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GIBBERELLIN A_{23} IN IMMATURE SEEDS OF *LUPINUS LUTEUS*

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Gibberellin-like substances have been shown to occur in immature seeds of *Lupinus luteus*[1-4] and an attempt was made to purify two active principles, which were tentatively named as Lupinus gibberellin I and II[5]. The former structure, whose provisional name was renamed as gibberellin A_{18}**, has been reported previously[6]. In this communication we wish to present evidence for the structure and stereochemistry of the latter C-20 Gibberellin, now named gibberellin A_{23}.

GA_{23}, (II) was isolated from the acidic fraction of the seeds in yield of ca. 0.04% by the same extraction procedure reported previously[6]. It was not crystallizable from the usual solvents, which gave an amorphous solid (m.p. 185-9°). However, this material was homogeneous on TLC and afforded only a single derivative on esterification and sodium borohydride reduction. It showed a purple fluorescent spot at R_{GA_3} 0.68 in system A*** and 0.60 in system B**** on TLC of silica gel G under UV light after spraying with 5% sulphuric acid-ethanol and heating, and showed a yellow spot with 0.5% aqueous solution of potassium permanganate.

The IR spectrum (KBr) indicated the presence of hydroxyls (3500-3300 br. cm^{-1}) and carboxyls (2800-2500 br. and 1708 br. cm^{-1}). On treatment with diazomethane (II) gave a

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** GA_1-GA_{15}, cf. ref. (7). The naming of GA_{16}-GA_{22} will be published elswhere in the near future by Drs. J. MacMillan and N. Takahashi.

*** System A: benzene-*n*-butanol-acetic acid, 70:25:5 v/v.

**** System B: ethyl acetate-chloroform-acetic acid, 15:5:1 v/v.

dimethyl ester (III), whose mass spectrum* exhibited a molecular ion peak at m/e 406. The IR spectrum of the ester (KBr) showed absorption attributable to hydroxyls (3500-3300 br. cm^{-1}), carbonyls (1713 br. cm^{-1}) and terminal methylene (1658 and 893 cm^{-1}). The compound (III) had NMR** signals ($CDCl_3$) at 1.20 ppm (3H, singlet, tertiary methyl), 3.65 and 3.73 ppm (3H each, singlets, two carbomethoxyls), 4.11 ppm (1H, multiplet, H-C-OH), 4.93 and 5.17 ppm (1H each, multiplets, terminal methylene), and 9.72 ppm (1H, singlet, aldehyde). The presence of two hydroxyl proton signals around 2.05 ppm was confirmed by shaking the solution of the sample with deuterium oxide. Under normal acetylation conditions (III) formed a monoacetate (IV), whose NMR spectrum ($CDCl_3$) showed a signal at 5.28 ppm (triplet, J=2.5 cps, H-C-OAc) and one hydroxyl proton signal around 2.22 ppm. This indicates that (II) contains one secondary and one tertiary hydroxyl group. These spectral data enable us to account for the number of oxygen atoms in the molecule, and the molecular formula $C_{20}H_{26}O_7$ may, thus, be reasonably assigned to the original acid (II). In addition to the signals described above, the resonance spectrum of (III) showed a pair of doublets, centered at 3.88 and 2.77 ppm (J=13 cps), which is assigned to the characteristic[8,9] gibbane 10:10a quartet. These results, together with its specific gibberellin-like activity on the growth promotion of rice and dwarf corn seedlings, suggest that (II) not only has five pendant carbon groups on a gibbane ring, but also retains the same stereochemistry as that of the known C-20 gibberellins[10-15] as shown in formula (I). The gibbane AB-quartet at such a low field indicates that one of the carboxyl groups is located at C-10 in β axial, and that the remaining carboxyl and aldehyde groups are placed either at C-1 or C-4a in α axial orientation respectively. Then the large paramagnetic shift of the C-10 α proton resonance may be visualized as being due to 1,3 diaxial transannular effects from the carbonyl function at C-1 and C-4a positions, similar to those of GA_{13}[11] and GA_{19}[14,15] (Bamboo gibberellin).

