Regulators of Cell Division in Plant Tissues
XXIX.* The Activities of Cytokinin Glucosides and Alanine Conjugates in Cytokinin Bioassays

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Abstract. In a number of cytokinin bioassays, the activities of the following compounds were compared: 3-, 7-, and 9-glucosides of 6-benzyl-aminopurine (BAP); 7- and 9-glucosides of zeatin; O-glucosides of zeatin, dihydrozeatin, and their ribosides; 9-alanine conjugates of zeatin, and BAP. The bioassays included the radish cotyledon, the *Amaranthus* betacyanin, the oat leaf senescence, and the tobacco pith callus. Cytokinin activity was markedly reduced by 7- and 9-glucosylation in nearly all bioassays, but 3-glucosylation of BAP and O-glucosylation of the zeatin sidechain usually had little effect on activity. However, there were two notable exceptions to this generalization: the activity of O-glucosylzeatin markedly exceeded that of zeatin in the oat leaf senescence assay; 9-glucosyl-BAP and free BAP were similarly active in retarding the senescence of radish leaf discs. The 9-alanine conjugate of zeatin (lupinic acid) and of BAP were markedly less active than zeatin and BAP, respectively, in all bioassays, but the responses evoked by these conjugates at high concentrations in the *Amaranthus* bioassay approached those caused by the corresponding base. The activities of several new compounds related to the alanine conjugate of BAP were also assessed. To serve as a guide in the selection of the most suitable bioassay for detection of the above-mentioned cytokinin conjugates, the lowest detectable amounts in selected bioassays have been compared.

In plant tissues, supplied cytokinin bases and ribosides are converted into a number of glucoside metabolites (for review, see Letham and Palni 1983). Metabolites of zeatin (Z) are 7-β-D-glucopyranosylzeatin (7GZ), 9-β-D-glucopyranosylzeatin (9GZ), O-β-D-glucopyranosylzeatin (OGZ) and its 9-riboside (OGZR), and the dihydro derivatives of these O-glucosides (OGDZ and OGDZR, respectively). Glucoside metabolites of 6-benzylaminopurine (BAP) are the 3-, 7-, and 9-β-D-glucopyranosides (3G-BAP, 7G-BAP, and 9G-BAP, respectively). In addition, Z is converted into the 9-alanine conjugate termed lupinic acid (LA), L-β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)-purin-9-yl]-alanine, and into the corresponding dihydro derivative, dihydrolupinic acid. The analogous alanine conjugate of BAP (Al-BAP) has also been identified as a metabolite of BAP. All the above-mentioned metabolites of Z have now been identified as endogenous compounds in plants (Letham and Palni 1983).

Determination of the cytokinin activities of the above-mentioned metabolites should contribute to our understanding of their physiological significance. Accordingly, in this paper, the activities of cytokinin glucosides and alanine conjugates are reported in several cytokinin bioassays including the oat leaf senescence, the radish cotyledon, the tobacco pith callus and the *Amaranthus* betacyanin.

Materials and Methods

Bioassays

Experimental details of the bioassays used are found in the following references: radish cotyledon (Letham 1971), *Amaranthus* betacyanin (Biddington and Thomas 1973), tobacco pith callus (Murashige and Skoog 1962), soybean callus (Miller 1968). In the above *Amaranthus* assay, which was normally used, betacyanin formation was induced in excised cotyledons. Occasionally, where indicated, a variant of the assay was used. In the modified procedure, surface-sterilized *Amaranthus* seed was placed directly on filter paper wetted with the cytokinin solution, which was prepared in the usual tyrosine-containing buffer. After 3 days in darkness at 22°C, the entire group of seedlings was extracted in the usual way for determination of A_{542} and A_{620}.

For the oat leaf senescence bioassay, oat seedlings (*Avena sativa* cv. Victory I) were grown in trays containing sterile potting mixture under a 16-h photoperiod of 100 $\mu Em^{-2}s^{-1}$ at 20°C and a dark temperature of 15°C. After 8 days growth, a leaf segment (length 1 cm) was excised 1 cm from the top of each primary leaf. The segments were placed with the abaxial surface downward in Petri dishes (diameter 5 cm, 15 segments per dish) containing one circle of Whatman No. 1 filter paper and cytokinin solution (1.0 ml). The dishes were wrapped in foil and left at 22°C for 4 days. The segments were then dropped into boiling 80% ethanol and kept at this temperature for 7 min. After dilution to a constant volume, the absorbance of the cooled solutions was measured at 665 nm.

