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SHORT COMMUNICATION

STRUCTURE-ACTIVITY RELATIONSHIPS IN OPTICALLY ACTIVE CYTOKININS

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Abstract—New optically active cytokinins have been synthesized and assayed for their effect on senescence in barley leaves. Enantiomers of S-configuration were shown to be more active than those of R-configuration.

INTRODUCTION

SINCE Miller and his co-workers observed that kinetin (6-furfurylaminopurine) induced cell division in a certain plant tissue, numerous kinetin analogues have been synthesized and assayed on several plant systems to correlate chemical structure with biological activity.[1-4] Based on such studies, certain minimum structural requirements have been found necessary for the cytokinin activity.[2-4]

Recently an optically active cytokinin, (−)-dihydrozeatin, was isolated from immature seeds of *Lupinus luteus* in our laboratory.[5,6] Of the two possible enantiomers, one mirror image is expected to be more active than the other as observed in the auxin activity of many enantiomeric plant-growth substances.[7,8] However, the effect of optical isomerism on cytokinin activity has not been examined. The purpose of this preliminary communication is to estimate the biological activity of N^6-(optically active alkyl and arylalkyl substituted)-adenines by the chlorophyll preservation test, one of the bioassays for cytokinins, and to provide information for the analysis of the structure/activity relationship, in particular, information on the stereospecific requirements of the side-chain attached to the purine ring.

RESULTS AND DISCUSSION

It may be assumed that the three-dimensional structure around the asymmetric carbon atom on the side-chain of cytokinins plays a significant role when the purine ring is oriented on some surface of the receptor site. Therefore, the smaller the distance between purine ring

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[6] K. KOSHIMIZU, S. MATSUBARA, T. KUSAKI and T. MITSUI, *Agr. Biol. Chem.* **31**, 795 (1967).
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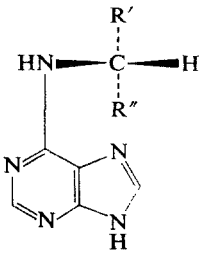
and asymmetric centre, the more the effect of asymmetric structure on biological response would be. In order to test this assumption, several new optically active cytokinins were prepared by means of the reaction of 6-chloropurine with optically active amines, which have an asymmetric carbon atom at the α-position. Their absolute configurations are summarized in Table 1. Kinetin, 6-benzylaminopurine and zeatin were prepared as standard samples.

TABLE 1. OPTICALLY ACTIVE CYTOKININS



Code name	Compound	R'	R''
S-V	S-(−)-6-(α-Hydroxymethyl-β-methylpropylamino)purine	—CH_2OH	—$CH(CH_3)CH_3$
R-V	R-(+)-enantiomer	—$CH(CH_3)CH_3$	—CH_2OH
S-L	S-(−)-6-(α-Hydroxymethyl-γ-methylbutylamino)purine	—CH_2OH	—$CH_2CH(CH_3)CH_3$
R-L	R-(+)-enantiomer	—$CH_2CH(CH_3)CH_3$	—CH_2OH
S-M	S-(−)-6-(α-Hydroxymethyl-γ-methylmercaptopropylamino)purine	—CH_2OH	—$(CH_2)_2SCH_3$
R-M	R-(+)-enantiomer	—$(CH_2)_2SCH_3$	—CH_2OH
S-PG	S-(−)-6-(α-Hydroxymethyl-α-phenylmethylamino)purine	—CH_2OH	—Ph
R-PG	R-(+)-enantiomer	—Ph	—CH_2OH
S-PA	S-(−)-6-(α-Hydroxymethyl-β-phenylethylamino)purine	—CH_2OH	—CH_2Ph
R-PA	R-(+)-enantiomer	—CH_2Ph	—CH_2OH
R-PE	R-(−)-6-(α-Methyl-α-phenylmethylamino)purine	—CH_3	—Ph
S-PE	S-(+)-enantiomer	—Ph	—CH_3

The chlorophyll preservation activity of these cytokinins is shown in Figs. 1 and 2. Although all the derivatives are active, the (−)-antipodes in the compounds with a hydroxymethyl group on the side-chain, are found to be more active. The more active antipodes possess the same absolute configuration, i.e the same arrangement of four substituents around a central asymmetric carbon atom: adenine, hydroxymethyl group at R', hydrogen atom and a substituent such as alkyl, aryl and arylalkyl at R'', as shown by the projection formula in Table 1. PE (where the hydroxymethyl group in PG is replaced with a methyl group), on the contrary, shows greater activity as the (+)-antipode, which has a phenyl and methyl group at R' and R'', respectively.

