Phytochemistry, Vol. 30, No. 8, pp. 2477–2486, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

SUBSTITUTED XANTHINES AND CYTOKININ ANALOGUES AS INHIBITORS OF CYTOKININ *N*-GLUCOSYLATION*

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(*Received in revised form* 19 *November* 1990)

Key Word Index—*Zea mays*; Gramineae; maize; *Raphanus sativus*; Cruciferae; radish; cytokinin metabolism; *N*-glucosylation; 3-substituted xanthines; 6-benzylaminopurine (BAP); 2-substituted 9-methyl-BAP; 7-benzylaminooxazolo[5,4-*d*]pyrimidine.

Abstract—A series of 3-substituted xanthines, 2-(2-hydroxy-2-methylpropylamino)-9-methyl-6-benzylaminopurine and 7-benzylaminooxazolo[5,4-*d*]pyrimidine were synthesized as potential inhibitors of cytokinin *N*-glucosylation. In maize leaf segments the latter compound was found to be the most effective inhibitor tested, inhibiting the formation of the 9-glucoside of 6-benzylaminopurine (BAP) and raising the amount of free BAP. *N*-glucosylation of BAP in radish leaves was found to be suppressed most effectively by 1,7-dimethyl-3-(3-methylbutyl)xanthine, 1,7-dimethyl-3-(5-hexenyl)xanthine and 1,7-dimethyl-3-(3-methyl-2-butenyl)xanthine. The first two compounds were also effective inhibitors in radish cotyledons and elevated the concentrations of both free BAP and BAP nucleotide. These results indicate that the structural requirements for effective inhibitors of *N*-glucosylation differ between the two species.

INTRODUCTION

Attempts to regulate the development and growth of plants often involves the supply of exogenous cytokinins. However, the effectiveness of exogenous cytokinins may be limited by rapid metabolic inactivation involving the formation of 7- and 9-glucosides, or oxidative cleavage of the N^6-sidechain [1]. To enhance growth responses to cytokinins it is desirable to suppress this inactivation. This could be achieved by either selectively inhibiting the inactivating enzymes, or by presenting the plant tissues with an active cytokinin derivative or analogue that is inert to the mechanisms of inactivation.

The formation of 7- or 9-glucosides is an important form of cytokinin inactivation in a number of plant tissues [1]. Hence, inhibitors of the responsible enzymes are of considerable interest, since they may provide a means of elevating the quantities of both endogenous and exogenous cytokinins and, thus, influence plant development. Based on the observations that theophylline and 1-methyl-3-isobutylxanthine (**1**) had each been demonstrated to inhibit *N*-glucosylation to varying degrees in radish cotyledons [2], it was decided to synthesize a series of 3-substituted xanthines and test their ability to inhibit *N*-glucosylation of 6-benzylaminopurine (BAP) in radish and maize leaves.

A 2-substituted BAP, 2-(2-hydroxyethylamino)-9-methyl-6-benzylaminopurine (**2**) has also been found to inhibit the enzyme purified from radish cotyledons which catalyses 7- and 9-glucosylation of cytokinins [3, 4]. The 9-methyl substituent blocked glucosylation of the imidazole ring while the 2-hydroxyethylamino group at position 2 enhanced binding to the enzyme. In the radish cotyledon expansion bioassay, **2** was found to be inactive as a cytokinin. When supplied together with suboptimal quantities of BAP, **2** enhanced the growth response to BAP, and the amount of free BAP in the tissue was also increased [5]. Subsequent studies have suggested that the inhibitory role of **2**, may be neutralized, in the cotyledons, by conversion to an *O*-glucoside [2]. Hence, the effectiveness of **2** as an inhibitor of *N*-glucosylation may be improved by sterically hindering *O*-glucosylation, utilizing a tertiary alcohol instead of a primary alcohol in the substituent at C-2 of the purine ring. Thus, we proposed to synthesize and test 2-(2-hydroxy-2-methylpropylamino)-9-methyl-6-benzylaminopurine (**3**).

An alternative approach to preventing 7- and 9-glucosylation is to modify the purine ring of the exogenously supplied cytokinin so that glucosylation becomes impossible. Alkylation of BAP at the N-9 position would at first sight seem to be a suitable modification. However, some such derivatives, for example, the 4-chlorobutyl derivative have been reported to be metabolically unstable as they are slowly dealkylated to BAP [6]. This problem could, perhaps, be avoided by a formal replacement of the nitrogen at position 9, with an oxygen. The resulting BAP analogue would be 7-benzylaminooxazolo[5,4-*d*]pyrimidine (**4**). Although such structural modification of the imidazole ring is likely to result in a reduction of cytokinin activity [7 and references therein], the degree of this reduction is not predictable. However, if supplied to plant tissues together with BAP, the oxazolopyrimidine may act as a competitive inhibitor of the glucosylating enzymes.

* Part 4 in the series 'Inhibitors of Cytokinin Metabolism'. For Part 3 see ref. [2].

RESULTS

Synthesis of 3-substituted xanthines

Alkylation of 1,7-methylxanthine (**5**) with the appropriate halide in DMF containing NaH yielded the 3-substituted 1,7-dimethylxanthines, **6**–**15**. Compounds **6** and **7** have been prepared previously by alkylation of **5** in aqueous ethanol containing KOH [8]. However, the yields obtained in the present study were markedly better (**6**, 89%, cf. lit. 44%; **7**, 63% cf. lit. 35%). The remaining compounds have not been reported previously. The UV spectra of these compounds remained unaltered with changes of pH (λ_{max} 273 nm), as would be expected for fully *N*-alkylated xanthines which can neither enolize nor ionize. The identity of the alkylation products was confirmed by NMR and mass spectra at both high and low resolution. The NMR spectra all featured singlet resonances for H-8 (ave. δ7.55), the NMe-7 (ave. δ4.03) and NMe-1 (ave. δ3.42). The methyl resonances were assigned by comparison with those observed in the NMR spectrum of 1-methylxanthine (δ3.40). The remaining signals were due to the 3-substituent.

The aromatic heterocyclic purine ring is very stable and not readily fragmented by electron impact. This property is reflected in the fact that the mass spectra of the 3-substituted xanthines generally have strong $[M]^+$ and the major fragments are derived from cleavages of the alkyl side chain. These fragmentation pathways are similar to those previously described for *N*-alkyluracils [9, 10]. The ion *m/z* 180, common to all the mass spectra of 1,7-dimethylxanthine derivatives, is derived from the loss of the alkyl substituent via a McLafferty rearrangement and entails transfer of a hydrogen to the purine moiety. Also common to these spectra are the ions resulting from cleavage of the side chain between C-1′ and C-2′. The shorter butyl and butenyl substituents of **8** and **12** tend to be homolytically fragmented to produce *m/z* 193. The longer and branched alkyl chains are cleaved between C-1′ and C-2′ with hydrogen transfer to the purine moiety and proceed via a four- or a five-membered transition state, yielding *m/z* 194. Fragmentation of the pentenyl derivative **9**, yielded both *m/z* 193 and 194 in almost equal abundance, whereas the hexenyl (**10**) and branched chain derivatives (**7**, **13**–**15**) yielded *m/z* 194 almost exclusively. In addition, the oxygenated sidechain of **12** may lose H_2O $[M-18]^+$ and CHOH $[M-30]^+$ or in the case of **15**, H_2O and CH_2OH $[M-31]^+$. The mass spectrum of **14** is dominated by the cleavage of the *cis*-diol in the alkyl substituent, affording *m/z* 223 $[M-COHMe_2]^+$. Subsequent fragmentation of the xanthine moiety (*m/z* 180, 193, 194) then proceeds *via* a retro-Diels–Alder reaction with expulsion of MeNCO involving the N-1 and C-2 atoms. These fragmentations parallel those described for caffeine and 1,7-dimethylxanthine [11, 12]. The formulae of all fragments were established by HR mass measurements. Trimethylsilylation of **14** produced a mixture of mono-TMSi and a di-TMSi derivatives which could be separated by GC. The base peak in the mass spectra of the mono-TMSi derivative appeared at *m/z* 295 $[M-COHMe_2]^+$ indicating a preferential silylation of the secondary alcohol over the sterically crowded tertiary alcohol.

