $\underline{J}$ =13), 4.31(H_K, d., $\underline{J}$ =1.0, +D₂O $\rightarrow$ s.), 4.62(H_J, d., $\underline{J}$ =2.5), 7.61(H_A, q., $\underline{J}$ =1.2).

Hemiacetal diacetate(13)

The hemiacetal(12) (7 mg) was dissolved in

acetic anhydride(0.2 ml) and pyridine(0.2 ml), and the reaction mixture was left at room temperature overnight. Crushed ice was added and the solution was extracted with ether. The usual work-up gave a resinous matter, which was purified by preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate 5:1) to afford a hemiacetal diacetate (13)(5.8 mg); uv, $\lambda_{\max}$ 272nm(ϵ 3500), sh. 227nm(ϵ 4600), end ab.(ϵ_{210} 8200); ir(CCl₄), 1762, 1752, 1728 (C=O), 1619(C=C), 1243, 1220, 1040, 932cm⁻¹; pmr(100 MHz), 0.88(3H, perturbed t.), 0.91(6H, d., $\underline{J}$ =6), 1.01(3H, d., $\underline{J}$ =7), 1.27(s., polymethylene), 1.78(3H, s.), 1.88(3H, d., $\underline{J}$ =1.5), 2.02(3H, s.), 3.61(H_P, d., $\underline{J}$ =2.5), 3.88(H_M, d., $\underline{J}$ =13), 4.35(H_L, d., $\underline{J}$ =13), 4.64(H_J, d., $\underline{J}$ =2.5), 5.53(H_K, s.), 7.53(H_A, q., $\underline{J}$ =1.5).

Bromohydrin(14) of diacetyl hexahydrohuratoxin

To a solution of diacetyl hexahydrohuratoxin (4)(40 mg) in acetic acid(1 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 3 hr, and extracted with a large amount of ether. The ether layer was washed with water

saturated with NaCl, and concentrated after drying over sodium sulfate to yield a resinous matter. It was purified by preparative thin-layer chromatography (silica gel GF₂₅₄, benzene-ethyl acetate, 2:1) to yield a bromohydrin(14) (30 mg); $[\alpha]_D^{22} +95.5^\circ$ ($c = 2.23$, CHCl₃), positive to the Beilstein test; uv, $\lambda_{\max}$ 239nm (ϵ 7600); ir(CCl₄), 3550, 3400(broad, OH), 1760-1740, 1710(C=O), 1638(C=C), 1222, 1135, 1062, 930cm⁻¹ pmr(100 MHz), 0.89(3H, perturbed t.), 0.92(3H, d., $\underline{J}=7$), 0.94(3H, d., $\underline{J}=7$), 1.08(3H, d., $\underline{J}=7$), 1.27(s., polymethylene), 1.78(3H, m.), 2.13, 2.18(3H each, s.) 2.32(H_P, d.d., $\underline{J}=10$, 2.5), 2.94(OH), 3.13(H_N, m.), 3.54(OH), 4.75(H_M, d., $\underline{J}=12$), 4.81(H_J, d., $\underline{J}=2.5$), 4.93(H_L, d., $\underline{J}=12$), 4.97(H_O, d., $\underline{J}=10$), 5.10(H_K, s.), 7.50(H_A, m.). The bromohydrin(14) was reconverted to diacetyl hexahydrohuratoxin by being heated in aqueous ethanol. It was confirmed by thin-layer chromatography and m.p. measurement.

Acid hydrolysis of hexahydrohuratoxin

A solution of hexahydrohuratoxin(2) (70 mg) in ethanol(8 ml) containing 2N-HCl(3 ml) was heated under reflux for 3 hr. Additional 1 ml of 2N-HCl

was added and the reaction mixture was refluxed for another 4 hr. It was neutralized with 5% aqueous Na_2CO_3 , and extracted with a large amount of ethyl acetate. The ethyl acetate layer was washed with water saturated with NaCl, dried over sodium sulfate and concentrated. The residue was chromatographed on a silicic acid(4 g)-Celite 545(8 g) column and eluted with chloroform containing an increasing ratio of methanol. The first fraction gave ethyl myristate, which was identified with the authentic sample by the combined gas chromatography-mass spectrometry; column, 5% SE-30 on Chromosorb-W(80-100 mesh), injection temperature 270°C , column temperature 160°C , carrier gas He, 8 ml/min., retention time, 9.5 min.

The subsequent fractions eluted with 6-10% methanol in chloroform were collected and purified by preparative thin-layer chromatography(silica gel GF₂₅₄, chloroform-methanol, 9:1) to yield the polyol(15)(15 mg); amorphous powder, positive to the Beilstein test; uv, λ_{max} 247nm(ϵ 6100); ir(Nujol), 3400(strong and broad, OH), 1695(C=O), 1635(C=C), 1125,1074, 1022, 931cm^{-1} ; pmr(d_5 -pyridine) 1.12(9H, d., $\underline{J}$ =6.5), 1.82(3H, m.), 3.89(1H, d., $\underline{J}$ =1.5), 4.35(3H), 4.66(2H,

broad s.), 5.27(1H, broad s.), 7.93(1H, m.).

Acetylation of the polyol

The polyol(15)(18 mg) was dissolved in pyridine (0.3 ml) and acetic anhydride(0.3 ml). The solution was left at room temperature overnight, and crushed ice was added into it. The mixture was extracted with ethyl acetate and the organic layer was washed with dil. HCl, dil. Na₂CO₃ and water, successively. After drying over sodium sulfate, the ethyl acetate was evaporated and the residue was separated by preparative thin-layer chromatography(silica gel GF₂₅₄, ether) into polyol diacetate(16)(6.5 mg), polyol triacetate A (17)(7.5 mg) and polyol triacetate B (18) (5 mg).

Polyol diacetate(16); uv, $\lambda_{\max}$ 244.5nm(ϵ 6600); ir(CHCl₃), 3548, 3430(OH), 1752, 1712(C=O), 1640(C=C) 1123, 1040, 990, 930cm⁻¹; pmr, 0.96(9H, d., $\underline{J}$ =6.5), 1.84(3H, m.), 2.15, 2.26(3H each, s.), 3.00(OH), 3.3 (H_P, d., $\underline{J}$ =2.0), 3.88(H_N,H_J, m.), 4.14(H_M, d., $\underline{J}$ =12) 4.38(H_O, s.), 4.58(H_L, d., $\underline{J}$ =12), 5.17(H_K, s.), 7.55 (H_A, m.).

Polyol triacetate A (17); m.p. 193-208°C(prisms

from ethyl acetate-n-hexane), positive to the Beilstein test; uv, $\lambda_{\max}$ 244.5nm(ϵ 7200) ; ir(CHCl_3), 3548, 3400(OH), 1755-1735, 1712(C=O), 1637(C=C), 1114, 1035-1015, 935-915 cm^{-1} ; pmr, 0.93, 0.96, 1.00 (3H each, d., $\underline{J}$ =6.5), 1.88(3H, m.), 2.18, 2.20, 2.27 (3H each, s.), 2.96(OH), 3.55(H_P , d., $\underline{J}$ =2.5), 3.81 (H_N , m.), 3.96(H_M , d., $\underline{J}$ =12), 4.26(H_O , d., $\underline{J}$ =4.0, + $\text{D}_2\text{O} \rightarrow$ s.), 4.63(OH, d., $\underline{J}$ =4.0), 4.70(H_L , d., $\underline{J}$ =12), 5.13(H_K , s.), 5.37(H_J , m.), 7.56(H_A , m.); mass spectrum, m/e (relative intensity) 542(M^+-18)(0.3), 524 (0.3), 499(0.6), 482(2.1), 464(1.8), 447(2.1), 440 (1.4), 439(1.5), 429(1.5), 422(2.1), 404(1.5), 387 (8.5), 369(4.0), 326(9.5), 309(4.5), 290(7.0), 255 (4.5), 109(16), 71(21), 69(17), 55(16), 43(100), 28(21).

Polyol triacetate B(18); uv, $\lambda_{\max}$ 242.5nm(ϵ 6900) ; ir(CHCl_3), 3552, 3475(OH), 1755, 1713(C=O), 1640 (C=C), 1120, 1045-1025, 992 cm^{-1} ; pmr, 0.87(3H, d., $\underline{J}$ =6.5), 0.96, 0.97(3H each, d., $\underline{J}$ =6.5), 1.75(3H, m.), 2.07, 2.14, 2.25(3H each, s.), 2.96(OH), 3.45(H_P , d., $\underline{J}$ =1.5), 3.76(H_N , m.), 3.80(OH), 3.99(H_M , d., $\underline{J}$ =12), 4.08(H_J , m.), 4.49(H_L , d., $\underline{J}$ =12), 4.75(OH), 5.21(H_K , s.), 5.35(H_O , broad s.), 7.56(H_A , m.).

Polyol orthoacetate(19)

The polyol triacetate A(17)(5 mg) was heated at 200-205°C for 3 min. under nitrogen gas, and allowed to cool to room temperature. The product was purified by preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate, 2:1) to yield the polyol orthoacetate(19)(3 mg); positive to the Beilstein test; uv, $\lambda_{\max}$ 240nm(ϵ 6000); ir(CHCl₃), 3545(OH), 1750, 1712(C=O), 1640(C=C), 1120, 1071, 1035, 920cm⁻¹; pmr, 0.94(6H, d., $\underline{J}$ =6.5), 1.10(3H, d., $\underline{J}$ =7), 1.63(3H, s.), 1.75(3H, m.), 2.13, 2.26(3H each, s.), 2.72(H_P, d., $\underline{J}$ =2.5), 2.85, 3.82(OH), 4.05(H_N, m.), 4.42(H_J, H_L, H_M), 4.56(H_O, s.), 5.32(H_K, s.), 7.50(H_A, m.); mass spectrum, m/e(relative intensity) 542(M⁺)(2.4), 524(0.1), 499(0.2), 482(1.0), 464(1.3), 447(2.5), 440(1.1), 439(1.0), 429(1.9), 422(1.5), 404(1.1), 387(8.0), 369(4.0), 326(6.5), 309(5.0), 290(11), 255(4.0), 109(15), 71(13), 69(17), 55(21), 43(100), 28(10).