Although a definite conclusion cannot be drawn until further evidence is available, some interpretations are possible as to the structure/activity relationships from the above results. Reviewing the common feature of the more active enantiomers in the two series of compounds tested, the substituent R' has a mobile electron system, e.g. lone-pair- and π-electrons. This leads us to assume that the hydroxymethyl or phenyl group as a hydrogen bond acceptor may assist in properly orienting the molecule on the surface of the primary site of action, which

may be in the chloroplast.[9] The substituents at R'', on the other hand, would assist the molecule in attaching itself through a hydrophobic bonding to the receptor surface. This is supported by the fact that the activity of S-M is less than that of S-L, since the hydrophobic

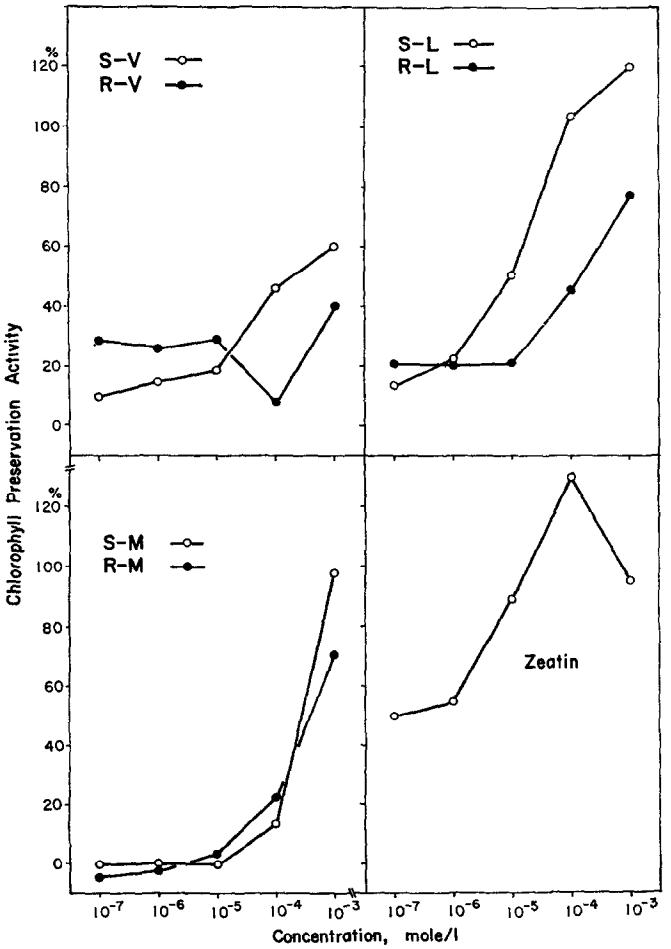


FIG. 1. EFFECT OF N^6-(OPTICALLY ACTIVE ALKYL SUBSTITUTED)ADENINES FOR RETARDING CHLOROPHYLL BREAKDOWN IN BARLEY LEAF SECTIONS.
The indices of chlorophyll preservation activity on ordinate are explained in the Experimental part. Code names of compounds are summarized in Table 1.

nature of the side-chain of S-M is considered to be less than that of S-L. The lower activity of S-V than of S-L, and of S-PA than of S-PG, also suggests that the hydrophobic nature of the substituents at R'' makes an important contribution to the receptor surface.

Further investigation of the biological activities of these compounds on other plant systems is in progress.

