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COMPARISON OF CYTOKININ ACTIVITIES OF 9-SUBSTITUTED N^6-BENZYLADENINES IN THE *CUCUMIS* AND *AMARANTHUS* BIOASSAYS

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; *Amaranthus tricolor*; Amaranthaceae; cytokinin activity; chlorophyll synthesis; amaranthin synthesis; bioassays; substituted N^6-benzyladenines.

Abstract—9-Substituted N^6-benzyladenines were tested for their ability to eliminate the lag phase in and promote chlorophyll synthesis in *Cucumis sativus* cotyledons and for their effectiveness in eliciting the dark biosynthesis of betacyanin in *Amaranthus tricolor* cotyledon–hypocotyl explants. The following general relationships were established for dose-responses: (a) 9-ribosidation brought about little (in *Amaranthus*) or no (in *Cucumis*) decrease in activity relative to the free base, (b) the presence of a 9-ribose 5′-phosphate group moderately depressed activity in *Amaranthus* but slightly enhanced activity in *Cucumis*, (c) the presence of a 9-ribose 3′, 5′-cyclic phosphate group depressed activity substantially in both systems, more so in *Amaranthus*, (d) 9-glucosylation greatly decreased activity, as did 7-glucosylation, while 3-glucosylation depressed activity to a much lesser extent, in both systems, (e) 9-substitution with cyclopentyl, methyl, methoxymethyl, and tetrahydropyranyl groups reduced activity, the first two substituents more so than the last two, and (f) alteration of the 9-riboside group to a 9-[2-*O*-β-hydroxyethylglycerol] moiety by oxidation–reduction led to complete (in *Amaranthus*) or nearly complete (in *Cucumis*) inactivation. Responses to hormone treatment were detectable after dark incubation times as short as 4 hr (in *Cucumis*) or 8 hr (in *Amaranthus*).

INTRODUCTION

Cytokinin-active substances are divided into four structural categories: purine derivatives, modified purine (aza and deaza purine) derivatives, urea (and amide) derivatives, and aminopyrimidines [1]. Active purine cytokinins are N^6-substituted adenines; 1-, 2-, 3-, 7-, and 9-monosubstituted adenines are only slightly active [1]. The structure–activity relationships of natural and unnatural N^6, 9-disubstituted adenines have been most thoroughly studied in the tobacco stem pith and soybean cotyledon callus bioassays [2–6]. These studies have shown that substitution in the 9-position of N^6-substituted adenines with ribose or ribose phosphates reduces biological activity substantially.

Glucosylation in the 9-position also reduces cytokinin activity in both the tobacco and soybean bioassays. The 9-β-D-glucopyranoside of N^6-benzyladenine is active only at high concentrations (≥1 μM) [7]. The 7-glucoside is likewise nearly inactive, but the 3-glucoside is nearly as active as the free base [7].

Substitution in the 9-position with unnatural alkyl or cyclic groups also affects cytokinin activity. Fox *et al.* [8] found that the 9-methoxymethyl, 9-propyl, and 9-cyclohexyl analogues of N^6-benzyladenine were 10–100 times less active than the free base in the tobacco and soybean bioassays. In a more thorough study of 9-substituted cytokinins, Hashizume *et al.* [9] synthesized and tested in the tobacco bioassay the activities of 9-methyl-, 9-ethyl-, 9-*n*-propyl-, 9-*n*-butyl-, 9-*n*-pentyl-, 9-*n*-hexyl-, and 9-*n*-heptyl-N^6-benzyladenine; cytokinin activity increased with the length of the 9-substituent up to 3 carbon atoms and then declined with longer chain lengths. Only the 9-*n*-propyl analogue approached the free base in biological activity. There is a dispute over the activity of 9-methyl-N^6-benzyladenine; while Kende and Tavares [10] found this analogue to be just as effective as the unsubstituted cytokinin in the soybean callus bioassay, Fox *et al.* [11], using the same bioassay, determined that the 9-methyl derivative was about 10 times less active than the free base.

Cytokinin structure–activity relationships depend in part on the biological response system used [12]. In the most complete studies, the tobacco and soybean callus bioassays have been used, and these have long (3–5 weeks) assay times. The purpose of this study was to compare biological activities of 9-substituted N^6-benzyladenines in two shorter-term response systems, the *Cucumis* chlorophyll and *Amaranthus* betacyanin bioassays, as a prerequisite to studies of cytokinin metabolism and its relationship to biological activity. The compounds which were tested for cytokinin activity are listed in Table 1. Preliminary reports of this work have appeared in refs. [13] and [14].