II-3. Determination of the Absolute Stereostructure of Huratoxin by X-ray Diffraction Analysis³⁶⁾

Formula I for huratoxin(1) is well consistent with all the chemical data. However, this evidence alone was not conclusive, because the structure was assigned on the assumption that the skeleton of 1 is formed via such a macrocyclic diterpene as casbene(27) derived from geranylgeranyl pyrophosphate. Therefore, X-ray diffraction analysis also carried out for structure elucidation simultaneously with the chemical studies.³⁶⁾

Huratoxin was converted to a number of derivatives containing a bromine atom, but all the attempts to obtain good crystals for X-ray analysis had failed of success until p-bromobenzoylcrepital(20) was produced as follows.

Huratoxin(1) was treated with p-bromobenzoyl chloride in pyridine to afford p-bromobenzoyl-huratoxin, which was ozonized with one molar equivalent of ozone in ethyl acetate at -70°C. The ozonide was cleaved reductively with triphenylphosphine, and the products were chromatographed over silica gel by stepwise elution with benzene

containing ethyl acetate to yield n-decanal, a small amount of p-bromobenzoylcrepital(20) and p-bromobenzoyl-C-16-nor-crepital(21), which was produced on oxidation with 2 molar equivalents of ozone, as well as the starting material. Then, 20 was recrystallized from ethyl acetate-n-hexane(4:1) to give colorless plates, m.p. 254-256°C, D_m 1.442 g/cm³. The single crystal is orthorhombic, space group $P2_1^2 2_1^2 2_1^2$, with four molecular 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. Independent reflections, 1704, were obtained from equiinclination Weissenberg photographs taken along the a and c axes with $CuK\alpha$ radiation.

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 20 Fig. 16³⁶⁾ shows the perspective view of the molecule along the c-axis, where all the atoms in the asymmetric unit of the crystal, excluding hydrogen, are given.

Thus, consisting of crepital and n-decanal,

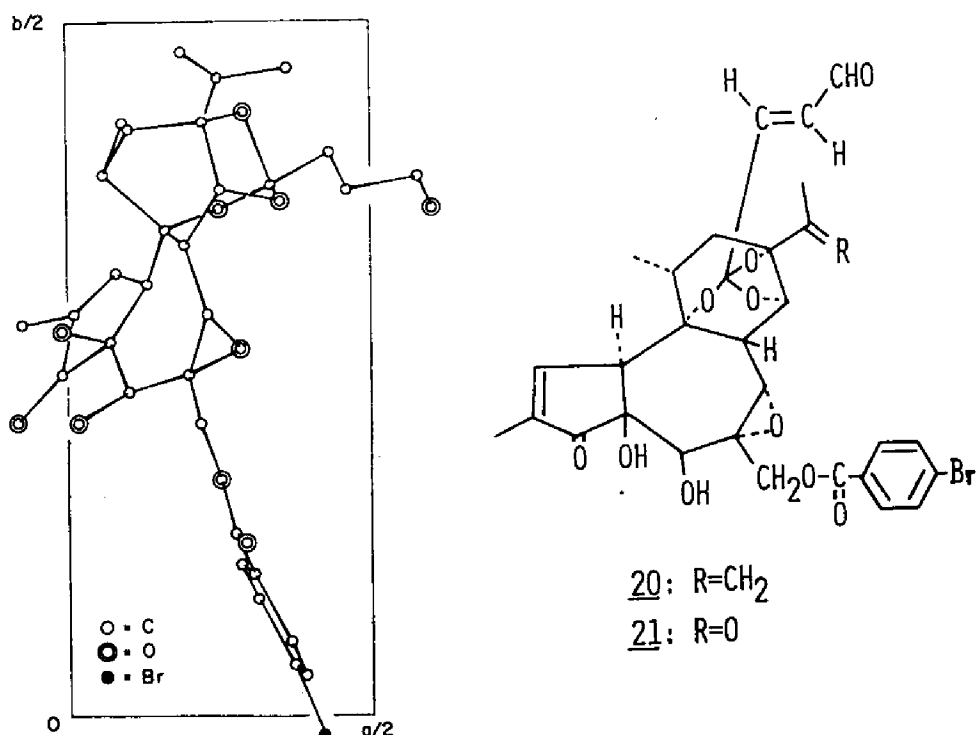


Fig.16. The molecule of p-bromo-benzoylcrepitol viewed along the c axis.³⁶⁾

huratoxin can be represented by the formula I. The coupling constant ($J_{AB}=15\text{Hz}$) of the ABX type splitting pattern of the α,β -unsaturated aldehyde group in 20 suggests the trans-configuration of the double bond which is consistent with the result of the X-ray analysis. Some experiments were carried out in order to determine the configuration of another double bond of the diene in 1. But the configu-

ration could not be established.

EXPERIMENTAL

p-Bromobenzoylcrepital(20)

p-Bromobenzoyl chloride(100 mg) in dry benzene (1.6 ml) was added to huratoxin(1)(110 mg) in dry pyridine(0.5 ml). The reaction mixture was left at room temperature for 3 hr. Crushed ice was added, and the reaction mixture was extracted with ether. The ether layer was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed over Florisil(100-200 mesh)(8 g), which was eluted with benzene containing ethyl acetate, the concentration of ethyl acetate being increased in 2% step. The fractions eluted with 8-10% ethyl acetate in benzene were further purified by preparative thin-layer chromatography(silica gel GF₂₅₄; benzene-ethyl acetate, 4:1), to yield p-bromobenzoyl-huratoxin(107 mg). To a solution of p-bromobenzoyl-huratoxin(107 mg) in ethyl acetate(10 ml) chilled to -70°C with dry ice and ethanol was added an ethyl acetate solution of 1.5 molar equivalents of ozone.

Subsequently, an ethyl acetate solution(5 ml) of 2.5 molar equivalents of triphenylphosphine(107 mg) was added into the solution of the ozonide. The reaction mixture was allowed to warm up to room temperature and left until the reaction mixture showed a negative potassium iodide-starch test. The solution was concentrated and chromatographed on a silicic acid(5 g)-Celite 545(8 g) column by elution with benzene containing ethyl acetate, a ratio of ethyl acetate being increased in 2% step. The fraction eluted with 6% ethyl acetate in benzene were further purified by preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate, 16:5) to yield p-bromobenzoylcrepitol(20)(24 mg); m.p. 254-256°C(colorless plates from ethyl acetate-n-hexane(4:1, v/v)), $[\alpha]_D +3.4^\circ$ ($c=1.37$, CHCl₃); uv, λ_{max} 244nm(ϵ 23,000); ir (CHCl₃), 3520(OH), 1725, 1700(C=O), 1630, 1594(C=C), 1273, 1120, 1070, 1012, 920cm⁻¹; pmr, 1.11(3H, d., $\underline{J}=7$), 1.80(6H, broad s.), 3.06(H_P, d., $\underline{J}=2.5$), 3.43(H_O, broad s.), 3.83(OH), 3.87(H_N, m.), 3.97(OH), 4.08(H_M, d., $\underline{J}=12$), 4.34(H_K, broad d., $\underline{J}=2.5$, +D₂O → s.), 4.53(H_J, d., $\underline{J}=2.5$), 4.97(H_G, m.), 5.03(H_F, m.), 5.06(H_L, d., $\underline{J}=12$), 6.65(1H, d.d., $\underline{J}=15$, 5.5),

6.79(1H, d.d., $\underline{J}$ =15, 2.5), 7.56(2H, d., $\underline{J}$ =8.5), 7.60 (H_A , m.), 7.91(2H, d., $\underline{J}$ =8.5), 9.70(1H, d.d., $\underline{J}$ =2.5, 5.5); Found: C, 57.63; H, 4.96. Calcd. for $C_{31}H_{31}O_{10}Br$: C, 57.86; H, 4.86%.

p-Bromobenzoyl-C-16-nor-crepital(21)

From the fraction eluted with 10% ethyl acetate in benzene was obtained p-bromobenzoyl-C-16-nor-crepital(21)(36 mg); amorphous powder; pmr, 1.20(3H, d., $\underline{J}$ =7), 1.80(3H, d.d., $\underline{J}$ =2.5, 1.0), 2.29(3H, s.), 3.07(H_P , d., $\underline{J}$ =2.5), 3.46(H_O , s.), 3.88(H_N , m.), 4.01(OH), 4.04(OH, d., $\underline{J}$ =3.0), 4.10(H_M , d., $\underline{J}$ =12), 4.35(H_K , d., $\underline{J}$ =3.0, $+D_2O \rightarrow$ s.), 4.85(H_J , d., $\underline{J}$ =2.5), 5.05(H_L , d., $\underline{J}$ =12), 6.66(1H, d.d., $\underline{J}$ =15, 5.5), 6.80(1H, d.d., $\underline{J}$ =15, 2.5), 7.54(H_A , m.), 7.56(2H, d., $\underline{J}$ =8.5), 7.91(2H, d., $\underline{J}$ =8.5), 9.70(1H, d.d., $\underline{J}$ =5.5, 2.5).

