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# ISOLATION OF A CYTOKININ, (−)-DIHYDROZEATIN, FROM IMMATURE SEEDS OF LUPINUS LUTEUS

Koichi Koshimizu, Toshiatsu Kusaki and Tetsuo Mitsui,

Department of Agricultural Chemistry, Kyoto University, Kyoto, Japan,

Satoshi Matsubara,

Biological Laboratory, Kyoto Prefectural University, Kyoto, Japan.

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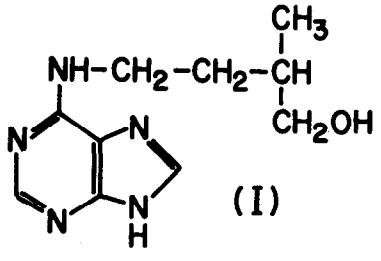
Evidence has been accumulated for the occurrence, in a number of higher plants, of substances with chemical and biological properties similar to kinetin (1). The first of naturally occurring cytokinins, zeatin, has been isolated from immature corn kernels (2) and identified as 6-N-(trans-4-hydroxy-3-methylbut-2-enylamino)purine (3-5). Recently 6-N-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine, which also possessed cytokinin activity, was isolated from yeast s-RNA as one of its constituent nucleosides (6).

In a search for cytokinins from plant sources, extracts of immature seeds of Lupinus luteus were shown to have strong cell-division promoting activity (7), and the partial purification of an active factor, tentatively named "Component L", was reported previously (8-10). We now wish to report the isolation of this factor, from lupin seeds, which has been identified as (−)-dihydrozeatin, (−)-6-N-(4-hydroxy-3-methylbutylamino)purine (I).




(I)

The extraction and purification procedures were followed by bioassay using tobacco callus as described previously (8,10). Methanol extracts obtained from 206 kg of immature lupin seeds with the pods were concentrated in vacuo. The aqueous concentrate, after acidification with hydrochloric acid, was extracted with ethyl acetate to remove soluble materials, and an active factor in the aqueous layer was

purified by charcoal adsorptions, silver precipitations and ion exchange chromatography. Final isolation of the factor from the purified fraction was accomplished by paper chromatography and it was precipitated as the picrate, which was recrystallized from ethanol to yield 15.8 mg of rods, m.p. 188-191°* (sinters above 176°), $\lambda_{max}^{EtOH}$ 265 and 358 mμ and $\lambda_{min}^{EtOH}$ 235 and 303 mμ. The factor gave a blue spot with silver nitrate-bromophenol blue reagent (11) on a chromatogram, suggesting that it is a purine derivative. It was indistinguishable from zeatin** on paper chromatograms developed with three different kinds of solvent systems, but the IR spectrum of the picrate of the factor was not identical with that of zeatin picrate. The NMR spectrum showed signals at δ ($d_5$-pyridine, 60Mc, p.p.m. from TMS) 1.15 (3H, doublet, J=6c.p.s., H-$\dot{C}$-C$\underline{H}_3$), 1.60-2.40 (3H, multiplets), 3.78 (2H, doublet, J=6c.p.s., H-C-C$\underline{H}_2$OH), 4.13 (2H, multiplet, >N-C$\underline{H}_2$-) and 8.98 (2H, singlet, protons at positions 3 and 5 of picric acid). In addition, two one-proton singlets at δ 8.47 and 8.78 were assigned to the protons at positions 2 and 8 of adenine and a broad four-proton signal at near δ 11 was attributable to the protons of the hydroxy and amino groups.

Treatment of an aqueous solution of the picrate with Dowex 1 (formate form) gave the free base, platelets (from ethanol-acetonitrile), m.p. 165-166°. The very small amount of crystalline material available has limited the investigation to the measurement of the optical rotation at 589 mμ, but the optical activity was demonstrated by the measurement of the ORD curve, which exhibited a negative plain dispersion. The UV absorption spectra, $\lambda_{max}^{0.1N\ HCl}$ 273 mμ, $\lambda_{max}^{EtOH}$ 269 mμ and $\lambda_{max}^{0.1N\ NaOH}$ 275 and 282 mμ (sh.) are indicative of $N^6$-(alkyl-substituted)adenine (12). All these data are consistent with the assignment of structure (I) to the cytokinin isolated.

The confirmation of this structure was made by synthesis of (±)-dihydrozeatin. The catalytic hydrogenation of zeatin synthesized by the method of Shaw et al. (3,4) afforded the products which after chromatography over

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* All melting points are uncorrected and were determined on a hot-stage equipped with a microscope.

** Kindly supplied by Professors D.S.Letham and G.Shaw for the chromatographic and spectroscopic studies.

Florisil and recrystallization from ethanol-acetonitrile gave (±)-dihydrozeatin $C_{10}H_{15}N_5O$*** in platelets, m.p. 167-168°, $\lambda_{max}^{0.1N\ HCl}$ 273 mμ, ε 16,400, $\lambda_{max}^{EtOH}$ 269 mμ, ε 17,600 and $\lambda_{max}^{0.1N\ NaOH}$ 275 mμ, ε 17,300 and 282 mμ (sh.), ε 13,600, δ ($d_5$-pyridine, 60Mc, p.p.m. from TMS) 1.13 (3H, doublet, J=6c.p.s., H-Ċ-C<u>H</u>$_3$), 1.60-2.40 (3H, multiplets, -C<u>H</u>$_2$-Ċ-<u>H</u>), 3.78 (2H, doublet, J=6c.p.s., H-Ċ-C<u>H</u>$_2$OH), 4.12 (2H, undissolved sextet, $A_2M_2X$, $J_{AM}$=7c.p.s., $J_{MX}$=6c.p.s., -CH$_2$-C<u>H</u>$_2$-NH-), 5.15-6.40 (2H, broad, -O<u>H</u> and >N<u>H</u>), 8.30 (1H, triplet, J=6 c.p.s., -N<u>H</u>-CH$_2$-), 8.35 and 8.82 (1H each, singlets, protons at positions 2 and 8 of adenine). It gave the picrate $C_{16}H_{18}N_8O_8$***, needles (from ethanol), m.p. 188-190° (sinters above 176°), $\lambda_{max}^{EtOH}$ 265 mμ, ε 19,500 and 358 mμ, ε 15,500 and $\lambda_{min}^{EtOH}$ 235 and 303 mμ, whose NMR spectrum was identical with that of the picrate of the natural product isolated. Although the substance isolated is optically active, the IR spectrum (KBr) of the factor was identical with that of racemic dihydrozeatin.

(−)-Dihydrozeatin picrate showed cell-division promoting activity at a concentration of 2 μg/l on bioassays with tobacco callus.

Full details of the isolation and biological activity will be published elsewhere. Synthesis of the optically active isomers is in progress.

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*** Satisfactory analyses were obtained for all compounds characterized by constitutional formula.

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