Phytochemistry, 1979, Vol. 18, pp. 819–824. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0501-0819 $02.00/0

THE COMPLEX OF *O*-GLUCOSYLZEATIN DERIVATIVES FORMED IN *POPULUS* SPECIES*

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(*Received* 6 *September* 1978)

Key Word Index—*Populus* spp.; cytokinin metabolism; *O*-glucosyl derivatives of zeatin.

Abstract—When zeatin was supplied to excised leaves of *Populus alba*, the principal metabolites formed were adenosine, *O*-β-D-glucopyranosyl-*cis*-zeatin (derived from *cis*-zeatin in the commercial zeatin used), *O*-β-D-glucopyranosylzeatin, and two new metabolites, namely, *O*-β-D-glucopyranosyldihydrozeatin and *O*-β-D-glycopyranosyl-9-β-D-ribofuranosyldihydrozeatin, the structures of which were confirmed by unambiguous synthesis. Chromatographic studies indicated that adenosine 5′-phosphate, zeatin 7-glucopyranoside, zeatin 9-glucopyranoside, dihydrozeatin and zeatin 9-riboside were minor metabolites. The principal metabolites of zeatin 9-riboside in *P. nigra* leaves were the new metabolites *O*-β-D-glucopyranosyl-9-β-D-ribofuranosylzeatin (synthesized chemically) and *O*-β-D-glucopyranosyl-9-β-D-ribofuranosyldihydrozeatin.

INTRODUCTION

Information concerning cytokinin levels and identity in leaves during development and senescence is very limited. The most detailed studies have been with leaves of *Populus* spp. [1–3]. The only cytokinin identified unambiguously in such leaves is 6-(*o*-hydroxybenzylamino)-9-β-D-ribofuranosylpurine which was extracted from *Populus* × *robusta* [4]. However chromatographic and other evidence have suggested that zeatin, zeatin riboside and a zeatin glucoside are also present in this species [2, 3]. Herein we report a study of the metabolism of exogenously supplied zeatin and zeatin riboside in poplar leaves.

RESULTS

Metabolites of zeatin in Populus alba *leaves*

The mature leaves of *Populus alba* were supplied with zeatin-[^{3}H] (**1**) through the transpiration stream. The leaf extract was chromatographed on paper (solvent A) and the distribution of radioactivity over the chromatogram was determined (Fig. 1). This revealed one broad major peak of radioactivity (R_f 0.32) and one minor peak (R_f 0.14). The zones 1–5 depicted in Fig. 1 were eluted for investigation of the metabolites present by co-chromatography with authentic compounds. Zone 1 (2% of total ^{3}H) which included the origin, is the region where nucleotide metabolites would occur. PC of the alkaline-phosphatase hydrolysate of zone-1 eluate indicated that 40% of the radioactivity was due to adenosine. Labelled zeatin riboside was not a hydrolysis product and hence zone 1 appeared to contain phosphates (presumably 5′) of adenosine, but not of zeatin riboside. Zone 2 (18% of total ^{3}H) included the minor radioactivity peak. The metabolites in this zone were not identified but did not include lupinic acid, a zeatin–alanine conjugate [5], which if present in the extract would occur in zone 2. During silica gel TLC of the eluate of zone 4 (11% of total ^{3}H) using solvents A and B, most of the radioactivity co-chromatographed with adenosine, adenine, zeatin 7-glucopyranoside and zeatin 9-glucopyranoside. Only 3% of the total ^{3}H was located in zone 5 in which zeatin, dihydrozeatin (**4**) and their ribosides would occur if present in the extract. TLC studies of the zone eluate, and particularly TLC on silica gel using solvent C, which separates zeatin and zeatin riboside from the corresponding dihydro compounds [6], indicated that the ^{3}H in zone 5 was due principally to zeatin, dihydrozeatin and zeatin 9-riboside. Zone 3 with an R_f (0.24–0.39) slightly less than that of adenosine contained the major peak of radioactivity and 48% of the total ^{3}H. *O*-β-D-Glucopyranosylzeatin (**2**) possesses a similar relative R_f [6]. However TLC indicated that zone 3 contained a complex of metabolites, some of which appeared to be new compounds. A procedure for the purification of the principal metabolites of zones 3 and 4 was devised. This yielded four UV-absorbing components Y1, Y2, Y3 and Y4, the identities of which are discussed below. When hydrolysed with a polystyrene sulphonic acid resin [7], Y2, Y3 and Y4 all yielded glucose which was identified with glucose oxidase [7].