II-4 Some Interesting Aspects of Huratoxin and its Derivatives in their Reaction and PMR Spectra

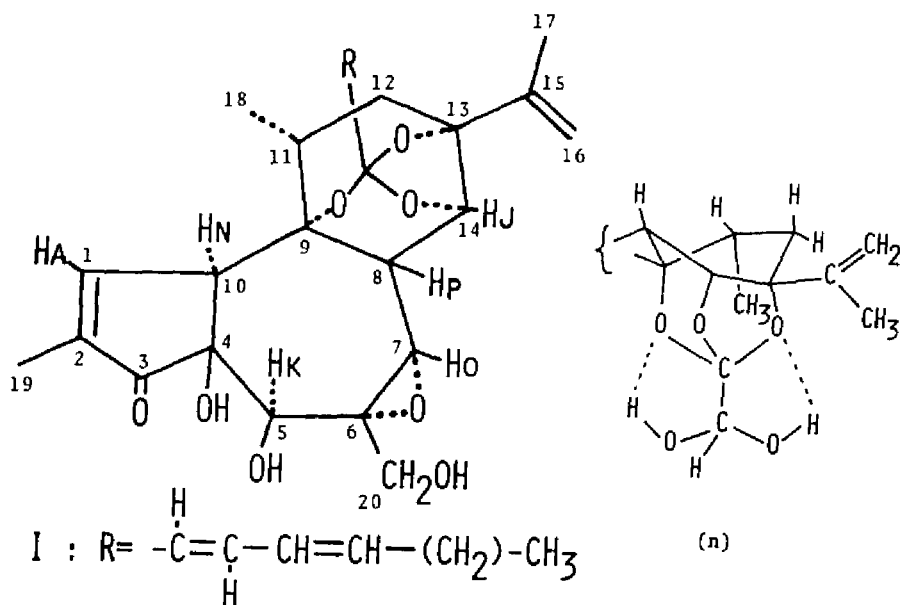
In terms of the absolute stereostructure of huratoxin, some phenomena which tended to obscure rather than aid our structural arguments could be comprehended.

One is that concerning the epoxide. The observation that H_O in huratoxin did not couple to any protons is now understood by the dihedral angle between H_O and H_P being near a right angle (Fig. 16)

Since in bromohydrin(14) the hydroxyl group resulting from the epoxide is tertiary, it is apparent that a bromine atom is introduced to C-7. A splitting of H_O ($J_{OP}=10\text{Hz}$) in 14 showed that inversion of the configuration of C-7 took place on the cleavage of the epoxide ring and that the entering bromine atom is located at the 7β -position.

Therefore, the bromine atom at C-7 and the hydroxyl groups at C-4 and C-5 have a severe steric interaction each other in the β -side, to which the facile reversibility of 14 to 4 may be attributed.

On the other hand, in the polyol(15) the



hydroxyl group resulting from the epoxide was secondary and a spin coupling between H_O and H_P was not observed. This indicates that the chlorine atom entered at C-6, and the hydroxyl resulting from the epoxide was located at C-7 with retention of the configuration.

Hexahydrofuratoxin(2) was heated under reflux in aqueous methanol containing sulfuric acid to yield a pentaol(22) as a major product as well as a small amount of a methoxyhydrin(24) which was also obtained as a major product by refluxing 2 in methanolic sulfuric acid. The spin coupling between H_O and H_P was not observed in 22 and 24 like in 15.

In these compounds which suffered the cleavage of the epoxide by refluxing in acidic conditions, the entering group was apparently located on C-6 and the hydroxyl group from the epoxide on C-7.

The second is the extreme stability of the orthoester function of huratoxin(1) against the acid hydrolysis. Generally, hydrolysis of orthoesters in acidic conditions is in equilibrium.^{48,49)} For instance, treatment of bersaldigenin-1,3,5-orthoacetate with 80% aqueous acetic acid at 90-100°C was reported to yield a 1:1 mixture of the orthoacetate and a bersaldigenin monoacetate.³⁰⁾ Hexahydrohuratoxin(2), however, was not hydrolyzed by refluxing in aqueous acetic acid, and afforded the acid hydrolyzate in a very poor yield by refluxing in aqueous ethanol containing hydrochloric acid. The extreme stability of the orthoester is now comprehensible as follows.

The conformation of the C-ring of 1 is a boat form as shown in Fig. 16. In the polyol(15) obtained by acid hydrolysis of the orthoester of 2, the isopropyl group would be obliged to assume an axial conformation, provided the C-ring changed to a chair

form. The bulky isopropyl group, however, tends to assume equatorial conformation, and then the C-ring must be converted to a skew-boat form, where severe non-bonded interactions take place among the three pseudo-axial hydroxyl groups. Consequently, the orthoester form is much more stable than the corresponding diterpene alcohol.

Some unusual aspects in the pmr spectrum of huratoxin(1) are also well comprehensible. The proton, H_N , appeared at a considerably lower field (3.83ppm) for the ordinary allylic methine proton. This is well explained as a result of the anisotropic effect of the epoxide due to the stereochemical relationship between H_N and the epoxide oxygen atom as illustrated in Fig. 16. The proton, H_p , is also sterically very near to the hydroxyl group at C-4, and is found at a lower field(2.95ppm) because of the anisotropic effect of the hydroxyl group.

The stability of the aldehyde hydrate(6) which was obtained on the ozonolysis of diacetylhuratoxin with three molar equivalents of ozone is explained in terms of the partial structure (n) where the hydrated aldehyde group is stabilized by hydrogen

bondings to the oxygen atoms of the orthoester function.

EXPERIMENTAL

Pentaol(21)

Hexahydrohuratoxin(2) (50 mg) was dissolved in 70% aqueous methanol(9 ml) containing 6N-H₂SO₄(0.8 ml). The reaction mixture was heated under reflux for 1 hr and extracted with a large amount of ethyl acetate after neutralization with 5%-Na₂CO₃. The ethyl acetate solution was concentrated and chromatographed over silicic acid(3 g)-Celite 545(4 g) eluted with benzene containing an increasing ratio(in 10% step) of chloroform, and subsequently with chloroform containing an increasing ratio(in 1% step) of methanol. The fraction eluted with 1% methanol in chloroform was purified by preparative thin-layer chromatography(silica gel GF₂₅₄, chloroform-methanol 10:1) to afford as a major product a pentaol(22)(16 mg); uv, $\lambda_{\max}$ 246nm(ϵ 6100); ir(CHCl₃), 3510(OH), 1695(C=O), 1632(C=C), 1118, 1038, 1008cm⁻¹; pmr, 0.89 (3H, perturbed t.); 0.92(6H, d., $J=7$), 1.13(3H, d.,

$\underline{J}=7$), 1.27(s., polymethylene), 1.82(3H, m.), 2.36(H_P , d., $\underline{J}=2.5$), 3.70(H_M , d., $\underline{J}=12$), 3.72(2H, OH), 3.80(H_N , m.), 4.05(H_L , d., $\underline{J}=12$), 4.12(H_O , s.), 4.34(H_K , s.), 4.41(H_J , d., $\underline{J}=2.5$), 4.49, 5.40(OH), 7.62(H_A , m.).

Pentaol triacetate(23)

The usual acetylation of the pentaol(15)(10 mg) with acetic anhydride(0.1 ml) and pyridine(0.1 ml), followed by purification with preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate 1:2) gave a pentaol triacetate(23)(8.4 mg); uv, $\lambda_{\max}$ 246.5nm(ϵ 5800); ir(CHCl₃), 3535(OH), 1760-1740, 1721(C=O), 1646(C=C), 1265-1245, 1102, 1050-1020, 890cm⁻¹; pmr, 0.89(3H, perturbed t.), 0.95(6H, d., $\underline{J}=7$), 1.12(3H, d., $\underline{J}=7$), 1.27(s., polymethylene), 1.82(3H, m.), 2.04, 2.13, 2.28(3H each, s.), 2.36(H_P , broad d., $\underline{J}=2.5$), 3.01, 3.71(OH), 4.22(H_N , m.), 4.26(H_L , H_M , broad s.), 4.44(H_O , broad s.), 4.46(H_J , d., $\underline{J}=2.5$), 5.20(H_K , s.), 7.27(H_A , m.).

Methoxyhydrin(24)

A solution of hexahydrohuratoxin(2)(70 mg)

in methanol(10 ml) containing 2 drops of conc.
 H_2SO_4 was refluxed for 30 min. and extracted with
 a large amount of ether. The subsequent usual
 work-up gave a resinous matter which was chromato-
 graphed on silicic acid(5 g)-Celite 545(10 g)
 column with stepwise elution of benzene containing
 an increasing ratio(5%) of ethyl acetate. The 20%
 ethyl acetate eluate fraction was purified by
 preparative thin-layer chromatography(silica gel
 GF₂₅₄, benzene-ethyl acetate 5:3) to yield as a
 major product a methoxyhydrin(24)(20.5 mg); uv,
 λ_{max} 245nm(ϵ 6600); ir($CHCl_3$), 3470(OH, broad), 1694
 (C=O), 1629(C=C), 1120, 1070, 1010, 990 cm^{-1} ; pmr,
 0.89(3H, perturbed t.), 0.92(6H, d., $\underline{J}$ =6.5), 1.12
 (3H, d., $\underline{J}$ =7), 1.27(s., polymethylene), 1.80(3H, m.),
 2.24(H_P , broad d., $\underline{J}$ =2.5), 3.60(3H, s.), 3.70(H_N ,
 m.); 4.12(H_L , H_M , broad s.), 4.23(H_K , m., +D₂O → s.),
 4.38(H_J , d., $\underline{J}$ =2.5), 4.50(H_O , broad s., +D₂O → s.),
 4.92, 5.41(OH), 7.58(H_A , m.).