Some aspects of the synthesis of the 3-substituted 1,7-dimethylxanthines merit comment. The production of 1,7-dimethyl-3-(3-methyl-2-buten-1-yl)xanthine (**13**) via alkylation of 1,7-dimethylxanthine required the production of a suitable alkyl halide. 3-Methyl-2-buten-1-ol was converted to the 1-iodide using a simple one-step treatment with iodotrimethylsilane [13]. The iodide was not isolated, the crude chloroform solution being used directly for the alkylation reaction. Iodination of 3-methyl-3-butene-1-ol resulted in the isomerization of the double bond (as evidenced by NMR data) so that **13** was the only alkylation product isolated. The isomerization was probably catalysed by hydrogen iodide which is produced during the reaction. An attempt to synthesize this iodide via the TMSi ether [13] avoiding the production of hydrogen iodide, similarly resulted in isomerization. The diol **14** was produced by a permanganate oxidation of **13** [14].

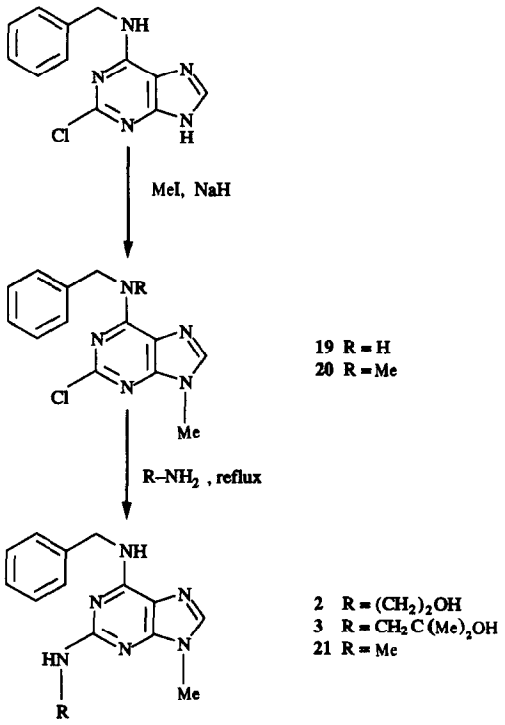
Table 1. The bathochromic shift of UV maxima is characteristic of the substitution pattern of disubstituted xanthines

Substituted xanthine	UV λ_{max} (nm)	
	Neutral pH	Basic pH
1,7-Dimethylxanthine (**5**)	270	293
1-Methyl-3-isobutylxanthine (**1**)	273	277
1,9-Dimethylxanthine	239, 271	250, 270

1-Methylxanthine is poorly soluble in DMF and so the alkylations were carried out in DMSO. A mixture of di- and mono-substituted derivatives was extracted after the reaction along with 1-methylxanthine. It is possible to direct alkylation to the 3-position by means of a protecting group at the 7-position [15], however, the mixtures of products were readily separated by HPLC and it was thought possible that inhibitory activity might be obtained from either 3 or 7-substituted 1-methylxanthines. Structural assignments for these xanthine derivatives (**16**–**18**) were based primarily on UV spectra which are diagnostic of the position of substitution on the xanthine ring [16] (see also Table 1). A marked bathochromic shift caused by basification is characteristic of a 1,7-disubstituted xanthine. While alkylation with 4-iodo-2-methyl-2-butene yielded a mixture of the 3- and 7-substituted products (**17** and **16**, respectively), reaction with benzyl bromide gave only the 7-benzyl derivative (**18**). The mass spectra of **16** and **17** were identical and did not provide information relevant to the positions of substitution.

Synthesis of 2-substituted BAP

When compound **2** was originally synthesized [3] both the 7- and 9-methyl derivatives were produced and these were separated by TLC. As the 9-methyl derivative (**2**) had been found to be a more effective inhibitor of cytokinin *N*-glucosylation when compared with the 7-methyl derivative, it was decided to modify the synthesis to minimize the yield of the 7-methyl isomer. It is known that adenine and N^6-substituted adenines can be alkylated almost exclusively at N-9 [17, 18], thus, by reversing the order of methylation and benzylation of 2,6-dichloropurine one would expect to favour the production of 2-chloro-9-methyl-6-benzylaminopurine (**19**) rather than the 7-methyl isomer. The synthesis of **2** and **3** was carried out as shown in Scheme 1. Methylation of 2-chloro-6-benzylaminopurine, in dry DMF containing NaH, yield-



Scheme 1. Synthesis of **2**, **3**, **19–21** from 2-chloro-6-benzylaminopurine, optimizing methylation at *N*-9.

ed **19** with only a trace of the 7-methyl isomer. The use of excess methylating reagent resulted in the methylation of the exocyclic nitrogen (**20**). Reaction of **19** with ethanolamine in DMF yielded **2**. An attempt to synthesize **3** as per the synthesis of **2**, but including traces of the hydrochloride salt of 2-aminomethyl-2-propanol, resulted in the exclusive production of **21**. The aminomethyl function is presumed to be derived from methylamine, a decomposition product of the solvent, DMF. Compound **3** was eventually synthesized following the use of the free base derived from the passage of the hydrochloride salt through an anion-exchange resin in the hydroxide form. When compared to the original synthesis of **2**, the preparation described here was much more convenient and TLC was not essential to isolate **19**.

Synthesis of 7-benzylaminooxazolo[5,4-d]pyrimidine

The oxazolo[5,4-*d*]pyrimidines have generally been prepared by ring closure of either suitably substituted pyrimidine or oxazole derivatives [19, and references therein]. However, the synthesis of 7-aminooxazolopyrimidine (**22**) has only been reported by Ferris and Orgel [20] and by Ohtsuka [21]. The latter procedure was reported to have the better overall yield and was therefore the method of choice. In a relevant preliminary study, methods for N^6-substitution of adenine were reviewed. Substitution of the exocyclic nitrogen of adenine has been achieved in five different ways. Firstly, adenine may be *N*-alkylated at position 1, which, when followed by an alkali-induced Dimroth rearrangement, produced the required 6-substituted aminopurine [22 and refs therein, 23–26]. Secondly, the exocyclic amine of adenine may be exchanged by heating in the presence of a secondary amine and the amine hydrochloride [27, 28]. It is also possible to acylate the amino group of adenine and to then reduce this intermediate with lithium aluminium hydride to yield a 6-substituted aminopurine [29–31]. Thirdly, nucleophilic substitution (aminolysis) of 6-chloropurine (prepared from adenine via hypoxanthine), by refluxing with the appropriate amine in *n*-butanol, will also yield the required adenine derivative [23, 32–36]. Finally, 9-(tetrahydropyran-2-yl)adenine can be directly alkylated at N^6 by reaction with an alkyl bromide in an aprotic solvent in the presence of NaH or potassium *t*-butoxide [37]. The 9-blocking group can then be cleaved with acid. Consideration of these procedures suggested that direct benzylation of **22** in the presence of NaH was probably the procedure to try initially.