In pyridine, a singlet at 1.20 ppm, ascribed to C-1β methyl protons in a deuteriochloroform solution of the methyl ester (III), was shifted to a lower field by 0.40 ppm, and a doublet at 2.77 ppm, assigned to the C-10aβ proton in deuteriochloroform, was deshielded by 0.60 ppm. This shows that a secondary hydroxyl is located at C-2 in a *cis*-1,3 diaxial relation to the

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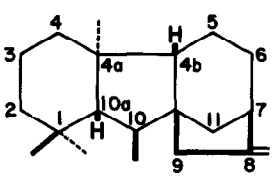
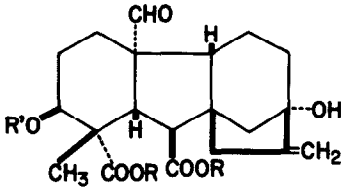
* Mass spectrum was determined on a Hitachi RMU-6D spectrometer with a direct inlet system.

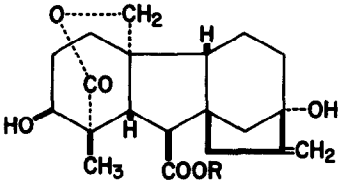
** NMR spectra were recorded on a Varian 60-A instrument with TMS as the internal standard.



(I)



(II) R=R'=H
(III) R=Me R'=H
(IV) R=Me R'=Ac



(V) R=H
(VI) R=Me

C-10a proton*. The presence of a tertiary hydroxyl at C-7* is confirmed by the evidence that the signals at 4.93 and 5.17 ppm in deuteriochloroform due to the terminal methylene protons in (III) shifted to 5.03 and 5.53 ppm in pyridine, respectively.

The mass spectrum of the ester (III) showed prominent peaks at m/e 374(M-32) and 346(M-60), which are commonly observed in the spectra of C-19[15-19] and C-20[19] gibberellin methyl esters. There were also significant peaks at m/e 378(M-28), 360(M-46), 342(M-64), 328(M-78), 318(M-88), 300(M-106), 286(M-120), 257(M-149) and 241(M-165). Those peaks can be explained by elimination of functional groups substituted on the gibbane ring. This general fragmentation pattern bears a resemblance to that reported for GA_{19} methyl ester[15], except for the prominent peaks at m/e 388 and 356. These two peaks are attributable to the ions formed by the loss of one molecule of water from the ions of M and M-32, respectively, due to the presence of a hydroxyl group at C-2 in (III). It is therefore possible to arrive at structure (II), a monohydroxy derivative of GA_{19}[14,15] at C-2, for GA_{23} on the basis of the above data, and this structure was further supported by the following evidence.

Reduction of the acid (II) with sodium borohydride in ethanol at room temperature yielded a monoacid (V), m.p. 237-9°, $[\alpha]_D^{20°}$ +29.5° (c, 0.576 in methanol). The IR spectra of the acid (V) (KBr) and the corresponding methyl ester (VI) ($CHCl_3$) showed absorptions attributable to δ-lactone (acid:1728 cm^{-1}), carboxyl (acid:2800-2500 br. and 1696 cm^{-1}) and hydroxyls (acid: 3390 and 3270 br. cm^{-1}, ester:3620 and 3460-3420 br. cm^{-1}) and terminal methylene (ester:1667 and 899 cm^{-1}). The NMR spectrum ($CDCl_3$) of the ester (VI) showed peaks at 1.19 ppm (3H, singlet, tertiary methyl), 3.70 ppm (3H, singlet, carbomethoxyl), 3.78 ppm (1H, multiplet,

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* Detail of these assignment, cf. ref. (9,14, 15).