III. Discussion on Biosynthesis of Macrocyclic Diterpenes and Related Novel Compounds

Up to the present, only a few macrocyclic diterpenes have been found in nature. Recently, from Euphorbia species has been reported the isolation of phorbol esters(30)^{37,50-53}) and its derivatives⁵⁴⁻⁵⁶) and of lathyrol esters⁴³⁻⁴⁵) whose structures are probably related to macrocyclic diterpenes. Interestingly, the diterpene part of huratoxin is quite similar to that of phorbol. The skeleton composed of 5,7,6-membered ring system present in phorbol(26) and huratoxin(1) can not be explained in terms of the usual biogenetic route from geranylgeranyl pyrophosphate established for common bi-, tri- or tetracyclic diterpenes.⁵⁷) Therefore, it should be assumed that these unusual diterpenes are biosynthesized through further cyclisation of such a macrocyclic diterpenes as casbene (27), which has been produced as a cyclisation product of geranylgeranyl pyrophosphate by the action of enzymes prepared from the seedlings of castor bean, Ricinus communis(Euphorbiaceae).⁴²)

Here the author wishes to briefly discuss the correlations of the structures of macrocyclic diterpenes and related compounds to their biogenesis.

Thunbergene(31)⁵⁸⁾ (originally named d-thumbelene⁵⁹⁾) has been isolated from Pinus thunbergii as the first macrocyclic diterpene(14-membered). This compound is identical with cembrene^{60,61)} and the Wienhaus hydrocarbon,⁶²⁾ and occurs in extracts of many other Pinus species (P. albicaulis,^{60,61)} P. menziesii,⁶³⁾ etc.). Its structure is represented as 31, but the stereochemistry of the trisubstituted double bonds has not been confirmed yet. This 14-membered diterpene hydrocarbon coexists⁶³⁾ with common bi-, tri- and tetracyclic diterpenes which are biosynthesized through the usual pathway from trans-geranylgeranyl pyrophosphate with a chair-chair conformation (Fig. 17). The hypothetical scheme to account for the biogenesis of this hydrocarbon has been proposed by Kobayashi et al.,⁵⁸⁾ who referred to that proposed by Hendrickson⁶⁴⁾ for 10- or 11-membered sesquiterpenes from farnesyl pyrophosphate.

Subsequently, Rowland et al. isolated 14-

membered diterpene alcohols(32-35) from Nicotiana tabacum.⁶⁵⁻⁶⁷⁾ Interestingly, all the compounds are present as diastereoisomers except 34.⁶⁷⁾ Further, incensole(36)⁶⁸⁾ and thunbergol(37)^{63,69)}, which are also 14-membered diterpene alcohols, were found in Boswellia carteri(Burseraceae) and Pseudotsuga menziesii, respectively. From the wood of Sciadopitys verticillata was isolated verticillol(38),⁷⁰⁾ a medium-cyclic(11-membered) diterpene, whose structure proposed is not conclusive.

Recently, Robinson and West⁴²⁾ have succeeded in the biosynthesis of casbene(27), a macrocyclic diterpene, as well as of the known cyclic diterpenes such as (+)-beyerene, (-)-kaurene, etc., using the extracts from the seedlings of castor bean, and proposed a hypothetical biogenetic pathway of 27 from geranylgeranyl pyrophosphate(Fig. 17).

These facts support the formation of macrocyclic diterpenes from geranylgeranyl pyrophosphate through the hypothetical biogenetic route and the possibility of the wide-spread occurrence of macrocyclic diterpenes and related compounds in higher plants.

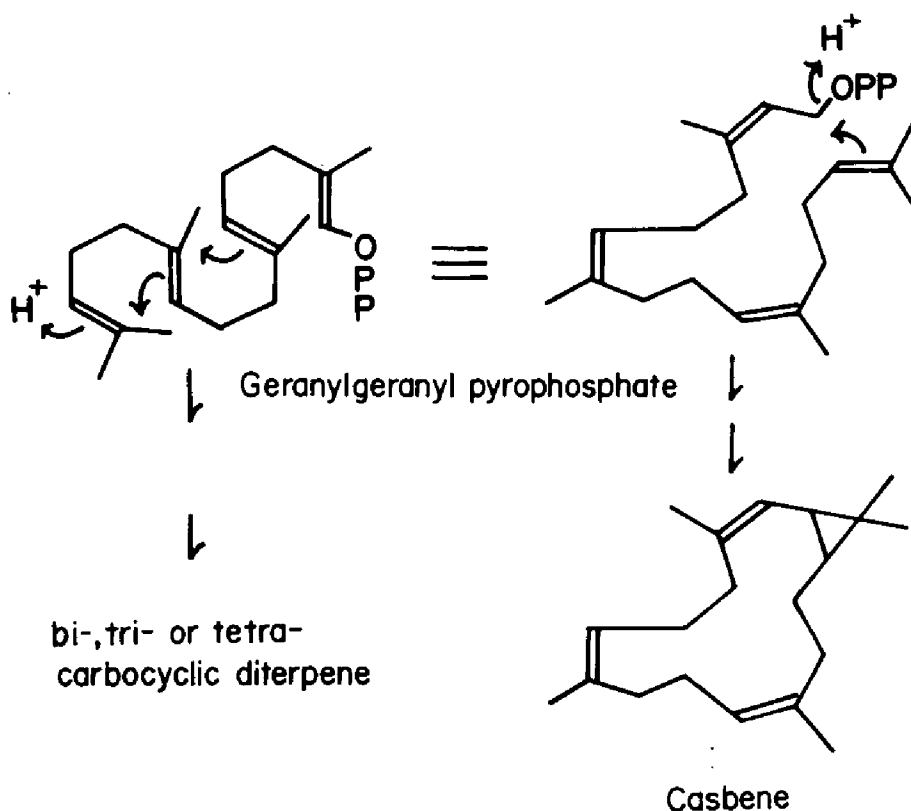


Fig. 17.

Phorbol(26)³⁷⁾ is a diterpene possessing a novel carbocyclic system. This skeleton seems to be formed by recyclisation of a certain macrocyclic diterpene. Recently from Euphorbia species have been isolated several novel diterpenes whose structures are very similar to that of phorbol: 12-desoxy-phorbol esters(39),⁵⁵⁾ 16-hydroxy-12-desoxy-phorbol esters(40),⁵⁶⁾ ingenol ester(41)⁷¹⁾ and a phorbol derivative(42)⁵⁴⁾ from E. triangularis,

E. cooperi, E. ingens and Croton rhamnifolius, respectively.

Moreover, 6,20-epoxy-lathyrol(28)^{43,44)} 7-hydroxy-lathyrol(43),⁴⁵⁾ bertiyadionol(29)⁴⁶⁾ and jatrophone(44)⁴⁷⁾ have been isolated quite recently from Euphorbia lathyris, a Bertya sp. nov. and Jatropha gossypifolia(Euphorbiaceae). They have a medium-ring(11- or 12-membered) which is supposed to be derived from such a macrocyclic diterpene as casbene(27).

Taxinine(45), a constituent of Taxus baccata⁷²⁾ and T. cospidata⁷³⁾ is also one of the new diterpenes whose formation can not be explained in terms of the common biogenetic pathway. An unusual cyclisation mechanism of geranylgeranyl pyrophosphate has been proposed by Kaneko et al.⁷⁰⁾ to account for its formation.

Thus, the isolation of huratoxin, one of the novel diterpenes, would provide additional support for the hypothesis that the macrocyclic diterpenes and related novel cyclic diterpenes are formed via macrocyclic diterpenes from geranylgeranyl pyrophosphate.

Quite recently, daphnetoxin(46)³¹⁾ and mezerein(47)^{32,33)} have been isolated as toxic principles of Daphne mezereum, D. laureola and D. gnidium (Thymelaeaceae). To our surprise, the diterpene part of huratoxin is essentially identical with that of daphnetoxin. The occurrence in Euphorbiaceae and Thymelaeaceae species of the same diterpene alcohol as orthoesters may give additional support for a close relationship^{31,32)} between the two families.

IV. Piscicidal Activity of Huratoxin and of its Several Derivatives

The sap of Hura crepitans killed all of the five loaches after 24 hr at a dose of 8 μ l in 1 liter of water, and killed only one of them at a dose of 2 μ l. The piscicidal activity of huratoxin and its derivatives was evaluated with killie-fish(Oryzias latipes)¹⁹⁾, and the experimental data are shown in Table 3 and 5. The 24- and 48-hr median tolerance limits estimated by straight line graphical interpolation are listed in Table 4, together with those of retenone, callicarpone and sodium pentachlorophenoxide. Huratoxin is about 10 times as toxic as

Table 3. 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 4. The Median Tolerance Limits of Huratoxin, Callicarpone, Rotenone and Sodium Penta-chlorophenoxide.

	TLm.			
	24 hr		48 hr	
	ppm	μM	ppm	μM
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
PCP-Na*	0.25	0.87	0.24	0.83

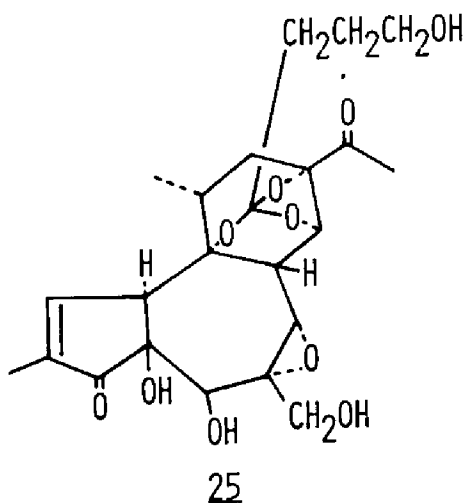
* The test condition is different from that of huratoxin, that is, the test solution in the case of callicarpone etc. is composed of 10 liter of water and an acetone solution(1 ml) of a test compound of a known concentration(in the case of PCP-Na, methanol solution was used).

Table 5. Toxicity of Huratoxin and its Derivatives to Killie-fish.

	Median tolerance limit			
	24hr.		48hr.	
	ppm	μM	ppm	μM
Huratoxin(1)	0.0014	0.0024	0.0011	0.0019
H ₆ -huratoxin(2)	0.0015	0.0025	0.0014	0.0024
H ₈ -huratoxin	0.12	0.20	0.11	0.19
Diacetylhuratoxin(3)	> 10	> 16	> 10	> 16
C-16-nor-H ₂ -crepitol(25)	7.5	16	7.5	16

rotenone to killie-fish.