Other leaf senescence bioassays were performed similarly using 1-cm segments excised from wheat leaves (cv. Gabo) and discs (diameter 5 mm) excised

from fully expanded radish (cv. Long Scarlet) cotyledons and leaves, immature radish leaves (about 60% of final size), and fully expanded Chinese cabbage (*Brassica pekinensis* Rupr.) leaves. Plants for these bioassays were grown in a greenhouse.

The significance of the difference between means at selected concentrations was assessed by analysis of variance using the Q method based on the Studentized range (Snedecor and Cochran 1967); the difference required for significance at the 5% level has been termed D(5%). In some instances, the refinement of this method involving sequential testing of means was employed.

Synthesis of Compounds

All glucosides of cytokinins were synthesized chemically by procedures detailed previously (Cowley et al. 1975, 1978; Duke et al. 1975, 1978, 1979; Letham et al. 1975, 1977), as were the alanine conjugates of Z and BAP (MacLeod et al. 1975, Duke et al. 1978, Letham et al. 1979).

New compounds related to the alanine conjugate of BAP were synthesized by Michael-type reactions involving addition of ethyl acrylate or acrylonitrile to the 9-position of BAP. These syntheses are outlined below.

Ethyl 3-(6-benzylaminopurin-9-yl)propionate

Benzene (7 ml) was added to a solution of BAP (0.90 g) in absolute ethanol (32 ml). After about 15 ml of the solvent had been distilled off under anhydrous conditions, sodium ethoxide (15 mg) and ethyl acrylate (1.4 ml) were added, and the mixture was heated under reflux for 18 h. The resulting solution was concentrated to about 4 ml and the product (1.1 g, mp 91°C) was crystallized by slow addition of hexane at -10°C. Found: C 62.6, H 5.8, N 21.6%; $C_{17}H_{19}N_5O_2$ requires: C 62.75, H 5.9, N 21.5%. Mass spectrum (*m/z* and relative intensities): 325 (M$^+$, 100), 324 (23), 309 (2), 296 (3), 280 (14), 248 (4), 225 (8), 224 (10), 220 (11). Pmr spectrum (D$_6$-DMSO, 100 MHz) δ:1.12 (3H, triplet, $J = 8$ Hz, CH$_3$), 3.04 (2H, triplet, $J = 8$ Hz, alkyl CH$_2$), 4.12 (2H, quartet, $J = 8$ Hz, ester CH$_2$), 4.52 (2H, triplet, $J = 8$ Hz, alkyl CH$_2$), 4.92 (2H, broad singlet, benzyl CH$_2$), 7.45 (5H, multiplet, benzyl aromatic H), 8.30 (1H, purine H), 8.42 (1H, purine H). UV spectrum (λ_{max}):pH 7,270 nm.

3-(6-benzylaminopurin-9-yl)propionic acid

A solution of the above ethyl ester (0.8 g) in 3 N HCl (25 ml) was heated under reflux for 3 h, adjusted to pH 4 with concentrated NaOH solution, then acidified to pH 3.1 with acetic acid, and finally left at 4°C for 24 h. The crude product was centrifuged down, washed with water, and then precipitated twice at 4°C from a solution (20 ml) in dilute NH$_4$OH by addition of formic acid to pH 3.1. The purified acid, 3-(6-benzylaminopurin-9-yl)propionic acid, was filtered off and washed with water (yield 0.48 g, mp 224–226°C). Found: C 61.0,

H 5.1, N 24.1%; $C_{15}H_{15}N_5O_2$ requires: C 60.6, H 5.1, N 23.6%. Pmr spectrum (D_6-DMSO, 100 MHz) δ:2.95 (2H, triplet, J = 8 Hz, alkyl CH_2), 4.45 (2H, triplet, J = 8 Hz, alkyl CH_2), 4.88 (2H, broad singlet, benzyl CH_2), 7.46 (5H, multiplet, benzyl aromatic H), 8.32 (1H, singlet, purine H), 8.42 (1H, singlet, purine H). UV spectrum (λ_{max}): pH 7, 271 nm.

9-(3-Aminopropyl)-6-benzylaminopurine

Solvent (about 8 ml) was distilled under anhydrous conditions from a solution of BAP (0.45 g) in ethanol (14 ml) and benzene (2 ml). After addition of sodium (10 mg) and acrylonitrile (0.40 ml), the mixture was heated under reflux for 24 h and then left at −5°C. The crystalline product that separated (0.28 g) was filtered off, washed with ethanol, and recrystallized from ethanol-petroleum ether to yield β-(6-benzylaminopurin-9-yl)propionitrile, mp 171–173°C. Preparative TLC of the mother liquid on silica gel yielded further nitrile (70 mg). Found: C 64.6, H 5.1, N 30.2%; $C_{15}H_{14}N_6$ requires: C 64.7, H 5.1, N 30.2%. Mass spectrum (*m/z*): 278 (M^+, 100), 277 (44), 262 (3), 225 (8), 224 (11), 201 (7), 173 (11), 106 (59), 91 (25). UV spectrum (λ_{max}): pH 1, 266 nm; pH 11, 270 nm.

