Phytochemistry, Vol. 21, No. 3, pp. 777–778, 1982.
Printed in Great Britain

0031-9422/82/030777-02$03.00/0

SHORT REPORTS

GUANOSINE 3′,5′-MONOPHOSPHATE IN FRUITS OF *EVODIA RUTAECARPA* AND *E. OFFICINALIS*

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(*Revised received* 13 *July* 1981)

Key Word Index—*Evodia rutaecarpa*; *E. officinalis*; Rutaceae; guanosine 3′,5′-monophosphate; cyclic GMP.

Abstract—Extraordinary high levels of cGMP activity were detected in the fruits of *Evodia rutaecarpa* and *E. officinalis*. The mature fresh fruits contained a cGMP-like substance in concentrations ranging from 10 to 35 mmol/g dry wt, as determined by both a competitive binding assay and radioimmunoassay. The partially purified cGMP-like substance from *E. rutaecarpa* showed the same chromatographic properties (TLC and columns) as authentic cGMP and was decomposed by cyclic nucleotide-specific phosphodiesterase.

While there have been several reports on the presence of adenosine 3′,5′-monophosphate (cAMP) in higher plants [1–3], there are only a few reports to show the temporary appearance of small amounts of guanosine 3′,5′-monophosphate (cGMP) in plants [4, 5]. We found cAMP in *Zizyphus jujuba* [6–8], a plant frequently used in Chinese medicine. In a continuation of these studies, we tested plants used in ancient Chinese medicine formulations for the possible presence of cGMP.

Boiling water extracts of 200 different plants were tested for cGMP activity by a competitive binding assay. Of these, the extracts of the fruits of *Evodia rutaecarpa* were positive. The active substance was partially purified by extraction with water and methanol followed by chromatography on Dowex 1×4 (Cl^-) and alumina (Woelm N. Super I) columns. The partially purified cGMP-like substance had the same TLC properties as authentic cGMP. After incubation with cyclic nucleotide-specific phosphodiesterase at 37° for 60 min, more than 95% of the activity disappeared and 5′-GMP was detected by TLC. On further purification by rechromatography on a Dowex 1×4 (Cl^-) column and TLC, the cGMP-like substance had the same UV and HPLC properties as authentic cGMP (Cyong, J., Takahashi, M. and Hanabusa, K., unpublished). These results indicate that the isolated substance is cGMP.

As measured by the competitive binding assay and radioimmunoassay, the cGMP levels in mature fresh fruits of *E. rutaecarpa* and *E. officinalis* ranged from 10 to 35 nmol/g dry wt. Moreover, both methods usually gave the same value on a given sample. Since boiling water extraction (×3) may not completely remove all the cGMP bound to the plant tissue, this value is semi-quantitative and a conservative estimate of the concentration of cGMP. Nevertheless these values are markedly higher than those reported for animal tissue [9]. Comparative studies on the physiological effects of the cGMP and medical use of this plant are currently in progress.

EXPERIMENTAL

Plant material. E. rutaecarpa and *E. officinalis*, imported from China, were purchased from Uchida Wakanyaku Co., Tokyo, Japan. Fresh samples were obtained from the Tokyo Metropolitan Botanic Garden.

Assay. Samples were diluted serially from 1/10 to 1/1000 with 50 mM acetate buffer, pH 4.0, and assayed for cGMP-like compounds by both the competitive binding assay [10] and radioimmunoassay [11]. Kits for these assays were purchased from Boehringer Mannheim, West Germany, and Yamasa, Japan, respectively.

Extraction and isolation. In a typical isolation, fruits (250 g) of *E. rutaecarpa* were extracted (×3) with boiling H_2O and the filtered extract concd *in vacuo*, then extracted (×3) with MeOH. The extract (100 ml) was concd *in vacuo* and passed through a column of Dowex 1×4 (Cl^-) (3×25 cm). After washing with 1.5 l. H_2O, cGMP activity was eluted with 1.5 l. 0.05 M HCl (each fraction 15 ml). The active fractions (29–49) were bulked, concd (1.05 g), taken up in 5 ml 0.05 M HCO_2NH_4, and then applied to a column of Al_2O_3 (Woelm N. Super I, 2×15 cm). After washing with 500 ml H_2O, the cGMP-like substance was fractionated (each fraction 5 ml) with 500 ml 0.05 M HCO_2NH_4 and the active fractions (28–70) bulked and concd *in vacuo* (110 mg).

Chromatographic comparison. The isolated cGMP-like substance and authentic cGMP had the same chromatographic behaviour in the following systems: cellulose, *t*AmOH–HCO_2H–H_2O (3:2:1) R_f 0.2; Si gel, EtOAc–C_6H_6–MeOH (1:1:3) R_f 0.38; Si gel, *iso*-PrOH–NH_4OH–H_2O (12:7:1) R_f 0.6.