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Table 1. Compounds tested for cytokinin activity

Chemical	Abbreviation
Adenine	Ade
Adenosine	Ado
Adenosine 5′-monophosphate	AMP
Adenosine 5′-diphosphate	ADP
Adenosine 5′-triphosphate	ATP
Adenosine 3′, 5′-cyclic monophosphate	cAMP
N^6, $O^{2'}$-Dibutyryladenosine 3′, 5′-cyclic monophosphate	diBcAMP
Inosine	Ino
Inosine 5′-monophosphate	IMP
N^6-Benzyladenine	bzl^{6}Ade
N^6-Benzyladenosine	bzl^{6}Ado
N^6-Benzyladenosine 5′-monophosphate	bzl^{6}AMP
N^6-Benzyladenosine 3′, 5′-cyclic monophosphate	bzl^{6}cAMP
N^6-Benzyladenine-3-β-D-glucopyranoside	bzl^{6}Ade-3-glc
N^6-Benzyladenine-7-β-D-glucopyranoside	bzl^{6}Ade-7-glc
N^6-Benzyladenine-9-β-D-glucopyranoside	bzl^{6}Ade-9-glc
9-Cyclopentyl-N^6-benzyladenine	cpn^{9}bzl^{6}Ade
9-Methyl-N^6-benzyladenine	met^{9}bzl^{6}Ade
9-Methoxymethyl-N^6-benzyladenine	mom^{9}bzl^{6}Ade
9-(Tetrahydropyran-2-yl)-N^6-benzyladenine	thp^{9}bzl^{6}Ade
2-O-[1(*R*)-(9-N^6-Benzyladenyl)-2-hydroxy-ethyl]glycerol	bzl^{6}Ado$^{ox\text{-}red}$

RESULTS AND DISCUSSION

Chemistry

Two new 9-substituted N^6-benzyladenines were synthesized for this study. 9-Cyclopentyl-N^6-benzyladenine was prepared by a procedure similar to the one described in ref. [8] for the 9-cyclohexyl derivative. I found that the use of $CHCl_3$ to remove the reaction by-product (benzylamine·HCl) facilitated subsequent crystallization of the 9-substituted cytokinin. The mass and ^{1}H NMR spectra of the new analogue fit precisely the pattern expected of its structure (see Experimental). Furthermore, the IR spectrum of cpn^{9}bzl^{6}Ade confirmed 9-substitution. Whereas a weak absorption band due to the acidic hydrogen at the 9-position was observed for free bzl^{6}Ade (IR ν_{max}^{KBr} cm^{-1}: 2800–2400), this band was not seen in the spectrum of cpn^{9}bzl^{6}Ade.

The mass and ^{1}H NMR spectra of bzl^{6}Ado$^{ox\text{-}red}$ were also consistent with its structure. In particular, the MS fragmentation pattern was consistent with that found for the oxidized–reduced, ribose-modified derivative of N^6-(Δ^2-isopentenyl)adenosine [15].

Cytokinin activity: dose-responses

The compounds Ade, Ado, AMP, ADP, ATP, cAMP, diBcAMP, Ino, and IMP were inactive when tested up to 44 μM in both the *Cucumis* and *Amaranthus* bioassays (data not shown in figures). All of these, except diBcAMP, lack an N^6-side chain. It was previously determined that diBcAMP induces betacyanin production in *A. tricolor* [16] and *A. paniculatus* [17] only at very high concentrations ($\geq$100 μM), even when theophylline is present.

Both bioassays exhibit excellent specificity for and sensitivity to cytokinins at low concentrations. Comparisons of biological activities have been made within two series of N^6-benzyladenine derivatives. The first series consists of bzl^{6}Ade, its 9-β-D-ribofuranoside (ribonucleoside), ribonucleoside 5′-monophosphate, ribonucleoside 3′, 5′-cyclic phosphate, and 9-β-D-glucopyranoside, as well as its 7- and 3-glucosides. All of these except the cyclic nucleotide have been identified as metabolites of radioactive, exogenous bzl^{6}Ade in a variety of higher plant tissues [12]. Responses were measured down to 4.4×10^{-4} μM, but the clear distinctions in biological effectiveness were observed at concentrations from 0.44 to 44 μM (Figs. 1 and 2). Over this range, the following relationships were observed. Relative to the unsubstituted free base, ribosidation in the 9-position decreased activity very slightly (observed only at 0.44 μM) in the *Amaranthus* bioassay and had no effect on activity in the *Cucumis* bioassay. The presence of a 9-ribose 5′-monophosphate group moderately depressed the *Amaranthus* response, except at the highest concentration, where it moderately enhanced activity (Fig. 2). The *Cucumis* response was slightly but consistently enhanced by the presence of the 9-ribose 5′-phosphate group (Fig. 1). The presence of a 9-ribose 3′, 5′-cyclic phosphate group depressed activity substantially in both systems, more so in the *Amaranthus* bioassay (Figs. 1 and 2). Glucosylation in the 9-position greatly decreased both the *Cucumis* and *Amaranthus* responses; glucosylation in the 7-position depressed activity to an even greater extent (Figs. 1 and 2). In the *Amaranthus* bioassay, the 7-glucoside was devoid of activity (Fig. 2). Glucosylation in the 3-position depressed activity to a significantly lesser degree than in the 7- or 9-positions (Figs. 1 and 2). In *Amaran-*