Y1. The UV spectra of Y1 (Table 1) were characteristic

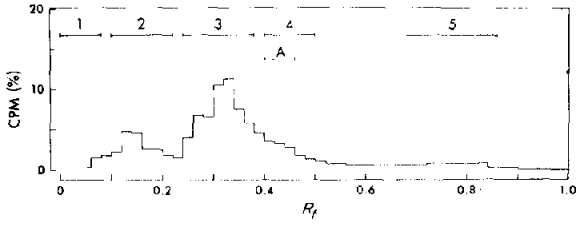


Fig. 1. The distribution of radioactivity over a paper chromatogram (solvent A) of extract of *P. alba* leaves supplied with zeatin-[^{3}H]. A denotes the position of co-chromatographed adenosine; zones eluted for examination of metabolites present are indicated by the numbers 1–5.

* Part IV in the series "The Structure and Synthesis of Cytokinin Metabolites". For Part III see Duke, C. C. and MacLeod, J. K. (1978) *Aust. J. Chem.* **31**, 2219.

Table 1. UV spectral characteristics of purified poplar metabolites

Metabolite	λ_{max}		
	80% EtOH	0.2 N NH_4OH in 80% EtOH	0.1 N HOAc
Y1	260	260.5	257.5
Y2	268	268	264
Y3	270	274.5 (284 sh)	273.5
Y4	270	275 (284 sh)	273

of a 9-substituted adenine. The MS and TLC established unequivocally that Y1 was adenosine.

Y2. The UV spectra of Y2 (Table 1) established that it was an N^6, 9-disubstituted adenine [8]. The MS of the underivatized compound indicated the presence of a dihydrozeatin (**4**) moiety, all peaks below *m/e* 222 being attributable to this structural feature. However, the molecular ion of Y2 was not evident in this spectrum. Hydrolysis of Y2 with β-glucosidase yielded a UV-absorbing product which was characterized by GC–MS of the per-TMSi derivative. The MS exhibited a molecular ion at *m/e* 641 suggesting that the hydrolysis product was dihydrozeatin 9-riboside (**7**, MW of tetra-TMSi derivative = 641). This possibility was substantiated by the presence of peaks in the MS at *m/e* 292, 293, 294, 322, 366, 408, 420, 423 and 348, which are attributable to the intact purine base fragment (*b*) of tetra-TMSi **7**, *b* + H, *b* − 2H, *b* + CH_2O, *b* + H + TMSi, *b* + 116, *b* + 128, *b* + 131, and *s* − H (*s* denotes the intact per-TMSi sugar fragment), respectively. All these ions are characteristic of purine ribosides and glucosides and have been rationalized structurally [9, 10]. Synthetic dihydrozeatin 9-riboside [11] was found to be indistinguishable from the Y2 hydrolysis product by MS of the per-TMSi derivative and by TLC on silica gel (several solvents) and on silica gel layers impregnated with $Na_2B_4O_7.10H_2O$ (solvent F).

The above observations indicated that Y2 was a glucoside of dihydrozeatin 9-riboside. This was confirmed by the MS of the per-TMSi derivative of Y2 which exhibited a molecular ion at *m/e* 1019. The glucosyl

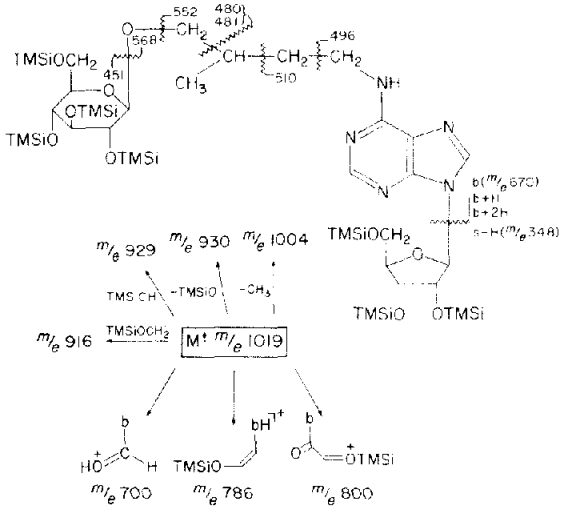


Fig. 2. A diagrammatic representation of the fragmentation revealed in the MS of hepta-TMSi Y2.