Compound **22** was synthesized as described in ref. [21]. Benzylation of **22** in DMF with NaH produced a mixture of disubstituted compounds, as evidenced by mass spectral data. Even when the calculated amount of NaH was used, disubstitution still resulted and the conversion of the mono- to the disubstituted derivative appeared to occur at a rate which greatly exceeded that of the initial alkylation. Transamination reactions also yielded only dibenzyl derivatives. Alkylation of **22** in a DMF solution of NaH with 1-bromo-3-methylbutane similarly yielded disubstituted derivatives. A Sandmeyer reaction [38], and a modified Sandmeyer reaction [39] with adenine, both produced 6-chloropurine; however, when applied to **22**, no 7-chlorooxazolopyrimidine was detected. Acetylation of **22** produced a mixture of both the mono- and diacetyl derivatives. As the diacetyl derivative was the major component of the mixture, such a procedure was also considered inadequate as a pathway to **4**.

Hexamethylphosphoramide (HMPA) has been reported [40] to be a solvent capable of facilitating the direct alkylation of aromatic amines by alkyl halides. When the *N*-arylation reaction was repeated using HMPA, plus a trace of 4-dimethylaminopyridine in the reaction mixture, a small amount of **4** was isolated. The structure of **4** was confirmed by spectroscopic analysis. High resolution mass measurements confirmed the formula of the $[M]^+$ (*m/z* 226) and of the strong benzylimine ion (*m/z* 106) in the mass spectrum. The ^{1}H NMR data proved to be consistent with the structure of **4**, the resonances assigned to the exocyclic amino proton (δ5.95) being readily exchanged with D_2O. These spectra eliminated the possibility that the product was 9-benzylhypoxanthine [41].

Metabolism of BAP in maize leaves and inhibition of N-glucosylation

The metabolism of [^{3}H]-ZR in maize seedling leaves is dominated by the activity of cytokinin oxidase [42]. Over a three day period, *ca* 56% of the extracted radioactivity could be accounted for as Ado, Ade or the Ade nucleotides. The only metabolite with an intact side chain that was conclusively identified was Z9G (2% extracted radioactivity). In comparison, the major metabolite produced when BAP was supplied to maize leaves, was BAP-9-G (45% extracted radioactivity). In studies with purified cytokinin oxidase isolated from *Zea mays* kernels [43] it was found that the BAP side chain was not susceptible to cleavage. However, the side chain is slowly cleaved *in vivo*

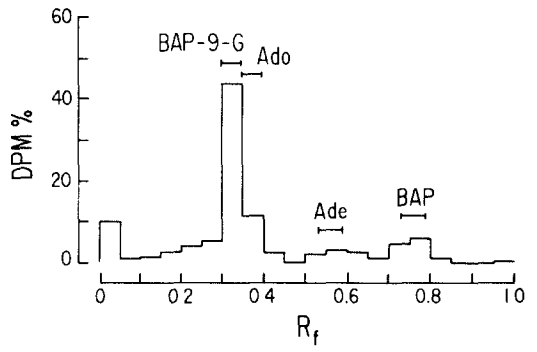


Fig. 1. Chromatography of radioactivity (silica gel TLC solvent C), extracted from maize leaf segments supplied with [^{3}H]BAP. The radioactivity in each TLC zone is expressed as a percentage of that eluted from the plate.

by maize leaf segments but the resultant Ado is only a minor metabolite (< 11% extracted radioactivity) (Fig. 1). Thus, it was decided to test the effectiveness of the synthesised compounds, in inhibiting the *N*-glucosylation of BAP, rather than ZR, in the maize leaf segments (Table 2).

A number of synthesized compounds inhibited appreciably the formation of BAP-9-G, namely, the xanthine derivatives **7**, **10**, **16** and **17** and the BAP analogue **4**, and these inhibitors also elevated the amounts of free BAP markedly. However, inhibition by the first four compounds was exhibited only at the highest concentrations tested, usually 5 mM. In the case of **10**, inhibition was very pronounced (87% reduction in the formation of BAP-9-G as compared with the control), but may have been associated with a toxic effect, as the incubation solution had become yellow after three days. This effect was only observed in the case of compound **10** supplied at 5 mM. Compounds **3** and **4** were the most effective inhibitors tested, in that they suppressed BAP-9-G formation and elevated the quantities of free BAP appreciably, even at 0.2 mM. Some inhibitors, especially **16** at 1 mM, elevated free BAP but did not inhibit formation of BAP-9-G and, hence, appeared to suppress BAP metabolism other than *N*-glucosylation.

The BAP derivative **2**, which had been found to be an effective inhibitor of cytokinin *N*-glucosylation in radish cotyledons, actually elevated the proportion of ^{3}H converted to BAP-9-G in maize leaves. In a separate experiment, **2** was compared with its 7-methyl isomer [3]. Again **2** increased the percentage of radioactivity due to BAP-9-G, but the 7-methyl compound did not affect this percentage, while it increased the ^{3}H due to free BAP from 14 to 19%.

Inhibition of N-*glucosylation of BAP in radish leaves and cotyledons*

With the exception of **3**, **17** and **4**, all compounds inhibited *N*-glucosylation of BAP appreciably in radish leaf discs, at least at the highest concentrations tested (Table 3). In the case of the 1-methylxanthines, **1** and **16**, this inhibition was accompanied by a marked elevation in the amount of free BAP, while that of BAP nucleotide was not appreciably altered. However, in the case of all 1,7-dimethylxanthines and also **2**, the quantities of both free BAP and BAP nucleotide were elevated markedly when *N*-glucosylation was suppressed. The most effective inhibitors of *N*-glucosylation in radish leaves were the three derivatives of 1,7-dimethylxanthine, **7**, **10** and **13**, which all showed significant activity at 0.2 mM (Table 3). These compounds were more effective than the BAP derivative **2** and the 1-methylxanthine derivative **1**, the most effective inhibitors of this type to date. At 5 mM, **10** elevated free BAP by 40-fold; 1-methylxanthine at 1 and 5 mM did not inhibit glucosylation. Compound **3**, an analogue of **2** in which the 2-substituent was modified to sterically suppress glucosylation at this site, was less effective than **2** itself.

