Lupinic Acid, a Purinyl Amino Acid and a Novel Metabolite of Zeatin

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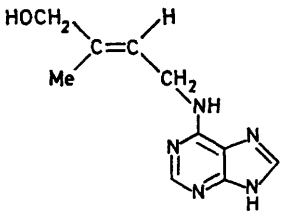
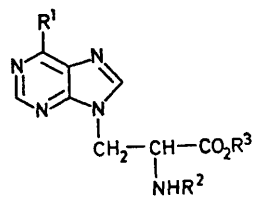
Summary The structure β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purin-9-yl]alanine has been assigned to lupinic acid, a novel zeatin metabolite isolated from *Lupinus angustifolius* seedlings.

IN plant tissues the principal known metabolites of the phytohormone zeatin (**1**) are 7- and 9-glycosides.[1] These metabolites were also formed when zeatin was supplied through the transpiration stream to 9-day-old lupin (*Lupinus angustifolius*) seedlings (roots excised), but the principal metabolites were two new compounds. One has tentatively been identified as *O*-β-D-glucopyranosylzeatin while the other, an amphoteric metabolite, is the subject of this report. Because of the quantity of material isolated (40 μg, purification detailed elsewhere) the techniques available for structure determination were limited to those discussed.

The metabolite reacted with ninhydrin (purple colour), exhibited u.v. spectra (λ_{max} at pH 3, 6, and 11: 266·5, 270, and 270 nm respectively) characteristic of (N^6, 9)-disubstituted adenines,[2] and moved towards the anode (mobility relative to AMP, 0·44) during paper electrophoresis at pH 10. The mass spectrum of the metabolite below *m/e* 220 was characteristic of an intact zeatin nucleus[3] except for a large *m/e* 44 (CO_2) peak, while significant higher mass ions were present at *m/e* 288 (1·5% R.I., $M^{+\cdot} - H_2O$), 271 (5·5, $M^{+\cdot} - H_2O - \cdot OH$), 262 (7, $M^{+\cdot} - CO_2$), 257 (3, $M^{+\cdot} - H_2O - \cdot CH_2OH$), 245 (53, $C_{12}H_{17}H_6$, $M^{+\cdot} - \cdot OH$),‡ 232 [3·5, $C_{10}{}^{13}CH_{15}N_6$ and 3·6, $C_{11}H_{14}N_5O$, $M^{+\cdot} - \cdot CH(NH_2)CO_2H$] and 231 (30, $C_{11}H_{15}N_6$, $M^{+\cdot} - CO_2 - \cdot CH_2OH$). This spectrum and the other physical data were rationalised in terms of structure (**2**). Confirmatory evidence for the proposed structure was obtained from the direct probe mass spectrum of the trimethylsilylated (TMS) derivative which showed ions at *m/e* 450 (2% R.I., $C_{19}H_{34}N_6O_3Si_2$, $M^{+\cdot}$), 435 (6, $M^{+\cdot}$ — Me), 361 (12, $M^{+\cdot}$ — ·OTMS), 360 (14, $M^{+\cdot}$ — TMSOH), 347 (19, $M^{+\cdot}$ — ·CH_2OTMS) and 333 (5, $C_{15}H_{25}N_6OSi$, $M^{+\cdot}$ — ·CO_2TMS) in accord with the *O*,*O*-bis-TMS derivative of (**2**).



(1)



(2) R^1 = *trans*-$HOCH_2(Me)C{=}C(H)CH_2NH$, $R^2 = R^3 = H$

(3) $R^1 = Cl$, $R^2 = COCF_3$, $R^3 = Me$

(4) $R^1 = Cl$, $R^2 = COCF_3$, $R^3 = H$

‡ Compositions are given where an ion has been accurately mass measured.

We believe this to be the first reported example of a naturally occurring purine derivative linked through one of its ring nitrogens to an amino acid. An analogous pyrimidine derivative, willardine, has been isolated from natural sources[4] but the only similar purines known are those which have been synthesised[5] as potential anti-metabolites.

Compound (**3**) was prepared by a Michael addition of 6-chloropurine to methyl-2-trifluoroacetamidoacrylate, followed by selective hydrolysis of the ester group to give (**4**). Condensation of (**4**) with *trans*-4-amino-2-methylbut-2-en-1-ol and deblocking of the amino function gave DL-lupinic acid m.p. 216—217 °C identical with the natural metabolite in all measurable respects, except for the as yet undetermined optical activity. Lupinic acid was also synthesised in good yield by addition of *O*-acetylzeatin to the same Michael acceptor, followed by hydrolysis of the protecting groups. Both routes utilise a new masked precursor for generating a β-substituted alanine requiring fewer and far less vigorous manipulations than reported procedures.[5]

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