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GLUCOSYLATION OF CYTOKININ ANALOGUES*

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Key Word Index—*Raphanus sativus*; Cruciferae; radish; metabolism; substituted purines; cytokinins.

Abstract—Glucosylation of adenine and 6-methylaminopurine was not detected in derooted 10-day-old radish seedlings. However, 4-(purin-6-ylamino)butanoic amide and 6-(3,4-dimethoxybenzylamino)purine (N^6-substituted adenines with negligible cytokinin activity), like the highly active cytokinin 6-benzylaminopurine, were converted to 7-glucopyranosides. The N^2-substituted guanine, 2-benzylaminopurin-6-one, and 6-benzylamino-2-(2-hydroxyethylamino)purine were also metabolized to glucosides which were probably 7-glucopyranosides. Hence glucosylation of purines is not restricted to N^6-substituted adenines with strong cytokinin activity. Although only *ca* 1.6% of 6-benzylamino-9-(4-chlorobutyl)purine taken up by the derooted seedlings could be accounted for as 7- and 9-glucosides, a considerable proportion was metabolized to these glucosides in cotyledons excised from 2-day-old radish seedlings. The high cytokinin activity of this 9-substituted compound may be a consequence of cleavage of the 4-chlorobutyl group at N-9.

INTRODUCTION

When the purine phytohormone zeatin, a cytokinin, and the active analogue 6-benzylaminopurine (BAP) are supplied to plant tissues, 7- and 9-glucopyranosides are formed as metabolites [1–6]. 7-β-D-Glucopyranosylzeatin has recently been identified as an endogenous cytokinin [7]. The only other known natural purine derivatives with a sugar at position 7 are a few compounds related to vitamin B_{12}, the sugar in each case being ribose [8]. Hence cytokinin 7-glucosides are unusual structurally. To provide information regarding the structural requirements for acceptance of a glucose moiety by purines, several compounds related to 6-benzylaminopurine were supplied to derooted radish seedlings. The seedling extracts were examined for the presence of glucoside and other metabolites. Radish seedlings are particularly suitable for such a study because 7- and 9-glucosides, together with relatively small amounts of the 3-glucoside, are the only metabolites which they form from 6-benzylaminopurine in appreciable amounts [9].

RESULTS

4-(Purin-6-ylamino)butanoic amide (**4**), 6-(3,4-dimethoxybenzylamino)purine (**5**), 6-benzylamino-2-(2-hydroxyethylamino)purine (**7**) and 2-benzylaminopurin-6-one (**8**, N^2-benzylguanine) were supplied to derooted radish seedlings through the transpiration stream. Relative to BAP, these compounds are weakly active or virtually inactive as cytokinins (see below). The seedling extracts, purified by *n*-BuOH extraction and chromatography on a cellulose phosphate column, were subjected to 2-D TLC on silica gel (solvent A or C followed by B). Comparison of these chromatograms with a chromatogram of identically purified 'control' extract (i.e. extract of seedlings supplied with water only) indicated that **5**, **7** and **8** were each converted to one major UV-absorbing metabolite; these metabolites were termed 5M, 7M and 8M respectively. However, **4** yielded two principal metabolites termed 4Ma and 4Mb. Each metabolite possessed an R_f which was much less than that of the compound supplied (Table 1) and therefore appeared to be a polar derivative. The above metabolites (50–100μg) were purified and their structures are discussed below. Aspects of the metabolism of adenine (**1**), 6-methylaminopurine (**2**) and 6-benzylamino-9-(4-chlorobutyl)purine (**6**) are also reported.