In the above studies, screening for inhibition was carried out using leaf discs rather than cotyledon discs, primarily because of the convenience of cutting many discs from one leaf rather than only four discs from any one cotyledon, given that metabolism of exogenous BAP is essentially the same in the two tissues. However, the inhibitory activities of **7**, **10** and **2** were also compared using radish cotyledons (Table 4). In this study, the radioactivity in BAP-9-G, -7-G and -3-G were determined in addition to that in free BAP and BAP nucleotide (BAPR-5′-P). It is evident that **2** preferentially inhibited formation of BAP-9-G which has been reported previously [2, 5] and explained in terms of effective inhibition of one of the two cytokinin glucosylating enzymes in radish cotyledons. These two enzymes have been shown to form the 7- and 9-glucosides of BAP in markedly different proportions, the ratios for 7-G/9-G being 1.5 for the major enzyme and 10–11 for the other [4]. The former enzyme has been characterized and **2** was designed to inhibit its action [3, 5]. In contrast to **2**, the new inhibitors **7** and **10** suppressed formation of BAP-7-G and BAP-9-G *ca* equally and also reduced formation of BAP-3-G. Thus, **7** and **10** seem to inhibit both glucosylation enzymes.

DISCUSSION

The metabolism of BAP in both maize and radish leaves, and radish cotyledons is very simple as it consists principally of *N*-glucosylation and to a much lesser degree, the interconversion of the base, riboside and nucleotide forms. Consequently, the potency of the synthetic compounds in preventing *N*-glucosylation of BAP was tested in these tissues. It was apparent from the results that the structural requirements for inhibitors in the two species were quite different. The xanthine derivative **1** and the BAP derivative **2**, which are known to inhibit *N*-glucosylation in radish cotyledons, had little effect in the maize leaf system. Similarly, compounds **7**, **10** and **13** which were observed in the present study, to suppress *N*-glucosylation markedly in radish, were ineffective inhibitors in maize. Conversely, compound **4** is an effective inhibitor of 9-glucosylation in maize, but has no activity in radish. It is noteworthy that substituent structures, which confer high cytokinin activity on N^6-substituted adenines in the radish cotyledon bioassay [44] are not optimal for enzyme inhibition by 3-substituted 1,7-dimethylxanthines. Thus, in the cotyledon bioassay, zeatin is much more active that 6-isopentylaminopurine, yet **15** with a zeatin side-chain at position 3 is a weak inhibitor relative to **7** with an isopentyl group at position 3.

In addition to being an inhibitor of 9-glucosylation, **4** could have anti-cytokinin activity, or it may be a cytok-

Table 2. Distribution of radioactivity, as assessed by silica gel TLC (solvent C), extracted from *Zea mays* leaf segments following incubation with [^{3}H]BAP in the presence of potential inhibitors of *N*-glucosylation

		Concentration (mM)	% Eluted radioactivity BAP-9-G	BAP
Control		—	45	13
Derivatives of 1-methylxanthine				
	Substituent			
1	3-CH_2CHMe_2	5	51	23
16	7-$CH_2CH{=}CMe_2$	3	27	51
16		1	46	31
17	3-$CH_2CH{=}CMe_2$	5	19	55
17		1	53	18
18	7-$CH_2C_6H_5$	2.5	51	16
Derivatives of 1,7-dimethylxanthine				
	Substituent			
6	3-$CH_2C_6H_5$	2.5	67	7
7	3-$(CH_2)_2CHMe_2$	5	31	46
7		1	48	13
8	3-$(CH_2)_2CH{=}CH_2$	5	70	8
9	3-$(CH_2)_3CH{=}CH_2$	5	67	9
10	3-$(CH_2)_4CH{=}CH_2$	5	6	67
10		1	54	12
11	3-$CH_2C(Me){=}CH_2$	5	67	17
12	3-$(CH_2)_4OH$	5	42	20
12		1	43	13
13	3-$CH_2CH{=}CMe_2$	5	54	21
13		1	58	14
14	3-$CH_2CHOHCOHMe_2$	5	48	13
15	3-$CH_2CH{=}C(CH_2OH)Me$	5	45	19
15		1	44	20
Derivatives of 9-methyl-BAP				
	Substituent			
2	2-$NH(CH_2)_2OH$	1	59	21
3	2-$NHCH_2C(Me)_2OH$	0.8	36	25
3		0.2	38	24
Derivatives of oxazolo[5,4-*d*]pyrimidine				
	Substituent			
4	7-$NHCH_2C_6H_5$	1	20	48
4		0.2	37	26

inin itself. If **4** is active as a cytokinin, then this would mean that 9-ribosylation is not a prerequisite for cytokinin activity [45]. However, unlike cytokinins such as BAP, **4** does not retard soybean leaf senescence [46]. Its activity in conventional cytokinin bioassays is not known at present.

While the inhibitors synthesized in this study were intended primarily as inhibitors of glucosylation in plant tissues, it is likely that they also inhibit other enzymes of cytokinin metabolism. This view is substantiated by the observation that **16** and **17** elevated BAP in maize and radish leaves, respectively, but did not inhibit glucosylation. In a recent study [46] it was found that compound **10** was an effective inhibitor of the conjugation of BAP with alanine in soybean leaves. Although it had no senescence-retarding activity when applied alone to soybean leaves, it enhanced the action of BAP which would normally be inactivated by alanine conjugation.

Caffeine and other related xanthine bases containing a 3-methylpyrimidine-2,4-dione moiety, constitute a well known group of inhibitors of cytokinesis, rapidly inducing cell-plate breakdown in the final stages of mitosis, although high concentrations are required [47]. Four of the 3-substituted-1,7-dimethylxanthines (**6**, **7**, **10** and **13**) have been shown to be more effective than caffeine as inhibitors of cell plate formation. The most effective was **7** which required a concentration one-tenth that of caffeine [48].

The synthetic xanthine derivatives synthesized in the present study may have, in common with the methylxanthines, cytokinins and related compounds, the ability to inhibit the action of mammalian adenosine-3,5-cyclic-monophosphate (cAMP) phosphodiesterase [49–53]. Hence these new xanthine derivatives may eventually prove to have biochemical applications in both plant and animal studies.

Past endeavour has yielded much information concerning cytokinin structure–activity relationships and a number of potent synthetic cytokinins are now known. It may now be more profitable to devote effort to

Table 3. Distribution of radioactivity as assessed by silica gel TLC (solvent C), extracted from radish leaf discs following incubation with [^{3}H]BAP in the presence of potential inhibitors of *N*-glucosylation