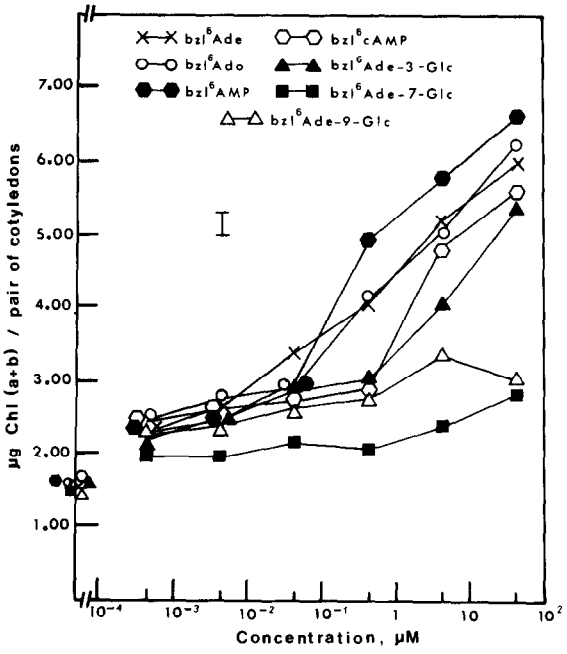

Fig. 1. Abilities of bzl⁶Ade and its nucleoside, nucleotide, cyclic nucleotide, and glucosides to eliminate the lag phase in and enhance chlorophyll synthesis in *C. sativus* cotyledons. The dark pretreatment and light periods were 14 hr and 2.5 hr, respectively. The bar represents an LSD (5%) of 0.30.

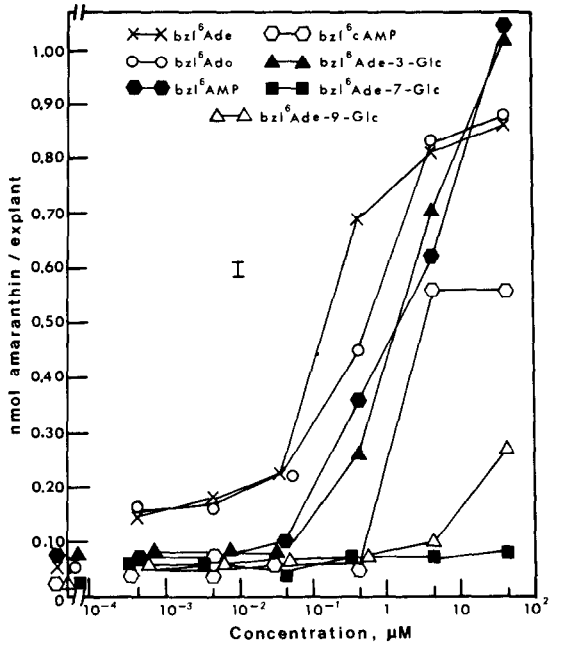

Fig. 2. Abilities of bzl⁶Ade and its nucleoside, nucleotide, cyclic nucleotide, and glucosides to induce the dark biosynthesis of amaranthin in *A. tricolor* cotyledon–hypocotyl explants. The dark incubation period was 24 hr. The bar represents an LSD (5%) of 0.03.

thus, 3-glucosylation even moderately enhanced activity at the highest concentration (Fig. 2).

The high activity of the cytokinin nucleotide is an interesting feature of the *Cucumis* bioassay, one which distinguishes it from the tobacco and soybean cell-division tests, in which the cytokinin base is much more active than either the nucleoside or nucleotide. Whether the nucleotide is an activated form of cytokinin, interacting closely with the action site(s) for the greening response, or whether it is rapidly accumulated and/or metabolized to active form(s) in this system, is not known. These possibilities should be examined even though cytokinin nucleotides appear to be bound, inactive or storage forms in other systems, most notably in light-sensitive lettuce seed germination ([18] and refs. cited therein). In any case, both the *Cucumis* and *Amaranthus* systems should be useful for the bioassay of cytokinin nucleosides and nucleotides; the *Cucumis* bioassay should be especially useful for detecting nucleotides. The activity associated with the cyclic nucleotide is due to the presence of the N⁶-benzyl side chain and is not a function of the cyclic phosphate group, since both cAMP and diB-cAMP are inactive. Similar findings have been reported for the tobacco bioassay [4].

As in the tobacco and soybean bioassays, glucosylation in the 9- and 7-positions greatly weakens cytokinin activity. It would be worthwhile to investigate the possibility that the 7- and 9-glucosides are storage or transport forms of cytokinin in *Cucumis* and *Amaranthus* seedlings. Convincing evidence for the 7-glucoside serving a storage role has been gathered for cytokinin-dependent tobacco cell cultures [19, 20]. The 3-glucoside is the only glucosylated form tested in *Cucumis* and *Amaranthus* with respectable activity. It would also be worthwhile to determine whether the 3-glucoside is an important metabolite in these species; it is a minor metabolite of bzl⁶Ade in de-rooted radish seedlings [21].

