Phytochemistry, Vol. 22, No. 6, pp. 1499–1500, 1983.
Printed in Great Britain.

0031–9422/83/061499–02$03.00/0

SHORT REPORTS

A FREE PURINE NUCLEOTIDE FROM *CICER ARIETINUM*

GEETA HANDA, JAGDEV SINGH*, L. N. NANDI and C. K. ATAL

Regional Research Laboratory, Jammu-Tawi, 180001, India

(*Revised received* 8 *November* 1982)

Key Word Index—*Cicer arietinum*; Leguminosae; theophylline-9-β-D-glucopyranosyl-6′-monophosphate.

Abstract—Theophylline-9-β-D-glucopyranosyl-6′-monophosphate has been isolated and characterized for the first time from a natural source by spectroscopic and chemical methods.

INTRODUCTION

In the course of an investigation of the antihyperlipidemic and stamina building activity of the spray-dried powder of *Cicer arietinum*, we found that the enhanced activity may be due to the presence of an additional compound besides pangamic acid (D-gluconodimethyl aminoacetic acid) reported by us in an earlier communication [1]. We now report, for the first time from a natural source the isolation of a free nucleotide characterized as theophylline-9-β-D-glucopyranosyl-6′-monophosphate. Although theophylline, a diuretic, cardiac stimulant and smooth muscle relaxant, has been reported to occur naturally, no reports are available about its occurrence as a nucleotide or nucleoside, except after synthesis [2].

RESULTS AND DISCUSSION

The nucleotide (B) mp 199–202° (d) was obtained as a dark grey amorphous powder after purification. IR ν_{max}^{KBr} cm^{-1}; 3400–3000 (–OH), 2850–2775, 2650–2400, 1260, 1060 (phosphate), 1690–1680 (>C=O), 1590 (double bond); ^1H NMR (60 MHz, D_2O): δ 2.8 (*s*, N–Me), 3.2 (*s*, N–Me), 7.6 (*s*, H-8); ^{13}C NMR (JEOL, FX90 Q, D_2O): δ 30.2 and 30.8 (2 × N–Me), 158.6 (C-2), 150.4 (C-4), 112.2 (C-5), 154.6 (C-6). 144.0 (C-8) indicated theophylline, and 102.2 (C-1′), 71.6 (C-2′), 72.3 (C-3′), 68.4 (C-4′), 74.2 (C-5′) and 63.2 (C-6′) the glucose moiety which is in accordance with the ^{13}C NMR data reported for theophylline and glucose [3, 4].

Mass spectra data (EI, 70 eV *m/z*) supported its indentity as theophylline-9-β-D-glucopyranosyl-6′-monophosphate although no M^+ peak was obtained. The peaks at *m/z* 181 $[B+2]^+$ (9.75), 243 (*s*) (2.83), 342 $[M-PO_3H]^+$ (9.6) are in agreement for the mass spectrum reported by Bieman [5] for nucleosides. The other prominent peaks appeared at *m/z* 404 $[M-18]^+$ (0.5), 393 $[(0.96)]^+$ M – 29, 319 (0.48), 281 (2.16), 169 (7.89), 131 (24.51), 119 (22.45), 105 (0.74), 100 (7.25), 96 (14.51), 81 (2.04), 69 (65.75), 68 (5.63), 67 (4.33) and 18 (bp 1.000).