		Concentration (mM)	% Eluted radioactivity BAPR-5'-P	Glucosides*	BAP
Control		—	8.8	83	1.0
Derivatives of 1-methylxanthine					
	Substituent				
1	3-CH_2CHMe_2	5	9	67	11
1		1	9	71	6
16	7-$CH_2CH{=}CMe_2$	3	13	53	11
16		1	11	74	56
17	3-$CH_2CH{=}CMe_2$	5	2	86	11
17		1	5	80	5
18	7-$CH_2C_6H_5$	2.5	15	69	4
Derivatives of 1,7-dimethylxanthine					
	Substituent				
6	3-$CH_2C_6H_5$	2.5	36	40	5
6		1	34	48	5
7	3-$(CH_2)_2CHMe_2$	5	41	14	25
7		1	36	41	9
7		0.2	25	61	4
8	3-$(CH_2)_2CH{=}CH_2$	5	38	29	13
8		1	30	54	5
9	3-$(CH_2)_3CH{=}CH_2$	5	33	34	11
9		1	31	47	8
10	3-$(CH_2)_4CH{=}CH_2$	5	18	13	39
10		1	33	49	12
10		0.2	23	63	5
11	3-$CH_2C(Me){=}CH_2$	5	31	45	6
12	3-$(CH_2)_4OH$	5	26	47	10
12		1	19	64	4
13	3-$CH_2CH{=}CMe_2$	5	42	22	21
13		1	28	53	6
13		0.2	23	67	4
14	3-$CH_2CHOHCOHMe_2$	5	22	63	3
15	3-$CH_2CH{=}C(CH_2OH)Me$	5	33	49	8
15		1	19	64	4
15		0.2	13	82	2
Derivatives of 9-methyl-BAP					
	Substituent				
2	2-$NH(CH_2)_2OH$	1	28	53	6
2		0.2	17	75	4
3	2-$NHCH_2C(Me_2)OH$	0.8	12	79	2
Derivative of oxazolo[5,4-*d*]pyrimidine					
	Substituent				
4	7-$NHCH_2C_6H_5$	1	17	75	3

*Radioactivity corresponding to BAP-9-G, -7-G and 3-G.

designing and synthesizing inhibitors of cytokinin inactivation. Such inhibitors could potentiate the activity of both endogenous and exogenous cytokinins and may eventually contribute to our ability to control plant development.

EXPERIMENTAL

EIMS were obtained at 70 eV, source temp. 240°. Underivatized compounds were introduced via the direct probe inlet. TMSi derivatives were introduced via a GC (1% OV-101 on 80-100 Gaschrom Q) temp. prog. 200 to 300° at 10° min^{-1}. NMR spectra were recorded at 100 MHz using TMS as int. standard. UV spectra were measured in both basic [75% EtOH–0.4 M NH_3 (aq)] and neutral solvents (70% EtOH). Mp are uncorr.

Chromatographic methods. HPLC systems used were (solvent proportions by vol, all contain 1% HOAc): A, μBondapak C_{18} (3.9 × 300 mm), 50% MeOH at 3 ml min^{-1}; B, column as A, 70% MeOH at 3 ml min^{-1}; C, μBondapak phenyl (3.9 × 300 mm), 15% MeOH at 1.5 ml min^{-1}; D, RCM_{100} C-8 cartridge (5 × 100 mm), 10% MeOH at 3 ml min^{-1}; E, Partisil 10 C-8 M9

Table 4. Distribution of radioactivity, as assessed by 2D-TLC, extracted from radish cotyledons following incubation with [^{3}H]BAP in the presence of potential inhibitors of *N*-glucosylation

Test compound	% Eluted radioactivity					
	BAP	BAPR-5'-P	BAP-9-G	BAP-7-G	BAP-3-G	Total G
Control	1 (1)*	5 (6)	29	48	4	81 (85)
7 (1 mM)	11 (11)	34 (35)	11	14	1	26 (30)
10 (1 mM)	11 (13)	35 (36)	12	16	2	30 (34)
2 (1 mM)	12 (12)	30 (31)	4	21	4	29 (35)

*Values in parentheses represent % radioactivity co-eluting in the 1D-TLC system D.

(9.4 × 500 mm), 60% MeOH at 4 ml min^{-1}; F, Techsil 10 C-8 (8 × 250 mm), 10% MeOH at 4 ml min^{-1}.

For TLC, layers spread with Merck silica gel 60 PF_{254}, 60 HF_{254} silanized or Serva cellose were employed. The following solvents were used: A, MeOH–$CHCl_3$ (1:9) with trace of NH_3; B, *n*-BuOH–HOAc–H_2O (12:3:5); C, *n*-BuOH–NH_3–H_2O (6:2:1, upper phase); D, H_2O; E, MeOH–H_2O (3:7); F, MeOH–H_2O (7:3). Silica gel/paraffin plates were made by impregnating silica gel (60 PF_{254}) plates with paraffin by developing with a 6% soln of paraffin in petrol and allowing the solvent to evap. 2D-TLC plates were developed firstly with solvent B and then C. Cytokinins and synthetic compounds were eluted from TLC with MeOH–HOAc–H_2O (10:1:10).

Scintillation counting. Normal phase TLC zones required for scientillation counting were scraped off the plates into scintillation vials and allowed to stand over night in H_2O (0.5 ml) before scintillant (5 ml) was added. The reverse phase TLC zones required for scintillation counting were allowed to stand in the dark for 24 hr in a mixt. of H_2O (0.5 ml) and scintillant (5 ml). Samples were counted in a toluene–Triton X-100 scintillant (2:1) containing 2,5-diphenyloxazole (2 g l^{-1}). Disintegrations per minute (dpm) were automatically calculated using the ext std channel ratio and a memory stored quench correction curve derived from a series of [^{3}H]-*n*-hexadecane stds quenched with pyridine.

[*G*-^{3}H]*BAP.* Synthesized according to ref. [54].

Alkyl derivatives of 1,7-*dimethylxanthine and* 1-*methylxanthine. Alkylation of* 1,7-*dimethylxanthine* (**5**). Compound **5** (Sigma) was dissolved in DMF, together with a 20% excess of NaH (55–60% oil suspension Fluka). The mixt. was then stirred at 60–70° for 20 min when the alkyl halide was added. The progress of the reaction was monitored by silica gel TLC (solvent A). At completion, the reaction mixt. was dild with H_2O and extracted (× 3) with EtOAc. The extract was washed with dil HCl, satd $NaHCO_3$ soln and H_2O before drying over Na_2SO_4. The solvent was then evapd and the residue recrystallized.

1,7-*Dimethyl*-3-*benzylxanthine* (**6**). Compound **5** was alkylated with benzyl bromide (Fluka) as described above to give **6** after recrystallization from $CHCl_3$, yield 40%. Mp 162–164°, λ_{max} 273.5 nm (base, neutral). EIMS *m/z* (rel. int.) 270 ([M]$^+$, 100), 184 (13), 165 (20), 150 (13), 136 (6). NMR ($CDCl_3$, 100 MHz) δ7.51 (1-H, *s*, H-8), 7.40 (5H, *m*, aromatic), 5.28 (2H, *s*, benzyl), 3.97 (3H, *s*, NMe-7), 3.40 (3H, *s*, NMe-1). HRMS for $C_{14}H_{14}N_4O_2$; calc. *m/z* 270.1118, found 270.1100.

1,7-*Dimethyl*-3-(3'-*methylbutyl*)*xanthine* (**7**). Compound **5** was alkylated with 1-bromo-3-methylbutane (Aldrich) to yield **7** after recrystallization from $CHCl_3$–*n*-hexane, yield 63%. Mp 116–118°. λ_{max} 275 nm (neutral, base). EIMS *m/z* (rel. int.): 250 ([M]$^+$, 53), 194 (100), 180 (72), 165 (15), 150 (15), 136 (87), 123 (32). NMR ($CDCl_3$, 100 MHz), δ7.62 (1H, *s*, H-8), 4.18 (2H, *m*, CH_2-1'), 4.05 (3H, *s*, NMe-7), 3.45 (3H, *s*, NMe-1), 1.68 (3H, *m*, CH_2-2' and CH-3'), 1.00 (*d*, 6H, J = 7 Hz, terminal methyls). HRMS for $C_{12}H_{18}N_4O_2$; calc. *m/z* 250.1431, found 250.1415.