The exceedingly strong toxicity of 1 may be well explained in terms of the structure where the highly oxygenated diterpene moiety is bonded through an ortho-ester linkage to the long aliphatic chain.



As illustrated in Table 5., the toxicity did not changed on hydrogenation of 1 to hexahydrouratotoxin (2). However, further hydrogenation to octahydro-uratotoxin, in which the α,β -unsaturated ketone was reduced to an allylic alcohol decreased the toxicity to 1/1000. The fact that the acetylation of the hydroxyl groups in 1 diminished the toxicity suggests that the highly oxygenated moiety is an active site of the molecule. An abrupt drop (ca. 1/10,000) in toxicity of C-16-nor-dihydrocrepitol (25), in which the C_{10} side chain is removed, suggests the significant role of the long aliphatic chain in the penetration of the molecule into the cells. The participation of the orthoester function in the toxicity has not been

revealed yet, but seems to be worth further investigation.

EXPERIMENTAL

Piscicidal Test¹⁹⁾

Oryzias latipes(killie-fish, himedaka 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- and 48-hr after their introduction. The experimental data are listed in Table 3. 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 4.

C-16-nor-dihydrocrepitol(25)

The ketoenal(5)(20 mg) in ethanol(10 ml) which was obtained by ozonolysis of diacetylhuratoxin(3), was hydrogenated over platinum catalyst(3 mg). The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was chromatographed over silicic acid(1 g)-Celite 545(2 g) by stepwise elution with benzene containing an increasing ratio(10%) of ethyl acetate. The fraction eluted with 50% ethyl acetate in benzene gave diacetyl C-16-nor-dihydrocrepitol(10 mg); uv, $\lambda_{\max}$ 240.5nm(ϵ 6600); ir(CHCl₃), 3560(OH), 1770-1735, 1710(C=O), 1635(C=C) cm⁻¹; pmr, 1.12(3H, d., $\underline{J}$ =7), 1.77(3H, d.d., $\underline{J}$ =2.5, 1.2), 2.02, 2.19, 2.28(3H each, s.), 3.01(H_p, d.,

$\underline{J}=2.5$), 3.30(OH), 3.36(H_O , s.), 3.63(H_M , d., $\underline{J}=12$), 3.74($-\overset{|}{\underset{|}{C}}_1-CH_2-CH_2-\underline{CH}_2-OH$, t., $\underline{J}=7$), 3.88(H_N , m.), 4.67(H_L , d., $\underline{J}=12$), 4.72(H_J , d., $\underline{J}=2.5$), 5.54(H_K , s.), 7.48(H_A , m.).

Diacetyl C-16-nor-dihydrocrepitol(7 mg) was dissolved in methanolic ammonia(2 ml), and left in a refrigerator overnight. The solution was concentrated in vacuo and the residue was purified by preparative thin-layer chromatography(silica gel GF₂₅₄, benzene-ethyl acetate, 1:6) to yield C-16-nor-dihydrocrepitol(25)(5 mg); uv, λ_{max} 243nm(ϵ 5900); ir($CHCl_3$), 3525(OH), 1718, 1697(C=O), 1630(C=C), 1359, 1130, 1080, 988 cm^{-1} ; pmr, 1.15(3H, d., $\underline{J}=7$), 1.78(3H, m.), 2.24(3H, s.), 2.92(H_P , d., $\underline{J}=2.5$), 3.42(H_O , s.), 3.6-3.9(5H), 4.24(H_K , broad s.), 4.79(H_J , d., $\underline{J}=2.5$), 7.53(H_A , m.).

V. Summary

Huratoxin(1), a piscicidal constituent, was isolated from the sap of Hura crepitans, and its chemical structure was elucidated on the basis of chemical and spectral evidence. The absolute stereostructure was established by the X-ray diffraction analysis with a heavy atom method.

Huratoxin is a naturally occurring novel orthoester of a diterpene-hexaol with a 2,4-tetradecadienoic acid. Up to date only a few naturally occurring orthoesters have ever been isolated.

The diterpene part of huratoxin is interesting in its biogenesis. The skeleton of 1 is not explained in terms of the same biogenetic sequence from geranylgeranyl pyrophosphate as that of common bi-, tri- or tetracyclic diterpenes. The diterpene part of huratoxin is assumed to be formed by recyclosation of such a macrocyclic diterpene as casbene (27), which has recently been reported as a cyclisation product of geranylgeranyl pyrophosphate with enzyme preparation of castor bean (Ricinus communis, Euphorbiaceae).

The exceedingly strong toxicity of 1, being about 10 times as toxic as rotenone, is also noticeable. It seems to be accounted for in terms of the structure where the highly oxygenated diterpene moiety is bonded through the orthoester linkage to the long aliphatic chain. The participation of the orthoester function in the toxicity has not been revealed yet.

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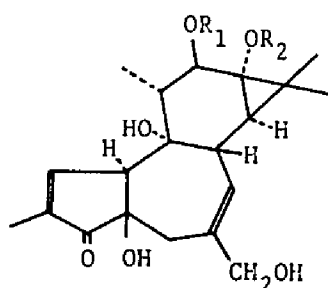
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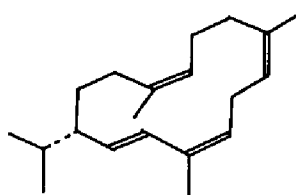
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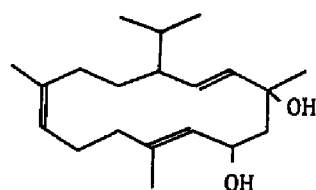


R_1 : acetyl, tiglyl or
(+)-S-2-methylbutyryl
 R_2 : caprylyl, capryl, lauryl,
myristyl or palmityl

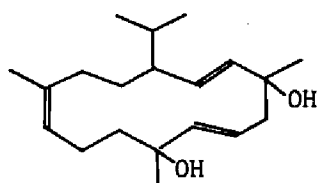
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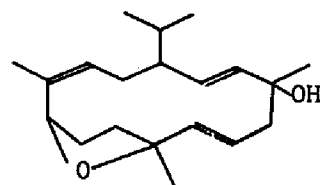
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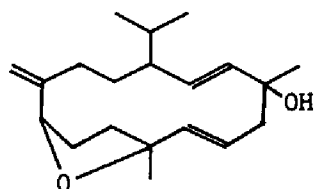
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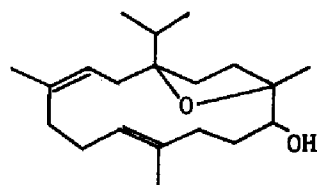
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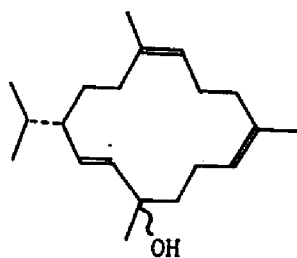
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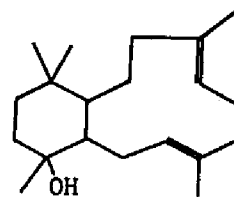
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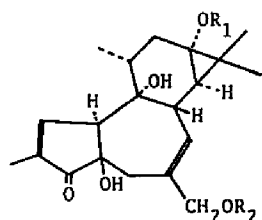
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37

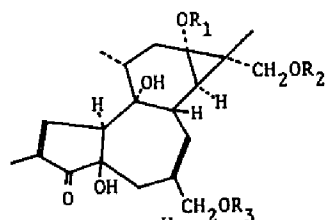


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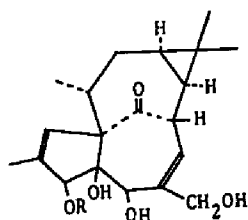
- i: $R_1 = \text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $R_2 = \text{Ac}$
 ii: $R_1 = \text{C}(\text{O})\text{C}(\text{CH}_3)=\text{CH}_2$, $R_2 = \text{Ac}$
 iii: $R_1 = \text{C}(\text{O})\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$, $R_2 = \text{Ac}$

39



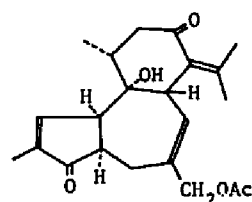
- i: $R_1 = \text{C}(\text{O})\text{C}(\text{CH}_3)=\text{CH}_2$
 $R_2 = \text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $R_3 = \text{H}$
 ii: R_1, R_2 as in i, $R_3 = \text{Ac}$

40

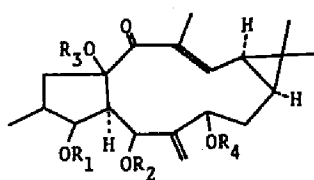


$R : -\text{CO}-(\text{CH}_2)_{14}-\text{CH}_3$

41

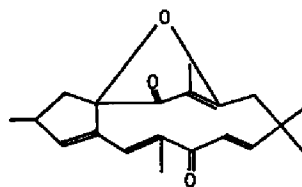


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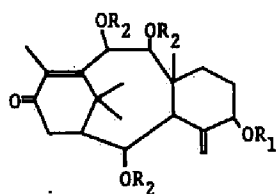


$R_1 \sim R_4 = 2\text{Ac}, 2\text{Bzo}$

43

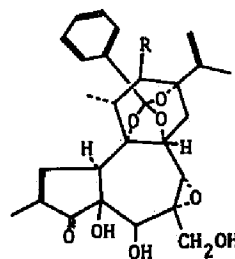


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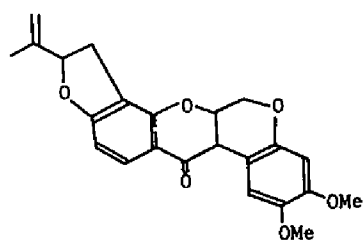