The mass spectrum of the TMSi derivative also gave no M^+ peak at *m/z* 782 but the peaks at *m/z* 527 $[M-CH_2(OPOTMSi)_2]^+$ (0.25), 361 (s) (2.5), 180 $[B+1]^+$ (14.0), 181 $[B+2]^+$ (11.2) confirmed it to be theophylline-9-β-D-glucopyranosyl-6′-monophosphate and is in agreement with the mass spectral fragmentation pattern proposed for TMSi derivatives of nucleotides by McCloskey *et al.* [6] and for sugar phosphates by Zinho *et al.* [7]. The glucopyranose TMSi peaks appeared at *m/z* 319 (0.8), 305 (0.8), 272 (15.1), 217 (10.2), 204 (17.6), 169 (1.0), 147 (109.4), 133 (5.3), 132 (2.2), 131 (8.1), 129 (4.4), 117 (5.2), 103 (2.7) and the theophylline [8, 9] fragments, besides the one quoted above, appeared at *m/z* 207 (3.3), 150 (1.9), 123 (4.3), 121 (2.7), 95 (20.5), 94 (0.22), 69 (2.2), 68 (52.5), 67 (3.1) and 28 (1000).

High resolution mass spectrometry further established its identity as theophylline-9-β-D-glucopyranosyl-6′-monophosphate ($C_{13}H_{19}N_4O_{10}P$). The fragments at exact mass spectra 362.9967 ($C_{11}H_{16}N_3O_9P$), 358.0585 ($C_{13}H_{18}M_4O_8$), 242.1717 ($C_{13}H_{18}N_4O_8$), 243.0041 ($C_6H_{15}O_8P$), 206.9954 ($C_8H_7N_4O_3$) coincided with the structure.

Hydrolysis with aq. H_2SO_4 (7%) indicated the presence of theophylline and glucose on PC. The presence of the C-9 glucoside linkage is based on UV λ_{max}^{HCl} (pH 1) 280 nm (theophylline UV $\lambda_{max}^{0.1\,M\,HCl}$ 274 nm) data. The fact that natural purine nucleotides have this linkage gives the added support [10]. Further chemical studies could not be carried out for lack of product.

EXPERIMENTAL

Bengal gram (*Cicer arietinum*, 10 kg) was soaked overnight with H_2O (20 l.) and extracted × 3 with hot H_2O (30, 20 and 20 l.) in an open steam pan. The combined H_2O extract was cooled, strained through fine muslin cloth, concd to *ca* 3 l. in an open steam pan, treated with an equal vol. of EtOH and centrifuged. The mother liquor was adjusted to pH 5 with HNO_3 (10%) and centrifuged. The mother liquor [11, 12] after removing the sediment was treated with $Hg(OAc)_2$ soln in HOAc and centrifuged. The sediment was suspended in EtOH–H_2O (1:1, 2 l.), H_2S passed through, centrifuged and the supernatant adjusted to

*Author to whom correspondence should be addressed.

pH 7 with dilute NH_4OH, treated with $Ba(OH)_2$ and centrifuged. The supernatant was adjusted to pH 7.5 with HOAc and passed through a column (6.6 × 75 cm) of Dowex-1 resin (Cl form, 400 mesh, 200 g) which had previously been washed with H_2O.

The adsorbed material was eluted successively with 2 mM (2.5 l., fractions 1–13), 20 mM (1.2 l., fractions 14–19), 0.2 M (0.6 l., fractions 20–22) and 1M HCl (1.5 l., fractions 23–31) at the rate of 1 ml/min collecting each fraction of *ca* 200 ml, pooling being done on the basis of UV at 280 nm. Each pooled fraction was concd *in vacuo* almost to dryness, dissolved in minimum amount of H_2O (20 ml) and passed over separate columns (2.54 × 40 cm) of activated charcoal (BDH, 18 g) uniformly mixed with Celite (Loba, 2 g). After thorough washing of each column with H_2O, the absorbed material was eluted with EtOH–H_2O (1:1) containing 1% NH_4OH (eluates: fractions 1–13, 300 ml; 14–19, 200 ml; 20–22, 100 ml; and 23–31, 200 ml). Each eluate was adjusted to pH 5 with HOAc and evaporated to dryness *in vacuo*. The fractions were marked as: (A) 1–13: 10 mg, dark brown resinous; (B) 14–19: 70 mg, dark grey amorphous powder; (C) 20–22: 8 mg, dark amorphous powder; and (D) 23–31: 30 mg white crystalline powder containing inorganic material. All samples had an identical UV λ_{max}^{HCl} pH 1, 280 nm. Sample (B) was the purest; UV λ_{max}^{HCl} pH 1, 280 nm $[\alpha]_D^{40}$ −56.64°, (H_2O; *c* 5.3). (A)–(D) had identical UV, NMR and MS and of their TMSi derivatives.