1,7-*Dimethyl*-3-(3'-*butenyl*)*xanthine* (**8**). Compound **5** was alkylated with 4-bromo-1-butene (Fluka) to yield **8** after recrystallization from $CHCl_3$–*n*-hexane, yield 60%. Mp 70–71°. λ_{max} 274 nm (base, neutral). EIMS *m/z* (rel. int.):, 234 ([M]$^+$, 19), 193 (30), 180 (22), 136 (100). NMR ($CDCl_3$, 100 MHz) δ7.65 (1H, *s*, H-8), 5.98 (1H, *m*, C-3' vinyl), 5.20 (2H, *m*, C-4' vinyls), 4.26 (2H, *m*, CH_2-1'), 4.08 (3H, *s*, NMe-7) 3.48 (3H, *s*, NMe-1) 2.60 (2H, *m*, CH_2-2'). HRMS for $C_{11}H_{14}N_4O_2$; calc. 234.11165, found 234.11151.

1,7-*Dimethyl*-3-(4'-*pentenyl*)*xanthine* (**9**). Compound **5** was alkylated with 5-bromo-1-pentene (Fluka) to yield **9** after recrystallization from $CHCl_3$–*n*-hexane, yield 82%. Mp 53–54°. λ_{max} 274 nm (base, neutral). EIMS *m/z* (rel. int.): 248 ([M]$^+$, 25), 194 (35), 193 (30), 180 (100), 136 (98), 123 (30). NMR ($CDCl_3$, 100 MHz) δ7.60 (1H, *s*, H-8), 5.96 (1H, *m*, C-4' vinyl), 5.12 (2H, *m*, C-5' vinyls), 4.10 (2H, *m*, CH_2-1'), 4.04 (3H, *s*, NMe-7), 3.44 (3H, *s*, NMe-1), 2.10 (4H, *m*, CH_2-2' and CH_2-3'). HRMS for $C_{12}H_{16}N_4O_2$; calc. 248.12730, found 248.12712.

1,7-*Dimethyl*-3-(5'-*hexenyl*)*xanthine* (**10**). Compound **5** was alkylated with 6-bromohexene (Fluka) to yield **10** after recrystallization from $CHCl_3$–*n*-hexane, yield 93%. Mp 65–67°. λ_{max} 274.5 nm (base, neutral). EIMS *m/z* (rel. int.): 262 ([M]$^+$, 100), 247 (10), 245 (6), 232 (2), 221 (8), 208 (3), 207 (3), 194 (22), 180 (28), 136 (5). NMR ($CDCl_3$, 100 MHz) δ7.57 (1H, *s*, H-8), 5.86 (1H, *m*, C-5' vinyl), 5.04 (2H, *m*, C-6' vinyls), 4.10 (2H, *m*, CH_2-1'), 4.02 (3H, *s*, NMe-7), 3.42 (3H, *s*, NMe-1), 2.14 (2H, *m*, CH_2-4'), 1.80 (2H, *m*, CH_2-2'), 1.50 (2H, *m*, CH_2-3'). HRMS for $C_{13}H_{18}N_4O_2$; calc. 262.1430, found 262.1429.

1,7-*Dimethyl*-3-(2'-*methyl*-2'-*propenyl*)*xanthine* (**11**). Compound **4** was alkylated with 3-chloro-2-methylpropene (Aldrich) to yield **11** after recrystallization from CH_2Cl_2–*n*-hexane, yield 63%. Mp 118–119°. λ_{max} 273.5 nm (base, neutral). EIMS *m/z* (rel. int.): 234 ([M]$^+$, 100), 219 (11), 177 (45), 165 (16), 162 (18), 149 (48), 136 (29), 123 (12), 108 (11). NMR ($CDCl_3$, 100 MHz) δ7.51 (1H, *s*, H-8), 4.78 (2H, *d*, J = 19 Hz, vinyls), 4.62 (2H, *s*, CH_2-1'), 4.0 (3H, *s*, NMe-7), 3.40 (3H, *s*, NMe-1), 1.80 (3H, *s*, vinyl methyl). HRMS for $C_{11}H_{14}N_4O_2$; calc. 234.11165, found 234.11195.

1,7-*Dimethyl*-3-(4'-*hydroxybutyl*)*xanthine* (**12**). Compound **5** was alkylated with 4-chloro-1-butanol (Fluka) to yield **12** after recrystallization from EtOH–*n*-hexane, yield 48%. Mp 109–110°. λ_{max} 273.5 nm (base, neutral). EIMS *m/z* (rel. int.): 252 ([M]$^+$, 12), 234 (11), 222 (18), 193 (33), 180 (100). HRMS for $C_{11}H_{16}N_4O_3$; calc. 252.1222, found 252.12321.

1,7-*Dimethyl*-3-(3'-*methyl*-2'-*butenyl*)*xanthine* (**13**). 3-Methyl-2-buten-1-ol (Aldrich) or 3-methyl-3-buten-1-ol (Aldrich) together with a slight excess of iodotrimethylsilane (Aldrich), were dissolved in dry $CHCl_3$ under N_2 in a reacti-vial containing 3

× 4 Å Linde molecular sieves. The mixt. was heated at 70° for 30–45 min, cooled and the $CHCl_3$ soln washed with aq. $Na_2S_2O_5$ soln and H_2O before drying over Na_2SO_4. The crude $CHCl_3$ soln of 4-iodo-2-methyl-2-butene was then used to alkylate **5** (as detailed above) to yield **13** after recrystallization from $CHCl_3$–*n*-hexane, yield 57%. Mp 113–114°. λ_{max} 274.5 nm (base, neutral). EIMS *m/z* (rel. int.): 248 ($[M]^+$, 21), 180 (100), 151 (3), 136 (2), 123 (45). NMR ($CDCl_3$, 100 MHz) δ7.56 (1H, *s*, H-8), 5.40 (1H, *m*, C-2′ vinyl), 4.72 (2H, *d* of *d*, $J = 8$ Hz, CH_2-1′) 4.02 (3H, *s*, NMe-7), 3.43 (3H, *s*, NMe-1), 1.90 (3H, *s*, vinyl methyl), 1.74 (3H, *s*, vinyl methyl). HRMS for $C_{12}H_{16}N_4O_2$; calc. 248.1273, found 248.1273.

1,7-*Dimethyl*-3-(3′-*methyl*-2′,3′-*dihydroxybutyl*)*xanthine* (**14**). A soln of **13** in *t*-BuOH–H_2O (3:1) at −10° was stirred vigorously and a cold alkaline soln of $KMnO_4$ (1.5 eq. in 60 mM NaOH) was added dropwise. The $KMnO_4$ colour was discharged after 5 min and the MnO_2 was then solubilized by adding a soln of $Na_2S_2O_5$. The soln was extracted (× 3) with EtOAc, evapd and the residue redissolved in 10% MeOH–0.2 M HOAc. Compound **14** was then purified by HPLC in system D. Yield 57%, mp 129–131°. λ_{max} 274 nm (base, neutral). EIMS *m/z* (rel. int.): 282 ($[M]^+$, 4), 267 (3), 252 (1), 237 (1), 223 (100), 193 (22). GC-EIMS di-TMSi derivative *m/z* (rel. int.): 426 ($[M]^+$, 1), 425 (2), 411 (6), 409 (4), 368 (11), 339 (9), 295 (22), 252 (100), 237 (20), 193 (7). GC-EIMS mono-TMSi derivative *m/z* (rel. int.): 354 ($[M]^+$, 1), 295 (100), 279 (9), 252 (59), 237 (26), 206 (11), 193 (16). HRMS for $C_{12}H_{18}N_4O_4$; calc. 282.1328, found 282.13153.

