Phytochemistry, Vol. 21, No. 7, pp. 1783–1784, 1982.
Printed in Great Britain.

0031-9422/82/071783-02$03.00/0

SHORT REPORTS

6-(2, 3, 4-TRIHYDROXY-3-METHYLBUTYLAMINO)PURINE, A CYTOKININ WITH HIGH BIOLOGICAL ACTIVITY

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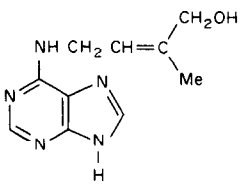
(*Revised received* 23 *October* 1981)

Key Word Index—Cytokinin; 6-(2, 3, 4-trihydroxy-3-methylbutylamino)purine; cell division.

Abstract—6-(2, 3, 4-Trihydroxy-3-methylbutylamino)purine, isolated from the oxidation of *cis*-zeatin with potassium permanganate, has been identified by ^{1}H NMR and high resolution mass spectrometry. Its activity as a cell division factor, when examined by the soybean callus assay in the concentration range 10^{-11}–10^{-5} M, equalled that of the parent compound.

INTRODUCTION

Zeatin, 6-(4-hydroxy-3-methyl-*trans*-2-butenyl-amino)purine (**1**), isolated by Letham [1, 2] some 18 years ago, is the biologically most active cytokinin tested to date. It will induce cell division in cultured



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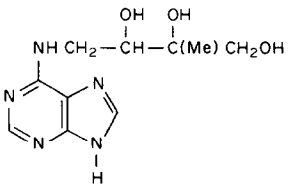

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carrot phloem tissue at a concentration of 5×10^{-5} μM [3, 4]. Subsequently, other cytokinins have been isolated and the structure–activity relationship of a great many of these investigated. In a recent review, Matsubara [5] has drawn renewed attention to the effect of the side-chain, which is attached to the N^6-position of the purine, on biological activity. The review highlights the importance of factors such as the absence of a terminal carboxyl group, the presence of a double bond at the 2, 3-position, introduction of a second methyl group at the 3-position, hydroxylation at the 4-position, and correct stereochemistry of the substituents attached to the double bond.

The obvious method to study the influence of structural effects on activity is the examination of a large number of zeatin analogues. In this regard, Leonard *et al.* [6] have made a significant contribution through their study of the influence of the position of the hydroxyl group in the side-chain on cytokinin activity. This group of researchers concluded the following:

Monohydroxy series: 4-hydroxy ≫ 3- and 2-hydroxy.
Dihydroxy series: 3, 4-dihydroxy > 2, 3-dihydroxy.

In general, a hydroxyl group at the 4-position of the side-chain of 6-isopentylaminopurine enhances activity whereas a hydroxyl at the 2-, 3-, or 2, 3-positions results in decreased activity. The 'missing' member of the series, 6-(2, 3, 4-trihydroxy-3-methylbutylamino)purine (**2**), has received scant attention and there is only one earlier reference to such a compound by Letham [7]. This was isolated from *Zea mays*, mp 224–227°, and it was also obtained synthetically by oxidation of *trans*-zeatin. The latter possessed only low cytokinin activity compared with the natural product.

RESULTS AND DISCUSSION

Our attention was first focused on the title compound during studies in which the metabolic fate of labelled zeatin in plant systems was investigated [8]. Separation of the labelled metabolites on a Sephadex LH-20 column [9] afforded a compound with identical chromatographic properties to one derived from the oxidation of *cis*-zeatin with alkaline potassium permanganate.

Since the quantity of *cis*-zeatin available to us was very limited, oxidations were carried out on a semi-micro scale and characterization of **2** was by ^{1}H NMR and high resolution mass spectrometry. Compound **2** is a white crystalline material, mp 230°.

A similar oxidation was carried out on 6-(3, 3-dimethylbutenylamino)purine, in order to test the scope of the oxidation and the subsequent isolation procedure. In this case the analogous diol, mp 251°, was isolated.

