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SHORT REPORTS

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

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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.

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γ-HYDROXYHOMOARGININE FROM PEA SEEDLINGS

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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*

sativa (Leguminosae) [5]. The occurrence of hydroxyarginines within the Leguminosae has been reviewed by Bell [6].

EXPERIMENTAL

Pea seedlings were grown in the dark in Petri dishes for 4 days and 200 g homogenised in 5 vol of 5% trichloroacetic acid. After standing for at least 7 days, the extract was centrifuged and the supernatant passed through a column (2 × 3 cm) of Dowex-50 W × 2, 200–400 mesh (H^+ form). The column was washed with H_2O and most of the amino acids were eluted with 1.5 M NH_4OH (50 ml) and discarded. The column was washed with H_2O (75 ml) until free of NH_4OH and the inorganic cations eluted with 0.4 M HCl (40 ml). The amine/lactone fraction was then eluted with 6 M HCl (40 ml) and dried. On dissolving in 0.1 M HCl (2 ml), 10 μl was subjected to TLC on cellulose CC-41 buffered at pH 2 with KCl–HCl, using phenol:pH 2 buffer (5:1) as solvent [7]. A yellow–brown spot suggesting a lactone was detected at R_f 0.56 with ninhydrin as chromogenic reagent. The spot was also Sakaguchi positive, indicating the presence of the guanidino group. On drying the amine fraction, 2 ml of 18 M NH_4OH was added to hydrolyse the lactone. After 18 hr, the soln was diluted with 8 vol H_2O and re-applied to the column (H^+ form). The unknown was eluted in 1.5 M NH_4OH, dried, dissolved in 0.1 M HCl (5 ml) and left for 24 hr to relactonize. By this means the unknown was obtained relatively free of contamination. The properties of the unknown (R_f, guanidino group, lactonization), in agreement with the properties of authentic material, suggested that it is γ-hydroxyhomoarginine. The yield was *ca* 20 mg. Attempts to crystallize the syrupy product obtained by concentrating the final fraction failed, as found previously for γ-hydroxyarginine [4]. This may be due to admixture of di and monohydrochlorides (E. A. Bell, personal communication). MS of the lactone at 220° gave peaks at *m/e* 374, 373, 313, 297 (all less than 1%), 275 (6), 259 (77), 168 (39), 165 (46), 151 (66), 96 (100), 59 (80), indicating dimerization. IR ν_{max}^{KBr} showed 1780 (γ-lactone) 1650, 1410, 1350, 1310, 1220, 1200, 1080, 1000, 970, 780 cm^{-1}. MS and IR of authentic (2 HCl) and extracted material were identical.

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CYANOLIPIDS IN *SAPINDUS EMARGINATUS* SEED OIL

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Key Word Index—*Sapindus emarginatus*: Sapindaceae; cyanolipids; triglycerides; argentation TLC; GLC; MS; fatty acid composition; double bond position.

Four types of cyanolipids, present individually or in pairs, have been identified in the seed lipids of the Boraginaceae [1,2] and some Sapindaceae species [3–8]. In this paper we report the isolation, identification and composition of one such cyanolipid, namely, the fatty acid diester of 1-cyano-2-hydroxymethylprop-l-ene-3-ol, in the oil of *Sapindus emarginatus* Vahl. (a soapnut). The fatty acid composition of the cyanolipid components is also compared with that of triglycerides.

S. emarginatus seed kernels contained 41% oil. On Si gel G TLC, the oil gave two spots (triglyceride, R_f 0.77 and cyanolipid, R_f 0.52) with Et_2O–petrol (40–60°) (PE) but only a single spot with C_6H_6. This TLC behaviour suggested that the cyanolipid is likely to be a diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol [5]. The oil was resolved into a triglyceride fraction (82%) and a cyanolipid fraction (16%) by preparative-TLC. The cyanolipid fraction gave ν_{max} 2220 cm^{-1} and ϵ 18845 at λ_{max} 208 nm. The PMR showed peaks at τ 9.14, 8.75, 8.05, 7.97, 7.67, 5.33, 5.13, 4.7 and 4.45 as also shown by the reference sample of fatty acid diesters of 1-cyano-2-hydroxymethyl-prop-1-ene-3-ol, isolated from *Cardiospermum halicacabum* seed oil [5]. The major M^+ peaks in the MS of the cyanolipids were *m/e* 671 and 669 followed by low intensity peaks corresponding to M^+ of 699, 697, 645, 641 and 615. Prominent peaks belonging to $R–C{\equiv}O^+$, $R–\underset{\underset{O}{\|}}{C}–O^+$, were also obtained. The picrate test was negative, as is the case with the similar cyanolipid of *Koelreuteria paniculata* seed oil [4]. GLC of the Me esters gave the composition reported in Table 1.

The total cyanolipid was separated into three fractions (26.8, 67.7 and 5.5%) by $AgNO_3$ TLC, based on the degree of unsaturation of the fatty acid moieties as shown by the magnitude of peak at τ 4.7. In the MS of the three fractions, the M^+ ions of decreasing intensity were *m/e* 669, 641 and 697; 671, 643, 615 and 699; and 645, 673, 617, 589 and 701. These fractions gave ϵ 16235, 18730 and 20507 at λ_{max} 208 nm, corresponding to the major M^+ peaks in the MS. The fatty acid compositions are recorded in Table 1. A higher proportion of eico-