(E)-1,7-*Dimethyl*-3-(4′-*hydroxy*-3′-*methyl*-2′-*butenyl*)*xanthine* (**15**) was synthesized as described in ref. [55].

1-*Methyl*-7-(3′-*methyl*-2′-*butenyl*)*xanthine* (**16**) *and* 1-*methyl*-3-(3′-*methyl*-2′-*butenyl*)*xanthine* (**17**). 1-Methylxanthine (Sigma) was alkylated (as described for **5**) in DMSO using one equivalent of NaH and 4-iodo-2-methyl-2-butene (prepared as described in the synthesis of **13**). The product was preparatively chromatographed on TLC (solvent A) and the monoalkylated products sepd by HPLC in system E. EIMS **16** and **17** *m/z* (rel. int.): 234 ($[M]^+$, 21) 166 (100), 109 (37). UV **16** λ_{max} 293.5 nm (base) 269.4 nm (neutral), UV **17** λ_{max} 276.5 nm (base) 273 nm (neutral).

1-*Methyl*-7-*benzylxanthine* (**18**). 1-Methylxanthine was alkylated with benzyl bromide (Fluka) and the monoalkylated product isolated as described for **16** and **17**. EIMS *m/z* (rel. int.): 256 ($[M]^+$, 70), 91 (100). UV λ_{max} 294.5 nm (base), 270 nm (neutral).

7-*Benzylaminooxazolo*[5,4-*d*]*pyrimidine* (**4**). Compound **22** (50 mg) (prepd as described in ref. [41] was dissolved in HMPA (500 μl, dist. stored over 4Å sieves), together with NaH (16 mg) and 15 μg 4-dimethylaminopyridine (1.5 μg/μl, pyridine soln). Benzyl bromide (45 μl) was added and the mixt maintained at 80° for two days. The mixt. was dil with H_2O and extracted with EtOAc. The extract was then subjected to prep. silica gel TLC (solvent A) and three UV absorbing zones, (1-B, 2-B, 3-B) at higher R_f than **22** eluted. These products were further purified by reverse phase HPLC; 2-B and 3-B were eluted with system D and 1-B eluted with system E. Compound **4** (2-B) was recrystallized from EtOH–H_2O, λ_{max} 209, 259 nm (base, neutral). EIMS *m/z* (rel. int.): 226 ($[M]^+$, 72), 210 (2), 198 (4), 170 (3), 149 (6), 121 (14), 106 (92), 91 (100). NMR ($CDCl_3$, 200 MHz) δ8.47 (1H, *s*, H-5), 7.94 (1H, *s*, H-2), 7.37 (5H, *m*, aromatic H), 5.94 (1H, *br m*, H-7 amino), 4.89 (2H, *br m*, benzylic H). HRMS for $C_{12}H_{10}N_4O$; calc. 226.0855, found 226.0848; for C_7H_8N, calc. 106.0657, found 106.0654. 1-B, 3-B were shown to be dibenzyl derivatives. EIMS (1-B) *m/z* (rel. int.): 316 ($[M]^+$, 5), 225 (100), 198 (2), 147 (5), 132 (3) 120 (8), 106 (20), 104 (9), 91 (80). EIMS (3-B) *m/z* (rel. int.): 316 ($[M]^+$, 34), 225 (47), 198 (1), 178 (2), 147 (1), 135 (9), 120 (5), 119 (5), 106 (5), 91 (100).

2-*Chloro*-9-*methyl*-6-*benzylmethylaminopurine* (**20**). 2-Chloro-6-benzylaminopurine (26 mg) was dissolved in DMF (1 ml) together with NaH (15 mg) and the mixt stirred at 70°. MeI (15 μl, Aldrich, 2.4 eq.) was added. After 2 hr the reaction mixt. was dild with H_2O and extracted into EtOAc. The extract was then subject to silica gel TLC, solvent A, and the zone corresponding to **20** was eluted with 20% MeOH–$CHCl_3$, yield 83%. λ_{max} 281 nm (base, neutral). EIMS *m/z* (rel. int.): 289 ($[M]^+$ Cl^{37}, 22), 287 ($[M]^+$ Cl^{35}, 64), 274 (Cl^{37}, 22), 272 (Cl^{35}, 67), 260 (Cl^{37}, 9), 258 (Cl^{35}, 27), 252 (2), 236 (3), 210 (5), 198 (Cl^{37}, 10), 196 (Cl^{35}, 30), 169 (8), 167 (4), 120 (10), 106 (2). HRMS for $C_{14}H_{14}Cl^{35}$, calc. 287.0938, found 287.0928; for $C_8H_{10}N$, calc. 120.0813, found 120.0813.

2-*Chloro*-9-*methyl*-6-*benzylaminopurine* (**19**). 2-Chloro-6-benzylaminopurine (24 mg) was methylated with MeI (6 μl, 1 eq.) in the presence of NaH (3 mg) in DMF as described for **20**. Yield 21 mg (81%), λ_{max} 274 nm, shoulder 266 nm (base, neutral). EIMS *m/z* (rel. int.): 275 ($[M]^+$ Cl^{37}, 17), 273 ($[M]^+$ Cl^{35}, 51), 238 (7), 237 (3), 198 (Cl^{37}, 2), 196 (Cl^{35}, 7), 168 (7), 147 (3), 133 (21), 119 (4) 106 (100), 91 (64), 79 (14), 77 (8), 65 (26). NMR (d_6-DMSO, 200 MHz) δ8.29 (1H, *s*, H-8), 7.49 (5H, *m*, aromatic H), 5.35 (1H, *br m*, H-6 amino), 4.83 (2H, *br m*, benzylic H), 3.86 (3H, *s*, NMe-9). HRMS for $C_{13}H_{12}N_5Cl^{35}$; calc. 273.0781, found 273.0775.

2-(2′-*hydroxyethylamino*)-9-*Methyl*-6-*benzylaminopurine* (**2**). Compound **19** (19.2 mg) was dissolved in DMF (2 ml) under N_2 together with excess ethanolamine. The mixt. was heated and stirred at 160° for 2.5 hr, then left overnight at room temp. The mixt was then dild with H_2O and extracted with H_2O satd *n*-BuOH. The *n*-BuOH extract was then washed with H_2O and the pH adjusted to 7.8 with 2M HCl. After a further H_2O wash the extract was subjected to silica gel TLC (solvent A) and the zone corresponding to **2** eluted with 50% MeOH–$CHCl_3$. Yield 18.1 mg (89%), mp 125–126°. λ_{max} 231, 288 nm (base, neutral). EIMS *m/z* (rel. int.): ($[M]^+$, 100), 281 (2), 280 (4), 279 (5), 268 (34), 267 (72), 254 (14), 163 (2), 149 (6), 106 (1). NMR (d_6-DMSO, 200 MHz) δ7.80 (1H, *s*, H-8) 7.25–7.48 (5H, *m*, aromatic H), 6.09 (1H, *br m*, C-6 amino H), 4.75 (2H, *br m* benzylic H), 3.65 (3H, *s*, NMe-9), 3.60 (2H, *d* of *t*, CH_2-1′), 3.44 (2H, *d* of *t*, CH_2-2′). HRMS for $C_{15}H_{18}N_6O$; calc. 298.1542, found 298.1529.