The above nitrile was reduced with lithium aluminium hydride-aluminium chloride, essentially by a method used previously for nitrile reduction (Letham et al. 1969), to yield 9-(3-aminopropyl)-6-benzylaminopurine, which was isolated as the crystalline hydrochloride. Found: M^+ 282 · 1585; $C_{15}H_{18}N_6$ requires 282 · 1593. Mass spectrum (*m/z*): 282 (M^+, 17), 252 (10), 239 (100), 238 (77), 226 (8), 225 (8), 224 (13). Pmr spectrum (D_6-DMSO, 200 MHz) δ: 2.16 (2H, multiplet, alkyl CH_2), 2.78 (2H, triplet, J = 7 Hz, alkyl CH_2), 4.31 (2H, triplet, J = 7 Hz, alkyl CH_2), 4.80 (2H, broad singlet, benzyl CH_2), 7.30 (5H, multiplet, benzyl aromatic H), 8.3 (5H, complex signal, two singlets due to purine C-2 and C-8 protons plus NH_3^+ signal). UV spectrum (λ_{max}): pH 7, 271 nm.

Reduction with lithium aluminium hydride alone under normal conditions gave a negligible yield of the required product apparently due to reversal of the Michael addition.

Results

N-Glucosides of Z and BAP

In the radish cotyledon (Figs. 1, 3), oat leaf senescence (Fig. 5), *Amaranthus* betacyanin (Figs. 8, 9) and tobacco callus (Table 1) bioassays, the 7- and 9-glucopyranosides of Z and BAP were markedly less active than the parent cytokinin bases. Furthermore, 7G-BAP and 9G-BAP were also much less active than BAP in the wheat leaf senescence bioassay and exhibited only weak activity even at 10 μM, while the lowest detectable concentration of BAP was between 0.05 and 0.2 μM. 9G-BAP and 9GZ also showed very weak activity relative to BAP and zeatin riboside (ZR), respectively, in the Chinese cabbage leaf senescence bioassay (results not detailed herein). However, in the bioassay
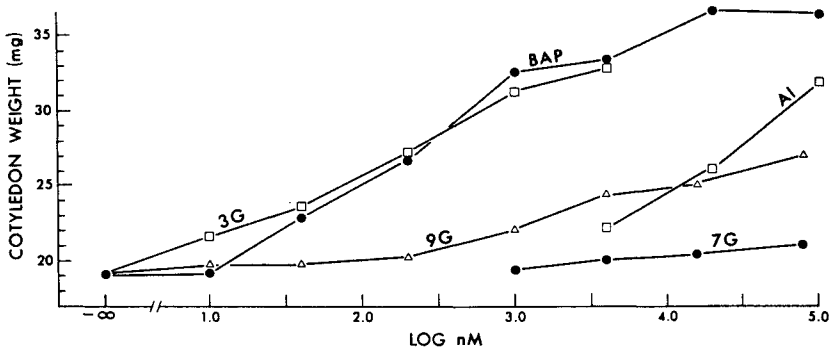
Fig. 1. A comparison of the activities of BAP, BAP alanine conjugate (Al), 3G-BAP (3G), 7G-BAP (7G), and 9G-BAP (9G) in the radish cotyledon bioassay. At 10 nM, D(5%) = 1.47; at 1,000 nM, D(5%) = 1.90.
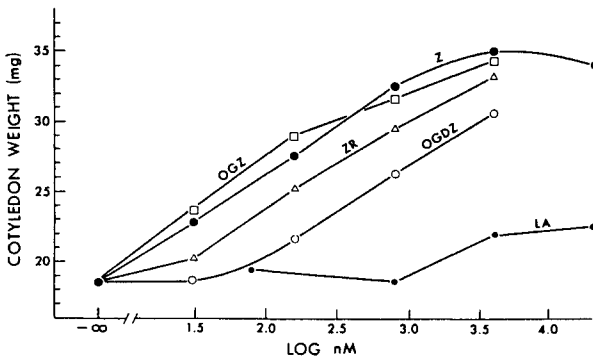

Fig. 2. A comparison of the activities of Z, ZR, OGZ, OGDZ, and LA in the radish cotyledon bioassay. At 800 nM D(5%) = 3.7; sequential testing established that the following paired means for 800 nM also differed significantly at the 5% level: Z and ZR; ZR and OGDZ.

based on the senescence of immature radish leaves (Fig. 7), the activity of 9G-BAP slightly exceeded that of BAP. When mature radish leaves were used for the senescence bioassay, BAP and 9G-BAP exhibited similar activity, and when fully expanded radish cotyledons were employed, the activity of 9G-BAP was appreciably less than that of BAP. In all of these senescence bioassays based on radish leaves or cotyledons, the activity of 7G-BAP was much less than that of 9G-BAP.