For the second series of compounds, it was generally found that substitution in the 9-position of bzl⁶Ade with cyclopentyl, methyl, methoxymethyl, and tetrahydropyran-2-yl groups reduced activity in both bioassays (Figs. 3 and 4). Although the extent of depressed activity varied from very slight to substantial, 9-substitution with cyclopentyl and methyl groups typically depressed activity more than substitution with methoxymethyl or tetrahydropyran-2-yl groups (Figs. 3 and 4). In agreement with ref. [11] the presence of a 9-methyl group clearly reduces activity relative to the free base. Oxidation–reduction of the 9-ribose group of bzl⁶Ado to a 9-[2-O-β-hydroxyethylglycerol] group resulted in nearly complete inactivation in the *Cucumis* bioassay (Fig. 3) and complete inactivation in the *Amaranthus* bioassay (Fig. 4). Previously, Chen *et al.* [22] found that the oxidized–reduced analogue of N⁶-(Δ²-isopentenyl)adenosine possessed only about 40% of the activity of the unaltered nucleoside in the tobacco bioassay. The depressed activity reported here for bzl⁶Ado$^{ox\text{-}red}$ is much more severe.

The biological response to exogenous cytokinin is, in part, a function of its uptake, transport, and metabolism. Young and Letham [23] have reasoned that susceptibility of the bond linking the purine

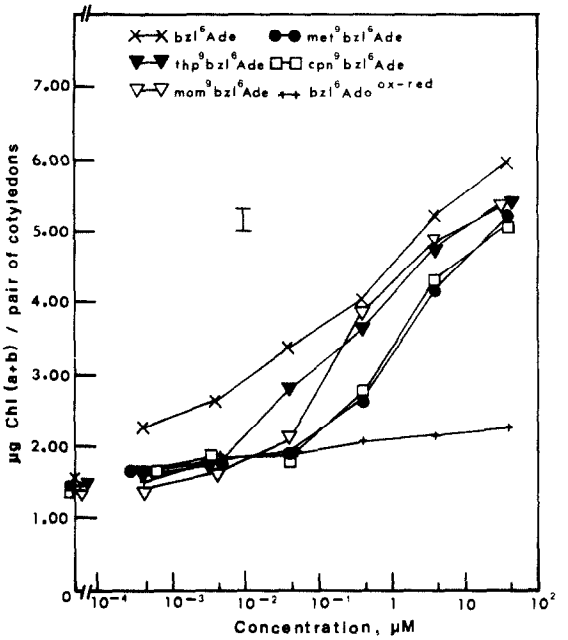

Fig. 3. Abilities of bzl⁶Ade and its 9-cyclopentyl, 9-methyl, 9-methoxymethyl, and 9-(tetrahydropyran-2-yl) analogues, and the oxidized–reduced derivative of bzl⁶Ado to eliminate the lag phase in and enhance chlorophyll synthesis in *C. sativus* cotyledons. The dark pretreatment and light periods were 14 hr and 2.5 hr, respectively. The bar represents an LSD (5%) of 0.30.

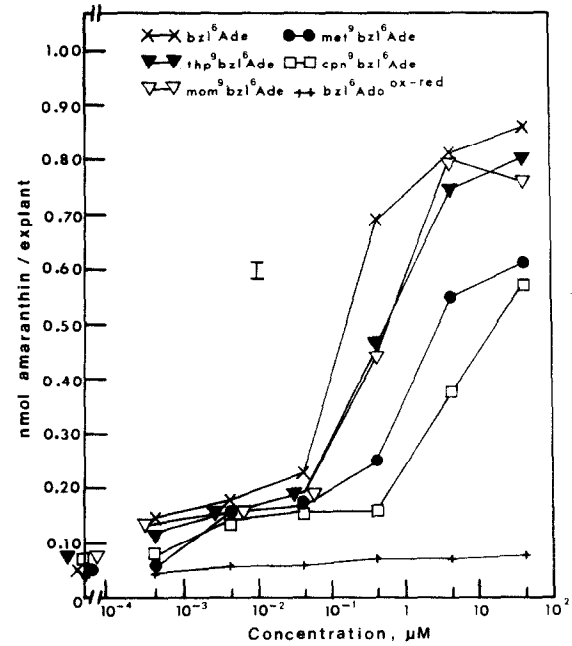

Fig. 4. Abilities of bzl⁶Ade and its 9-cyclopentyl, 9-methyl, 9-methoxymethyl, and 9-(tetrahydropyran-2-yl) analogues, and the oxidized–reduced derivative of bzl⁶Ado to induce the dark biosynthesis of amaranthin in *A. tricolor* cotyledon–hypocotyl explants. The dark incubation period was 24 hr. The bar represents an LSD (5%) of 0.03.

nitrogen to the 9-substituent might reflect its susceptibility to enzymatic cleavage in plant tissues. Since the 9-tetrahydropyranyl group is readily cleaved by acid treatment *in vitro*, it was suggested that the high activity of 9-tetrahydropyran-2-yl-N^6-(Δ^2-isopentenyl)adenine in the radish cotyledon expansion bioassay is dependent on the enzymatic removal of the 9-substituent *in vivo*. Subsequently, Fox *et al.* [11, 24] found that the 9-methyl analogue of bzl⁶Ade was metabolically unstable in tobacco and soybean tissue cultures and was readily converted to several products, among them the free base. Even so, the proposal that the biological activity of a 9-substituted cytokinin depends on the ability of the tissue to remove the 9-substituent warrants further investigation in other systems. Preferably, these systems should have short response times to cytokinin, so that metabolism might be correlated with biological activity.

