Short Communication

Yukiyoshi OGAWA*: Properties of Gibberellin-Like Substances from
Seeds of *Lupinus luteus***

小川幸持*: ルピナス種子におけるジベレリン様物質の特性について**

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Evidence for the occurrence of gibberellin-like substances in *Lupinus* seeds has been reported by several investigators¹⁻⁴). West and Reilly⁵), after considering Rf values, suggested that the *Lupinus*-gibberellin is identical with or closely related to gibberellin A₈. In the present paper preliminary observations concerning this question are reported.

The ethanol extract obtained from seeds of *Lupinus luteus* (ca. 4 kg), after removal of alcohol, was adjusted to pH 3 with 4 N hydrochloric acid and then extracted with ethyl acetate. The ethyl acetate fraction was extracted with pH 8 phosphate buffer solution. The buffer extract was then adjusted to pH 3 with the hydrochloric acid

Table 1. Rf values of *Lupinus*-gibberellins and gibberellins A₁ to A₉
on thin layer chromatogram.

Compounds	Solvents	<i>iso</i> -Propanol (4) Water (1)	<i>n</i> -Butanol (3) 4.5 N Ammonia (1)
<i>Lupinus</i> -1		0.35	0.05
<i>Lupinus</i> -2		0.35	0.05
Gibb. A ₁		0.57	0.18
A ₂		0.50	0.18
A ₃		0.57	0.22
A ₄		0.67	0.41
A ₅		0.58	0.41
A ₇		0.65	0.43
A ₈		0.48	0.10
A ₉		0.70	0.65

and extracted with ethyl acetate. This extract (ca. 3 g) was chromatographed on a column of charcoal-Celite with aceton-water mixtures as described by MacMillan *et al*⁶). Each of the fractions was examined for its growth promoting activities by means of the rice seedling test⁷). Activity was found in the fractions eluted with 55% (L-1, 200 mg) and 50% acetone (L-2, 150 mg). Each fraction was rechromatographed on a column of Celite-silicic acid with chloroform containing increasing proportions of ethyl acetate. Activities corresponding to those of L-1 and L-2 were found in the

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fractions containing 50–55% and 60–65% ethyl acetate in chloroform respectively. Attempts were made to crystallize these substances from ethyl acetate-petroleum, and colourless powders of L-1 (40 mg, mp. 231–234°) and L-2 (20 mg, mp. 205–210°) were obtained. The leaf sheath of rice treated with 5 mg/l of these *Lupinus*-gibberellins was twice as long as that of the water control. Thin layer chromatography of *Lupinus*-gibberellins and gibberellins A₁–A₉ was carried out on a layer of silica gel G at room temperature (30–35°). Spots of these substances were detected by spraying with sulphuric acid, heating to 120° and viewing under ultra violet light⁸⁾. Concur-

Fig. 1. Histograms of the activities of 5 μ g of *Lupinus*-gibberellins (L-1 and L-2) on thin layer chromatograms using a mixture of *n*-propanol and 3 N ammonium hydroxide (5:1). The positions of *Lupinus*-gibberellins and gibberellins A₁–A₉ (except A₆) are shown in the lower part. They were detected under ultra violet light followed by spraying with sulphuric acid and heating.

Table 2. Infra-red absorption maxima, 1690–1800 cm⁻¹ (KBr).

Compounds	Absorption maxima (cm ⁻¹)	
	Carbonyl	5-ring lactone
<i>Lupinus</i> -1	1700	—
<i>Lupinus</i> -2	1690	—
Gibb. A ₁	1720	1740
A ₃	1720 (shoulder)	1750
A ₈	1710 (shoulder)	1740

rently the positions corresponding to the spots of the *Lupinus*-gibberellins on the chromatogram were assayed on the rice seedling. The results are shown in Table 1 and Fig. 1. The R_f values of the *Lupinus*-gibberellins are different from those of gibberellins A₁–A₉. Infra-red spectra of *Lupinus*-gibberellins were determined as KBr

pellets. Table 1 shows the absorption maxima in the frequency range 1690 to 1800 cm^{-1} for the *Lupinus*-gibberellins and gibberellins A₁, A₃ and A₈. The positions of the maxima around 1700 cm^{-1} are consistent with the presence of the maxima around 1700 cm^{-1} are consistent with the presence of carbonyl radical, probably carboxylic acid. The failure of absorption by the *Lupinus*-gibberellins at 1750 cm^{-1} indicates the absence of the 5-ring lactone which is assignable to the gibberellins A₁-A₁₁⁹⁾. It may be concluded that the *Lupinus*-gibberellins are entirely different from gibberellin A₈ which is almost without effect on the rice seedling, and chemically new substances which are different from the known gibberellins A₁-A₁₁.

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