$R_1 = \text{cinnamoyl}, R_2 = \text{Ac}$

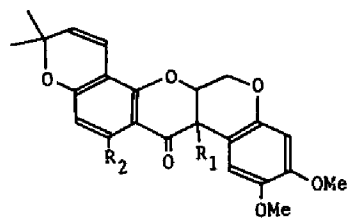
45



46: $R = \text{H}$
 47: $R = \text{O}-\text{C}(\text{O})-\text{C}(\text{H})=\text{C}(\text{H})_2-\text{Ph}$



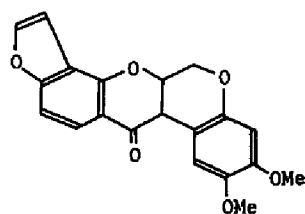
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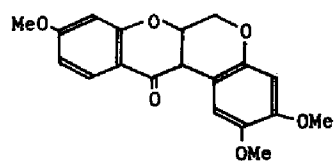
49: $R_1, R_2 = H$

51: $R_1 = OH, R_2 = H$

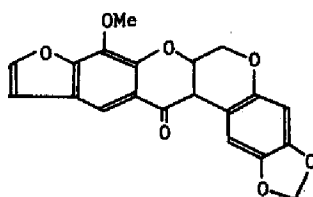
52: $R_1 = H, R_2 = OH$



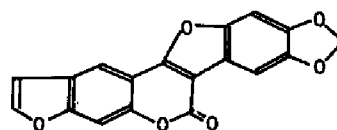
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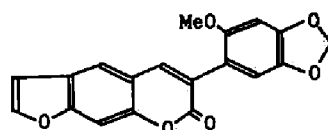
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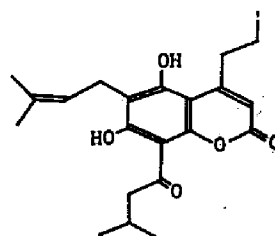
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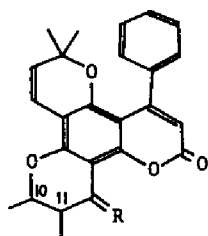
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56



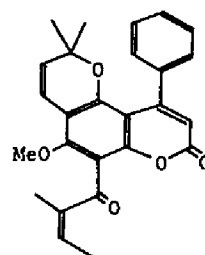
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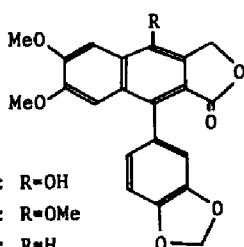
58: R=O (10,11 cis)

59: R=O (10,11 trans)

60: R=H, OH



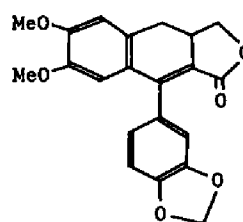
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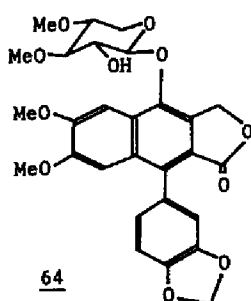
62: R=OH

65: R=OMe

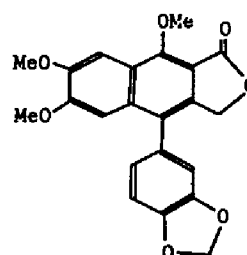
66: R=H



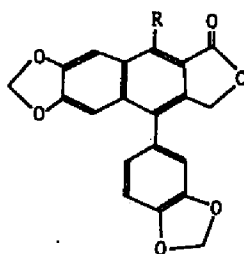
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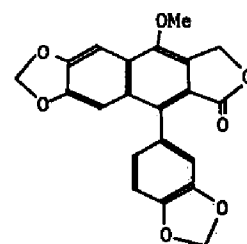


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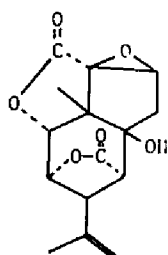


68: R=OMe

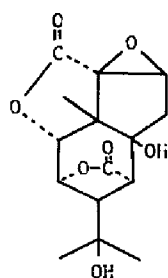
69: R=H



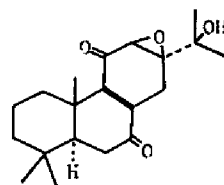
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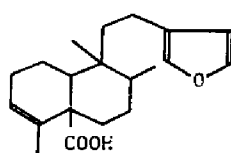
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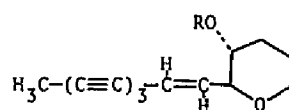
72



73



74



75: R=H

76: R=Ac

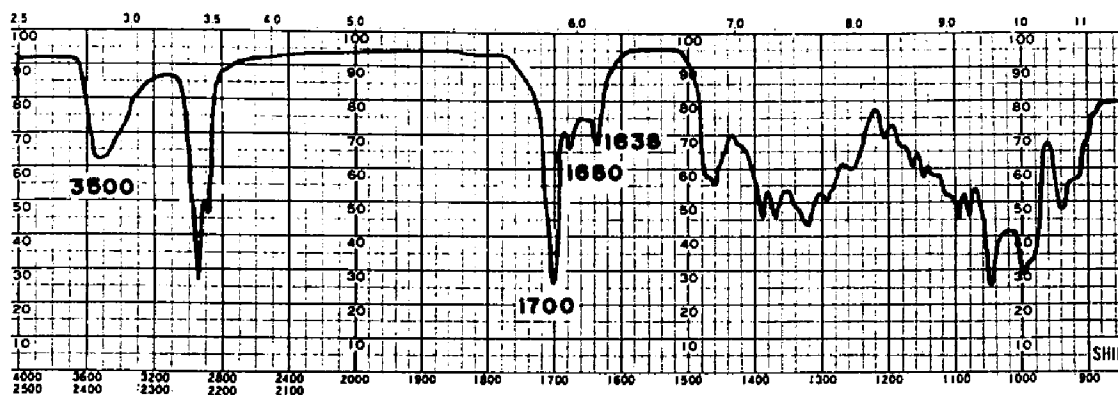


Fig. 2. Infra Red Spectrum of Huratoxin(1) (CCl_4)

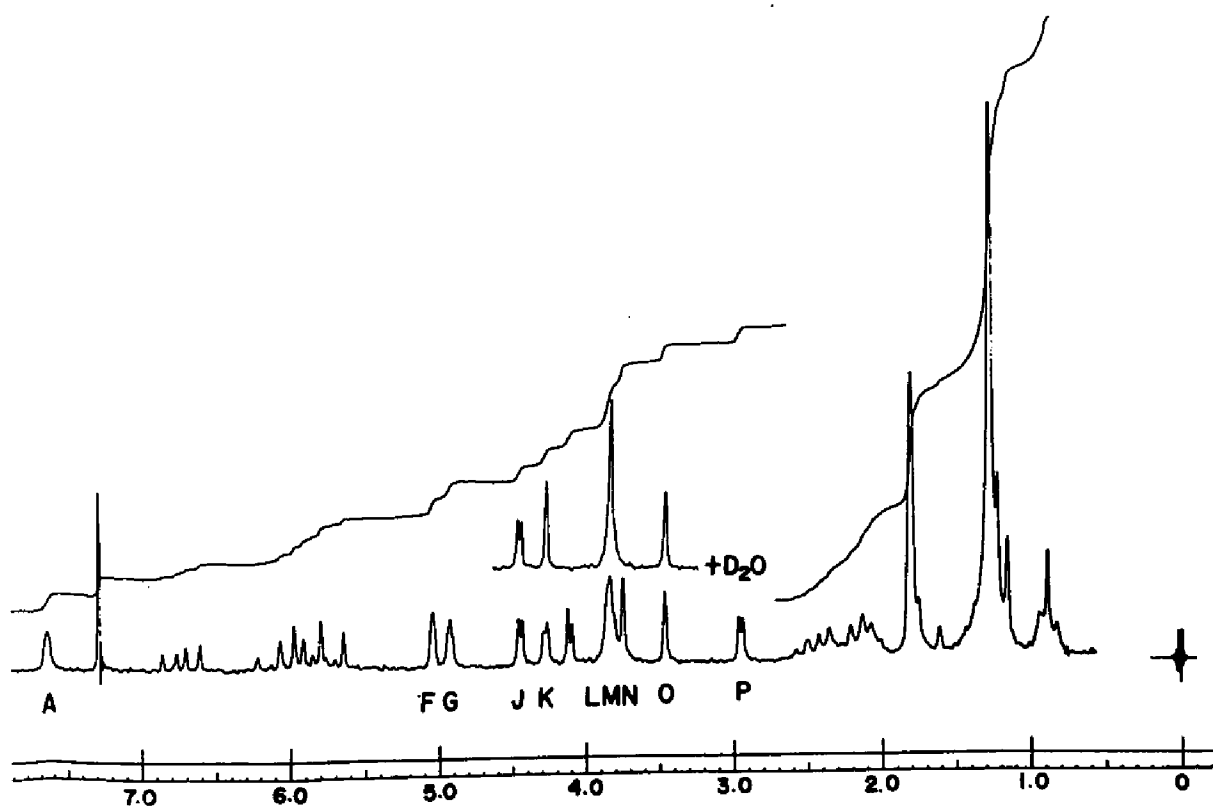


Fig. 3. PMR Spectrum of Huratoxin(1) (CDCl_3 , 100MHz)