Cytokinin activity: time-course

That the *Cucumis* and *Amaranthus* systems are well suited to the study of short-term cytokinin activity and metabolism is evidenced by their relatively rapid response times (Figs. 5–8). With the exception of bzl⁶Ado$^{ox\text{-}red}$, responses to all forms tested were measured after dark incubation times as short as 4 hr in *Cucumis* cotyledons, and as short as 8 hr in *Amaranthus* explants (Figs. 5–8). Previously, minimum dark pretreatments with bzl⁶Ade of 6 hr for the *Cucumis* response [25] and 8 hr for the response in *A. tricolor* [26] were established. Time-course responses in this study were followed for compounds at 4.4 µM. The incubation time optima were 20–24 hr for the *Cucumis* bioassay (Figs. 5 and 6) and at least 48 hr in the *Amaranthus* bioassay (Figs. 7 and 8; periods longer than 48 hr were not tested). Piattelli *et al.* [26] have observed a maximum response in *A. tricolor* explants at 48 hr. The reasons for the unexpectedly elevated responses to bzl⁶cAMP at 20 and 24 hr, and to cpn⁹bzl⁶Ade and met⁹bzl⁶Ade at 24 hr, in the *Cucumis* system are not known, but perhaps relate to their uptake and/or metabolism.

Closing remarks

In this study, I have not attempted to rank biological activities in a specific order since concentration optima were not generally reached (they were ≥44 µM). Previously, Tahbaz [27] tested a series of naturally occurring and synthetic cytokinin bases and corresponding nucleosides in the *Cucumis* bioassay. Although an optimum of 10 µM was obtained for N^6-(Δ^2-isopentenyl)adenine, the optima for all other compounds were not reached even at 100 µM. In contrast with the tobacco bioassay, in which cytokinin bases, nucleosides, and nucleotides reach maximum activity at 0.1–1.0 µM [4], the concentration optima in the *Cucumis* and *Amaranthus* bioassays are much higher. Further work is required to establish these optima.

Variability in these bioassays can be kept to a quite low level (see LSD bars in figures) by using seeds of a single lot, selecting plants of a uniform size, and maintaining sterile assay conditions. Furthermore, in the *Cucumis* bioassay, it is especially important to remove all hypocotyl tissue, to leave cotyledon pairs

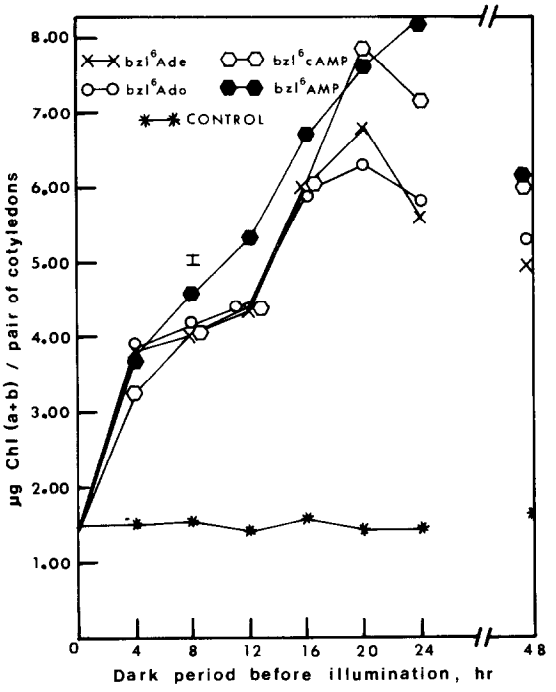

Fig. 5. Time-course of the greening response in *C. sativus* cotyledons to bzl^{6}Ade and its nucleoside, nucleotide, and cyclic nucleotide. All compounds were applied at 4.4 μM. The illumination period was 2.5 hr. The bar represents an LSD (5%) of 0.11.

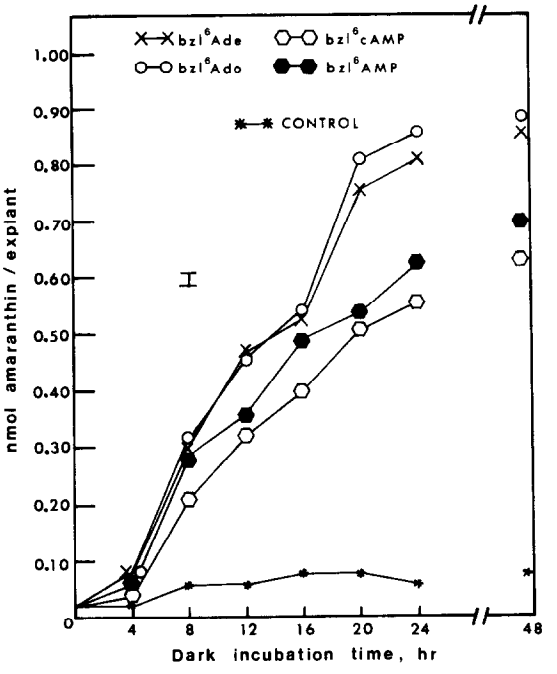

Fig. 7. Time-course of the dark amaranthin biosynthesis induced in *A. tricolor* explants by bzl^{6}Ade and its nucleoside, nucleotide, and cyclic nucleotide. All compounds were applied at 4.4 μM. The bar represents an LSD (5%) of 0.02.