moiety could have been attached to the oxygen of the N^6 side-chain or to the riboside residue at N-9. The fragmentation pattern revealed in the MS of per-TMSi Y2 was in accord with the former structure (see Fig. 2) but was generally inconsistent with the latter. In particular, the *m/e* 348 peak was strongly indicative of a tri-TMSi ribosyl fragment ion and therefore of an unsubstituted 9-ribosyl moiety in Y2. The relative intensities of the *m/e* 204 and 205 peaks in the mass spectrum of per-TMSi Y2 indicated that the glucosyl moiety was a glucopyranosyl, and not a glucofuranosyl, residue [9]. Hence Y2 was assigned the structure 6-(4-β-D-glucopyranosyloxy-3-methylbutylamino)-9-β-D-ribofuranosylpurine, i.e. *O*-β-D-glucopyranosyl-9-β-D-ribofuranosyldihydrozeatin (**6**). This was established unequivocally by a comparison of Y2 with **6** prepared by unambiguous synthesis (see below); the two compounds were indistinguishable by TLC on silica gel and cellulose, and by MS of the underivatized compounds and per-TMSi ethers.

Y3. The UV spectral characteristics (Table 1) indicated that Y3 was a 6-(monosubstituted amino)purine. The MS (M^+ at *m/e* 381) was essentially identical to that of *O*-β-D-glucopyranosylzeatin (**2**), previously identified as a metabolite of zeatin in lupin seedlings [6, 12]. Hydrolysis of Y3 with β-glucosidase yielded a product which co-chromatographed with zeatin during TLC. The very low mobility of Y3 (equal to that of synthetic **2**) during paper electrophoresis (0.025 M borate buffer, pH 9.2) indicated that the glucose moiety was in the pyranose form. Y3 and synthetic **2** [13] were not separable by TLC on silica gel or cellulose. Hence Y3 was identified as *O*-β-D-glucopyranosylzeatin (**2**).

Y4. The UV spectra for Y4 (Table 1) are indicative of 6-(monosubstituted amino)purines. The MS showed paired peaks at *m/e* 383 and 381 due to molecular ions, at *m/e* 366 and 364 (M^+ − OH ions), at *m/e* 352 and 350 ($M^+ - CH_2OH$) and at *m/e* 250 and 248 ($M^+ - C_5H_9O_4$), suggesting that Y4 was a mixture of a zeatin glucoside and a dihydrozeatin glucoside. The prominent peaks at *m/e* 160, 188 and 202 were characteristic of a zeatin moiety [14], while the peak at *m/e* 162 was attributable to a dihydrozeatin structure. Y4 was hydrolysed with β-glucosidase and the hydrolysate subjected to two-dimensional TLC on silica gel (solvent C three times, solvent D twice). This system clearly separates zeatin, *cis*-zeatin and dihydrozeatin (for R_f data, see ref. [6, 15]). These and other TLC studies established that the hydrolysate contained two UV-absorbing compounds; one co-chromatographed with *cis*-zeatin, the other with dihydrozeatin. The ratio of *cis*- to dihydro-zeatin was *ca* 3:1. When Y4 was subjected to TLC on cellulose (solvent A, plate developed twice), it was separated into two UV-absorbing components of very similar R_f (0.21 and 0.19). The compound of R_f 0.21 co-chromatographed with synthetic *O*-β-D-glucopyranosyldihydrozeatin (**5**) while the other, after hydrolysis with β-glucosidase, co-chromatographed with *cis*-zeatin. During paper electrophoresis (0.025 M borate buffer, pH 9.2), the compounds of R_f 0.21 and 0.19 both exhibited a very low mobility equal to that of synthetic **5**; hence both were glucopyranosides.

The above evidence established that Y4 was a mixture of *O*-β-D-glucopyranosyldihydrozeatin (**5**) and *O*-β-D-glucopyranosyl-*cis*-zeatin (*cis* isomer of **2**). The formation of the latter metabolite is attributable to gluco-

sylation of the *cis*-zeatin present in the commercial sample of zeatin supplied to the leaves. NMR indicated a *trans* to *cis* ratio of 3:1 in the zeatin sample.

