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Short Communication

Inhibitors of Cytokinin Metabolism II. Inhibition of Cytokinin–Alanine Conjugation in Soybean Leaves and Associated Effects on Senescence

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Abstract. A group of xanthine derivatives and a novel analog of 6-benzylaminopurine (BAP) were tested for their ability to inhibit conjugation of cytokinin with alanine in soybean leaves. 3-(5-Hexenyl)-1,7-dimethylxanthine was found to be the most effective xanthine derivative. Although it had no senescence-retarding activity when applied alone to soybean leaves, it enhanced the action of BAP.

6-Benzylaminopurine (BAP) is an effective retardant of senescence in soybean leaves (Zhang et al. 1987a), where it is metabolized principally to the 9-alanine conjugate (Zhang et al. 1987b). This metabolite is essentially inactive in retarding senescence (Zhang and Letham 1988). Hence, the activity of BAP in soybean leaves could possibly be enhanced by suppression of alanine conjugation.

Certain 9-substituted derivatives of BAP are more effective than BAP in retarding soybean leaf senescence, and this has been attributed to their ability to gradually release free BAP while being resistant to alanine conjugation (Zhang and Letham 1988). Another possible way to enhance BAP activity is to supply compounds which directly inhibit alanine conjugation. Auxins (including 2,4-D) have been found to suppress conversion of BAP to the alanine conjugate (termed 9Ala-BAP) both with purified enzyme (Parker et al. 1986) and in leaf tissue (Zhang et al. 1987b). Hence, 2,4-D has now been assessed for its ability to enhance the senescence-retarding activity of BAP and a search for other inhibitors of alanine conjugation has been conducted. Certain substituted xanthines are known to inhibit conversion of cytokinins to 7- and 9-glu-

cosides in radish cotyledons (Hocart 1985), while the BAP analog, 7-benzylaminooxazolo[5,4-d]pyrimidine (BOP), is an inhibitor of the conversion of BAP to the 9-glucoside in maize leaf segments (Hocart 1985). Since some xanthines also inhibited forms of cytokinin metabolism other than *N*-glucosylation (Hocart 1985), BOP and a selection of substituted xanthines were tested for their ability to suppress conversion of BAP to 9Ala-BAP in soybean leaf discs.

Materials and Methods

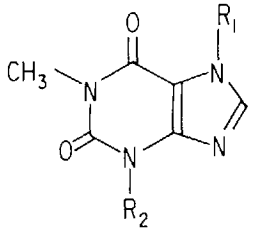
The synthesis of xanthine derivatives and BOP have been described previously (Hocart 1985). Procedures for the metabolism studies employing excised leaf discs and the bioassays using intact soybean (*Glycine max* (L.) Merr., cv. Anoka) leaves have also been detailed previously (Zhang et al. 1987b; Zhang and Letham 1988). In metabolism studies, [^{3}H]BAP was supplied at 7.5 μM. Formation of 9Ala-BAP was monitored by TLC using layers spread with Merck silica gel 60 PF_{254} (solvent *n*-butanol/14N NH_4OH/water, 6:1:2 upper phase).

Results and Discussion

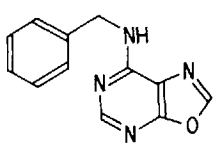
The structural formula of the xanthine derivatives tested are presented in Fig. 1. Compounds X1 to X4 are 3-substituted 1,7-dimethylxanthines, X5 and X7 are 7-substituted 1-methylxanthines, while X6 is a 3-substituted 1-methylxanthine. The substituents in all these compounds, if at position N^6 of adenine, would confer cytokinin activity.