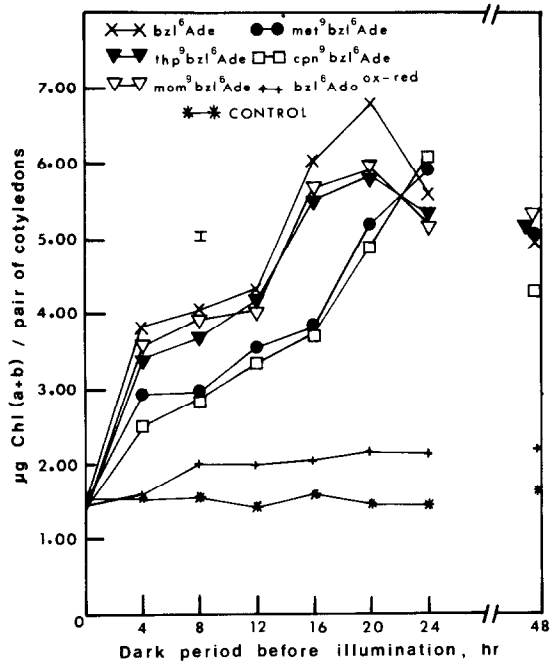

Fig. 6. Time-course of the greening response in *C. sativus* cotyledons to bzl^{6}Ade and its synthetic 9-substituted derivatives. All compounds were applied at 4.4 μM. The illumination period was 2.5 hr. The bar represents an LSD (5%) of 0.11.

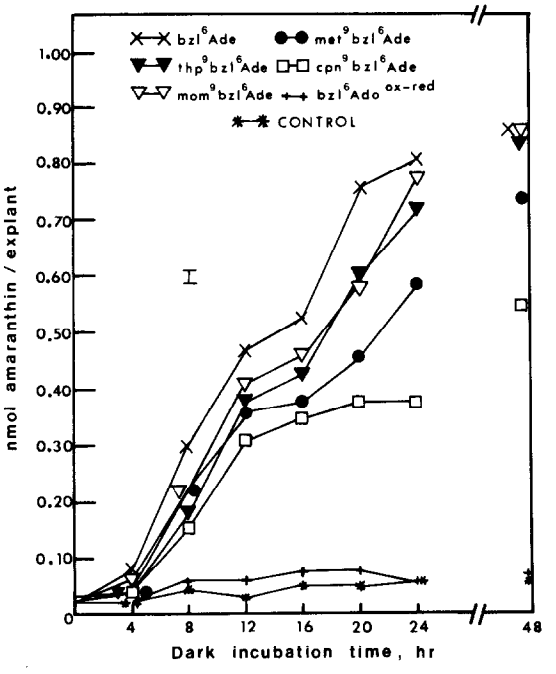

Fig. 8. Time-course of the dark amaranthin biosynthesis induced in *A. tricolor* by bzl^{6}Ade and its synthetic 9-substituted derivatives. All compounds were applied at 4.4 μM. The bar represents an LSD (5%) of 0.02.

attached, and to use a minimum volume of medium so as not to submerge the tissue (Fletcher, R. A., personal communication, and unpublished observations). In the *Amaranthus* bioassay, care must be taken to handle the explants gently and to guard against their desiccation during excision and transfer. Sources of variability in the *Amaranthus* bioassay are discussed in greater detail by Elliott [28, 29].

Uniform, reproducible, quick and differential responses to both metabolic and non-metabolic forms of cytokinin make the *Cucumis* and *Amaranthus* systems attractive not only for bioassays, but also for the study of the relationship of biological activity to the uptake and metabolism of cytokinins. I have already exploited the *Cucumis* system in a comprehensive study of the modes of metabolism of bzl^{6}Ade, its 9-riboside, and its 9-methyl analogue and their relationships to biological activity. A preliminary report of the findings has appeared [30], and a full report will be prepared in the near future. The comparative biological efficacies of compounds established herein are the foundation for such a study.

EXPERIMENTAL

Compounds. Ade, Ado, AMP, ADP, ATP, cAMP, diBcAMP, Ino, IMP, bzl^{6}Ade, and bzl^{6}Ado were purchased from commercial sources. Free acid forms of bzl^{6}AMP and bzl^{6}cAMP were gifts from Dr. G. B. Chheda, Roswell Park Memorial Institute, Buffalo, NY, U.S.A. The 3-, 7-, and 9-glucosides of bzl^{6}Ade were gifts from Dr. D. S. Letham, Australian National University, Canberra.

