Isolation and characterization of a new glucopyranosyl derivative of 6-(3-methyl-2-butenylamino)purine from sweet potato tubers

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Received 14 May 1982

Cytokinin *Glucosyl cytokinin* Ipomea batalas L. *9-β-D-glucopyranosyl/6-chloropurine* *9-β-D-glucopyranosyl/6-(3-methyl-2-butenylamino)* *Puriné* *9-β-D-ribosyl* cis-*zeatin*

1. INTRODUCTION

As a part of a continuing study of the isolation and characterization of cytokinins, we reported the isolation and identification of *cis*-zR from sweet potato tubers (*Ipomoea batatas* L.) [1]. We have now found, using HPLC and the *Amaranthus* betacyanin bioassay, that in addition to *cis*-zR several other cytokinins are present in the extract of the tubers. We report here the isolation and characterization of a new glucopyranosyl derivative of i^6Ade from sweet potato tubers.

2. MATERIALS AND METHODS

2.1. *Plant materials*

Sweet potato (*Ipomoea batatas* L. cv. Kohkei No. 14) tubers (3.75 kg) were used.

2.2. *Purification of cytokinins from the tubers*

Procedures similar to those in [1] were employed.

2.3. *HPLC and GC/MS*

The instruments and analytical methods used in this study were similar to those in [1].

Abbreviations: *cis*-zR, 9-β-D-ribosyl *cis*-zeatin; i^6Ade, 6-(3-methyl-2-butenylamino)purine; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; GC/MS, gas chromatography–mass spectrometry; TMS, trimethylsilyl; *m/z*, mass-to-charge ratio; M, molecular ion

2.4. *Synthesis of 9-β-D-glucopyranosyl 6-(3-methyl-2-butenylamino)purine*

This compound was synthesized from 3-methyl-2-butenylamine hydrochloride (10 mg, 0.1 mmol) and 9-β-D-glucopyranosyl 6-chloropurine (16 mg, 0.05 mmol, m.p. 187–192°C) as described below.

A mixture of the two compounds in *n*-butanol (5 ml) was refluxed or 2 h in the presence of triethylamine (0.1 ml, 0.07 mmol). The residue obtained after rotary evaporation of *n*-butanol was dissolved in a few ml ethanol and loaded on a silica gel colum (25 × 0.7 cm i.d., Merck, 70-230 mesh); the column was eluted with ethyl acetate containing 20% ethanol. Fractions (15 ml/tube) of the effluent were collected by an automatic fraction collector and each fraction was examined for UV-absorbing constituents by TLC (Merck silica gel plate GF254; solvent, ethylacetate–ethanol (3:1, v/v). The fractions containing 9-β-D-glucopyranosyl 6-(3-methyl-2-butenylamino)purine (R_F 0.3) were combined and, after removal of the solvents, yielded an oil (75% yield based on 9-glucopyranosyl 6-chloropurine), which was crystallized from an aqueous ethanol solution. The crystals with an m.p. of 224–226°C had the following spectral properties: UV_{max} in 80% ethanol 269 nm (ε 24 400); MS *m/z* 365 (M), 322 (M-43), 297 (glucosyl adenine), 232 (B + 30), 203 (B + H), 188, 160 (B-43), 135 (adenine).

9-β-D-Glucopyranosyl 6-chloropurine was prepared by fusion reaction of 6-chloropurine with penta-*O*-acetyl-D-glucopyranose. Coupling was effected by addition of *bis*-(*p*-nitrophenyl) hydrogen phosphate [2] to a melt of the above compounds, followed by heating at 155°C for 20 min at 15 mm pressure. The reaction product was dissolved in

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

chloroform and, after filtration, the chloroform solution was subjected to silica gel chromatography (40 cm × 2.5 cm i.d.; solvent, ethyl-acetate–benzene (2:1, v/v). The fractions containing 9-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-glucopyranosyl 6-chloropurine as determined by TLC were combined. After removal of the solvents, the compound was deacetylated in methanolic ammonia at 1°C overnight. Upon removal of the solvent a viscous material was obtained. Crystallization from ethanol yielded the desired compound: m.p. 191–195°C; UV_{max} in 95% ethanol 266 nm (sh 250 nm, ε 8000); MS *m/z* 319 (M), 183, 154 (6-chloropurine), 119 (purine); ^{1}H-NMR δ 8.83 (s, 1H, purine 2-H), 8.80 (s, 1H, purine 8-H), 5.62 (d, 1H, J = 9 Hz, ribose 1′-H). The overall yield was 9%.