H-Ċ-OH), 4.91 and 5.22 ppm (1H each, multiplets, terminal methylene). It is interesting to note that the spectrum lacked a feature highly reminiscent of the quartet due to the 10:10a protons in the known gibberellins. The two proton signals appeared only as a singlet at 2.79 ppm*, although the spectrum of the free acid (V) in pyridine showed an AB-quartet centered at 3.32 and 3.35 ppm (J=13 cps). In addition, at the expense of an aldehydic proton signal observed in (III), a pair of doublets appeared at 4.09 and 4.48 ppm (J=12 cps) in (VI). These signals assigned to the quartet due to $-CH_2OCO$ correspond to those observed in GA_{15}[13]. The chemical shift of tertiary methyl protons at C-1 was also fairly consistent with that of GA_{15}, which is deshielded by the β-carbonyl group belonging to the lactone ring. This indicates that the oxygen of the δ-lactone ring is attached to the carbon substituted at position 4a. The aldehyde and carboxyl groups in (II) are then placed at C-4a and C-1 in a *cis*-1,3 diaxial position, respectively.

GA_{23} showed no response to 2,4-dinitrophenylhydrazine in acidic ethanol[22] on TLC, although it has a free aldehyde group.

GA_{23} and its derivative (V), tentatively named as 2,7-dihydroxy GA_{15}, exhibited about the same activity as GA_1 on the growth of rice seedlings.

Acknowledgment

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References

1. Y.Murakami, *Bot. Mag.(Tokyo)*, 72, 438 (1959).
2. Y.Ogawa and S.Imamura, *ibid.*, 73, 125 (1960).
3. Y.Hirono, Y.Ogawa and S.Imamura, *Plant & Cell Physiol.*, 1, 81 (1960).
4. Y.Ogawa, *ibid.*, 4, 85 (1963).
5. Y.Ogawa, *Bot. Mag.(Tokyo)*, 79, 197 (1966).
6. K.Koshimizu, H.Fukui, T.Kusaki, T.Mitsui and Y.Ogawa, *Tetrahedron Letters*, 2459 (1966).
7. B.D.Cavell, J.MacMillan, R.J.Pryce and A.C.Sheppard, *Phytochemistry*, 6, 867 (1967).

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* A similar signal, due to the 10:10a protons, has been observed at 2.85 ppm as a two proton singlet on the spectrum of GA_{22} methyl ester[20,21] (Canavaria gibberellin II methyl ester) in deuteriochloroform.

8. N.Sheppard, *J. Chem. Soc.*, 3040 (1960).

9. J.R.Hanson, *ibid.*, 5036 (1965).

10. B.E.Cross and K.Norton, *ibid.*, 1570 (1965).

11. R.H.B.Galt, *ibid.*, 3143 (1965).

12. B.E.Cross, *J. Chem. Soc. C*, 501 (1966).

13. J.R.Hanson, *Tetrahedron*, 23, 733 (1967).

14. S.Tamura, N.Takahashi, N.Murofushi, S.Iriuchijima, J.Kato, Y.Wada, E.Watanabe and T.Aoyama, *Tetrahedron Letters*, 2465 (1966).

15. N.Murofushi, S.Iriuchijima, N.Takahashi, S.Tamura, J.Kato, Y.Wada, E.Watanabe and T.Aoyama, *Agr. Biol. Chem.*, 30, 917 (1966).

16. N.S.Wulfson, V.I.Zaretskii, I.B.Papernaja, E.P.Serebryakov and V.F.Kucherov, *Tetrahedron Letters*, 4209 (1965).

17. N.Takahashi, N.Murofushi, S.Tamura, N.Wasada, H.Hoshino, T.Tsuchiya, T.Aoyama and H.Morita, *ibid.*, 895 (1967).

18. N.Takahashi, N.Murofushi, T.Yokota and S.Tamura, *ibid.*, 1065 (1967).

19. N.Murofushi, N.Takahashi, S.Tamura, N.Wasada, H.Hoshino, T.Tsuchiya, S.Sasaki and M.Itagaki, Third symposium on Mass Spectrometry of Organic Compounds, Kyoto University, October 1967.

20. N.Murofushi, S.Iriuchijima, N.Takahashi, S.Tamura, J.Kato, Y.Wada, E.Watanabe and T.Aoyama, *Planta*, 75, 279 (1967).

21. S.Tamura, N.Takahashi, N.Murofushi and T.Yokota, Sixth International Conference on Plant Growth Substances, Carleton University, July 1967.

22. D.Waldi, *Thin-Layer Chromatography*, E.Stahl ed., p. 490, Springer-Verlag, Berlin (1965).