REFERENCES

1. Singh, J., Handa, G., Rao, P. R. and Atal, C. K. (1983) *J. Ethno-Pharmacol* **14** (in press).
2. Sandanori, I., Akira, M., Toru, S. and Tetsuo, S. (1971) *Chem. Abstr.* **75**, 20917f.
3. Nicolai, C. and Hilderbrand, K (1974), *Z. Natuforsch. Teil C* 476.
4. Herz, W., Grisebach, H. and Kirby, G. W. (eds.) (1979) in *Progress in the Chemistry of Organic Natural Products.* Vol. 36, p. 168. Springer, Berlin.
5. Bieman, K (1962) *J. Am. Chem. Soc.* **84**, 2005.
6. McCloskey, J. A., Lamson, A. M., Tsuboyama, K., Kruger, P. M. and Stiliwel, R. N. (1968) *J. Am. Chem. Soc.* **90**, 4182.
7. Zinho, M., William, R. and Sherman, R., (1970) *J. Am. Chem. Soc.* **92**, 2105.
8. Spiteller, G. and Spiteller, M. (1962) *Friedman, Montash* **93**, 634.
9. Budzikiewicz, H. Djerassi, C. and William, D. (1964) in *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. 1, p. 214. Holden Day, New York.
10. Falconer, R., Gullard, J. M. and Leonard, F. (1939) *J. Chem. Soc.* 1784.
11. Baddiley, J. and Mathias, A. P. (1954) *J. Chem. Soc.* 2723.
12. Cabib, E., Leloir, L. F. and Cardini, C. E. (1953) *J. Biol. Chem.* **196**, 717.

Phytochemistry, Vol. 22, No. 6, pp. 1500–1503, 1983.
Printed in Great Britain.

0031-9422/83/061500-04$03.00/0

ARABINOGALACTAN-PROTEINS FROM DOUGLAS FIR AND LOBLOLLY PINE

JOHN F. BOBALEK and MORRIS A. JOHNSON

The Institute of Paper Chemistry, P.O. Box 1039, Appleton, WI 54912, U.S.A.

(*Received* 28 *August* 1982)

Key Word Index—*Pseudotsuga menziesii; Pinus taeda*; Pinaceae; Douglas fir; loblolly pine; arabinogalactan-proteins; β-lectins; chemotaxonomy; structural variation during development.

Abstract—Arabinogalactan-proteins, identified as β-lectins by their precipitation with β-glucosyl Yariv antigen, were isolated from the dry seeds, stratified seeds, 2-week-old seedlings, 2-month-old seedlings, 2-year-old saplings, and 1-month-old callus of loblolly pine and Douglas fir. Two-way analyses of variance revealed that certain β-lectin parameters differed significantly from one another depending on the species and developmental state of a sample's origin.

INTRODUCTION

The β-lectins [1] are a class of arabinogalactan-proteins (AGPs) which precipitate with β-glycosyl Yariv antigen [1,3,5-tri-(*p*-β-D-glycosyloxyphenylazo)-2,4,6-trihydroxybenzene]. The function of these AGPs remains unexplained, although their distribution through virtually all phyla of green land plants [1, 2] implies that they may have some fundamental role in plant physiology. It has been suggested that the β-lectins may be cell-surface components involved in the expression of identity of individual plants, tissues, or cell types [3]. This paper describes the first statistically designed study to evaluate compositional variations in the β-lectins in various tissues of *Pseudotsuga menziesii* and *Pinus taeda* (Douglas fir, D, and loblolly pine, L, respectively), with the goal of determining their potential as developmental or chemotaxonomic markers.