Metabolites 4Ma, 5M, 7M and 8M

MS indicated that all these metabolites were hexose sugar conjugates of the purines supplied. Thus, in the

Table 1. R_f values and spectral characteristics of metabolites

Metabolite	Relative R_f*	λ_{max} (nm) 80% EtOH	H_2O
4Ma	0.36	276†‡	281 (pH 3)
4Mb	0.68	269‡	266 (pH 3)
5M	0.39	280.5‡	283 (pH 3)
7M	0.24	232, 304	230, 301 (pH 7.6); 231, 252, 290 (pH 1)§
8M	0.37	246, 293	253.5, sh 285 (pH 1); 289 (pH 13)

* R_f of metabolite/R_f of purine supplied, Si gel TLC (solvent B).

† Inflection at 291 nm.

‡ Spectrum unaltered by addition of NH_4OH (final conc 0.4 N).

§ A at 290 nm (pH 1)/A at 301 nm (pH 7.6) = 1.33; A at λ_{min} 273 nm (pH 1)/A at λ_{min} 270 nm (pH 7.6) = 2.57.

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Table 2. Mass spectra of metabolites (only the principal ions and those of diagnostic significance are presented)

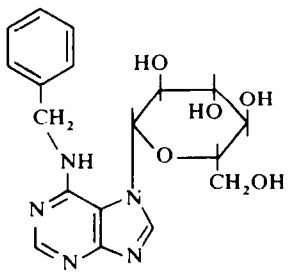
Metabolites	*M/e* and relative intensity (%)
4Ma	365($M^+ - NH_3$, 0.5%), 324(0.2), 220(*b** + H, 6), 203(30), 174(6), 162(100), 160(20), 148(87), 135(14), 120(20), 119(21)
4Mb	352(M^+, 1), 294(5), 263(2), 249(4), 232(4), 220(*b* + H, 5), 203(34), 174(8), 162(84), 160(21), 148(100), 135(13), 120(25), 119(21)
5M	447(M^+, 0.3), 430(0.2), 429(0.1), 356(0.3), 285(*b* + H, 64), 270(20), 254(10), 238(6), 166(23), 151(100), 135(7), 120(10)
7M	446(M^+, 0.1), 428(0.1), 416(0.1), 415(0.1), 402(0.1), 284(*b* + H, 32), 266(30), 265(15), 254(27), 253(68), 240(16), 161(19), 135(9), 134(10), 106(17), 91(100)
8M	403(M^+, 0.3), 241(*b* + H, 65), 240(15), 164(4), 136(7), 135(8), 110(5), 109(6), 108(6), 106(45), 91(100)
TMSi derivatives†	
4Ma (tetra-TMSi)	670(4%), 655(2), 567(1), 219(4), 220(3), 221(10), 249(5), 335(2), 450(3), 361(6), 162(6), 293(6), 363(9), 368(4), 598(1), 612(5)
5M (tetra-TMSi)	735(49), 720(4), 632(1), 284(14), 285(29), 286(10), 314(3), 400(3) 450(1), 361(4), 151(40), 357(4), 358(4), 428(8)
7M (penta + hexa)	
penta-TMSi	806(35), 791(17), 703(5), 355(9), 356(11), 357(4), 385(4), 471(5), 450(4), 361(30), 91(81), 117(15), 271(11) {253(14), 240(21), 690(20)
hexa-TMSi	878(5), 863(4), 775(9), 427(4), 428(4), 429(4), 457(3), 543(3) {325(9), 312(9), 762(10)
8M (tetra + penta)	
tetra-TMSi	691(24), 676(3), 588(<0.2); 240(1), 241(12), 242(4), 270(2), 356(3), 450(3), 361(11), 91(32), 106(8), 271(6)
penta-TMSi	763(20), 748(2), 660(1), 312(6), 313(28), 314(14), 342(4), 428(6),

* *b* denotes the intact purine base fragment.