The cytokinin activities of BAP glucosides with a modified 7- or 9-β-D-glucopyranosyl moiety were also determined. Activity exhibited by 7-β-D-glucofuranosyl-BAP was similar to that of the 7-β-D-glucopyranoside (7G-BAP) in the radish cotyledon bioassay. However, 9-α-D-glucopyranosyl-BAP was considerably less active than the β-glucoside (9G-BAP) in this assay. The activity of synthetic 7G-BAP equaled that of the natural 7-glucoside metabolite isolated from radish cotyledons (Parker and Letham 1973). This supports the structural assignment made to this metabolite (Cowley et al. 1978).

Unlike 7G-BAP and 9G-BAP, 3G-BAP exhibited activity that equaled or closely approached that of BAP in several bioassays including the radish cotyledon (Fig. 1) and those based on retardation of senescence of oat (Fig. 5), radish (Fig. 7), and Chinese cabbage leaves, and radish cotyledons (results of
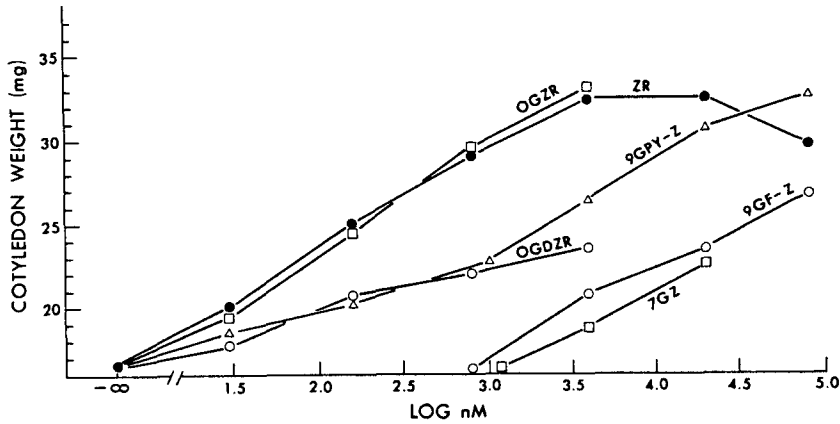
Fig. 3. A comparison of the activities of ZR, OGZR, OGDZR, 9-β-D-glucopyranosylzeatin (9GPY-Z, termed 9GZ in text), 9-β-D-glucofuranosylzeatin (9GF-Z), and 7GZ in the radish cotyledon bioassay. At 4 μM, D(5%) = 4.0; sequential testing established that the following paired means at this concentration also differed significantly at the 5% level: 9GPY-Z and OGDZR; OGDZR and 9GF-Z.

last two senescence assays are not detailed herein). In the wheat leaf senescence assay, 3G-BAP was somewhat more active than BAP at concentrations of 1 and 10 μM. However, in the *Amaranthus* betacyanin bioassay based on excised cotyledons, 3G-BAP was appreciably less active than BAP (Fig. 8), but the difference was less marked in the assay involving intact seedlings in which 9G-BAP was essentially inactive.

O-Glucosides of Z and Related Compounds

Unlike 7- and 9-glucosylation of the purine ring, O-glucosylation of the isoprenoid sidechain of Z and ZR did not markedly alter cytokinin activity in the radish cotyledon bioassay (Figs. 2, 3), the tobacco callus bioassay (Table 1),
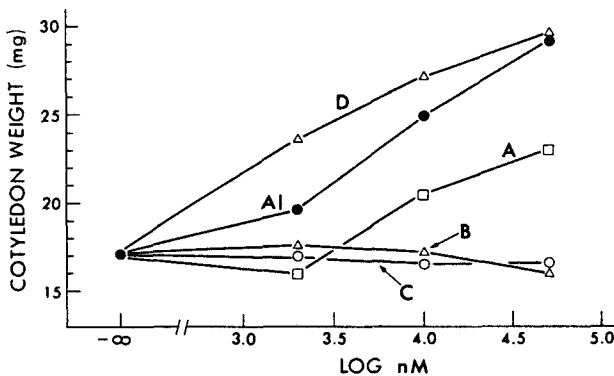