2-*Aminomethyl*-9-*methyl*-6-*benzylaminopurine* (**21**). 2-Aminomethyl-2-propanol hydrochloride (Aldrich) was dissolved in H_2O and the pH adjusted to 7–8 with 4 M NaOH. The soln was extracted with H_2O satd *n*-BuOH and the extract evapd to obtain the free base plus traces of the HCl salt. Compound **19** (20 mg) was dissolved in DMF (2 ml) under N_2 together with excess dried extract. The mixt. was heated and stirred at 150° for 2.5 hr, then extracted and chromatographed as described for **2**. EIMS *m/z* (rel. int.): 268 ($[M]^+$, 100), 253 (16), 239 (16), 224 (9), 191 (4), 163 (17), 148 (7), 134 (24), 119 (6), 106 (27).

2-(2′-*hydroxy*-2′-*methylpropylamino*)-9-*Methyl*-6-*benzylaminopurine* (**3**). 2-Aminomethyl-2-propanol HCl (Aldrich) was converted to the free base by passage through a column of Dowex 1X8-200 in the hydroxide form and eluted with deionized H_2O as the free base. Compound **21** (20 mg) was dissolved in 2-aminomethyl-2-propanol (2 ml) under N_2 and the mixture heated and stirred overnight at 255–260°. Compound **3** was extracted and isolated as described for **2** and recrystallized from Me_2CO–EtOAc–*n*-hexane. Yield 20 mg (84%). λ_{max} 232, 291 (base, neutral). EIMS *m/z* (rel. int.): 326 ($[M]^+$, 22), 311 (3), 309 (3), 268 (91), 267 (71), 251 (7), 240 (2), 191 (2), 177 (7), 163 (24), 148 (9), 133 (4), 121 (5), 105 (12), 91 (100). NMR ($CDCl_3$, 200 MHz) δ7.40 (1H, *s*, H-8), 7.25–7.39 (5H, *m*, aromatic proton), 5.91 (1H, *br m*, H-6 amino), 5.23 (1H, *br m*, H-2 amino), 4.77 (2H, *br d*, benzylic H), 3.64 (3H, *s*, NMe-9), 3.40 (2H, *d*, $J = 6.2$ Hz, CH_2-1′), 1.66 (1H, *br s*, OH), 1.25 (6H, *s*, Me-2′ and Me-3′). HRMS for $C_{17}H_{22}N_6O$; calc. 326.1855, found 326.1847.

Metabolism of [^{3}H] *BAP in maize leaves.* Maize plants (*Zea mays* L., F1 hybrid, Iochief) were grown in sterile soil under

ambient conditions. The first leaf of 10-day-old seedlings was excised and 4 × 1 cm segments were placed, lower surface uppermost, on a filter paper in a Petri dish. A soln of [^{3}H]BAP (5 μM in 2.5 ml H_2O) was then applied to the filter paper. The segments were incubated under light (700 lux) for 3 days and then blotted dry, rinsed thoroughly with dist H_2O, and again blotted dry, before being weighed (1.5 g). Cytokinins were extracted as described previously [42] to minimize the action of non-specific phosphatases. The extract was chromatographed on silica gel TLC (solvent C), using BAP, BAP-9-G, Ado and Ade as markers (Fig. 1). The BAP, BAP-9-G and Ado zones were eluted and further analysed. Aliquots of the BAP-9-G eluate were rechromatographed on cellulose TLC (solvent C) and also on silanised silica gel (solvent F) and it was determined that 90% of the radioactivity co-chromatographed with authentic BAP-9-G. An aliquot of the BAP-9-G eluate was hydrolysed in 0.5 M HCl at 95° for 1 hr. The hydrolysis product co-chromatographed with BAP on silica gel TLC (solvent C). The eluate from the BAP zone and the Ado zone were also rechromatographed using both cellulose (solvent C) and silanized silica gel (solvent F) TLC, and it was found that 80 and 70% of the radioactivity co-chromatographed with BAP and Ado, respectively.

Assessment of potential inhibitors of N-*glucosylation in maize leaves.* The centres of six leaves from 15-day-old *Zea mays* seedlings were cut into 5 mm segments which were bisected longitudinally; six halved segments, one from each of the 6 leaves, were used in each assay. The segments were each placed on a 20 μl drop of test soln in plastic Petri dishes with the adaxial surface downwards. The sides of the Petri dish and the edges of the lid were lined with moist filter paper and the dishes were wrapped in clear plastic film. The test soln used was Tween 80 (0.05%) containing 7.5 μM [^{3}H]BAP plus test compound. The segments were left for 2.5–3 days in the light (700 lux) at 23° when the segments were washed sequentially with Tween 80 (0.05%), a soln of Tween 80 (0.05%) containing unlabelled BAP (40 μM), and H_2O. The segments were then blotted dry and extracted. The extracts were then analysed by silica gel TLC (solvent C) using BAP and BAP-9-G as markers and the radioactivity co-chromatographing with each was then assessed (Table 2). The presence of inhibitors resulted in a reduction of uptake of [^{3}H]BAP as assessed by the total extd radioactivity. However, in the most extreme case this was no more than 50% and would make no difference to the metabolism of BAP *per se* (unpublished data, Letham). Hence, the results are expressed in terms of % extracted radioactivity. A similar reduction in uptake was noted for radish tissue (see below).

Assessment of potential inhibitors of N-*glucosylation in radish leaves and cotyledons.* Leaf discs were excised from fully expanded radish leaves (*Raphanus sativus* L. cv Long Scarlet) cut from 40–50-day-old radish seedlings grown aseptically on nutrient agar. Each assay was conducted as described previously for maize leaf segments using 6 discs (diam. 6.5 mm) per Petri dish, each set being excised from the same 6 leaves, 1 disc per leaf. Each disc was placed on a 20 μl soln of [^{3}H]BAP (7.5 μM) in Tween 80 (0.05%). After a 3 day incubation, the tissue was washed, extracted (as described for maize leaves) and the extracts analysed by silica gel TLC (solvent C) using BAP, BAP-3-G, -7-G, -9-G and BAPR 5′-P as markers. The radioactivity co-chromatographing with these markers was then assessed (Table 3). Cotyledons were excised from 15-day-old radish seedlings and 4 discs (diam. 4.5 mm) cut from each cotyledon. The assay was conducted as described for radish leaves, with the exception that only 10 μl of [^{3}H]BAP soln was applied to each disc. The tissue extract was analysed by both silica gel TLC solvent C, and by 2D-TLC which effected the resolution of the BAP *N*-glucosides (Table 4).

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