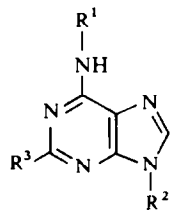
† All spectra showed peaks at *m/e* 73 (base peak), 103, 129, 147, 169, 204, 217 and 319. Values between large brackets (*m/e* 450–271) for 7M and 8M indicate ions which would be derived from both TMSi derivatives. Ions are presented in the following sequence: M^+, $M^+ - CH_3$, $M^+ - CH_2OTMSi$; (space) *b*, *b* + H, *b* + 2H, *b* + CH_2O, *b* + 116; (space) *s* − H, *s* − TMSiOH, *s* denoting the intact TMSi-hexose substituent; (space) other ions

spectra of the underivatized metabolites 5M, 7M and 8M (Table 2), the ions of highest mass occurred at *m/e* values equal to the MW of the supplied purine plus 162 (i.e. plus $C_6H_{10}O_5$). In the cases of the metabolites 5M (M^+, *m/e* 447) and 8M (M^+, *m/e* 403), accurate mass measurements established the formulae $C_{20}H_{25}N_5O_7$ and $C_{18}H_{21}N_5O_6$, respectively for these ions. The spectra of 5M and 7M both showed an $M^+ - H_2O$ ion and in the case of 7M, ions at *m/e* 416 and 415 were attributable to loss of CH_2O and $\cdot CH_2OH$ from M^+. Since the MS of **4** showed a strong $M^+ - NH_3$ ion (see Experimental), the ion of greatest mass in the MS of 4Ma (*m/e* 365) could be accounted for by loss of NH_3 from the M^+ of a hexose conjugate of **4** (MW 382). Loss of CH_2CONH_2 from the M^+ of 4Ma would give the ion of *m/e* 324. The MS of 4Ma, 5M, 7M and 8M all exhibited a prominent ion (*b* + H, Table 2) characteristic of purine glycosides [10] arising from cleavage of the hexose moiety with H transfer to the charged purine fragment. At lower *m/e* values, the spectra closely resembled those of the respective parent compounds. Hence each metabolite possessed an intact moiety of the purine supplied to the plant.

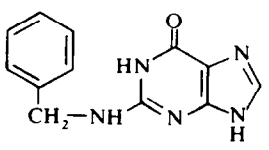
The MS of the TMSi derivatives of 4Ma, 5M, 7M and 8M confirmed that these metabolites were hexose conjugates. 4Ma and 5M both yielded tetra-TMSi derivatives, whereas 7M was converted to a mixture of penta and hexa derivatives and 8M to a mixture of tetra and penta derivatives. The formation of these mixtures is attributable to incomplete silylation of a site in the purine moiety; in the case of 8M the additional site of silylation is probably the enol form of the 6-keto group. Each TMSi spectrum showed a prominent M^+ at the *m/e* value calculated for a hexose conjugate of the supplied purine (see Table 2). In addition ions were present with the *m/e* values for a base fragment *b*, *b* + H, *b* + 2H, *b* + CH_2O, *b* + 116, *s* − H (*m/e* 450), and *s*(TMSiOH (*m/e* 361) where *s* represents the intact TMSi-sugar



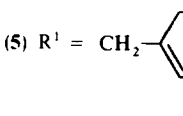
(1)



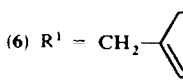
(2) $R^1 = R^2 = R^3 = H$
(3) $R^1 = CH_3$, $R^2 = R^3 = H$
(4) $R^1 = CH_2 \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH_2$, $R^2 = R^3 = H$



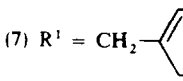
(8)



(5) $R^1 = CH_2$—(OMe, OMe), $R^2 = R^3 = H$



(6) $R^1 = CH_2$—(), $R^2 = CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot Cl$, $R^3 = H$



(7) $R^1 = CH_2$—(), $R^2 = H$, $R^3 = NH \cdot CH_2 \cdot CH_2 \cdot OH$

substituent. These fragment ions are characteristic of per-TMSi derivatives of purine 7- and 9-glucosides [11]. All spectra also exhibited prominent ions at *m/e* 73, 103, 129, 147, 169, 204, 217 and 319 which are present in the MS of most carbohydrate TMSi derivatives. 7M and 8M, like BAP 7- and 9-glucosides, gave a fragment ion at *m/e* 271; this has been attributed to *s* − 2TMSiOH [11].