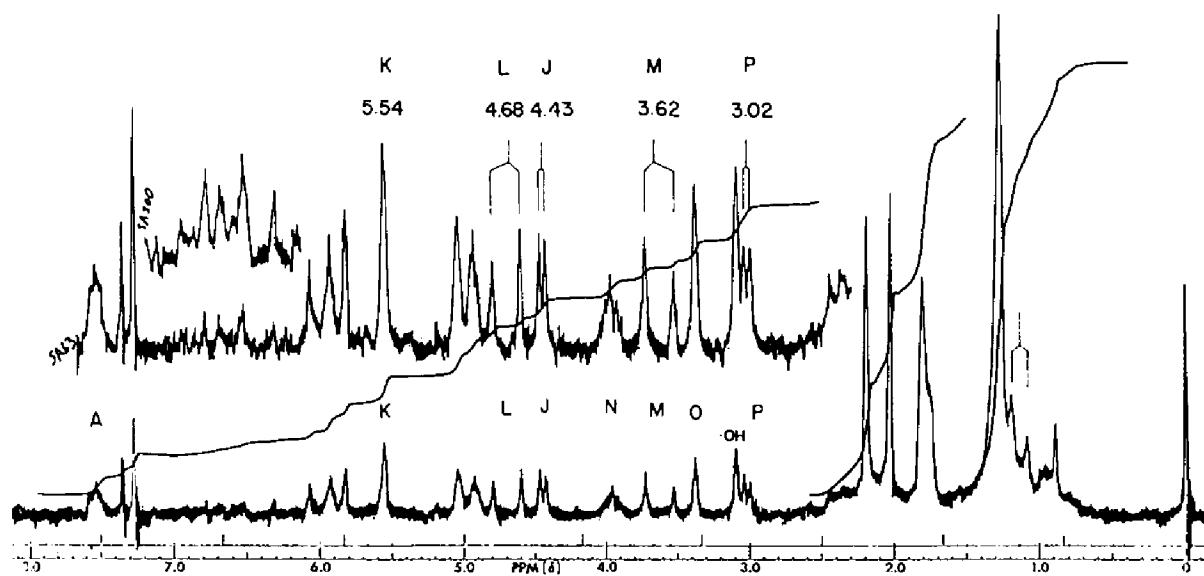
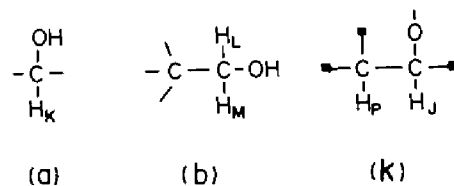


Fig. 4. PMR Spectrum of Diacetyluratonin(3) (CDCl_3 , 60MHz)

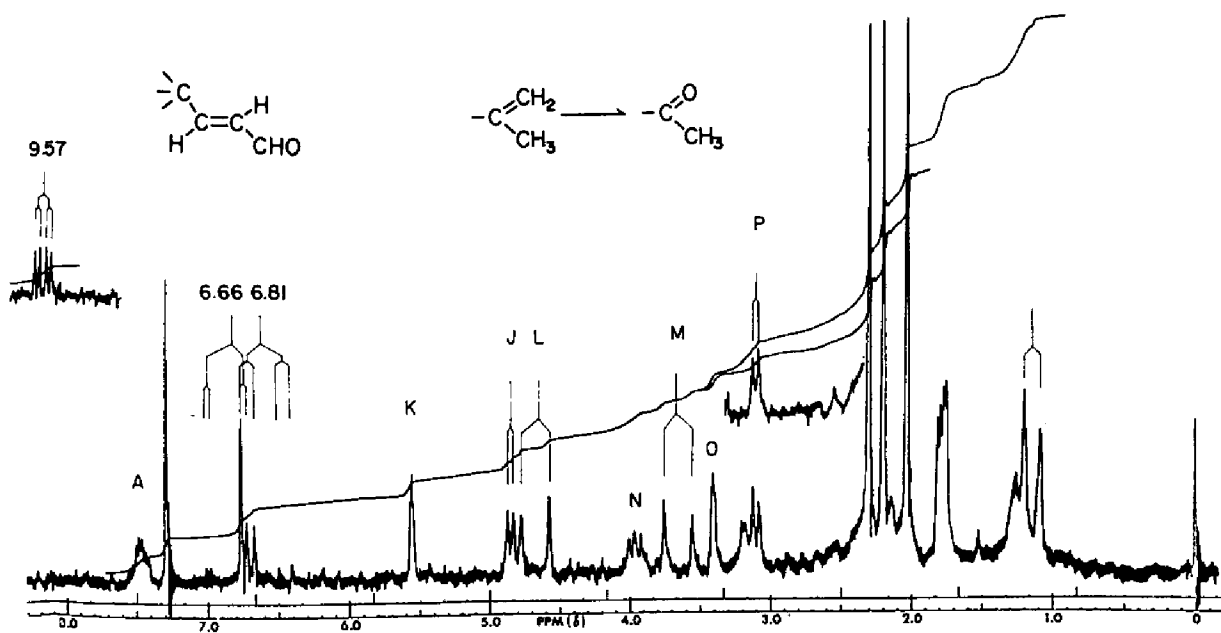
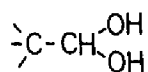


Fig. 5. PMR Spectrum of Ketoenal(5) (CDCl_3 , 60MHz)

Aldehyde hydrate



(M⁺-18) 520

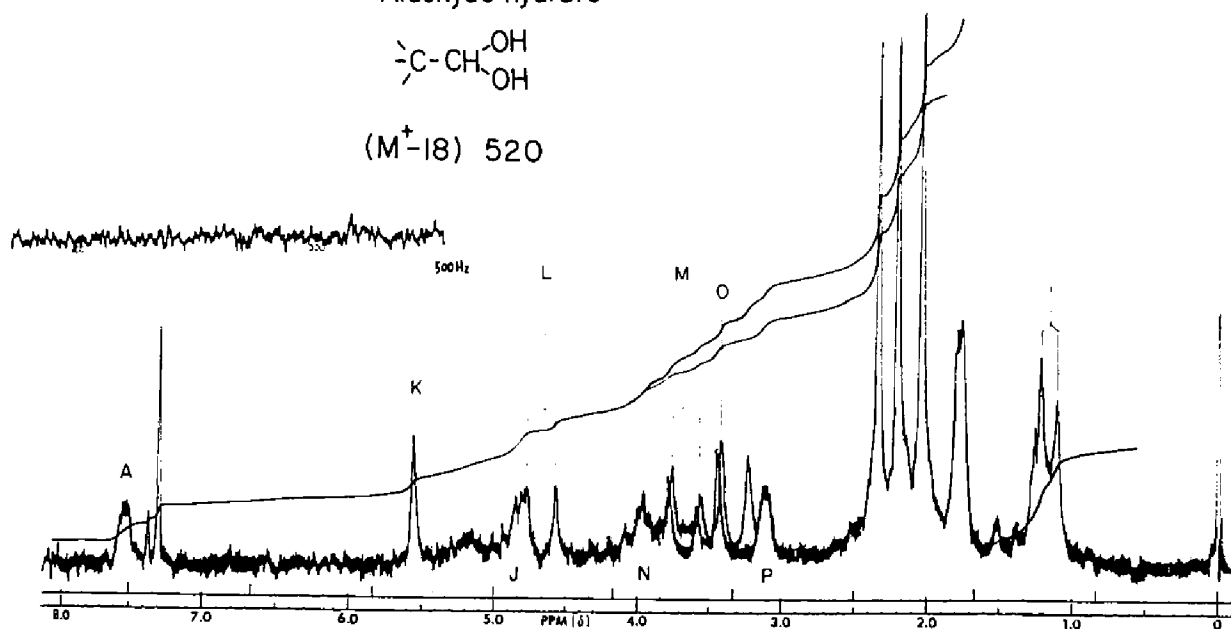


Fig. 6. PMR Spectrum of Aldehyde hydrate(6) (CDCl₃, 60MHz)

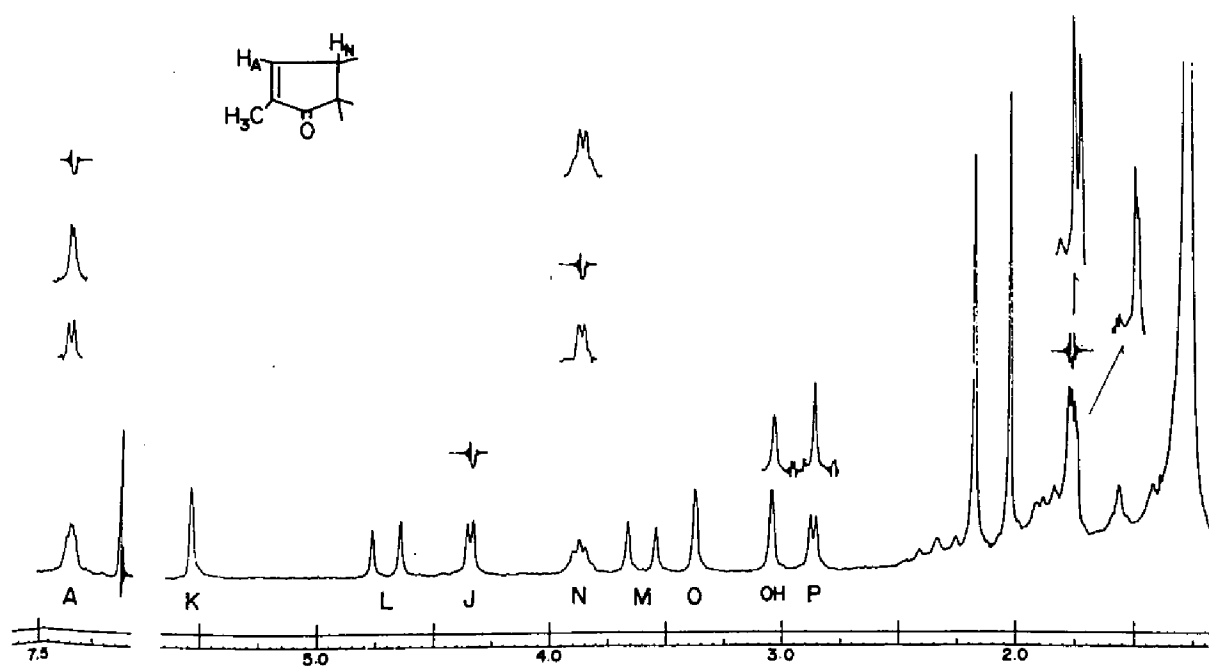


Fig. 7. Spin-decoupling Experiments of Diacetyl hexahydrohuratoin(4) (CDCl₃, 100MHz)