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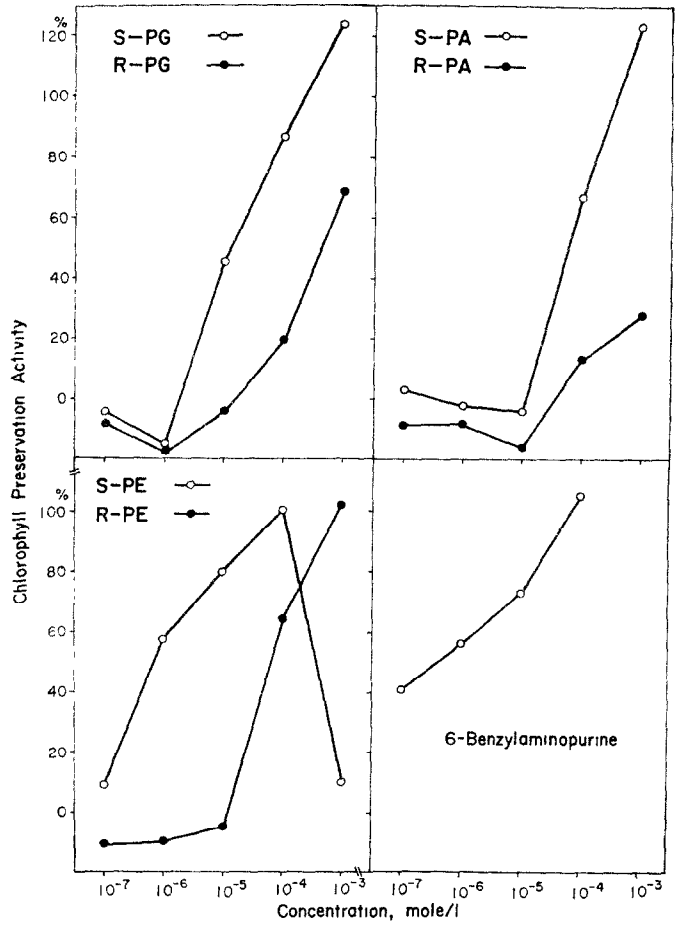

FIG. 2. EFFECT OF N^6-(OPTICALLY ACTIVE ARYLALKYL SUBSTITUTED)ADENINES FOR RETARDING CHLOROPHYLL BREAKDOWN IN BARLEY LEAF SECTIONS.

The indices of chlorophyll preservation activity on ordinate are explained in the experimental part. Code names of compounds are summarized in Table 1.

EXPERIMENTAL

Chlorophyll Preservation Test

Barley (*Hordeum distichum* var. Asahi 19) seedlings were grown in moist Vermiculite contained in wooden trays in a greenhouse in daylight at 20° for 2 weeks. The first leaves were selected for uniformity of width, and a piece of each leaf was cut between the 3rd and 4th cm from the top of the blade. Five sections were floated, with the upper epidermis upward, on 1 ml of an aqueous solution of a test compound contained in a capped vial. After 48 hr of incubation at 25° in darkness, the sections from each vial were extracted with 5 ml of 80% ethanol at 70° for 15 min. The absorbance of each solution was measured at 665 nm; each fraction was tested in duplicate and the test was repeated twice. Standards were treated with water or 10^{-5} M kinetin. The amount by which chlorophyll in treated sections exceeded that in water-treated sections was expressed as a percentage of the amount by which chlorophyll of 10^{-5} M kinetin-treated sections exceeded that of water-treated sections. The index for water-treated sections was, therefore, zero, and that for sections treated with 10^{-5} M kinetin was 100.

