Phytochemistry, 1976, Vol. 15, p. 1565. Pergamon Press. Printed in England.

SHORT REPORTS

N^6-(Δ^2-ISOPENTENYL)ADENOSINE FROM CROWN GALL TUMOR TISSUE OF *VINCA ROSEA*

CHONG-MAW CHEN*, RICHARD L. ECKERT* and JAMES D. MCCHESNEY†
*Science Division, University of Wisconsin-Parkside Kenosha, Wisconsin 53140; †Medicinal Chemistry Department, Kansas University Lawrence, Kansas 66045, U.S.A.

(*Revised received* 29 *March* 1976)

Key Word Index—*Vinca rosea*; Apocynaceae; crown-gall tumor; cytokinin; N^6-(Δ^2-isopentenyl)adenosine.

Plant. Vinca rosea L.46 line of crown-gall tumor tissue. *Source.* This culture was originally supplied by Dr. C. O. Miller of Indiana University and was grown as described [1]. *Previous work.* The presence of N^6-(Δ^2-isopentenyl)adenosine (i^6Ado) in other plant tissues either free or as a constituent of *t*RNA is well documented [2,3]. Zeatin riboside or 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)-9-β-D-ribofuranosyl purine, one of the i^6Ado derivatives, has been isolated from this strain of tissue [1].

Present work. The procedure for analyzing subnormal levels of i^6Ado in plant tissues was as previously described [4]. The tumor tissue (45-day-old) was extracted with EtOH. The aq solution was then oxidized with $NaIO_4$, and reduced by [^{3}H]-$NaBH_4$. The solution was lyophilized and the residue was dissolved in 35% EtOH. Isolation and purification of the oxidized-reduced i^6Ado ([^{3}H]-$i^6Ado^{ox\text{-}red}$) was achieved by Sephadex LH-20 column in 35% EtOH followed by pc [4,5].

As a control, authentic i^6Ado was also oxidized–reduced, and purified under the conditions used for the tissue extracts. This product after purification provided the 2-*O*(1R-(9-N^6-(Δ^2-isopentenyl) adenyl-2-hydroxyethyl)glycerol ($i^6Ado^{ox\text{-}red}$). $\lambda_{max}H_2O$ 268 nm (ϵ 19 500). The structure of this compound was characterized by MS:*m/e* 337 (M^+) [i^6Ado(335) + 2]; 322 (−15), loss of Me; 294 (−43), loss of $C(CH_3)_2$ and H; 306 (−31) and 246 (−91), loss of CH_2OH and $C_3H_7O_3$ from 2-*O*-β-hydroxyethylglycerol moiety; 203, free base; 188 free base less Me; 160 (−177), loss of 2-*O*-β-hydroxyethylglycerol and $C(Me)_2$. Further fragmentation of the N^6-(Δ^2-isopentenyl)adenine side chain yields ions at *m/e* 148, 135 and 119. The breakdown of the 2-*O*-β-hydroxyethylglycerol yields ions at *m/e* 103, 60 and 45.

One of the [^{3}H]-labeled samples from the tissue extract was identical with synthetic $i^6Ado^{ox\text{-}red}$ PC (4 solvent systems and in GLC R_t [4,5]. Another [^{3}H]-labeled sample has R_f on PC and R_t on GLC similar to oxidized–reduced zeatin riboside.

Based on the recovery and the sp act of the control samples after final purification, the conc of i^6Ado from two separate experiments is 84 and 117 nmol; while zeatin riboside is 432 and 526 nmol/kg of fr tissue, respectively.

Biological significance. i^6Ado and its derivatives are naturally occurring cytokinins which promote cell division and cell differentiation in plant tissues.

Acknowledgement—This work was supported by National Science Foundation Grant No. BMS 72-02067 AO1 to C-M C.

REFERENCES

1. Miller, C. O. (1975) *Proc. Natl Acad. Sci.* **72**, 1883.
2. Skoog, F. and Armstrong, D. J. (1970) *Ann. Rev. Plant Physiol.* **21**, 359.
3. Chen, C. M. and Hall, R. H. (1969) *Phytochemistry* **8**, 1687.
4. Chen, C. M., Hartnell, G. F., Smith, O. C. and Francis, W. S. (1974) *Anal. Biochem.* **60**, 441.
5. Chen, C. M., Smith, O. C. and Hartnell, G. F. (1974) *Can. J. Biochem.* **52**, 1154.

Phytochemistry, 1976, Vol. 15, pp. 1565–1566. Pergamon Press. Printed in England.

γ-HYDROXYHOMOARGININE FROM PEA SEEDLINGS

TERENCE A. SMITH and GRAHAM R. BEST
Long Ashton Research Station, University of Bristol, Long Ashton, Bristol, BS18 9AF, England

Key Word Index—*Pisum sativum*; Leguminosae; pea; γ-hydroxyhomoarginine.

We wish to report the identification of γ-hydroxyhomoarginine in peas. *Threo*-γ-hydroxy-L-homoarginine [1] has previously been found in several *Lathyrus* species where it is formed by hydroxylation of homoarginine [2]. The lower homologue, γ-hydroxyarginine, is known from *Vicia* species [3] and from *Lens culinaris* [4]. Hydroxylysine, an amino acid closely related to hydroxyhomoarginine, has been found in the roots of *Medicago*