Metabolism of zeatin riboside in leaves of Populus nigra

Extracts of dark green leaves of *Populus nigra* supplied with zeatin 9-riboside-[^{3}H] were chromatographed on paper (solvent A). Two major zones of radioactivity were present on the chromatogram at R_f values of 0.20–0.40 and 0.60–0.80, and contained 49 and 33%, respectively, of the total radioactivity. TLC of the eluate of the latter zone on silica gel (solvents A and C) established that 58% of the ^{3}H in the zone co-chromatographed with zeatin 9-riboside, 29% with dihydrozeatin 9-riboside, 5% with zeatin and 3% with dihydrozeatin.

When the eluate of the paper chromatogram zone of R_f 0.20–0.40 was subjected to silica gel TLC (solvent A), 75% of the ^{3}H occurred in a zone at R_f 0.17 (cf. R_f of co-chromatographed zeatin 7-glucopyranoside, 0.23). TLC studies (silica gel, solvents A, C and D) of the β-glucosidase hydrolysate of the eluate of the zone at R_f 0.17 indicated that the ^{3}H present was due to dihydrozeatin 9-riboside and to zeatin 9-riboside (ratio 2:1). Hence 9-ribosides of *O*-glucosylzeatin and *O*-glucosyldihydrozeatin appeared to be major metabolites in the *P. nigra* extracts. This was confirmed as outlined below. The extract was fractionated on a column of cellulose phosphate by the method used for *P. alba* extracts. The fraction eluted by 0.3 N NH_4OH was subjected to TLC on silica gel (solvent A) and the required glucoside zones eluted for further TLC on cellulose (solvent A). This yielded two radioactive zones of R_f 0.15 and 0.21; during TLC (cellulose, solvents A and B), the ^{3}H in the former co-chromatographed with synthetic **3** and in the latter with synthetic **6**. The eluate of the R_f-0.15 zone and synthetic **3** were co-crystallized to constant specific activity. Hence **3** and probably **6** (ratio 1:2) were major metabolites of zeatin 9-riboside in *P. nigra*. TLC studies indicated that **2** and **5** were present as minor metabolites.

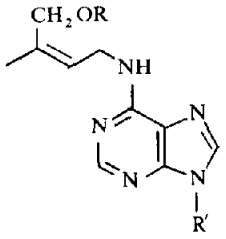
The chemical synthesis of O*-glucosyl metabolites*

Condensation of 4-amino-2-methylbut-*trans*-2-enyl-β-D-glucopyranoside [13] with 6-chloropurine and 6-chloro-9-β-D-ribofuranosylpurine gave *O*-β-D-glucopyranosylzeatin (**2**) and *O*-β-D-glucopyranosyl-9-β-D-ribofuranosylzeatin (**3**), respectively. Reduction of **2** and **3** with palladium on charcoal yielded **5** and **6**, respectively, as major products. The minor products from each reduction were isolated by TLC and identified by MS as 6-isopentylaminopurine and 6-isopentylamino-9-β-D-ribofuranosylpurine. These products conceivably arose from hydrogenolysis of glucose from the zeatin side-chain followed by reduction of the isoprenoid double bond.

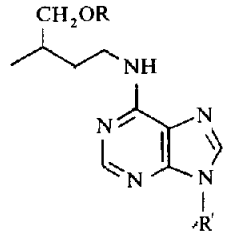
The major products of the reduction (**5** and **6**) would each be expected to be a mixture of two possible epimers at C-3 of the side chain, although ^{1}H NMR and ^{13}C NMR spectra of these compounds showed only a single set of signals for the protons and carbon atoms at or near this centre. This suggested that induced stereospecific reduction of the double bond could have occurred due to the proximity of the *O*-β-D-glucosyl substituent. To determine if such reduction had actually occurred, **5** was hydrolysed to dihydrozeatin (**4**). ORD measurements taken over the range 320–589 nm [16] showed no rotation indicating that the dihydrozeatin was racemic and that **5** was a 1:1 mixture of the two epimers at C-3 in the N^6 side chain. Consequently **6** would also be expected to be a 1:1 mixture of epimers since the 9-ribofuranosyl substituent induces no significant asymmetry on catalytic reduction of the side chain in zeatin 9-riboside [17].

