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ISOLATION AND STRUCTURE OF A NOVEL GIBBERELLIN IN BAMBOO SHOOTS (PHYLLOSTACHS EDULIS)

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Up to now the presence of seven characterized gibberellins has been established (1,2) in higher plants. They were usually isolated from immature seeds, but exceptionally gibberellin A_1 (GA_1) was isolated from water sprouts of citrus tree by Kawarada and Sumiki (3). In 1963, Kato (4) and Koshimizu et al. (5) demonstrated the presence of a new gibberellin-like substance in young shoots of bamboo (Phyllostachs edulis), which shows extremely rapid growth. In this paper we wish to report the isolation of a new gibberellin from this plant and propose the structure VI. Water extract obtained from 44 tons of bamboo shoots by bleaching process in canning industry was concentrated

in vacuo. The residue was extracted with ethyl acetate, which was then treated in the usual way to afford acidic fraction. This was successively purified through following procedures: counter current distribution (10 transfers, pH 5.4 sated. phosphate buffer-ethyl acetate), charcoal chromatography (elution with acetone-water, 7:3,v/v), silicic acid adsorption chromatography (elution with benzene-ethyl acetate, 1:1, v/v). Active material thus obtained was crystallized from acetone-hexane into 14 mg of colorless rods.

In dwarf maize leaf-sheath growth test, this compound showed the same order of activity as GA_1 on d-5 and as GA_3 on d-2, while its activity on d-1 was one hundredth of those of GA_1 and GA_3. We tentatively named it Bamboo Gibberellin.

It shows dimorphism, form A (unstable) which shrinks at 124-126°C and form B (stable) which melts at 236-237°C. Infrared spectra of both forms are different from each other but on treatment with diazomethane they give an identical methyl ester, m.p. 140°C.

The high resolution mass spectrum* of this ester exhibited a molecular ion peak at m/e 390, and through its elemental composition analysis the molecular formula $C_{22}H_{30}O_6$ was assigned. Its NMR spectrum** in deuterochloroform showed

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* Mass spectrum was determined by JMS-01S high resolution mass spectrometer, equipped with direct inlet system. Electron accelerating voltage was 26 eV.

** NMR spectra were measured by JNM-4H-100 at 100 mc. Chemical shifts are expressed in δ-value in p.p.m. from tetramethylsilane as internal standard.

two methoxyl signals at δ 3.69 and 3.77, indicating that the free acid must be dibasic. Consequently, the molecular formula $C_{20}H_{26}O_6$ was assigned to the original acid. These results, together with its characteristic biological activity, indicate that this acid is a novel C_{20} gibberellin.

The infrared spectra of the acid and its methyl ester reveal the presence of a hydroxyl (acid: (form A) 3480 cm^{-1}, (form B) 3370 cm^{-1}; ester: 3500 cm^{-1}), three carbonyls (acid: (A) 1742, 1718, 1680 cm^{-1}, (B) 1720, 1695 cm^{-1} (intensity of the latter corresponds to two carbonyls); ester: 1727, 1713 cm^{-1} (intensity of the latter corresponds to two carbonyls)) and an exocyclic methylene (acid: (B) 887 cm^{-1}; ester: 884 cm^{-1}). A sharp 1H singlet at δ 9.73 and 3H singlet at δ 1.15 in the NMR spectrum of the ester in deuterochloroform indicate the presence of an aldehyde and a tert-methyl, respectively. The presence of an exocyclic methylene is confirmed by two somewhat broad 1H singlets at δ 4.98 and 5.20, but no other olefinic protons are observed. Thus, bamboo gibberellin must be tetracyclic. These evidences, together with its characteristic gibberellin-like activity, and emission of fluorescence under ultraviolet light on treatment of sulfuric acid after heating, suggest that it not only contains gibbane skeleton (I) but also retains the structural features* common to gibberellins.

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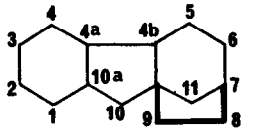
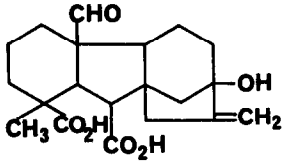
* Most of known C_{19} gibberellins carry a methyl and a lactone at C-1, a carboxyl at C-10 and an exocyclic methylene at C-8 on gibbane ring. C_{20} gibberellins contain an additional one carbon substituent at C-4a.