Fig. 4. A comparison of the activities of BAP-alanine conjugate (Al) and related compounds in the radish cotyledon bioassay. The compounds are: A—9-(3-aminopropyl)-6-benzylaminopurine; B—ethyl 3-(6-benzylaminopurin-9-yl)propionate; C—3-(6-benzylaminopurin-9-yl)propionic acid; D—β-(6-benzylaminopurin-9-yl)-N-trifluoroacetylalanine methyl ester. At 2 μM and 10 μM, D(5%) = 3.6 and 3.9, respectively.
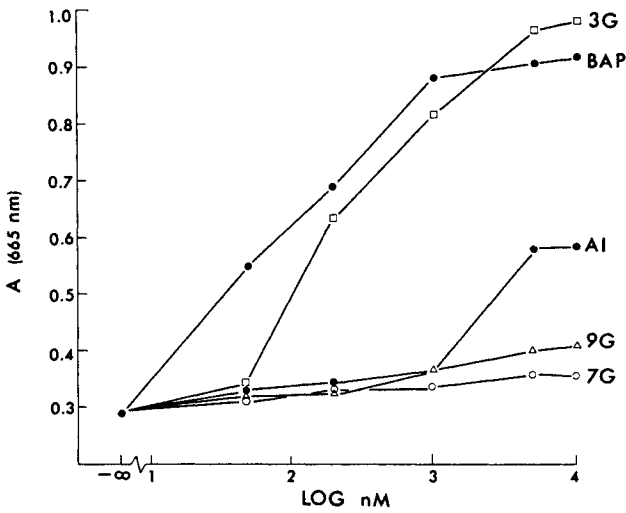
Fig. 5. A comparison of the activities of BAP, 3G-BAP (3G), BAP-alanine conjugate (Al), 7G-BAP (7G), and 9G-BAP (9G) in the oat leaf senescence bioassay. At the highest tested concentration, D(5%) = 0.08.

Table 1. The increments in weight of tobacco callus explants induced by zeatin, zeatin glycosides, and lupinic acid.

Concentration (nM)	Increment in mean explant weight (g)[a,b]						
	Z	ZR	OGZ	OGZR	OGDZ	OGDZR	9GZ
50	2.26	0.94	1.92	1.48	2.79	1.42	0.00
5	1.26	0.43	1.46	0.44	1.55	0.68	0.00
0.5	0.22	0.00	0.00	0.00	0.09	0.00	0.00

[a] Each value is derived from the weight of 6 explants; the mean weight of control explants was 0.46 g.

[b] In this assay at 100, 10, and 1 nM, lupinic acid induced increments of 0.36, 0.36, and 0.00 g, respectively.

and the *Amaranthus* betacyanin bioassay (Fig. 9). Compared with BAP, Z is ineffective as a retardant of oat leaf senescence. However, the activity of OGZ in the oat leaf bioassay exceeded that of Z (Fig. 6) and the following activity sequence applied: BAP >> OGZ > Z = dihydrozeatin (DZ) = OGDZ. It is noteworthy that OGDZ and OGDZR were less active than OGZ and OGZR, respectively, in both the radish cotyledon (Figs. 2, 3) and *Amaranthus* (Fig. 9) bioassays.

Synthetic OGZ and the natural metabolite isolated from lupin leaves (Parker et al. 1978) were compared in the radish cotyledon assay over the concentration range 0.5 to 30 μM. No difference was detected confirming the structural assignment made to the natural glucoside.