DISCUSSION

Cytokinin metabolites with a glucose moiety at positions 7 or 9 of the purine ring have been isolated from a number of plant tissues and synthesized chemically [18, 19]. However the glucoside metabolite **2**, with the sugar moiety conjugated to the N^6 side-chain of exogenously supplied zeatin, has been isolated relatively recently, first from lupin leaves [6, 12] and then from soybean tissue [20]. The present paper establishes that leaves of *Populus* spp. metabolize exogenously supplied zeatin and zeatin 9-riboside to a complex of *O*-glucosides, i.e. compounds **2**, **3**, **5** and **6**. Since this work was reported in a preliminary manner [21], **2**, **3** and **5** have been reported to be endogenous cytokinins [22–24], although comparisons of the natural compounds with synthetic glucosides were not made. Compounds with cytokinin activity detected in other plant species by bioassay of chromatographed extracts, but not purified and characterized chemically, also appear to be *O*-glucosides of zeatin or closely related compounds. On hydrolysis with almond β-glucosidase which does not hydrolyse cytokinin 7- or 9-glucosides, they yield cytokinin activity



1 R = R′ = H
2 R = Glc
R′ = H
3 R = Glc
R′ = Rib



4 R = R′ = H
5 R = Glc
R′ = H
6 R = Glc
R′ = Rib
7 R = H
R′ = Rib

which co-chromatographs with zeatin or zeatin 9-riboside [3, 25–29]. Hence *O*-glucosides of zeatin or related compounds now appear to be widely distributed plant metabolites. An unidentified cytokinin in *Populus* × *robusta* leaves, which appears to be a glucoside [2], exhibits chromatographic behaviour on Sephadex LH-20 similar to that of **2** and **5**. The unidentified cytokinin may be either of these glucosides.

In some plant species, cytokinins which appear to be *O*-glucosides of zeatin or related compounds account for most of the extractable cytokinin activity in mature or senescing leaves [2, 25, 26]. Cytokinin glucosides have not been detected in xylem sap, and hence are probably not supplied directly to the leaf from the root, a principal site of cytokinin biosynthesis [30]. The leaf cytokinin glucosides may form in the leaf itself by metabolism of xylem sap cytokinins of which zeatin 9-riboside is the most common [30]. Our results detailed herein show that poplar leaves are capable of rapidly metabolizing both zeatin 9-riboside (probably the principal cytokinin in xylem sap of *Populus* × *robusta* [31]) and zeatin to the complex of *O*-glucosyl derivatives and hence are consistent with this suggestion.

The physiological significance of the *O*-glucoside metabolites of zeatin is unknown at present. However, it is noteworthy that they are present in detectable amounts in cultured plant tumour tissue only when the medium contains reduced nitrogen, a condition which markedly elevates the zeatin and zeatin riboside level [22]. Furthermore, the *O*-glucosides appear to be the dominant cytokinins in leaves which have been actively accumulating cytokinin [24, 26]. Hence the *O*-glucosides may be storage forms of zeatin. However, the probable presence of *O*-glucosylzeatin in honeydew of aphids feeding on *Salix babylonica* [32] raises the possibility of a role in phloem transport of cytokinins. The methods for synthesizing **2**, **3**, **5** and **6** reported herein, and the TLC procedures for separating these closely related compounds (see also ref. [21]), should facilitate physiological studies of this potentially important group of zeatin metabolites.

EXPERIMENTAL

Chromatographic methods. The following solvents were used: (A) *n*-BuOH–14 N NH_4OH–H_2O (6:1:2, upper phase); (B) *n*-BuOH HOAc–H_2O (12:3:5); (C) MeOAc–EtOH–2,2-dimethoxypropane (90:10:1) with 5 drops of formic acid per 100 ml (for details concerning preparation and use of this solvent, see ref. [6]); (D) $CHCl_3$–MeOH (9:1); (E) *n*-BuOH satd with H_2O (conc NH_4OH added to bottom of PC tank); (F) EtOH–H_2O (2:1) satd with $Na_2B_4O_7.10H_2O$. All compounds on chromatograms were located under UV light, the Si gel containing a fluorescent indicator activated at 254 nm. Si gel was used for all TLC and PLC mentioned below. When determination of MS of PC eluates was required, acid-washed Schleicher and Schull 2040b paper was used after it had been rewashed exhaustively with 20% EtOH.