This assumption was supported by following physico-chemical data. Some differences in NMR spectra of gibberellins measured in deuterochloroform and pyridine have been correlated with their structural features by Hanson (6). He pointed out that in the NMR spectra of gibberellins carrying a hydroxyl at C-2, the signal of methyl protons at C-1 in deuterochloroform shifts to lower field by about 0.3 p.p.m. in pyridine and, similarly, the signal assigned to one of the exocyclic methylene protons in deuterochloroform shifts to lower field by about 0.5 p.p.m. in pyridine when C-7 carries a hydroxyl.



I



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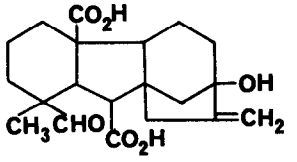
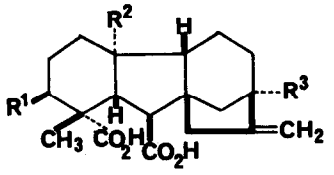


III



IV R^1=H R^2=CH_3 R^3=H

V R^1=OH R^2=CO_2H R^3=H

VI R^1=H R^2=CHO R^3=OH

According to this information, NMR spectra of the methyl ester of bamboo gibberellin in deuterochloroform and pyridine were compared in details. A singlet at δ 1.15 in deuterochloroform assigned to C-1 methyl protons shows only slight shift to δ 1.28 in pyridine, suggesting the absence of hydroxyl at C-2. Two 1H singlets at δ 4.98 and 5.02 in deuterochloroform due to the exocyclic methylene shift to δ 5.10 and 5.57 in pyridine. Thus the presence of hydroxyl at C-7 has been confirmed. Two 1H doublets characteristic of protons at C-10a and C-10 are observed at δ 3.88 and 2.43 (J=12 c.p.s.) in deuterochloroform, δ 4.16 and 2.46 (J= 14 c.p.s.) in pyridine. These chemical shifts of the protons at C-10a are unusually low as compared with those in C_{19} gibberellins, but features of these chemical shifts and coupling constants are quite similar to those of GA_{13} (V) (7) which carries carboxyl groups at C-1, C-4a and C-10. This similarity suggest the presence of three carbonyl functions at C-1, C-4a and C-10 in bamboo gibberellin.

The high resolution mass spectrum of the ester also gives good informations about the structure of bamboo gibberellin. Wulfson et al. (8) reported that mass spectra of methyl ester of GA_1, A_3 and A_4 containing hydroxyl at C-2 and carboxyl at C-10 give a peak with moderate intensity at m/e M-18 and very intense ketene peak at m/e M-32. Complete lack of a peak at m/e 372 (M-18) as well as the presence of a very prominent peak at m/e 358 (M-32) in the spectrum of bamboo gibberellin methyl ester indicate the absence of hydroxyl at C-2 and presence of a carboxyl at C-10 in

bamboo gibberellin. The peak at m/e 362 may be due to elimination of CO from formylgroup accompanying with rearrangement of a hydrogen atom. Most of intense peak higher than m/e 225 can be explained by elimination of substituents on the gibbane skeleton.

The structures II and III are only compatible with the evidences cited above. All gibberellins already known contain carboxyl or its equivalents at C-1 and C-10, but substituents at C-4a are quite variable depending upon biogenetical stages. Namely, every C_{19} gibberellin has no one carbon substituent at C-4a, while among C_{20} gibberellins, GA_{12} (IV) (9) contains methyl and GA_{13} carboxyl there. Recently Cross et al. (10) demonstrated that GA_{13} is an intermediate between GA_{12} and GA_3 by feeding experiment using Gibberella fujikuroi. Bamboo gibberellin is also most likely to be a key intermediate in biological transformation from C_{20} to C_{19} gibberellins. On the basis of above consideration, structure II is proposed to bamboo gibberellin and its stereochemistry is expected to be the same as that of GA_{13} as shown in VI. Following observations support this assignment of stereochemistry. In the infrared spectrum of the free acid of form B, the carbonyl bands, probably due to aldehyde and carboxyl groups, appear at 1692 cm^{-1}(broad), whereas these bands shift to normal frequencies in the spectrum of the ester. This can be explained by assuming the presence of internal hydrogen bond in the acid between aldehyde and carboxyl groups in cis-1,3-diaxial position. Further, the large coupling constant, J=12--14 c.p.s., between the protons

at C-10 and C-10a presumably indicates that these protons are situated in trans configuration because dihederal angle between these two protons in this configutation is nearly 180°. Thus the structure VI has been proposed to bamboo gibberellin. This is the first demonstration not only for the presence of C_{20} gibberellins in higher plants but also for the isolation of gibberellin from normally growing plants.

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