Synthesis of Raphanatin and its 6-Benzylaminopurine Analogue

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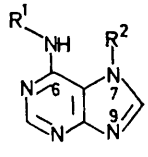
Summary The complete structures of raphanatin, the stable zeatin metabolite isolated from de-rooted radish seedlings, and the analogous 6-benzylaminopurine metabolite from the same plant system, have been confirmed by synthesis to be the 7-β-D-glucopyranosides of zeatin and 6-BAP respectively.

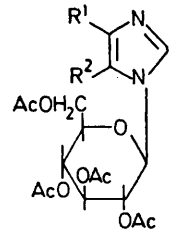
RAPHANATIN (**1**) was isolated as the major stable metabolite of the natural cytokinin, zeatin, when the latter was fed exogeneously to excised radish seedlings.[1] Although it was readily identified from its u.v. and mass spectral characteristics and acid hydrolysis as the 7-glucosyl derivative of zeatin, no evidence could be obtained concerning the glucose ring size or the stereochemistry of the sugar linkage, owing to the small quantity isolated (*ca.* 70 μg and its stability to both α- and β-glucosidase. Based on mass spectral evidence, Fox[2] proposed the glucofuranosyl structure for the 7-glucoside of the synthetic cytokinin, 6-benzylaminopurine (6-BAP), which he isolated as a stable metabolite of 6-BAP from a number of plant systems. We have recently shown[3] that these 7-glucosyl metabolites of zeatin and 6-BAP are different from the synthetic 7-β-D-glucofuranosides of zeatin and 6-BAP respectively and that their t.l.c. mobilities on silica gel plates impregnated with borate are consistent with their possessing a glucopyranose ring.

7-β-D-Glucopyranosylzeatin (**1**) was synthesised unambiguously using essentially the same route previously described by us[3] for the synthesis of the 7-β-D-glucofuranosides of zeatin and 6-BAP. The best conditions for the initial fusion reaction between penta-*O*-acetylglucopyranoside and 4-bromo-5-nitroimidazole necessitated the use of conc. H_2SO_4 as catalyst in place of chloroacetic acid.[3,4] This gave as the major product the β-linked compound (**3**) as determined by its u.v. spectral characteristics[4] and by the ^{1}H n.m.r. coupling constant ($J_{1,2}$ *ca.* 8·5 Hz) of the anomeric proton in the de-acetylated imidazole glucoside.



(1) R^1 = *trans*-$HOCH_2(Me)C{=}CHCH_2$;
R^2 = β-D-glucopyranosyl

(2) R^1 = $PhCH_2$;
R^2 = β-D-glucopyranosyl



(3) R^1 = O_2N; R^2 = Br

(4) R^1 = EtOCH=NH; R^2=NC

Conversion of (**3**) into the ethoxymethyleneamine (**4**) proceeded in good yield using reported procedures,[4] with all intermediates fully characterised. Treatment of (**4**) with 5 equiv. of *trans*-4-amino-2-methylbut-2-enol in methanol

at room temperature followed by addition of acetic acid gave, after evaporation and heating of the residue at 100 °C, the desired 7-β-D-glucopyranoside of zeatin (**1**). This reaction, involving ring closure and Dimroth rearrangement, represents a new and convenient route to 7-glycosides of N^6-substituted adenines. This compound had physical and spectroscopic characteristics (u.v., mass spectra, t.l.c. mobility with and without borate) identical with the natural metabolite raphanatin to which we therefore assign structure (**1**).

Using benzylamine in place of *trans*-4-amino-2-methylbut-2-enol, we prepared compound (**2**) in high yield from (**4**). This compound was identical with the 7-glucosyl metabolite of 6-BAP isolated by us from de-rooted radish seedlings which in turn showed the same u.v. and mass spectral characteristics (including the mass spectrum of its trimethylsilyl derivative) as the 7-glucoside of 6-BAP reported by Fox.[2] From a large-scale experiment, we were able to isolate *ca.* 1 mg of this metabolite and ascertain the ^{1}H n.m.r. coupling constant ($J_{1,2}$ *ca.* 8·5 Hz) for the anomeric proton. This was identical with the J value for the anomeric proton in the n.m.r. spectrum of the synthetic compound (**2**) thereby establishing unequivocally the β-stereochemistry of the *N*-glucoside linkage in the 6-BAP and, by inference, the zeatin metabolites.

Our early efforts to synthesise the 7-β-D-glucopyranosides of zeatin and 6-BAP *via* initial direct alkylation of 6-chloropurine with acetobromoglucose–K_2CO_3 in acetone, Me_2SO, or $HCONMe_2$ led to 9-substituted products only. Using propylene carbonate–K_2CO_3 at room temperature, however, we have recently been able to achieve up to 20% substitution at the 7-position. After separation of the 7- from the more abundant 9-β-substituted isomer, deacetylation, and displacement of the 6-chloro-substituent by benzylamine, compound (**2**) was obtained which was identical with that synthesised by the alternative imidazole route.

Complete characterisation of this stable metabolite of zeatin will now permit a more detailed study of its proposed role in plant metabolism[5] as a natural storage form of the active cytokinin.

(*Received, 7th October* 1975; *Com.* 1146.)

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