Chromatogram zones were eluted as follows: PC and TLC for determination of radioactivity by liquid scintillation counting, with H_2O in counting vials at 25° for 18 hr or at 55° for 3 hr; PC for rechromatography, with 0.15 N HOAc (allowed to flow down zones); PC for MS, with 80% EtOH; PLC, exhaustively with MeOH–H_2O–HOAc (80:20:1).

Mass spectrometry. Concentrated eluates of PC zones were evapd onto the direct inlet probe of an AEI MS902 mass spectrometer. MS at 70 eV were obtained by flash evaporation of the sample into a hot source (> 270°). Preparation of TMSi derivatives and GC–MS were performed as before [9].

Enzymic hydrolyses. Nucleotides were converted to nucleosides by hydrolysis with alkaline phosphatase as described previously [33]. Glucosides were hydrolysed in acetate buffer (0.03 M, pH 5.3) with almond β-glucosidase (0.3 mg/ml, Sigma Chemical Co.) for 3 hr at 35°.

Uptake of labelled cytokinins and tissue extraction. The petioles of excised mature leaves of *Populus alba* (collected in Canberra in early March) were placed in an aq. zeatin-[G-^{3}H] soln (8 μM; 180 mCi/mmol) and left under continuous white fluorescent light (700 lx) in a gentle air current. After 24 hr, the leaves were transferred to 1 μM zeatin-[^{3}H] for a further 100 hr. Petioles of dark green *Populus nigra* (var. italica) leaves collected in autumn were placed in an aq. soln of zeatin 9-riboside-[8-^{3}H] (4 μM; 350 mCi/mmol) and left for 100 hr as above.

All extracts were prepared by the following method. Leaves were dropped into 80% MeOH (20 ml/g tissue) at 65°, held at this temp. for 5 min, cooled rapidly and then blended with the solvent. The evapd extract was suspended in 50% EtOH (1.0 ml/g tissue); centrifugation yielded a supernatant for chromatography.

Purification of principal metabolites of zeatin. Unlabelled zeatin (100 μM soln) was supplied to mature leaves (220 g) of *P. alba* for 48 hr as above. The leaves were then transferred to a 10 μM zeatin soln for 96 hr prior to extraction. The evapd leaf extract was suspended in water (150 ml) and adjusted to pH 3.0. The filtered soln was percolated through a column of Whatman P1 cellulose phosphate (NH_4^+ form equilibrated to pH 3.0; 50 g) which was washed with 0.03 N HOAc (1.8 l) and then H_2O (300 ml) and finally eluted with 0.3 N NH_4OH (2.4 l.). To the evapd column eluate was added eluate of the major broad zone of radioactivity on a paper chromatogram of extract of leaves supplied with zeatin-[^{3}H] (see Fig. 1). This radioactivity provided a basis for fractionation of the column eluate. PLC using solvent A yielded two zones of radioactivity at R_f 0.37 (zone A, 61% of ^{3}H) and 0.46 (zone B, 21% of ^{3}H). PLC of zone B eluate (solvent B) followed by PC (solvent A) yielded purified metabolite Y1, the major metabolite in zone B; MS *m/e* (rel. int.): 267 (M^+, 2%), 237 (4), 178 (16), 164 (44), 148 (4), 136 (57), 135 (100).

Zone-A metabolites were purified by PLC (solvent B), the principal zone of radioactivity (R_f 0.37, 69% of ^{3}H) being eluted for PC (solvent A). The resulting major broad radioactive zone (R_f 0.36, 78% of ^{3}H) was resolved by TLC (solvent A; plates developed twice) into 3 principal radioactive UV-absorbing components of R_f 0.16, 0.20 and 0.24, containing 25, 39 and 26% respectively of the chromatographed ^{3}H. PC (solvent E) of the three components yielded purified metabolites Y2 (25 μg), Y3 (90 μg) and Y4 (100 μg), respectively. MS *m/e* (rel. int.) of Y2 (**6**): 426 (1.2%), 412 (1.7), 384 (1.7), 383 (1.9), 382 (2.5), 366 (1.4), 354 (1.5), 353 (1.9), 352 (5.5), 337 (1.0), 336 (3.4), 294 (1.3), 292 (1.4), 281 (1.3), 280 (1.0), 278 (1.6), 276 (1.1), 265 (1.1), 264 (3.5), 263 (1.3), 262 (1.1), 250 (40), 222 (21), 221 (9), 220 (45), 204 (39), 190 (10), 162 (48), 161 (5), 160 (7), 149 (30), 148 (100), 136 (38), 135 (40), 121 (8), 120 (9), 119 (19); Y3 (**2**): MS identical to that recorded [6] for *O*-β-D-glucopyranosylzeatin except minor peak at *m/e* 314 absent; Y4: 383 (0.2), 381 (0.3), 366 (0.2), 364 (0.5), 352 (0.3), 350 (0.5), 250 (4), 248 (2), 222 (2), 221 (1), 220 (7), 219 (2), 218 (2), 202 (100), 201 (17), 200 (8), 188 (13), 186 (10), 185 (8), 175 (2), 174 (3), 173 (4), 162 (7), 160 (21), 148 (30), 136 (31), 135 (28), 120 (10), 119 (22).