Alanine Conjugates

In the radish cotyledon, oat leaf senescence, and *Amaranthus* bioassays, the alanine conjugate of BAP (Al-BAP) and the analogous conjugate of zeatin
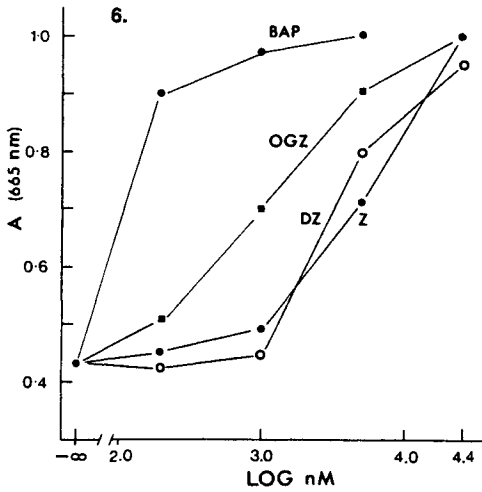
Fig. 6. A comparison of the activities of BAP, Z, DZ, and OGZ in the oat leaf senescence bioassay. In a comparison of the means for control segments and segments treated with cytokinin at the lowest concentration (200 nM), D(5%) = 0.055; at 5 μM, D(5%) = 0.09.
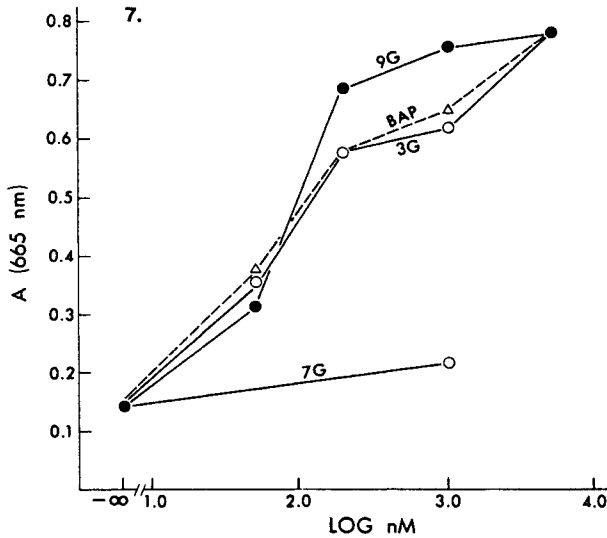

Fig. 7. A comparison of the activities of BAP, 3G-BAP (3G), 7G-BAP (7G), and 9G-BAP (9G) in a senescence bioassay using immature radish leaves.

(lupinic acid) were markedly less active than BAP and zeatin, respectively (Figs. 1, 2, 5, 8, and 9; Table 1 footnote). In the wheat leaf senescence bioassay over the concentration range 1–10 μM, Al-BAP exhibited very weak activity that was barely detectable. However, BAP retarded senescence markedly at these concentrations; the lowest detectable concentrations of the two compounds were 1 μM and 0.1 μM, respectively. The synthetic lupinic acid used in these experiments was racemic (DL), while the natural acid is known to be the L-isomer (Duke et al. 1978). It was not possible to compare the synthetic racemate with the natural L-acid directly, but a comparison of the L-compound with Z was made using the radish assay. The activity of this L-acid, relative to Z, was very similar to that shown in Fig. 2 for the DL-mixture.
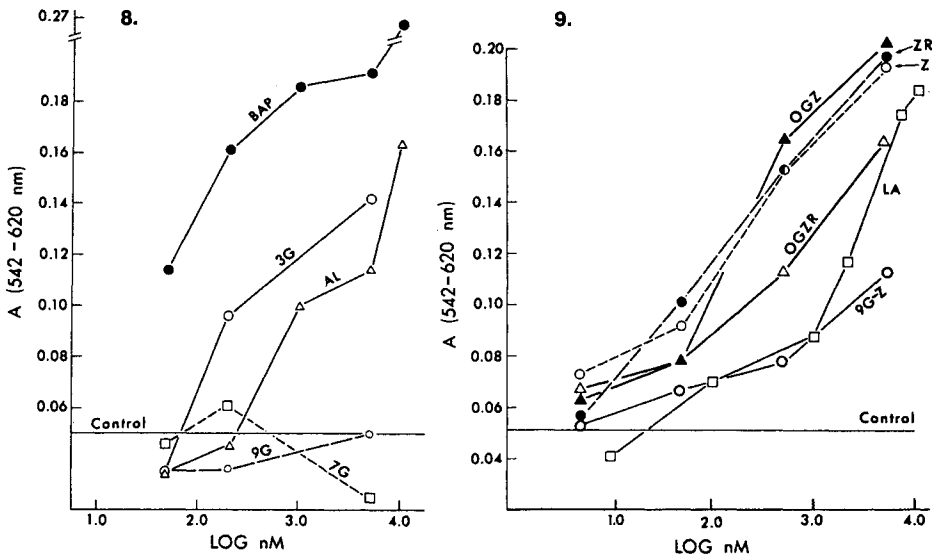
Fig. 8. A comparison of the activities of BAP, 3G-BAP (3G), 7G-BAP (7G), 9G-BAP (9G), and BAP-alanine conjugate (Al) in the *Amaranthus* betacyanin bioassay. Fig. 9. A comparison of the activities of Z, ZR, OGZ, OGZR, 9GZ, and LA in the *Amaranthus* betacyanin bioassay. In this assay, LA, OGDZ, and OGDZR exhibited very similar activity.