*Preparation of Compounds**

S- and *R*-Aminoalcohols—valinol, leucinol, methionınol, phenylglycinol and phenylalaninol—were prepared by the reduction of corresponding L- and D-amino acid methyl esters with $LiAlH_4$.[10–12] S-(−)- and *R*-(+)-α-Phylethylamine† were obtained according to the method of Theilacker and Winkler.[13] The appropriate amine was, then, treated with 6-chloropurine[14] in butanol at 150° for 2 hr. Compounds thus obtained were purified by column chromatography on Florisil developed with benzene–ethanol, then recrystallized to give the corresponding purine derivatives as follows:

S-(−)-6-(α-*Hydroxymethyl-β-methylpropylamino)purine*, S-V (Table 1)‡, m.p. 173–174°, sintering above 160° (from acetonıtrile–ethanol), $[\alpha]_D^{28}$ −53·7 (2·59), λ_{max}^{EtOH} 269 nm (ε 17·5 × 10³), $\lambda_{max}^{0\cdot1N\ NaOH}$ 275 nm (ε17·5 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 275 nm (ε 17·2 × 10³). (Found: C, 54·33; H, 6·83; N, 31·97. $C_{10}H_{15}ON_5$ required: C, 54·28; H, 6·83; N, 31·66 per cent.)

R-(+)-6-(α-*Hydroxymethyl-β-methylpropylamino)purine*, R-V (Table 1)‡, m.p. 173–174°, sintering above 160° (from acetonıtrile–ethanol), $[\alpha]_D^{28}$ + 53·1 (3·11), λ_{max}^{EtOH} 269 nm (ε 17·8 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 18 0 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 275 nm (ε 17·6 × 10³). (Found: C, 54·40; H, 6·85; N, 31·72. $C_{10}H_{15}ON_5$ required: C, 54·28; H, 6·83; N, 31·66 per cent.)

S-(−)-6-(α-*Hydroxymethyl-γ-methylbutylamino)purine*, S-L (Table 1), m.p. 172–173°, sintering above 164° (from acetonitrile–ethanol), $[\alpha]_D^{23}$ −58·1 (2·96), λ_{max}^{EtOH} 269 nm (ε 18·0 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 18·4 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 275 nm (ε 17·8 × 10³). (Found: C, 56·25; H, 7·33; N, 29·53. $C_{11}H_{17}ON_5$ required: C, 56·15; H, 7·28; N, 29·77 per cent.)

R-(+)-6-(α-*Hydroxymethyl-γ-methylbutylamino)purine*, R-L (Table 1), m.p. 172–173°, sintering above 164° (from acetonitrile–ethanol), $[\alpha]_D^{26}$ + 59·9 (2·99), λ_{max}^{EtOH} 269 nm (ε 17·7 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 18·1 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 275 nm (ε 17·5 × 10³). (Found: C, 56·13; H, 7·40; N, 29·55. $C_{11}H_{17}ON_5$ required: C, 56·15; H, 7·28; N, 29·77 per cent.)

S-(−)-6-(α-*Hydroxymethyl-γ-methylmercaptopropylamino)purine*, S-M (Table 1), m.p. 172–173°, sintering above 158° (from ethanol), $[\alpha]_D^{24}$ −59·2 (2·98), λ_{max}^{EtOH} 270 nm (ε 17·8 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 17·7 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 276 nm (ε 17·3 × 10³). (Found: C, 47·56; H, 6·08; N, 27·84. $C_{10}H_{15}ON_5S$ required: C, 47·41; H, 5·97; N, 27·65 per cent.)

R-(+)-6-(α-*Hydroxymethyl-γ-methylmercaptopropylamino)purine*, R-M (Table 1), m.p. 171–172°, sintering above 158° (from ethanol), $[\alpha]_D^{24}$ + 59·7 (3·02), λ_{max}^{EtOH} 270 nm (ε 18·0 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 17·9 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 276 nm (ε 17·4 × 10³). (Found: C, 47·56; H, 6·08; N, 27·49. $C_{10}H_{15}ON_5S$ required: C, 47·41; H, 5·97; N, 27·65 per cent.)

S-(−)-6-(α-*Hydroxymethyl-α-phenylmethylamino)purine*, S-PG (Table 1), m.p. 195–196° (from water), $[\alpha]_D^{20}$ −79·8 (1·01), λ_{max}^{EtOH} 270 nm (ε 19·3 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 274 nm (ε 18·3 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 277 nm (ε 19·3 × 10³). (Found: C, 61·42; H, 5·16; N, 27·20. $C_{13}H_{13}ON_5$ required: C, 61·16; H, 5·13; N, 27·44 per cent.)