In the soybean leaf disc system, all the compounds tested had some inhibitory effect on 9Ala-BAP formation (Table 1). At 0.2 mM, X1 evoked a marked effect, was the most active inhibitor among the tested compounds, and elevated the level of free BAP twofold. The next most effective was X7, which was more active than the isomeric compound X6 and the benzyl analog X5. At 0.2 mM, some compounds, namely, X2, X6, and BOP, did not appreciably affect the extent of alanine conjugation, but still elevated the BAP level (Table 1). Hence, these compounds must have effects on BAP metabolism other than an inhibition of alanine conjugation. BOP at 1 mM both markedly elevated the level of free BAP and suppressed conjugation. Xanthine itself had no inhibitory activity at 1 mM.

Since 2,4-D and X1 were effective in inhibiting the alanine conjugation of BAP applied to soybean leaf discs, these two compounds were tested in the soybean leaf senescence assay, especially to examine their influence on the activity of applied BAP. 2,4-D at 25 μM had no effect when applied alone or with BAP (Table 2). However, the xanthine derivative, X1, at 200 μM potentiated the action of BAP, but was inactive when supplied alone (Table 2). While X1 may be an inhibitor of alanine conjugation which is fairly specific and as a consequence enhances the action of BAP, 2,4-D probably affects other aspects of metabolism and these override the desirable effect of its inhibition of 9Ala-BAP formation.



Compound	R_1	R_2
X1	$-CH_3$	$-(CH_2)_4CH=CH_2$
X2	$-CH_3$	$-(CH_2)_2CH(CH_3)_2$
X3	$-CH_3$	$-(CH_2)_4OH$
X4	$-CH_3$	$-CH_2CHOH-C(CH_3)_2OH$
X5	$-CH_2-C_6H_5$	$-H$
X6	$-H$	$-CH_2CH=C(CH_3)_2$
X7	$-CH_2CH=C(CH_3)_2$	$-H$



BOP

Fig. 1. Structural formula for compounds tested for ability to inhibit conversion of BAP to the alanine conjugate.

The use of X1 to enhance the senescence-retarding activity of BAP may exemplify a new approach to the control of senescence and plant development. Much is known concerning cytokinin structure–activity relationships and many potent cytokinins have been prepared. In the future it may be worth-

Table 1. Effect of substituted xanthines and BOP on 9Ala-BAP formation in soybean leaf discs supplied with [^{3}H]BAP (7.5 μM).

Compound and concentration	Extracted ^{3}H (dpm disc^{-1})	% of DPM 9Ala-BAP	% of DPM BAP	% inhibition of 9Ala-BAP formation
C[a]	867	42.2	16.9	
X1 0.2 mM	870	27.9	34.4	33.9
X1 1.0 mM	673	29.9	37.7	29.1
X2 0.2 mM	1051	42.8	23.6	0.0
X2 1.0 mM	1045	36.1	29.4	14.5
X3 0.2 mM	858	36.1	23.0	14.5
X3 1.0 mM	953	39.4	23.3	6.6
X4 0.2 mM	913	44.8	18.9	0.0
X4 1.0 mM	755	31.8	24.1	24.6
X5 0.2 mM	778	35.9	23.7	14.9
X5 1.0 mM	726	34.7	25.8	17.8
X6 0.2 mM	768	39.8	24.8	5.7
X7 0.2 mM	912	30.5	29.5	27.7
BOP 0.2 mM	823	42.6	22.5	0.0
BOP 1.0 mM	703	32.1	39.5	23.9

[a] [^{3}H]BAP only.

Table 2. Effect of 2,4-D (25 μM) and X1 (200 μM) on leaf chlorophyll retention of intact soybean plants (cv. Anoka) in the presence and absence of BAP (10 μM).

Compound[a]	C[b]	2,4-D	X1	BAP	2,4-D + BAP	X1 + BAP
A_{665}	0.144	0.150	0.142	0.256	0.252	0.355

Similar results were obtained in several other experiments.

[a] All compounds were dissolved in 0.2% DMSO containing 0.05% Tween 80.

[b] C, control, DMSO and Tween 80 only.

while to devote effort to designing effective inhibitors of cytokinin inactivation as these may potentiate the action of both exogenous and endogenous cytokins, especially when the cytokinin concentration is suboptimal.

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