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A NEW CYTOKININ FROM POPULUS ROBUSTA

R. Horgan, E.W. Hewett*, J.G. Purse and P.F. Wareing

Department of Botany and Microbiology, University College of Wales, Aberystwyth, SY23 3DA, U.K.

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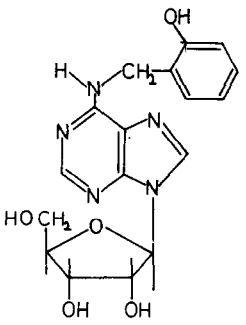
We wish to report the isolation and identification of a new cytokinin, from the leaves of P. robusta. The structure has been confirmed by synthesis as 6-(o-hydroxybenzylamino)-9-β-D-ribofuranosylpurine(I).

This is the first isolation of a naturally occurring cytokinin having an aromatic side chain. Synthetic compounds of this type have long been known to be active as cytokinins and Kuraishi[1] has shown that synthetic 6-(o-hydroxybenzylamino)-purine is an active cytokinin in at least one bioassay system.

An aqueous methanol extract of poplar leaves (180 g) was passed through a column of Zeocarb 225 (H^+ form) at pH 2.5. The basic fraction retained on the column was eluted with 5 M ammonium hydroxide. This fraction was further purified by paper chromatography (sec-butanol: 25% NH_4OH (4:1)) and reverse phase partition chromatography (LH-20 Sephadex in 35% aqueous ethanol)[2].



I

Measured	Composition	Measured	Composition
373.1413	$C_{17}H_{19}N_5O_5$	148.0644	$C_6H_6N_5$
241.0956	$C_{12}H_{11}N_5O$	135.0544	$C_5H_5N_5$
224.09266	$C_{12}H_{10}N_5$	119.0358	$C_5H_3N_4$
178.0742	$C_7H_8N_5O$	108.0447	$C_5H_4N_4$
164.0566	$C_6H_6N_5O$	106.0412	C_7H_6O

Table 1

* Present address: Plant Diseases Division, D.S.I.R., Private Bag, Auckland, New Zealand.

Cytokinin activity was located by a soybean callus bioassay.[3] The biologically active fractions from the Sephadex column were bulked and examined by G.L.C. as T.M.S. derivatives.

Preparative G.L.C. showed that all the biological activity was confined to one peak on the chromatogram. The homogeneity of this peak was confirmed by four low resolution G.C.M.S. scans taken at different points on the peak. Examination of these mass spectra showed a parent ion at m/e 661 and a series of ions indicating a ribosylpurine type of compound.

50 μg of the compound (estimated by G.L.C.) was isolated by preparative G.L.C. and hydrolysed with 0.01 M acetic acid to remove the T.M.S. groups. The material thus obtained exhibited the following u.v. spectral characteristics: λ_{max} (EtOH neutral) 266 nm, λ_{max} (EtOH pH 2) 260 nm, λ_{max} (EtOH pH 11) 265 nm, indicative of a N^{-6} substituted adenosine.

A low resolution mass spectrum of this material showed principal peaks at m/e 373, 284, 270, 241, 224, 178, 164, 148, 135 (base peak), 121, 120, 119, 108 106, 78, 66 and suggested that the compound was a N^{-6}(hydroxybenzyl) adenosine. This was confirmed by high resolution mass spectrometry (Table 1).

The position of the side chain OH group was determined by comparison of the low resolution mass spectra of the three synthetic isomers[4] with that of the natural product and was confirmed by co-chromatography on a G.L.C. system which clearly separated the three isomers.

REFERENCES AND NOTES

1. S. Kuraishi, Scientific Papers of the College of General Education University of Tokyo, 9, 67-104 (1959).
2. E.W. Hewett and P.F. Wareing, Planta (in press).
3. C.O. Miller. In Biochemistry and Physiology of Plant Growth Regulators, p.33, Runge Press, Ottawa, 1968.
4. Prepared by reaction of 6-chloropurine-9-β-D-riboside with the relevant hydroxybenzylamine in refluxing n-butanol.

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