The bioassay that exhibited the greatest response to the alanine conjugates was the *Amaranthus* betacyanin (Figs. 8, 9). At higher concentrations, the response evoked by lupinic acid approached that of Z and greatly exceeded that of 9GZ (Fig. 9). Similarly, the activity of Al-BAP approached that of 3G-BAP and was markedly greater than that of 9G-BAP (Fig. 8).

The activities of Al-BAP and the following related synthetic compounds were compared in the radish assay (see Fig. 4): 9-(3-aminopropyl)-6-benzylaminopurine (A), ethyl 3-(6-benzylaminopurin-9-yl)propionate (B), 3-(6-benzylaminopurin-9-yl)propionic acid (C), and β-(6-benzylaminopurin-9-yl)-N-trifluoroacetylalanine methyl ester (D). In these compounds, the substituents at position 9 of BAP are as follows: A—$CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$; B—$CH_2 \cdot CH_2 \cdot COOCH_2CH_3$; C—$CH_2 \cdot CH_2 \cdot COOH$; D—$CH_2 \cdot CH$ $(NH \cdot CO \cdot CF_3) \cdot COOCH_3$. Compound D was appreciably more active than Al-BAP in the radish cotyledon bioassay (Fig. 4). However, compound C and its ethyl ester (compound B), which lack an amino group, were devoid of activity. Compound A, which lacks a carboxyl group, was weakly active relative to Al-BAP. Hence, the weak activity of Al-BAP appears to depend on the presence of an intact α-amino acid moiety.

Differences between Bioassays

The full range of available zeatin conjugates was tested in three bioassays and the activity sequences are compared below.

Radish cotyledon: Z = OGZ > ZR = OGZR > (OGDZ, 9GZ)
   > OGDZR > (LA, 7GZ)
Amaranthus betacyanin: Z = ZR = OGZ > OGZR
   > (OGDZ, OGDZR, LA) > 9GZ
Tobacco callus: (Z, OGZ, OGDZ) > (ZR, OGZR, OGDZR) > LA
   > 9GZ

Sidechain reduction of O-glucosides diminished cytokinin activity markedly in the radish cotyledon and *Amaranthus* bioassays, but had little effect in the tobacco callus bioassay. Apart from this difference, these three bioassays exhibited very similar trends in activity with the zeatin metabolites listed. In the three bioassays mentioned, the activity of OGZ could not be distinguished from that of Z, but in the oat leaf senescence assay OGZ was markedly more active than Z.

To facilitate studies of endogenous cytokinins in plant extracts, the sensitivities of selected bioassays for the detection of Z and its metabolites are compared in Table 2, where the lowest detectable concentrations and amounts are presented. Table 2 also includes results for the soybean bioassay based on values in a recent separate paper from this laboratory (Palni et al. 1983). Because of the insensitivity of the oat leaf senescence bioassay to Z (lowest detectable concentration and amount, 1 μM and 219 ng, respectively) and its derivatives, this bioassay is of limited value for assaying plant extracts and accordingly is not included in Table 2. While the values for the least detectable amount of Z in the four bioassays of Table 2 are similar (range 2–6 ng), the values for particular metabolites of Z vary greatly. For example, the values for 9GZ range from 47 to > 476 ng. None of the bioassays detected 7GZ effectively.

Discussion

O- and N-Glucoside metabolites and alanine conjugates of cytokinins have been purified from a number of plant tissues, but the μg-amounts obtained have been insufficient to define the cytokinin activity of these compounds. The present study was made possible by the availability of synthetic compounds, but in discussion of the results, the following points should be kept in mind: (1) the synthetic alanine conjugates are racemates (DL), while the naturally formed metabolites are known to be L isomers; (2) the synthetic products OGDZ and OGDZR are also racemic, while the natural metabolites are presumably O-glucosides of (S)-DZ and (S)-dihydro-ZR, since the natural isomer of DZ has an (S) configuration (Fujii and Ogawa 1972). Results presented herein for one bioassay suggest that the stereochemical difference between natural and synthetic lupinic acid does not appreciably alter cytokinin activity. It has not been possible to compare natural and synthetic products in the cases of OGDZ and OGDZR. However, if O-glucosides are active only after cleavage of the glucosyl moiety (see below), then the synthetic (RS)-O-glucosides would be expected to be somewhat more active than the natural compounds, since (R)-DZ and (R)-dihydro-ZR are more active than the natural (S)-antipodes (Matsubara et al. 1977). In the radish cotyledon (Letham 1972) and *Amaranthus*

Table 2. A comparison of the sensitivities of four cytokinin bioassays for zeatin and its metabolites.