Synthesis of bzl^{6}Ade analogues substituted in the 9-position with cyclopentyl, methyl, methoxymethyl, and tetrahydropyran-2-yl groups. The 9-substituted 6-chloropurine (2 mmol) was refluxed with a 2-fold excess of benzylamine in H_2O for 12 hr. The mixture was then cooled and taken to dryness at 30° by rotary film evapn. The residue was treated with $CHCl_3$, and ppt. (benzylamine·HCl) was removed by filtration. The soln was evapd to dryness, and the product was dissolved in EtOH. The derivatives were crystallized from satd, aq. EtOH solns at 5°.

Synthesis of bzl^{6}Ado$^{ox\text{-}red}$. An analogue of bzl^{6}Ado in which the 2′, 3′ C–C bond of the ribose group is oxidatively cleaved with periodate and the resulting dialdehyde then reduced with borohydride to a 2-O-β-hydroxyethylglycerol group was prepd by the method in refs. [31, 32].

Mps: uncorr; UV: aq. solns; ^{1}H NMR: 10% solns, TMS as int. standard except where indicated otherwise; MS: 90–145°.

9-Cyclopentyl-N^6-benzyladenine. Yield, 75%; mp 108–109°; UV $\lambda_{max}^{H_2O}$ nm (log ε): 270 (4.30); ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.52–2.52 (9H, *m*, $C_5\underline{H}_9$), 4.92 (2H, *d*, $J = 6$ Hz, N^6–$C\underline{H}_2$), 6.70 (1H, *m*, N^6–$\underline{H}$), 7.33 (5H, *m*, $C_6\underline{H}_5$), 7.50 (1H, *s*, purine C-8), 8.40 (1H, *s*, purine C-2); EIMS (probe) 70 eV, *m/z* (rel. int.): 293 $[M]^+$ (100), 224 $[M - C_5H_9]^+$ (98), 209 $[M - C_5H_9 - NH]^+$ (17), 197 $[M - C_5H_9 - HCN]^+$ (21), 106 $[M - C_5H_9 - C_5H_2N_4]^+$ (95), 91 $[M - C_5H_9 - C_5H_2N_4 - NH]^+$ (92), 69 $[M - C_5H_2N_4 - NH - C_7H_7]^+$ (94); (Found: C, 69.49; H, 6.44; N, 24.01. $C_{17}H_{19}N_5$ requires C, 69.62; H, 6.48; N, 23.90%).

9-Methyl-N^6-benzyladenine. Yield, 87%; mp 134–135° (lit. [11] 138°); (Found: C, 65.38; H, 5.48; N, 29.42. Calc. for $C_{13}H_{13}N_5$: C, 65.27; H, 5.44; N, 29.29%). The UV, ^{1}H NMR, and mass spectra of this compound have been published [11]; the compound synthesized for this study had spectra consistent with the published ones.

9-Methoxymethyl-N^6-benzyladenine. Yield, 40%; mp 118–120° (lit. [8] 118–118.5°); UV $\lambda_{max}^{H_2O}$ nm (log ε): 269 (4.31); ^{1}H NMR (60 MHz, $CDCl_3$): δ 3.36 (3H, *s*, CH_2–O–$C\underline{H}_3$), 4.90 (2H, *d*, $J = 6$ Hz, N^6–$C\underline{H}_2$), 5.50 (2H, *s*, $C\underline{H}_2$–O–CH_3), 6.70 (1H, *m*, N^6–$\underline{H}$), 7.35 (5H, *m*, $C_6\underline{H}_5$), 7.69 (1H, *s*, purine C-8), 8.43 (1H, *s*, purine C-2); EIMS (probe) 70 eV, *m/z* (rel. int.): 269 $[M]^+$ (100), 224 $[M - C_2H_5O]^+$ (98), 209 $[M - C_2H_5O - NH]^+$ (18), 197 $[M - C_2H_5O - HCN]^+$ (20), 106 $[M - C_2H_5O - C_5H_2N_4]^+$ (96), 91 $[M - C_2H_5O - C_5H_2N_4 - NH]^+$ (85), 45 $[M - C_5H_2N_4 - NH - C_7H_7]^+$ (82); (Found: C, 62.59; H, 5.54; N, 26.21. Calc. for $C_{14}H_{15}N_5O$: C, 62.45; H, 5.58; N, 26.02%).

9-(Tetrahydropyran-2-yl)-N^6-benzyladenine. Yield, 23%; mp 112–114° (lit. [33] 108–110°); UV $\lambda_{max}^{H_2O}$ nm (log ε): 269 (4.33); ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.23–2.12 (6H, *m*, tetrahydropyran C-3, C-4, C-5), 3.33–4.33 (3H, *m*, tetrahydropyran C-2, C-6), 4.83 (2H, *d*, $J = 6$ Hz, N^6–$C\underline{H}_2$), 6.80 (1H, *m*, N^6–$\underline{H}$), 7.27 (5H, *m*, $C_6\underline{H}_5$), 7.72 (1H, *s*, purine C-8), 8.33 (1H, *s*, purine C-2); EIMS (probe) 70 eV, *m/z* (rel. int.): 309 $[M]^+$ (100), 224 $[M - C_5H_9O]^+$ (99), 209 $[M - C_5H_9O - NH]^+$ (97), 197 $[M - C_5H_9O - HCN]^+$ (97), 106 $[M - C_5H_9O - C_5H_2N_4]^+$ (98), 91 $[M - C_5H_9O - C_5H_2N_4 - NH]^+$ (99), 85 $[M - C_5H_2N_4 - NH - C_7H_7]^+$ (97); (Found: C, 65.75; H, 6.13; N, 22.79. Calc. for $C_{17}H_{19}N_5O$: C, 66.02; H, 6.15; N, 22.65%).