4Ma, 5M, 7M and 8M, when hydrolysed with a sulphonic acid resin, yielded glucose which was identified with the specific enzyme glucose oxidase. In the MS of the TMSi derivatives of these metabolites, the ion at *m/e* 204 was markedly more intense than that at *m/e* 205; hence the glucose moiety possessed a pyranose ring [11]. The above observations established that these metabolites were all glucopyranosides of the purine supplied to the seedlings. The site of the glucosidic linkage remained to be established. The UV spectra of 4Ma and 5M (Table 1) were characteristic of N^6,7-disubstituted adenines [12] and hence these metabolites were 7-glucopyranosides. Certain features of the MS of the TMSi derivatives of 4Ma and 5M were in accord with this conclusion. For example, as in the MS of the per-TMSi derivative of the 7-, but not the 9-glucopyranoside of BAP [11], M^+ was more intense than $M^+ - 15$; the ion at *m/e* 450 was very weak; a prominent $b + 144$ peak was present at *m/e* 363 (4Ma) and *m/e* 428 (5M).

In metabolite 7M, the glucose moiety could be attached to the oxygen of the C-2 side chain, or to N-3, N-7 or N-9 of the purine ring. Because of steric hindrance due to the C-2 and N^6 substituents, and since only 3-, 7- and 9-glucosides are formed in radish seedlings from BAP, N-1 was not regarded as a site for acceptance of a glucosyl moiety. MS established unequivocally that the side-chain hydroxyl was not substituted. Firstly, an ion was evident in the MS of underivatized 7M at *m/e* 402; this was attributable to loss of a CH_2CH_2OH group from M^+ with hydrogen transfer to the purine fragment. Secondly, in the MS of the per-TMSi 7M, prominent ions were present at *m/e* 117 (attributable to $\overset{+}{C}H_2CH_2OTMSi$), 690 and 762, and metastable peaks established the transitions: hexa-TMSi 7M → 762, penta-TMSi 7M → 690. These transitions indicated loss of a CH_2CH_2OTMSi moiety from the M^+ with hydrogen transfer to the purine fragment and confirmed that the C-2 side chain was not glucosylated. Change in pH from 7.6 to 1 caused a marked hypsochromic shift in the UV spectrum of 7M which was accompanied by a hyperchromic effect (Table 1). In contrast, 9-substituted 2,6-diaminopurines [13], 2,6-diamino-9-β-D-glucopyranosylpurine (spectra determined with synthetic sample) and 3-substituted 2,6-diaminopurines [14] do not exhibit this spectral change; indeed, the 9-substituted compounds show a marked bathochromic shift in λ_{max} in response to pH change from 7.6 to 1. Hence 7M was probably a 7-glucopyranoside. The relative intensities of the M^+ and $M^+ - 15$ ions in the MS of per-TMSi 7M were in accord with this assignment. The UV spectra of 8M at pH 1, 7 and 13, and particularly the shifts in λ_{max} in response to change in pH, were markedly different from those found for N^2-methylguanosine, and from those recorded for 3-substituted guanines [15, 16], O^6-substituted guanines [17, 18] and 1-methylguanine [19]. However, the spectra of 8M were strikingly similar to those recorded for 7-alkylguanines [20] and 7-β-D-ribofuranosylguanine [15, 21]. Hence 8M appeared to be a 7-glucoside of **8**. Since the UV spectra of **8** are very similar to those of guanine over the pH range 1–13, the presence of an N^2-benzyl group in 8M is unlikely to invalidate this conclusion.