Zeatin metabolites	Approximate lowest detectable concentration (nM) and amount (ng, in parentheses)							
	Radish cotyledon		*Amaranthus* betacyanin		Tobacco callus		Soybean callus	
Z	10	(6)	5	(2)	0.5	(3)	0.5	(3)
ZR	30	(26)	15	(8)	3.0	(26)	<1.0	(<9)
OGZ	10	(10)	30	(17)	1.0	(10)	4.0	(38)
OGZR	30	(38)	30	(23)	3.0	(39)	5.0	(64)
OGDZ	100	(96)	180	(103)	1.0	(10)	5.0	(48)
OGDZR	100	(129)	180	(140)	2.0	(26)	5.0	(64)
7GZ	4000	(3800)	ND[a]		ND		>50	(>475)
9GZ	30	(29)	180	(103)	>50	(>476)	30	(285)
LA	2000	(1500)	180	(83)	10	(77)	ND	

[a] ND = not determined.

(Conrad 1971) bioassays, the activity of Z exceeds that of (RS)-DZ. In these bioassays, decreased activity due to sidechain reduction is also evident with the O-glucoside pairs, OGZ and OGDZ, OGZR and OGDZR (Figs. 2, 3, 9).

In the bioassays used in the present study, the O-glucosides of Z and ZR usually exhibited activity similar to Z and ZR respectively. A very large and stable N^6-substituent would be expected to abolish cytokinin activity (Letham 1978). Hence, OGZ and related O-glucosides are unlikely to be active *per se*. Indeed the glucosyl moiety of exogenous O-glucosides is known to be cleaved in plant tissues (Letham and Palni 1983), and the activity of these metabolites is almost certainly a consequence of this cleavage. Endogenous O-glucosides appear to be storage forms of Z and related compounds (see discussion by Letham and Palni 1983). The activity of OGZ exceeds that of Z in one assay, the oat leaf senescence bioassay. Z is known to be degraded in oat leaf segments by isoprenoid sidechain cleavage, since adenosine is a major metabolite (Tao et al. 1983). To account for the enhanced activity of OGZ, it is reasonable to suggest that the O-glucosyl moiety renders the sidechain resistant to cleavage by cytokinin oxidase during movement to the subcellular site of action where hydrolysis of the glucosyl residue releases Z, an active molecule.

Cytokinin 7- and 9-glucosylation markedly reduced activity in all bioassays used with one exception, the radish leaf senescence, in which 9G-BAP and BAP exhibited similar activity. High activity for 9G-BAP, similar to that of BAP, has been reported once previously and concerned the growth of resting apple buds (Benes et al. 1965). Cytokinin 7- and 9-glucosides are very stable metabolites in plant tissues (Letham and Palni 1983), and the reduction in activity probably reflects the inability of most plant tissues to convert these N-glucosides into free bases. Since 3-alkyl derivatives of BAP are essentially inactive (Skoog et al. 1967), the high activity of 3G-BAP in diverse bioassays is probably due to cleavage of the 3-glucoside moiety to release free BAP. Such cleavage has been demonstrated in radish cotyledons (Letham et al. 1982).

The alanine conjugates LA and Al-BAP were only weakly active and three

synthetic compounds related to Al-BAP were even less active than this conjugate (Fig. 4). However the trifluoroacetyl derivative of the methyl ester of Al-BAP exhibited activity that exceeded that of Al-BAP. In plant tissues, LA exhibits great stability (Parker et al. 1978, Palni et al. 1983), and the low cytokinin activity of the alanine conjugates is probably due to an inability to cleave the alanine moiety readily and release the free cytokinin base.

In the tobacco callus and radish cotyledon bioassays, cytokinin ribosides have been found to be less active than the corresponding bases (Letham 1978, Matsubara et al. 1977). This generalization also applies to O-glucosyl derivatives of Z and DZ, since the activity of OGZ exceeds that of OGZR, while OGDZ is more active than OGDZR (Table 1, Figs. 2, 3).

The abilities of four bioassays to detect Z and its metabolites are compared in Table 2. The results show that detection of some Z metabolites would be facilitated greatly by selection of a particular bioassay. Thus, for detection of 9GZ, the most suitable bioassay is the radish cotyledon. The bioassay that exhibits the greatest sensitivity for OGDZ is the tobacco callus. The *Amaranthus* bioassay is preferable for the detection of LA. No bioassay effectively detected 7GZ and mass spectrometric methods provide the only effective means for detecting this glucoside and for accurately quantifying all metabolites. Studies of endogenous cytokinins in plant extracts should be based on an integrated use of selected bioassays and mass spectrometric methods.

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