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STRUCTURE AND SYNTHESIS OF UNUSUAL CYTOKININ METABOLITES

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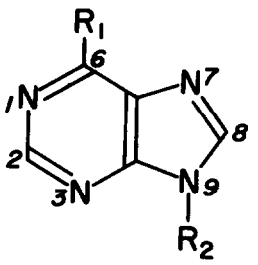
There have been several recent reports [1-5] of the isolation from a range of plant tissues of 7- and 9-glucosides as stable metabolites of the cytokinins, zeatin and 6-benzylaminopurine (6-BAP). From an analysis of the mass spectrum of the trimethylsilyl (TMS) derivative of the 7-glucosyl metabolite of 6-BAP, Fox [1] suggested that the sugar moiety possessed the furanose structure. On the other hand, it has been shown that 9-glucosylzeatin, the principal metabolite of zeatin in Zea mays seedlings, and 6-benzylamino-9-glucosylpurine, a major metabolite of 6-BAP in de-rooted radish seedlings, could not be differentiated from the synthetic 9-β-D-glucopyranosyl derivatives of zeatin (1) and 6-BAP (3) respectively [3]. The minute quantities (<100μg) of isolated metabolites restricted measurements to u.v., mass spectral and t.l.c. comparisons.

We have synthesised the 9-β-D-glucofuranosides of zeatin (2) and 6-BAP (4) by standard methods [6,7,8] and compared their u.v. and mass spectral, g.c.-m.s. (permethyl and TMS derivatives) and t.l.c. (with and without borate) characteristics with those of the synthetic [3] 9-β-D-glucopyranosides (1) and (3) and the 9-glucosyl metabolites of zeatin and 6-BAP respectively. The results confirmed that the metabolites were 9-glucopyranosides. As the 9-substituted metabolites were not hydrolysed by either α- or β-glucosidase, the nature of the anomeric linkage in the 9-glucosyl metabolite of 6-BAP was established as β using the above criteria by its identity with the synthetic 9-β-D-glucopyranoside (3) and dissimilarity to the synthetic 9-α-D-glucopyranoside of 6-BAP (5). The latter compound (5) was prepared by fusion of the triphenylsilyl derivative of 6-chloropurine with α-D-tetra-O-acetylglucopyranosyl-bromide, separation of the 9-α from the 9-β-anomer (formed in the ratio 2:3) and subsequent



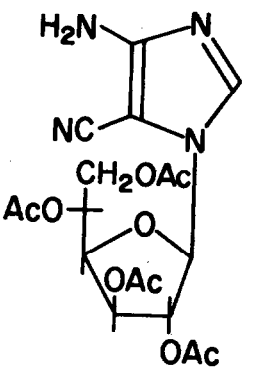
(1) R_1 = $HOCH_2$, H, CH_3, C=C, CH_2NH- ; R_2 = β-D-glucopyranosyl-

(2) R_1 = " ; R_2 = β-D-glucofuranosyl-

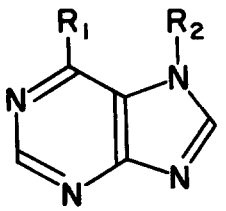
(3) R_1 = $C_6H_5CH_2NH$- ; R_2 = β-D-glucopyranosyl-

(4) R_1 = " ; R_2 = β-D-glucofuranosyl-

(5) R_1 = " ; R_2 = α-D-glucopyranosyl-



(11)



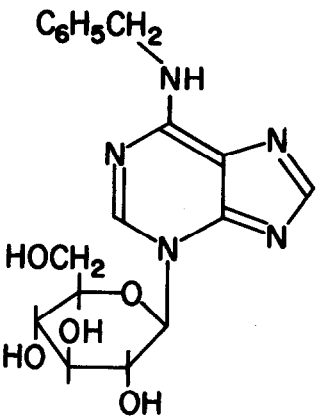
(6) R_1 = $HOCH_2$, H, CH_3, C=C, CH_2NH- ; R_2 = β-D-glucopyranosyl-

(7) R_1 = " ; R_2 = β-D-glucofuranosyl-

(8) R_1 = $C_6H_5CH_2NH$- ; R_2 = β-D-glucopyranosyl-

(9) R_1 = " ; R_2 = β-D-glucofuranosyl-

(10) R_1 = CH_3S- ; R_2 = β-D-glucofuranosyl-



(12)

displacement of the 6-chloro-substituent and concomitant de-acetylation with benzylamine in refluxing n-butanol. A mixture of the α- and β-anomers (5) and (3) in the ratio (1:2) was also obtained in good yield on condensing α-D-tetra-O-acetylglucopyranosylbromide with the 9-tricarbonylcyclohexa-2,4-dienyliron derivative of 6-BAP (obtained by treating 6-BAP with $[C_6H_7Fe(CO)_3]BF_4$) in DMF at 90° followed by treatment with methanolic ammonia.

7-β-D-Glucofuranosyl-6-methylthiopurine (10) was prepared via ring closure of 4-amino-5-cyano-1-tetra-O-acetyl-β-D-glucofuranosylimidazole (11) using the procedure of Townsend *et al*,[9] substituting penta-O-acetylglucofuranose [10] for tetra-O-acetylribofuranose in the first step. Displacement of the 6-methylthio substituent in (10) using neat benzylamine at 140° for 24 hours gave 6-benzylamino-7-β-D-glucofuranosylpurine (9) while displacement with *trans*-4-amino-2-methylbut-2-enol in refluxing n-butanol afforded 7-β-D-glucofuranosylzeatin (7). Both cytokinin glucofuranosides (7) and (9) were also synthesised in better yield from the ethoxymethyleneimine of the above imidazole (11) and utilising benzylamine or *trans*-4-amino-2-methylbut-2-enol to effect pyrimidine ring closure[11] to the 1-substituted adenine derivatives. A modified Dimroth rearrangement [12] of these intermediates provided the desired compounds, identical in all respects with the 7-β-D-glucofuranosyl derivatives of zeatin and 6-BAP obtained by displacement of the 6-methylthio group. The u.v. spectra of these two compounds were consistent with their possessing an N^6,7-disubstituted adenine chromophore [13].

Comparison of the u.v., mass spectral (including g.c.-m.s. of the TMS and permethyl derivatives) and t.l.c. characteristics of the synthetic 7-β-D-glucofuranosides of zeatin (7) and 6-BAP (9) with the 7-glucosyl metabolites of zeatin (raphanatin)[2] and 6-BAP[3] respectively established that these were not identical. In particular the behaviour of the metabolites on t.l.c. plates impregnated with borate buffer strongly supported the assignment of a glucopyranose structure to the sugar moiety in both compounds, in contradiction to the proposal by Fox. Synthesis of the 7-glucopyranosides (6) and (8) by the above route has proved unsatisfactory and alternative procedures are being examined.

A minor but highly active metabolite of 6-BAP from excised radish seedlings has been identified as 6-benzylamino-3-β-D-glucopyranosylpurine (12). The metabolite was slowly cleaved by almond β-glucosidase and showed the characteristic u.v. spectral absorptions for an N^6,3-disubstituted adenine. 6-Benzylaminopurine was condensed with α-D-tetra-O-acetyl-glucopyranosyl bromide in DMF at 100° [14] and the major product separated and deacetylated to provide synthetic 6-benzylamino-3-β-D-glucopyranosylpurine (12) identical in all respects

including biological activity with the isolated metabolite. This is the first reported instance of the isolation from plant tissue of a compound with a glycosidic linkage at the 3-position of a purine ring. The biological activity of the cytokinin glucosides and their role in plant metabolism will be reported elsewhere.

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