Hence, in summary, 4Ma and 5M have been identified unequivocally as 4-(7-glucopyranosylpurin-6-ylamino)-butanoic amide and 6-(3,4-dimethoxybenzylamino)-7-glucopyranosylpurine, respectively. 7M and 8M were both glucoside metabolites, and were probably 7-glucosides, i.e. 6-benzylamino-7-glucopyranosyl-2-(2-hydroxyethylamino)purine and 2-benzylamino-7-glucopyranosylpurin-6-one, respectively.

Metabolite 4Mb

4Mb was characterized by UV spectra (Table 1) as an N^6,9-disubstituted adenine [12] and did not yield glucose when hydrolysed with a sulphonic acid resin. The *m/e* value of the M^+ (352) equalled the MW calculated for a riboside of **4**. The fragment ions (Table 2) at $b + 30$ and $b + 44$ are characteristic of purine ribosides [10]. The ions of *m/e* 294 and 232 are attributable to $M^+ - CH_2CONH_2$ and to $(b - NH_3) + 30$, respectively. The MS of 4Mb below *m/e* 220 indicated the presence of an intact **4** moiety. 4Mb and zeatin riboside possessed an identical electrophoretic mobility in borate buffer (pH 9.2), further confirming that 4Mb was a riboside. Hence 4Mb was probably the 9-riboside of **4**.

Metabolism of adenine, 6-methylaminopurine and 6-benzylamino-9-(4-chlorobutyl)purine

An extract of derooted seedlings supplied with adenine (**2**) was purified by *n*-BuOH extraction and chromatography on cellulose phosphate and was then subjected to 2D-TLC on silica gel (solvents A and B), synthetic 7- and 9-β-D-glucopyranosyladenine being used as markers. Examination of the chromatograms under UV light failed to detect these glucosides as metabolites, although the same procedures readily detected metabolites 4Ma, 4Mb, 5M, 7M and 8M. Similar methods also failed to detect glucoside metabolites of 6-methylaminopurine (**3**). TLC established that unmetabolized exogenously-supplied **2** and **3** were prominent UV-absorbing components of the purified extracts obtained above. Adenine-[2-^{3}H] was also supplied to the derooted seedlings and the seedling extract was again purified by *n*-BuOH extraction and cellulose phosphate fractionation. TLC studies of the purified extract on silica gel (solvents A and B) and DEAE–cellulose impregnated with $Na_2B_4O_7$ (solvent E) indicated that if 7- and 9-glucopyranosyladenine were formed at all as metabolites, they accounted for less than 0.24% of the extracted radioactivity. Free adenine was the principal ^{3}H-labelled compound in the purified extract.

6-Benzylamino-9-(4-chlorobutyl)purine-[^{3}H] (**6**) was supplied as a saturated solution to derooted 10-day-old radish seedlings (experiment A), to cotyledons excised from 2-day-old radish seedlings when the solution of **6** contained BAP at 14 μM (experiment B), and to these cotyledons in the absence of exogenous BAP (experiment C). The extract obtained in A was purified by *n*-BuOH extraction and cellulose phosphate fractionation. All the ^{3}H was extracted from aqueous solution by *n*-BuOH and all was retained on the cellulose phosphate column and eluted by 0.3 N NH_4OH. Two dimensional silica gel TLC studies of the eluate (solvents A and B) established that radioactivity co-chromatographed with the

synthetic 7- and 9-β-D-glucopyranosides of BAP (1.4 and 0.23% of total extracted, respectively); 0.66% was attributable to BAP and 83.6% to unmetabolized **6**. In experiment B, unlabelled BAP was supplied to excised cotyledons to generate a 'trap' of BAP glucosides. In contrast to experiment A, the 7- and 9-glucosides of BAP co-chromatographed during 2-D TLC with 27.5 and 19.6%, respectively, of the extractable radioactivity; only 1.6% was attributable to BAP, and 41.2% to unmetabolized **6**. In experiment C, TLC studies indicated that the 7- and 9-glucosides were again major metabolites and accounted for 11 and 8%, respectively of the extractable ^3H. Two UV-absorbing components responsible for this proportion of the ^3H were purified and converted to TMSi derivatives for characterization by MS. The two MS were identical to those reported [11] for the 7- and 9-β-D-glucopyranosides of BAP and identity was further established by TLC and GLC.