Sensitivity to phosphodiesterase. The isolated cGMP-like substance (50 pmol/ml) was incubated at 37° for 60 min with 5.4 munits of cyclic nucleotide-specific phosphodiesterase (EC 3.1.4.17) (Boehringer) in the presence of 5 mM $MgCl_2$ at

pH 8.6. The reaction was stopped by heating to 100° for 2 min. Almost 95% of the cGMP activity was lost. The formation of 5'-GMP was detected by TLC (Si gel, C_6H_6–EtOAc–MeOH, 1:1:3).

Acknowledgements—This work was supported by a Grant-in-Aid from the Ministry of Health and Welfare, Japan, and also by a donation from Tsumura-juntendo Co. Ltd., Tokyo, Japan.

REFERENCES

1. Lin, P. P.-C. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 439.
2. Brown, E. G., Al-Najafe, T. and Newton, R. P. (1979) *Phytochemistry* **18**, 9.
3. Newton, R. P., Gibbs, N., Moyse, C. D., Wievers, J. L. and Brown, E. G. (1980) *Phytochemistry* **19**, 1909.
4. Haddox, M. K., Stephenson, J. H. and Goldberg, N. D. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 552.
5. Lundeen, C. V., Wood, H. N. and Braun, A. C. (1973) *Differentiation* **1**, 225.
6. Cyong, J., Hanabusa, K. and Otsuka, Y. (1979) *Proc. Wakan-Yaku Symp.* **12**, 1.
7. Cyong, J. and Hanabusa, K. (1980) *Phytochemistry* **19**, 2747.
8. Hanabusa, K., Cyong, J. and Takahashi, M. (1981) *Planta Med.* **42**, 380.
9. Goldberg, N. D., O'Dea, R. F. and Haddox, M. K. (1973) *Adv. Cyclic Nucleotide Res.* **3**, 165.
10. Cyong, J. and Okada, H. (1975) *Methods Immunol.* **4**, 1183.
11. Asakawa, T., Russell, T. R. and Ho, R.-J. (1976) *Biochem. Biophys. Res. Commun.* **68**, 682.

Phytochemistry, Vol. 21, No. 3, pp. 778–780, 1982.
Printed in Great Britain

0031-9422/82/030778-03$03.00/0

ENZYMATIC EXTRACTION AND LINKAGE ANALYSIS OF PECTIC POLYSACCHARIDES FROM ONION

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(*Received* 12 *June* 1981)

Key Word Index—*Allium cepa*; Liliaceae; onion; cell walls; pectic polysaccharides; extraction; methylation analysis.

Abstract—Pectin lyase was superior to polygalacturonase for the extraction of onion cell wall pectic polysaccharides. Exhaustive treatment of onion tissue with pectin lyase solubilized 89% of the total uronides of the tissue. The galacturonides released from the tissue were separated into three fractions (10.7, 5.3 and 84%, in order of MW) by gel filtration on Sephadex G-100. The low MW fraction was a mixture of oligogalacturonides. High and intermediate MW fractions were purified by DEAE-Sephadex column chromatography. The intermediate MW fraction was a rhamnogalacturonan II type component which contained 3- and 3,4-linked rhamnose. Methylation analysis showed that the pectic polysaccharides of onion resembled those of potato tuber.

INTRODUCTION

Homogalacturonans or homogalacturonan regions of pectic polysaccharides in the cell wall contribute to tissue coherence by the ability of their chains to form bundles [1]. If galacturonan chains are greatly modified by insertion of rhamnosyl residues and branching, the cell walls lack cohesion [2]. Darvill *et al.* [3] isolated highly branched and complex pectic polysaccharides (rhamnogalacturonan II, RG-II) from the cell wall of suspension-cultured sycamore cells. Recently Ishii [4] has isolated a RG-II type component from potato tuber cell walls by purified polygalacturonase (PG) treatment of the tissue. It was characterized by the existence of 3- and 3,4-linked rhamnosyl residues.

The cell walls of onion are known to contain pectic polysaccharides [5, 6]. It is of interest to know whether monocotyledonous plants contain RG-II type components in the cell wall.

RESULTS AND DISCUSSION

Pectin lyase (PL) solubilized 62.5% of the total uronides from onion tissue in 6 hr at 30°, while PG only solubilized 18%. Under the same condition 1.3% of the tissue uronides were released without enzyme. It has been demonstrated that susceptibility of plant tissue to enzymatic attack depends largely on enzyme specificity but not on enzyme concentration [7]. Therefore, PL which is specific for methyl-galacturonide linkages is essential for the extraction of