Derivatives of Y2. Y2 was trimethylsilylated to give a mixture of hepta- and hexa-TMSi derivatives, the reaction solution being evapd on the direct inlet probe of the mass spectrometer using a stream of N_2. MS *m/e* > 340 (rel. int.): 1019 (M^+

hepta-TMSi, 1.8%), 1018 (0.6), 1004 (5), 947 (M^+ hexa-TMSi, 0.8), 932 (3), 931 (1.0), 930 (1.0), 929 (0.9), 916 (1.6), 802 (0.4), 801 (0.4), 800 (0.8), 786 (0.6), 700 (1.5), 672 (1.0), 671 (0.8), 670 (1.0), 642 (0.5), 628 (1.5), 598 (6), 568 (16), 552 (22), 510 (3), 497 (2), 496 (2), 481 (2), 480 (4), 464 (4), 451 (1), 450 (2), 361 (4), 360 (3), 348 (4), 73 (100). Y2 (6 μg) was also hydrolysed with β-glucosidase. The hydrolysate was chromatographed (TLC, solvent A) and the UV-absorbing product eluted and converted to the TMSi derivative for GC–MS. MS *m/e* > 290 (rel. int.): 641 (M^+, 0.6%), 626 (3.6), 552 (0.5), 551 (0.6), 538 (0.6), 536 (0.6), 510 (1.6), 498 (0.7), 497 (0.7), 496 (0.6), 483 (0.8), 463 (0.5), 462 (0.7), 423 (1.6), 420 (1.0), 408 (4), 366 (4), 348 (4), 322 (42), 294 (10), 293 (3), 292 (7), 73 (100).

Synthesis of O-β-D-*glucopyranosyldihydrozeatin* (**5**). Compound **2** (74.2 mg) synthesized as before [13] was dissolved in MeOH (50 ml), 10% Pd on charcoal (25 mg) added, and the mixture stirred for 48 hr in an atmosphere of H_2. The catalyst was then removed by centrifugation and the solvent evapd from the soln and washings to give a colourless resin (63 mg). By PLC (solvent A), a by-product (7.0 mg, M^+ *m/e* 205) was purified and the product **5** (56.4 mg) isolated as a colourless resin, $[\alpha]_D^{22}$ − 15.9° and $[\alpha]_{546}^{22}$ − 20.0° (H_2O, *c* 1.0). λ_{max} nm (log ε): EtOH, 269.5 (4.18); 0.1 N aq. HCl, 272.7 (4.16); 0.1 N aq. NaOH, 275.0 (4.18) with sh 282 (4.06). ^{1}H NMR (100 MHz, D_2O): δ 8.50 (1H, *s*, purinyl), 8.44 (1H, *s*, purinyl), 4.80 (1H, *d*, *J* = 7.5 Hz, anomeric), 4.36–3.58 (10H, *m*), 2.5–1.7 (3H, *m*), 1.39 (3H, *d*, *J* = 6.5 Hz, CH_3). MS *m/e* (rel. int.): 383 (M^+, 3%), 366 (0.7), 352 (2), 264 (1), 250 (36), 222 (13), 221 (6), 220 (40), 204 (33), 162 (38), 148 (100), 136 (32), 135 (32), 119 (21). (Found: *m/e* 383.1800. $C_{16}H_{25}N_5O_6$ requires: 383.1805).