Cytokinin activity of compounds **4**, **5**, **6**, **7** *and* **8**

The cytokinin activities of **5**, **6**, **7** and **8** in the radish cotyledon bioassay [22] were compared with the activity of BAP; **5** and **8** were virtually inactive, while **7** exhibited weak activity. In contrast, the activity of **6** was similar to that of BAP. The lowest detectable concentration of **4** in the bioassay was 5 μM, at which concentration the moderately active analogue 6-butylaminopurine induced a marked growth response. Hence the amide group on the N^6 substituent greatly reduces cytokinin activity and renders **4** only weakly active.

DISCUSSION

In previous studies [1, 3, 4], it was found that zeatin and BAP are converted to 7-β-D-glucopyranosides in radish seedlings. The present investigation establishes that 7-glucosylation is not confined to N^6-substituted adenines with high cytokinin activity. Thus two N^6-substituted adenines (**4** and **5**), an N^2-substituted guanine (**8**) and a substituted 2,6-diaminopurine (**7**), which were either essentially inactive as cytokinins or very weakly active, were all converted to metabolites which appeared to be 7-glucopyranosides. However, adenine and 6-methylaminopurine did not appear to be glucosylated. Hence acceptance of a 7-glucosyl residue by an adenine moiety appears to be greatly enhanced by, or dependent on, an N^6-substituent of 5–7 carbon atoms, but this need not confer cytokinin activity.

Several 9-alkyl derivatives of cytokinins have been tested for cytokinin activity; in bioassays in which heating was avoided during media preparation, the derivatives were all less active than the unsubstituted cytokinins [23]. In view of this, the high activity of the 9-substituted cytokinin **6** was unexpected. It is important to determine whether 9-alkyl groups of cytokinins are metabolically stable, or whether they are cleaved with release of the corresponding cytokinin base unsubstituted at N-9, this being the active molecule. If such groups are stable, ribosylation at N-9 would be prevented. It could then be concluded that riboside and nucleotide formation is not necessary for cytokinin action, and that the mechanism of cytokinin action does not involve incorporation of intact cytokinin molecules into *t*RNA, as has been proposed [24]. In the only reported study of metabolism of a 9-alkylpurine cytokinin, the methyl group of 9-methyl-BAP-[CH_3-^{14}C] was metabolized to $^{14}CO_2$ [25]. It was concluded that the methyl group was cleaved yielding free BAP but no acceptable evidence for this was presented. Hence the metabolism of **6** in radish seedlings reported here is particularly significant.

Although free BAP was not conclusively identified as a metabolite of **6** in radish seedlings, the 7- and 9-β-D-glucopyranosides of BAP were isolated and their identity was established unequivocally by MS. Formation of these glucosides is dependent on prior cleavage of the 4-chlorobutyl group at N-9 to give BAP. The high activity of **6** is probably partly due to gradual conversion to free BAP. Other 9-alkyl cytokinins are probably also dealkylated. Hence the assumption that the activity of such compounds does not depend on formation of nucleoside derivatives [26] is not acceptable. When only one or a few cytokinin applications are practicable for evoking a response (e.g. the promotion of apple fruit growth [27, 28]), use of compounds which slowly release highly active cytokinin molecules may be desirable and give an enhanced response. 9-Alkyl cytokinins such as **6** may be useful for this purpose.

EXPERIMENTAL

Chromatography solvents. A: *n*-BuOH–HOAc H_2O (12:3:5); B: *n*-BuOH–14 N NH_4OH–H_2O (6:1:2, upper); C: MeCOEt–HOAc–H_2O (16:1:4); D: *n*-BuOH satd H_2O; E: solvent D satd with $Na_2B_4O_7 . 10H_2O$; F: MeCOEt satd H_2O.

