

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

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Cytokinin	Glucosyl cytokinin	<i>Ipomea batatas</i> L.	9- β -D-glucopyranosyl/6-chloropurine
9- β -D-glucopyranosyl/6-(3-methyl-2-butenylamino)		Purine	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^6 Ade 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].

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 for 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 column (25 $\times$ 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

Abbreviations: *cis*-zR, 9- β -D-ribosyl *cis*-zeatin; i^6 Ade, 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

chloroform and, after filtration, the chloroform solution was subjected to silica gel chromatography (40 cm $\times$ 2.5 cm i.d.; solvent, ethylacetate–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); ¹H-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

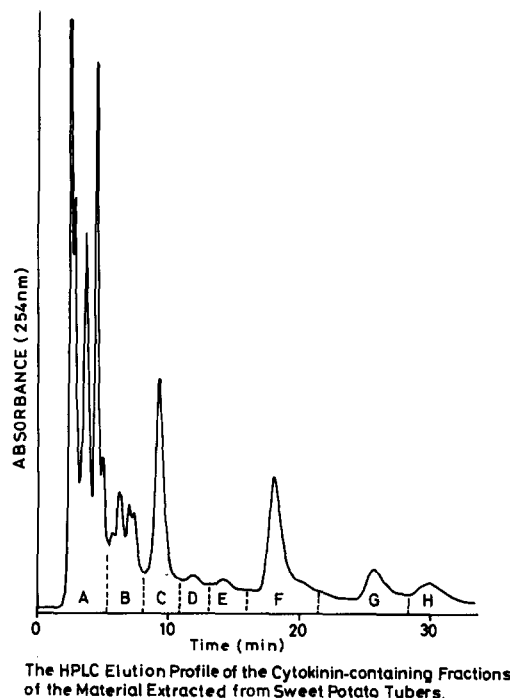


Fig.1. The HPLC elution profile of the cytokinin-containing fractions of the material extracted from sweet potato tubes.

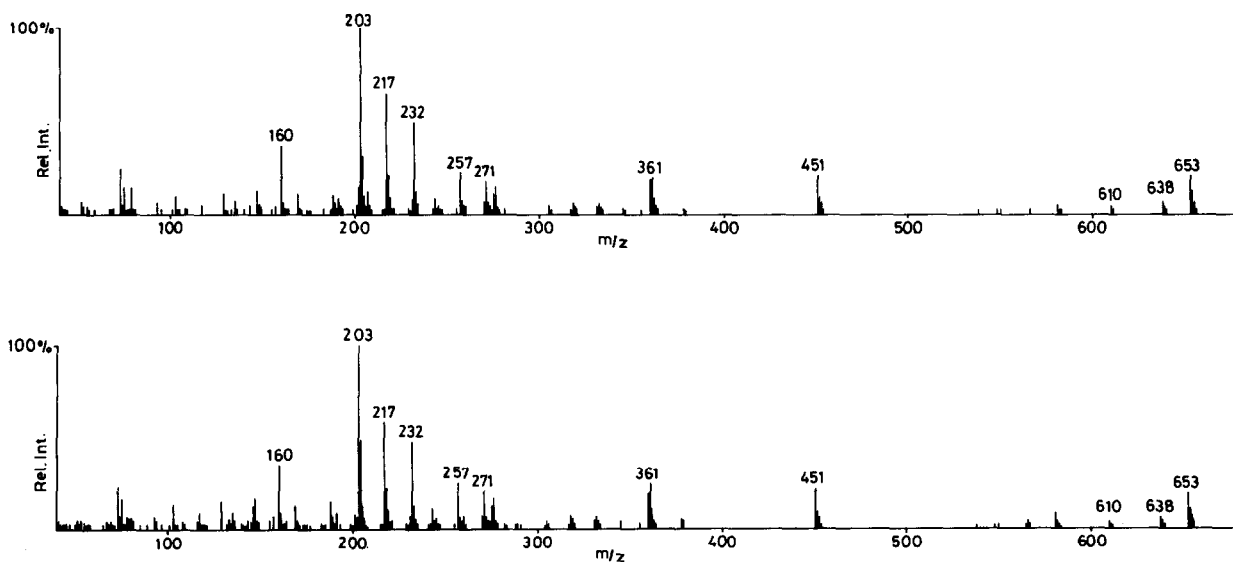


Fig.2. Mass spectra of TMS derivatives of synthetic (upper) and isolated (bottom) 9- β -D-glucopyranosyl 6-(3-methyl-2-butenylamino)purines at an ionizing energy of 20 eV.

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^6 Ade 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^6 Ade 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 $\sim 1\%$ 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].

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