Tetrahedron Letters No.22, pp. 2459-2463, 1966. Pergamon Press Ltd.
Printed in Great Britain.

A NEW C_{20} GIBBERELLIN IN IMMATURE SEEDS OF LUPINUS LUTEUS

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(Received 22 March 1966)

Extracts of immature seeds of Lupinus luteus have been found to possess biological activities similar to the gibberellins (1-4). It was suggested from the chromatographic behavior and the characteristics of infrared spectra that the active principles were different from C_{19} gibberellins (5). We wish to report the isolation of a new gibberellin, "Lupinus gibberellin-I", from the seeds, and to propose the structure shown as I for the gibberellin.

The method of isolation followed that of MacMillan et al. (6). The fraction eluted from the charcoal-celite column with water containing 59-62 % acetone was rechromatographed on silica-celite. Elution with 50-55 % ethyl acetate in chloroform yielded 35 mg. of Lupinus gibberellin, (L_1), from 60 kg. of the seeds. L_1, (I), which formed needles from acetone, m.p. 240-242°, gave elementary analysis

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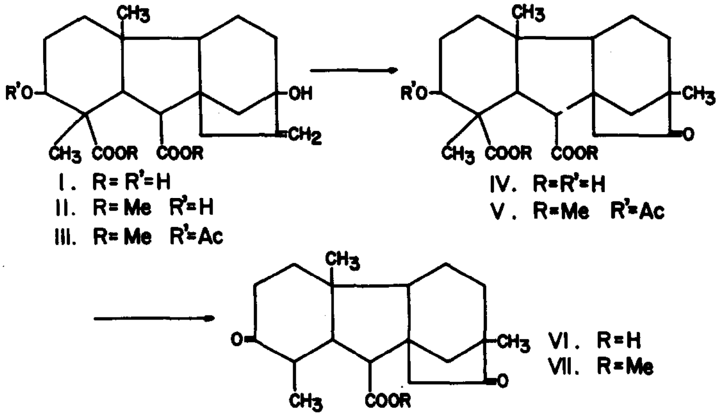
consistent with the formula $C_{20}H_{28}O_6$.

The infrared (I.R.) absorption (nujol) at 3400-3100 br., 2500 br., 1703 and 1673 cm^{-1}, and no ultraviolet absorption between 250 and 350 mμ indicated the presence of hydroxyl and carboxyl group in L_1. The I.R. spectrum of the corresponding methyl ester (II) (KBr) showed absorption attributable to hydroxyl (3550-3300 br. cm^{-1}), ester carbonyl (1725 br. cm^{-1}) and terminal methylene (1667 and 894 cm^{-1}). Under normal acetylation conditions, the methyl ester formed a monoacetate (III) which had I.R. absorption ($CHCl_3$) at 3585 (tertiary hydroxyl), 1720 br. (ester carbonyl), 1665 and 896 cm^{-1} (terminal methylene), and had nuclear magnetic resonance (N.M.R.) signals at 0.70 and 0.97 δ (two tertiary methyls), 2.07 δ (acetate), 3.67 and 3.69 δ (two carbomethoxyls), 4.87 and 5.10 δ (terminal methylene, multiplet) and 5.29 δ (H-$\overset{|}{\underset{|}{C}}$-OAc, triplet, J=2.5 c.p.s.). In addition, a pair of doublets centered at 2.26 and 3.26 δ (J=12 c.p.s.) was assigned to the 10, 10a AB-quartet which is a characteristic feature of the gibbane skeleton in the known gibberellins and their derivatives (7-9). The presence of a tertiary hydroxyl proton signal at 2.11 δ was confirmed by shaking the solution of the sample with deuterium oxide. L_1,(I), has therefore five pendant carbon groups on a gibbane ring as GA_{12} (10) and GA_{13} (11) which were isolated from fermentations of Gibberella fujikuroi.

Treatment of L_1 with dilute hydrochloric acid at 100° gave a keto-acid (IV), and methylation of the keto-acid



I. R=R'=H
II. R=Me R'=H
III. R=Me R'=Ac

IV. R=R'=H
V. R=Me R'=Ac

VI. R=H
VII. R=Me

produced a dimethyl ester which readily formed a monoacetate (V). The resultant monoacetate (V) showed the I.R. absorption band (CCl_4) at 1740 br. cm^{-1}, and the N.M.R. signals at 0.67, 0.97 and 0.98 δ (three tertiary methyls), 2.10 δ (acetate), 2.40 (doublet, J=12 c.p.s.) and 3.38 δ (doublet, J=12 c.p.s.) (10, 10a protons), 3.69 δ (two carbomethoxyls) and 5.32 δ ($H-\overset{|}{\underset{|}{C}}-OAc$, triplet, J=2.5 c.p.s.). Comparison of the spectra of III and V showed that the acid treatment caused the rearrangement which is analogous to the Wagner-Meerwein rearrangement of GA_1 (12, 13), GA_5 (14), GA_6 and GA_8 (6) to their keto-acids.

The presence of a gibbane AB-quartet in L_1 derivatives required a carboxyl group at C-10, and the rearrangement of L_1, (I), to the keto-acid (IV) suggested that the tertiary hydroxyl group was placed at C-7 and the terminal methylene group at C-8.

The keto-acid (IV) on treatment with the Jones reagent was oxidized to a diketo-acid which was decarboxylated at 100° to give a diketo-monoacid (VI). The N.M.R. spectrum of the corresponding methyl ester (VII) showed signals at 0.83 δ ($=CH-CH_3$, doublet, J=6 c.p.s.), 0.98 and 1.02 δ (two tertiary methyls) and 3.67 δ (carbomethoxyl). The hydroxyl group was thus placed at C-2 which was adjacent to C-1 bearing both a tertiary methyl group and a carboxyl group.

Lupinus gibberellin-I was assayed on rice seedlings (15). It increased the second leaf sheath length to ca. 200 % of the control at an optimum concentration of 10 mg./l.

The N.M.R. spectra were measured on a Varian A60 spectrometer in carbontetrachloride with tetramethylsilane as internal reference.

We thank Dr. T. Shingu for the measurements of N.M.R. spectra and Prof. M. Nakajima for the I.R. absorption spectra.

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