MS were taken at 70 eV. TMSi derivatives were prepared by dissolving the sample in Py (10–20 μl) and heating at 60° (1 hr) with BSTFA–TMCS (99:1, 100 μl). The silylation reaction mixture was evapd onto the direct inlet probe using a stream of N_2.

Synthesis of 4-(*purin-6-ylamino*)*butanoic amide* (**4**). 6-Chloropurine (0.38 g), 4-aminobutanoic amide hydrochloride (0.80 g), Et_3N (4.0 ml) and *n*-BuOH (20 ml) were heated at 100° for 8 hr. The cooled (2°) reaction soln was filtered and subjected to PLC (Si gel, solvent D). Elution of the principal UV-absorbing zone with MeOH and crystallization from EtOH–H_2O yielded **4** which decomposed at >250°, λ_{max} (pH 11) 274, sh 283 nm. MS *m/e* (rel. int.): 220 (M^+, 12), 203 (21), 162 (100), 160 (15), 148 (49), 135 (8), 120 (10), 119 (14). (Found: *m/e*: M^+, 220.1070; $M^+ - NH_3$, 203.0804. $C_9H_{12}ON_6$ and $C_9H_9ON_5$ require: 220.1072 and 203.0807, respectively).

Synthesis of 6-*benzylamino*-9-(4-*chlorobutyl*)*purine* (**6**). Under anhydrous conditions, 1-bromo-4-chlorobutane (0.46 g, 2.7 mmol) was added to a stirred soln of 6-benzylaminopurine (0.56 g, 2.5 mmol) and NaH (66 mg, 2.7 mmol) in dry DMF (7 ml). After being stirred for 18 hr at room temp., the soln was evapd and the residue subjected to PLC (Si gel, solvent F). The principal UV-absorbing zone (R_f 0.74) was eluted with EtOAc; crystallization from EtOH–H_2O yielded **6** (0.40 g), mp 96°, λ_{max} nm: 272 (EtOH) and 269 (0.1 N HCl). MS *m/e* (rel. int.): 317 (M^+, 33), 315 (M^+, 100), 280 (69), 266 (9), 253 (8), 252 (8), 238 (7), 225 (19), 224 (34), 210 (11), 148 (18), 121 (9), 120 (13), 119 (12), 106 (85), 91 (59). (Found: C, 61.3; H, 5.6; N, 22.2; Cl, 10.7. $C_{16}H_{18}N_5Cl$ requires: C, 60.9; H, 5.7; N, 22.2; Cl, 11.2%). Repetition of the synthesis with 6-benzylaminopurine-[G-^3H] yielded ^3H-labelled **6** (4 mCi/mmol).

Synthesis of 6-*benzylamino*-2-(2-*hydroxyethylamino*)*purine* (**7**). 2,6-Dichloropurine was condensed with benzylamine (excess) in refluxing *n*-PrOH for 2 hr (cf. [29] where different conditions were used for similar condensations). The crude product was washed with H_2O and crystallized from EtOH–DMF to give 6-benzylamino-2-chloropurine, mp 249–250°; $\lambda_{max}^{H_2O}$ nm: 277 (pH 1), 279 (pH 11). MS *m/e* (rel. int.): 261 (M^+, 35), 259 (M^+, 100), 224 (13), 182 (7), 154 (7), 119 (9), 106 (29), 100 (7), 91 (25).