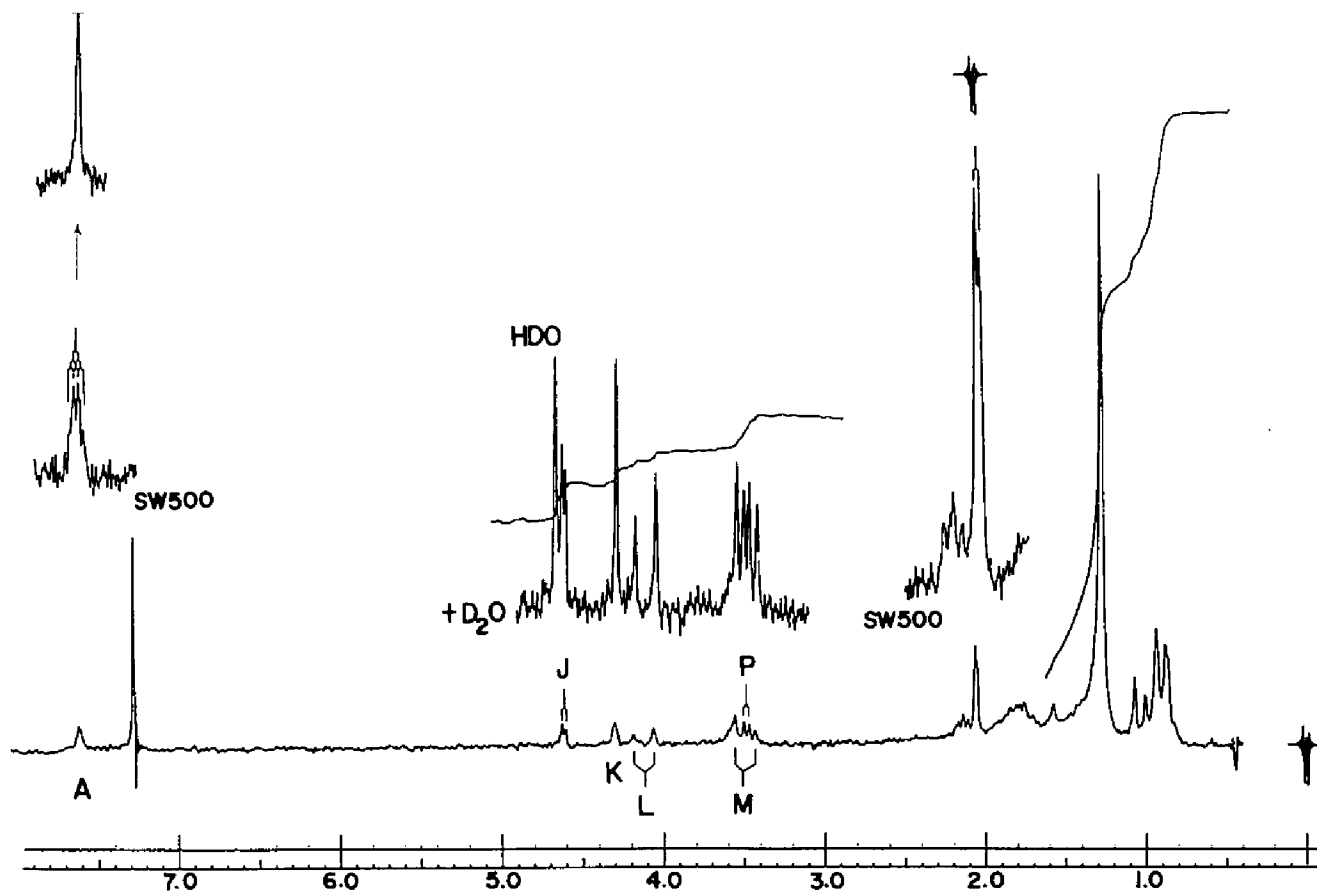


Fig. 10. PMR Spectrum of Hemiacetal(12) (CDCl_3 , 100MHz)

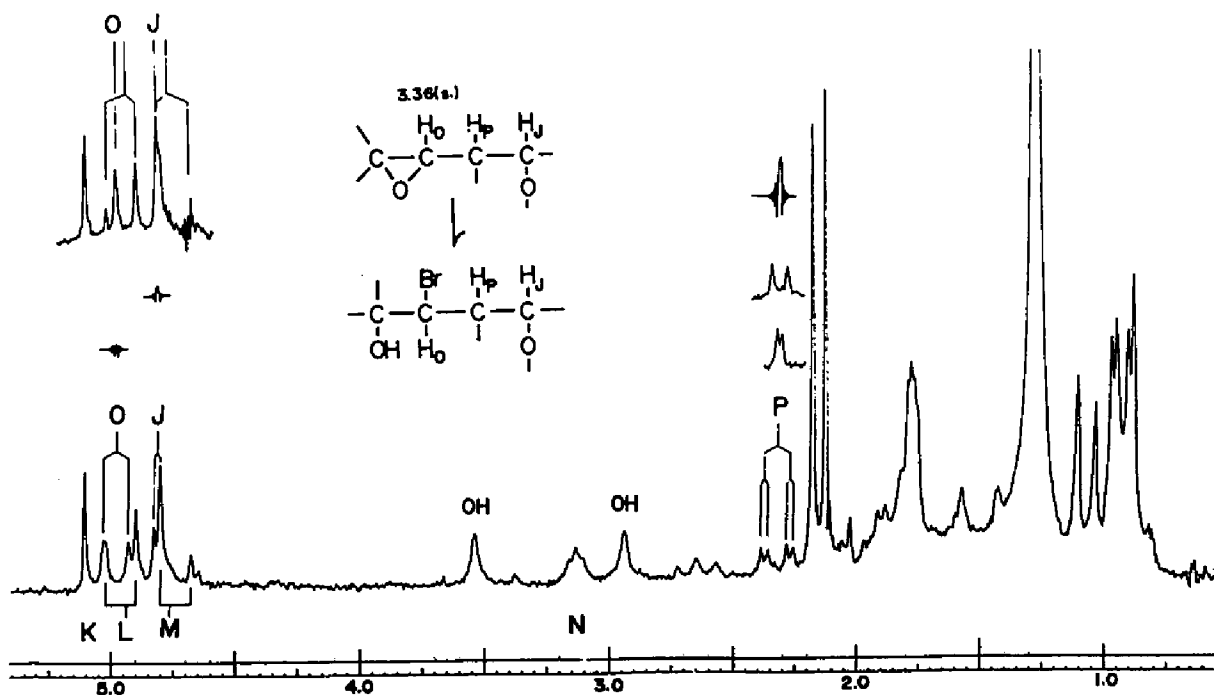


Fig. 12. Spin-decoupling Experiments of Bromohydrin(14) (CDCl_3 , 100MHz)

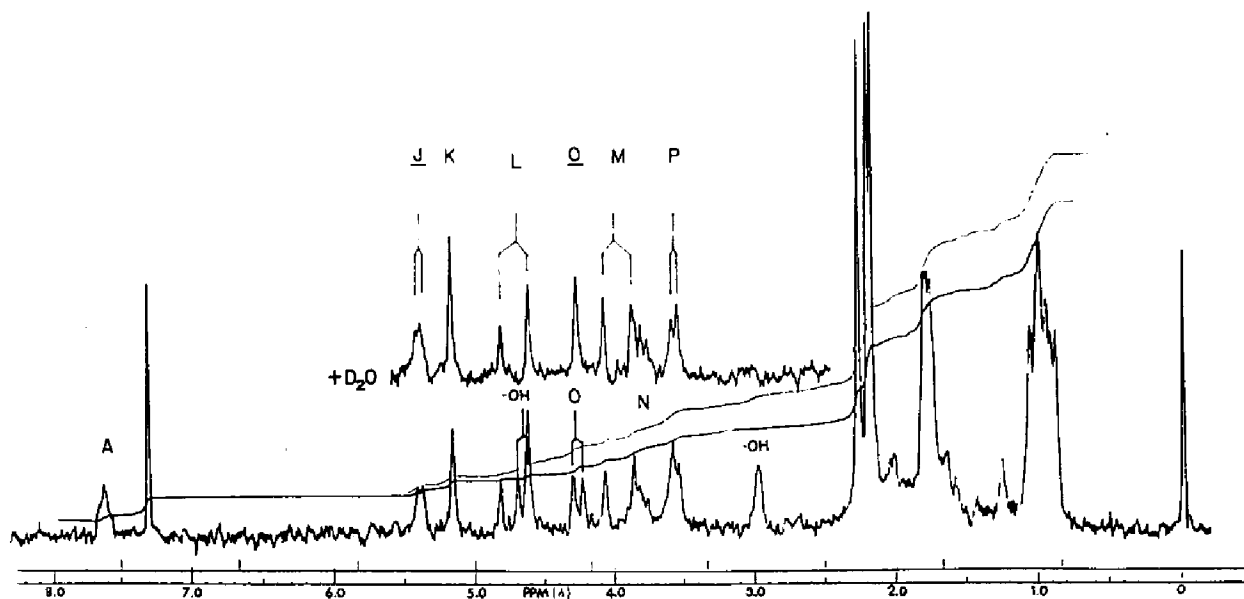


Fig. 13. PMR Spectrum of Polyol triacetate A (17) (CDCl₃, 60MHz)