2-O-[1(R)-(9-N^6-Benzyladenyl)-2-hydroxyethyl]glycerol. Yield, 63%, after further purification by prep. TLC on Si gel developed in EtOAc–*n*-PrOH–H_2O (4:1:2, upper phase). The band at R_f 0.46 was eluted with MeOH, taken to dryness, dissolved in H_2O, and lyophilized to yield a white, very hygroscopic powder, which was dried for 2 days *in vacuo* over P_2O_5 at 65°. The product sublimed at 180° and decomposed above 280°. UV $\lambda_{max}^{H_2O}$ nm (log ε): 269 (4.32); ^{1}H NMR (60 MHz, D_2O, DSS as int. standard): δ 3.33–4.33 (11H, *m*, 2-O-β-hydroxyethylglycerol $\underline{H}$s), 4.67 (*s*, H-D exchange), 7.28 (5H, *m*, $C_6\underline{H}_5$), 8.25 (2H *d*, $J = 6$ Hz, N^6–$C\underline{H}_2$), 8.70 (1H, *s*, purine C-2); EIMS (probe) 70 eV, *m/z* (rel. int.): 359 $[M]^+$ (100), 343 $[M - NH - H]^+$ (15), 331 $[M - H - HCN]^+$ (12), 328 $[M - CH_2OH]^+$ (13), 285 $[M - H - C(Me)_2 - CH_2OH]^+$ (15), 268 $[M - C_3H_7O_3]^+$ (99), 253 $[M - C_3H_7O_3 - Me]^+$ (99), 224 $[M - C_5H_{11}O_4]^+$ (99), 209 $[M - C_5H_{11}O_4 - NH]^+$ (99), 197 $[M - C_5H_{11}O_4 - HCN]^+$ (97), 106 $[M - C_5H_{11}O_4 - C_5H_2N_4]^+$ (99), 91 $[M - C_5H_{11}O_4 - C_5H_2N_4 - NH]^+$ (99).

Preparation of test solns. Compounds were prepd as aq. solns buffered at pH 6.0 with 2 mM KPi buffer for the *Cucumis* bioassay, or as aq. solns containing 0.1 mg/ml L-tyrosine buffered at pH 6.3 with 13 mM NaPi buffer for the *Amaranthus* bioassay. The concns of all test solns were checked by UV spectrophotometry using published or calculated ε values. All solns were filter-sterilized.

The Cucumis *bioassay.* The procedure was basically that of refs. [25, 34], with the following modifications and clarifications. *C. sativus* L. var. Chicago Pickling seeds (Wetsel Seed Co., Waynesboro, VA, U.S.A.) of a single lot were used; they were surface-sterilized, germinated and grown under aseptic conditions for 5 days at 28 ± 1° in darkness. Expts were set up under a dim green safelight; only seedlings with stems 7 ± 1 cm in length were selected, and cotyledon pairs were left intact. Excised cotyledon pairs were transferred immediately to distilled H_2O and were then dealt randomly to test solns. Incubation dishes were 60 × 15 mm, each containing 2.0 ml of test soln. Incubation was at 28 ± 1° also. For the illumination step (2.5 hr), cool-white fluorescent light of 3280 ± 108 lx was used. Total chlorophyll was determined by the method of ref. [35].

The Amaranthus *bioassay.* The procedure was that of refs. [26, 36], with the following modifications and clarifications. *A. tricolor* L. var. Early Splendor seeds

(Burpee Seed Co., Warminster, PA, U.S.A.) of a single lot were used. Seeds were surface-sterilized and grown under sterile conditions for 2 days. Growth and incubation temp. was the same as for the *Cucumis* bioassay. For expts, cotyledon–hypocotyl explants were excised (2 mm below the cotyledonary node) under dim green light. Explants were transferred quickly to filter paper wetted with distilled H_2O to avoid desiccation and were subsequently distributed randomly to incubation dishes. Each dish contained one filter paper circle (4.25 cm diam.) wetted with 0.5 ml of test soln. Amaranthin was extracted by 5 cycles of freezing and thawing in H_2O. The $(A_{537}-A_{620})$ value was used to calculate the amount of pigment, using an ϵ value of 5.66×10^4 [37].

Experimental design. Dose-responses were determined over a concn range from 4.4×10^{-4} to 44 μM at ten-fold increments, using dark incubation times of 14 hr and 24 hr for the *Cucumis* and *Amaranthus* bioassays, respectively. For each concn, 3 dishes containing 10 tissue pieces each were used. Responses to compounds applied at 4.4 μM were followed over a time-course from 4 to 48 hr; all compounds were run in duplicate. The data were analysed by analysis of variance, and LSD values were calculated according to ref. [38].

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