Using the soybean callus bioassay [10] it could be shown that **2** and its parent compound, *cis*-zeatin gave a similar response in the concentration range 10^{-11}–10^{-5} M. (Fig. 1). This activity is considerably higher than that found for the compounds obtained by Letham [7]. The formation of oxidation products such as **2**, and others related to it, from a range of cytokinins with unsaturated side-chains, is likely to have far-reaching implications in our understanding

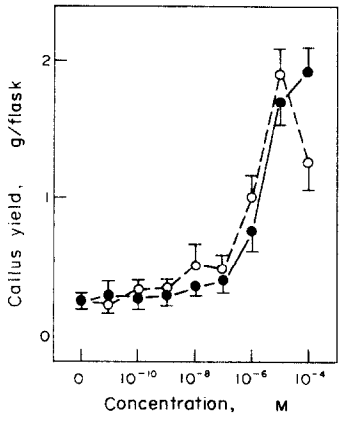

Fig. 1. Comparison of activity of 6-(2, 3, 4-trihydroxy-3-methylbutylamino)purine (—●—) with *cis*-zeatin (—○—).

of the mode of action of these hormones in plant growth. Such products may indeed prove to be the functional metabolites derived from the naturally occurring cytokinins.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were measured at 80 MHz and mass spectra at 70 eV direct inlet.

Oxidation and separation procedure. *Cis*-zeatin (30 mg) suspended in H_2O (3 ml), was dissolved completely by the addition of 3 drops of 0.5 N NaOH. An equimolar quantity of $KMnO_4$ was added and the reaction allowed to proceed at 25° for 30 min. Reaction was terminated by the addition of EtOH and the resultant ppt was filtered off. The filtrate, after concentration (2 ml), was fractionated on a Sephadex LH-20 column using aq. MeOH (90 : 10) as eluant. Fractions of 40 ml were collected and the title compound eluted between 520–680 ml. Concentration of the eluates *in vacuo* yielded a small quantity (4.1 mg) of white needles, mp 230° ^{1}H NMR (80 MHz FT, NaOH): δ 1.25 (3H, *s*, Me), 3.66 (2H, *dd*, CH_2OH), 3.86 (3H, *m*, $-NHCH_2CHOH$), 7.96 (1H, *s*, arom. CH), 8.14 (1H, *s*, arom. CH). EIMS (probe) 70 eV, *m/z* (rel. int.): 254 $[M+1]^+$ (3.4), 253 $[M]^+$ (3.4), 222 $[M-CH_2OH]^+$ (8.5), 178 $[M-C(OH)Me{\cdot}CH_2OH]^+$ (85.0), 149 (59), 148 (100), 136 (24), 135 (34), 121 (18), 120 (29), 119 (22), 108 (12), 93 (10), 81 (3); high resolution 253.1186 (calc. for $C_{10}H_{15}N_5O_3$: 253.1176), 222.0963 (calc. for $C_9H_{12}N_5O_2$: 222.0991), 178.0739 (calc. for $C_7H_8N_5O$: 178.0729).

The oxidation product from 6-(3, 3-dimethylbutenylamino)purine had mp 25°. EIMS (probe) 70 eV, *m/z* (rel. int.): 237 $[M]^+$ (3.6), 222 $[M-CH_2OH]^+$ (3.2), 178 (85), 149 (40), 148 (100), 136 (23), 135 (34), 121 (12), 120 (18), 119 (16), 108 (10), 93 (9), 81 (4).

Acknowledgements—The authors wish to thank the Council for Scientific and Industrial Research, and the Natal University Research Fund for financial assistance.

REFERENCES

1. Letham, D. S. (1963) *Life Sci.* **2**, 569.
2. Letham, D. S., Shannon, J. S. and McDonald, I. R. C. (1964) *Proc. Chem. Soc.* 230.
3. Letham, D. S. (1967) *Planta* **74**, 228.
4. Skoog, F., Hanzi, H. Q., Szweykowska, M., Leonard, N. J., Carraway, K. L., Fujii, T., Helgeson, J. P. and Loeppky, R. W. (1967) *Phytochemistry* **6**, 1169.
5. Matsubara, S. (1980) *Phytochemistry* **19**, 2239.
6. Leonard, N. J., Hecht, S. M., Skoog, F. and Schmitz, R. Y. (1969) *Proc. Natl Acad. Sci., U.S.A.* **63**, 175.
7. Letham, D. S. (1973) *Phytochemistry* **12**, 2445.
8. Van Staden, J. (1981) *Physiol. Plant.* **53**, 269.
9. Hutton, M. J. and Van Staden, J. (1981) *Ann. Botany* **47**, 527.
10. Miller, C. O. (1965) *Proc. Natl Acad. Sci., U.S.A.* **54**, 1052.