The foregoing product (250 mg) and ethanolamine (700 mg)

were heated at 140° for 5 hr in a sealed evacuated tube. Excess ethanolamine was evaporated *in vacuo*, the solid residue washed with H_2O and dissolved in dil. HCl. The ppt. obtained by neutralization was crystallized from EtOH–H_2O to yield **7**, mp 189–190°. λ_{max} nm: 231, 289 (80% EtOH); 235, 250, 287 (0.1 N HCl). MS *m/e* (rel. int.): 284 (M^+, 26), 266 (7), 265 (6), 254 (19), 253 (40), 240 (12), 135 (7), 134 (8), 119 (5), 106 (17), 91 (100). (Found: C, 59.1; H, 5.7; N, 29.5. $C_{14}H_{16}ON_6$ requires: C, 59.1; H, 5.7; N, 29.6%).

Other syntheses. **5** was prepared by condensation of 6-chloropurine with an excess of 3,4-dimethoxybenzylamine in refluxing *n*-BuOH, the product being crystallized from EtOH–H_2O, mp 249°; MS *m/e* (rel. int.): 285 (M^+, 100), 270 (31), 254 (15), 238 (12), 166 (25), 151 (93). **8** was synthesized by published methods [30]; MS *m/e* (rel. int.): 241 (M^+, 73), 240 (13), 164 (5), 136 (6), 135 (10), 106 (44), 91 (100).

Uptake of compounds and extraction of tissue. The ends of the hypocotyls of derooted 10-day-old radish (*Raphanus sativus* cv Long Scarlet) seedlings were placed in solns (70 μM) of compounds **3**, **4**, **5**, **7** and **8** for 55 hr under continuous fluorescent light (700 lx). Adenine (**2**) was given as a 140 μM soln for 55 hr; adenine-[2-^{3}H] (500 mCi/mmol; 10 μM) was taken up for 12 hr and the seedlings were then transferred to H_2O for 12 hr prior to extraction. ^{3}H-Labelled compound **6** (solubility < 70 μM) was supplied as a satd soln to the derooted seedlings for 74 hr and also to cotyledons, excised from 2-day-old radish seedlings, for 4 days under the conditions described previously for cytokinin bioassay [22].

All tissue was extracted by being dropped into 80% MeOH (10 ml/g of tissue) at 65°, held at this temp. for 5 min, cooled rapidly and finally homogenized with the solvent. The filtered extract was evapd *in vacuo*. When extracting tissue which had received compound **6**, $CaCO_3$ was added to the MeOH (0.3 g/100 ml).

Purification of extracts and isolation of metabolites. The evapd extract was suspended in H_2O, which was then clarified by centrifugation, and shaken with 4 equal vols of *n*-BuOH. An aq. soln of the fraction extracted by *n*-BuOH was adjusted to pH 3 and percolated through a column of Whatman P1 cellulose phosphate (1 g/equiv of 10 g tissue; NH_4^+ form equilibrated to pH 3) which was washed with 0.03 N HOAc (discarded) and eluted with 0.3 N NH_4OH. To isolate metabolites of **4**, **5**, **6**, **7** and **8**, the evapd eluate was subjected to 2-D PLC on Si gel (Merck PF_{254}) using solvents A or C and B. The required spots (detected under UV light) were eluted with MeOH–H_2O (4:1) and rechromatographed on paper (washed exhaustively with 20% EtOH) using solvent D, the tank being satd with NH_3. The metabolite spots were eluted with purified 80% EtOH for UV and MS. Metabolite 5M required additional purification by PC with solvent C.

Hydrolysis of metabolites to glucose. Metabolite (10 μg) was dissolved in 0.1 N HOAc (0.1 ml) and the soln was stirred with the cation-exchange resin (Zeo-Karb 225, Permutit SRC 14, H^+ form, 10 mg) for 15 min. The suspension was then heated at 120° (autoclave) for 1 hr and finally centrifuged. The supernatant was evapd onto a Si gel thin-layer and chromatographed with solvent C. The developed chromatogram was dried, placed in an atmosphere containing NH_3 to neutralize HOAc, redried and finally sprayed with a glucose oxidase–chromogen soln [31] to detect glucose (red-brown spot, R_f 0.16).

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