3. RESULTS AND DISCUSSION

The HPLC elution profile of the cytokinin-containing fractions of the material extracted from sweet potato tubers is presented in fig.1. Eight UV-absorbing peaks (fraction A–H) were obtained as shown in the figure, and the cytokinin activity of each fraction was examined at various concentrations (0.1–0.0001%) after removal of the solvent. Four fractions (C–F) exhibited cytokinin activity

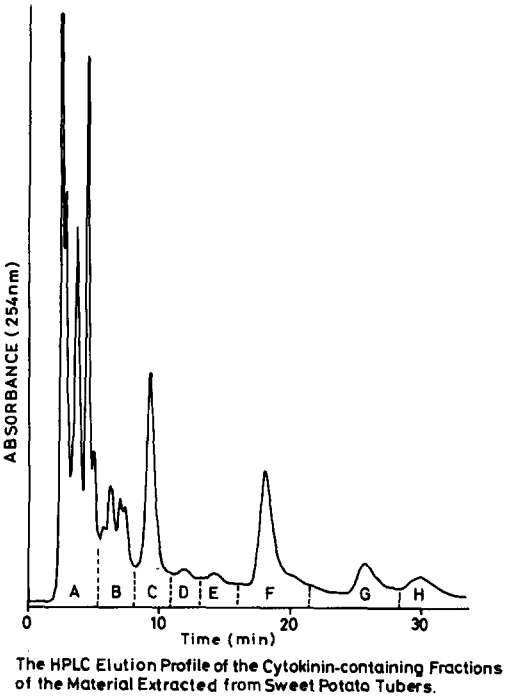

Fig.1. The HPLC elution profile of the cytokinin-containing fractions of the material extracted from sweet potato tubes.

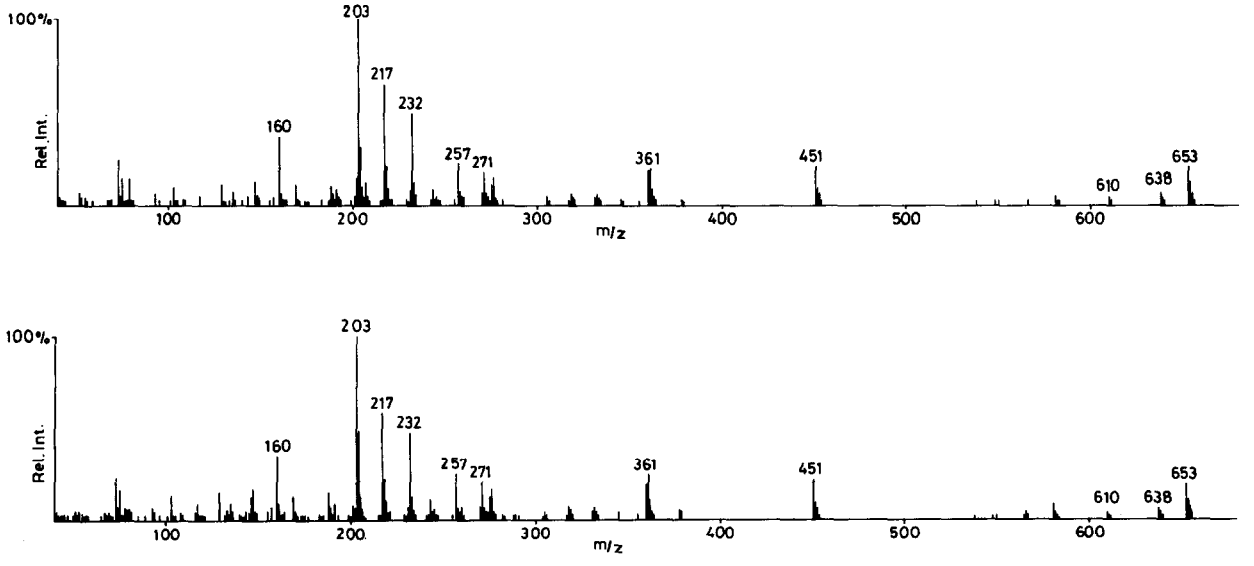

Fig.2. Mass spectra of TMS derivatives of synthetic (upper) and isolated (bottom) 9-β-D-glucopyranosyl 6-(3-methyl-2-butenylamino)purines at an ionizing energy of 20 eV.

using the *Amaranthus* betacyanin bioassay [3]. Fraction D was identified as *cis*-zR on the basis of retention times on HPLC and GC, and the similarity of the mass spectrum with that in [1]. Here, the characterization of fraction F is described.