Acid hydrolysis of **5**. A soln of **5** in N HCl (42 mg in 5 ml) was heated at 80° for 18 hr. The aq. HCl was removed under red. pres. and H_2O was added to the residue and evapd *in vacuo* to remove the remaining acid. PLC (solvent A) of the residue yielded unhydrolysed compound **5** (4.9 mg, after purification on Sephadex G-10) and a hydrolysis product (19.2 mg) as a solid, $[\alpha]_{320}^{22}$ to $[\alpha]_{589}^{22}$ 0° (H_2O, *c* 0.64). Recrystallization of the latter from EtOAc–EtOH gave colourless crystals (mp 166–168°) identified as (±)-dihydrozeatin (**4**) by mmp and MS using an authentic sample [34].

Synthesis of O-β-D-*glucopyranosyl*-9-β-D-*ribofuranosylzeatin* **3**. The acetic acid salt of 4-amino-2-methylbut-*trans*-2-enyl-β-D-glucopyranoside (125 mg) synthesized as before [13, 21], 6-chloro-9-β-D-ribofuranosylpurine (115 mg), MeOH (1.5 ml) and NEt_3 (0.2 ml) were sealed in a cooled (liquid N_2), evacuated glass tube which was then heated at 90° for 5 hr. Purification of the reaction product by PLC (solvent A) gave crude **3** (116 mg). Crystallization from MeOH yielded colourless crystals of **3**, mp 158–160° (Found: C, 48.9; H, 6.2; N, 13.5. $C_{21}H_{31}N_5O_{10}$ requires: C, 49.1; H, 6.1; N, 13.6%);

$[\alpha]_{22}^{\lambda}$:	589	546	520	440	360	310 nm
	−42.2	−51.7	−61.2	−82.2	−135.5	−234°

(H_2O, *c* = 1.2). λ_{max} nm (log ε): EtOH, 269 (4.29); 0.1 N aq. HCl, 265.5 (4.30); 0.1 N aq. NaOH, 269 (4.29). ^{1}H NMR (100 MHz, D_2O): δ 8.64 (1H, *br s*, purinyl), 8.52 (1H, *s*, purinyl), 6.42 (1H, *d*, *J* = 6 Hz, riboside anomeric H), 6.10 (1H, *br t*, —CH=C), 4.85 (1H, *d*, *J* = 7.5 Hz, glucoside anomeric H), 4.9–3.6 (15 H, *m*), 2.23 (3H, *s*, CH_3—). MS of hepta-TMSi **3** *m/e* > 300 (rel. int.): 1017 (M^+, 2%), 1002 (9), 914 (0.5), 550 (100), 478 (38), 462 (10), 316 (15).

Synthesis of O-β-D-*glucopyranosyl*-9-β-D-*ribofuranosyldihydrozeatin* **6**. Compound **3** (86 mg) was dissolved in MeOH (50 ml), 10% Pd on charcoal (43 mg) added, and the mixture was stirred for 16 hr in an atmosphere of H_2. The catalyst was then removed by centrifugation and the solvent evapd from the soln and washings to give a colourless resin (83 mg). PLC (solvent A) yielded a by-product (6.3 mg, M^+ *m/e* 337) and **6** (50 mg) as a colourless resin with MS identical to that of metabolite Y2. MS of per-TMSi **6**: M^+ at *m/e* 1019 (Found: *m/e* 1019.5004. $C_{42}H_{89}N_5O_{10}Si_7$ requires: 1019.4993) and spectrum identical to that of per-TMSi Y2. $[\alpha]_D^{22}$ − 42.8° and $[\alpha]_{546}^{22}$ − 52.7° (H_2O, *c* 1.5). λ_{max} nm (log ε): EtOH, 268 (4.16); 0.1 N aq. HCl, 264 (4.20); 0.1 N aq. NaOH, 269 (4.18). ^{1}H NMR (100 MHz, D_2O): δ 8.62 (1H, *br s*, purinyl), 8.48 (1H, *s*, purinyl), 6.39 (1H, *d*, *J* = 6 Hz, riboside anomeric H), 4.85 (1H, *d*, *J* = 7.8 Hz, glucoside anomeric H), 4.95–4.65 (2H, *m*), 4.45–3.63 (13H, *m*), 2.55–1.70 (3H, *m*, $—CH_2—CH\langle$), 1.40 (3H, *d*, *J* = 6.3 Hz, CH_3—).

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