R-(+)-6-(α-*Hydroxymethyl-α-phenylmethylamino)purine*, R-PG (Table 1), m.p. 195–196° (from water), $[\alpha]_D^{20}$ + 82·2 (1·00), λ_{max}^{EtOH} 270 nm (ε 19·3 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 274 nm (ε 18·3 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 277 nm (ε 19·3 × 10³). (Found: C, 61·01; H, 5·10; N, 27·29. $C_{13}H_{13}ON_5$ required: C, 61·16; H, 5·13; N, 27·44 per cent.)

S-(−)-6-(α-*Hydroxymethyl-β-phenylethylamino)purine*, S-PA (Table 1), m.p. 217–218°, sublimed above 200° (from ethanol), $[\alpha]_D^{26}$ −132 (0·287), λ_{max}^{EtOH} 270 nm (ε 18·5 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 269 nm (sh.), 275 nm (ε 16·8 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 277 nm (ε 15·9 × 10³). (Found: C, 62·19; H, 5·72; N, 25 71. $C_{14}H_{15}ON_5$ required: C, 62·44; H, 5·61; N, 26 01 per cent.)

R-(+)-6-(α-*Hydroxymethyl-β-phenylethylamino)purine*, R-PA (Table 1), m.p. 217–218°, sublimed above 200° (from ethanol), $[\alpha]_D^{26}$ + 138 (0·301), λ_{max}^{EtOH} 270 nm (ε 19·2 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 269 nm (sh.), 275 nm (ε 17·1 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 277 nm (ε 16·2 × 10³). (Found: C, 62·52; H, 5 78; N, 25·62. $C_{14}H_{15}ON_5$ required: C, 62·44; H, 5·61; N, 26·01 per cent.)

R-(−)-6-(α-*Methyl-α-phenylmethylamino)purine*, R-PE (Table 1), m.p. 163–165°, sublimed above 156° (from acetonitrile), $[\alpha]_D^{28}$ −57·9 (2·99), λ_{max}^{EtOH} 266 nm (sh.), 270 nm (ε 14·3 × 10³), $\lambda_{max}^{0\cdot1NNaOH}$ 275 nm (ε 13·7 × 10³), 282 nm (sh.), $\lambda_{max}^{0\cdot1NHCl}$ 276 nm (ε 13·7 × 10³). (Found: C, 65·10; H, 5·56; N, 29·42. $C_{13}H_{13}N_5$ required: C, 65·25; H, 5·48; N, 29·27 per cent.)

* All m.ps are uncorrected and were determined on a hotstage equipped with a microscope and polarizer. The optical rotations were recorded in EtOH on a Yanagimoto photo-magnetic direct reading polarimeter Model OR-20, and the u.v. spectra on a Hitach EPS-3T spectrophotometer.

† Absolute configuration: W. Leithe, *Chem. Ber.* **64**, 2827 (1931); R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia* **12**, 81 (1956).

‡ The i.r. spectra of these two enantiomers were different, although the NMR spectra in d_5-pyridine were superimposable.

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S-(+)-6-(α-*Methyl-α-phenylmethylamino)purine*, S-PE (Table 1), m.p. 163–165°, sublimed above 156° (from acetonitrile), $[\alpha]_D^{28}$ +58·8 (3·11), λ_{max}^{EtOH} 266 nm (sh), 270 nm (ϵ 14·2 × 10³), $\lambda_{max}^{0\ 1NNaOH}$ 275 nm (ϵ 13·6 × 10³), 282 nm (sh.), $\lambda_{max}^{0\ 1NHCl}$ 276 nm (ϵ 13 7 × 10³) (Found: C, 65·37; H, 5·68; N, 29·51. $C_{13}H_{13}N_5$ required: C, 65·25; H, 5·48; N, 29·27 per cent.)

Kinetin,* 6-benzyladenine* and zeatin* were prepared according to the methods of Daly and Christensen,[15] and Shaw *et al.*[16]

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* Satisfactory analyses were obtained for all compounds characterized by their constitutional formula.

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