Removal of solvent from fraction F resulted in a trace of semi-solid residue. The residue was dried at 90°C for 20 min in vacuo and silylated by heating for 1 h at 90°C in anhydrous pyridine, hexamethyldisilazane and trimethyl chlorosilane. The low resolution mass spectrum was obtained using a Shimadzu 9000 instrument (20 eV, ion source 270°C), with samples introduced by a gas chromatograph (1.5% OV-1). The mass spectrum of the TMS derivative of F indicates that the ion at m/z 653 is the molecular ion (relative intensity 19.7%), which is easily recognized by the accompanying M-CH_3 ion (m/z 638, relative intensity 6.3%). In TMS derivatives of carbohydrates and related compounds, loss of a methyl radical from the TMS group generate the M-CH_3 ion, whose mass is used to corroborate the M_r-value [4]. The ion at m/z 610 appears to be M-C_3H_7 ion which suggests the presence of the N^6-isopentenyl group on the adenine ring. The ions at m/z 319, 217, 204, 169, 147, 129, 103 and 75 are the well-documented ion species characteristic of TMS derivatives of carbohydrates and related compounds [5,6].

In addition, the presence of the ions at m/z 451, 361, 271 suggests that compound F is per-trimethylated 9-glucosyl purine [7]. Also, the presence of ions m/z 653 (M), 638 (M-CH_3), 610 (M-C_3H_7) suggests the presence of N^6-isopentenyl adenine [7].

To obtain information on the ring size of the glucose, the relative intensities of the ions at m/z 204 and 205, which have the greatest diagnostic value for assignment of sugar ring size [7], were compared. Observations that the ion at m/z 204 is more intense (relative intensity 49.4%) than m/z 205 (12.5%), and that the ion at m/z 202 (B) is more intense than m/z 203 (B + H) indicated that the glucose residue has a pyran ring. Thus, close examination of the mass spectrum suggested that compound F is 9-glucopyranosyl 6-(3-methyl-2-butenylamino)purine.

Therefore, we synthesized the compound as above, and compared the mass spectra, retention times on GC and HPLC, and UV spectra with those of the naturally-occurring compound. These physical parameters were identical for natural and synthetic glucopyranosyl derivatives. Thus, compound F has been identified as 9-β-D-glucopyranosyl 6-(3- methyl-2-butenylamino)purine.

This new cytokinin constitutes the first 9-glucosyl derivative of i^6Ade isolated from a natural source, although the corresponding ribofuranosyl derivative is ubiquitous in plant species and is found in a variety of natural sources as a constituents of Ser- or Tyr-tRNA, as well as in the free state [8].

Several glucosyl cytokinins including *O*-β-D-glucosyl dihydrozeatin [9], *O*-β-D-glucosyl zeatin [10,11], 7-β-D-glucopyranosyl zeatin [12,13], 9-β-D-ribofuranosyl *O*-β-D-glucosyl zeatin [10,11] have been identified from a variety of plants. However, these compounds are all derivatives of zeatin, either *O*-glucosides or 7-glucosyl derivatives. The level of these cytokinins *in vivo* appears to be under phytochrome control [14].

Besides, several articles have reported the presence of glucose-containing cytokinins and have shown that glucose derivatives are formed as primary metabolites from cytokinins fed to plant tissues [15]. In [16,17] i^6Ade was converted to the 7-glucosyl derivative in tobacco-cell suspension cultures; it was suggested that cleavage of the isopentenyl side chain was protected by the presence of the glycosidic linkage. The new 9-glucosyl derivative exhibited cytokinin activity in the *Amaranthus* bioassay, although the activity was ~ 1‰ of that of N^6-benzyladenine on a molar basis. The exact role of 9-glucosyl cytokinin in plants remains to be determined. It can be readily transported in plant tissue, as shown in [18] using synthetic 6-benzylamino-9-glucofuranosyl purine. This glucosyl cytokinin can also serve as a storage form which can be activated when needed [17,19,20].

ACKNOWLEDGEMENTS

T.H. acknowledges support from the Japanese Ministry of Education and Culture (Specified Project Research Grant). We are also grateful to Professor N. Watanabe of the University Farm for supplying the sweet potato tubers.

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