Short Communication

Structure of a Tuber-inducing Stimulus from Potato Leaves (*Solanum tuberosum* L.)

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Several workers have postulated the existence of a specific tuber-inducing stimulus in potato leaves. Grafting experiments brought forward evidence for the existence of the substance,^{1,2)} which was formed in the leaves under proper conditions and translocated to the stolon tip. Recently, we have confirmed the occurrence of a tuber-inducing stimulus in potato leaves using the bioassay of a potato single-node stem segment culture,³⁾ and isolated the active substance (**1**, 2.7 mg) from potato leaves (100 kg).⁴⁾ This substance (**1**) showed tuber-inducing activity *in vitro* at a concentration of 0.01 mg/l ($c \ 3 \times 10^{-8}$ M). In this paper, we report the structural elucidation of this compound (**1**).

Field desorption mass spectrometry (FD-MS) of the active substance (**1**) showed a molecular ion peak at m/z 388. The peak at m/z 163 suggested **1** to be a glycoside. Acetylation of **1** by acetic anhydride-pyridine resulted in an acetyl derivative (**2**). A molecular ion peak in FD-MS was shown at m/z 556, indicating that **2** was a tetraacetate derivative, and that **1** was a monoglycoside. Acid treatment (2 M HCl, 110°C, 30 min) of **1** gave glucose, which was recognized by GC analysis (2% OV-1 on Gasport-H) after silylation with TMS-1-C reagent. The silyl derivative (**3**) showed the same retention time as that of authen-

tic glucose. The presence of a β -glucose moiety was revealed by the ¹H-NMR spectrum (δ 4.26, $J=8.0$ Hz) and ¹³C-NMR spectrum (δ 104.4). In addition to these signals, those due to a glucose moiety were observed: ¹H-NMR spectrum (270 MHz, CD₃OD), δ 3.16 (C₂-H), 3.26 (C₄''-H and C₅''-H), 3.35 (C₃''-H), 3.68 (C₆''-Ha) and 3.88 (C₆''-Hb); ¹³C NMR spectrum (67.9 MHz, CD₃OD), δ 63.1 (C₆''), 72.0 (C₄''), 75.4 (C₂''), 78.2 (C₃''), 78.4 (C₅'').

The ¹H NMR spectrum gave 16 protons due to the aglycone (**4**): δ 1.88 (1H, m), 2.00~2.05 (1H, m), 2.05~2.20 (2H, m), 2.24 (2H, m), 2.3~2.35 (1H, m), 2.35~2.45 (4H, m), 2.80 (1H, m), 3.55 (1H, m), 3.86 (1H, m) and 5.47 (2H, m). The ¹³C NMR spectrum gave eight carbons due to the aglycone (**4**): $5 \times -CH_2-$ (δ 24.5, 27.0, 29.1, 36.5 and 70.5), $1 \times -CH$ (δ 54.4), $-HC=CH-$ (δ 128.6 and 130.2). The ¹H-NMR COSY spectrum (270 MHz, CD₃OD) indicated that the signal at δ 5.47 (2H) assigned to two *cis*-olefinic protons was coupled with the protons at δ 2.35~2.45, 2.30~2.35 and 2.05~2.20. The two signals at δ 3.86 (1H) and 3.55 (1H) were assigned to a methylene group next to the oxygen, they being coupled with each other and the protons at δ 2.35~2.45. The signal at δ 2.30~2.35 was coupled with the proton at δ 2.35~2.45. This led to the partial structure I (Fig. 1). Another coupling was observed between δ 1.88, 2.00~2.05 and 2.24, which led to partial structure II. HOHAHA experiments (500 MHz, CD₃OD) for **1** showed partial structure III, and combined I and II with III.

The result of high-resolution mass spectrometry (HR-MS) of **2** indicated the molecular formula to be C₂₆H₃₆O₁₃ (M⁺, MW=556.2093). The molecular formula of **1** must have been C₁₈H₂₈O₉ (MW 388). The fragmentation ion at m/z 415 (C₁₉H₂₇O₁₀, M⁺ - C₇H₉O₃) suggested that glucose was connected to the oxygen of partial structure I.

During the isolation procedure, **1** was absorbed by a Dowex 1 $\times$ 4 [AcO⁻] column and eluted by 2 M acetic acid. The dehydration ion

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Fig. 1. Partial Structures I, II and III.

peak of 2 was observed at m/z 538 ($C_{26}H_{34}O_{12}$), and it is reasonable to assign at least one carboxyl group for the aglycone (4). The molecular formula, $C_{18}H_{28}O_9$, indicates that the remaining group of the aglycone (4) was $-CO-$. Considering the chemical shifts of a possible structure made up of partial structures I, II and III, $-COOH$ and $-CO-$, the structure of the active substance (1) was finally determined to be 3-oxo-2-(5'- β -D-glucopyranosyloxy-2'-z-pentenyl)-cyclopentane-1-acetic acid. The aglycone (4) was named as tuberonic acid. A phenylalanine derivative of 4 has been isolated from *Praxelis clematidea*.⁵⁾

The tuber inducing activity tended to be weakened during the isolation procedure, and the isolated active substance (1) readily changed to another inactive compound. This means that two substituents on the cyclopentanone were in the *cis* orientation and epimerization at the carbon next to the ketone group occurred to form a stable *trans*-isomer.

The tuber-inducing activity of five related compounds was tested by the bioassay of a potato single-node stem segment culture.^{3,4)} Jasmonic acid (5),^{6,7)} its methyl ester (6)⁷⁻¹⁰⁾ and cucurbic acid (7)¹¹⁾ showed the tuber-inducing activity at a concentration of 10^{-5} M (Fig. 2).

1. R = β -D-glucose
 2. R = β -D-tetraacetyl glucose
 4. R = H (tuberonic acid)
- Chemical structure

Compounds	Activity
	Jasmonic acid (5) +
	Methyl jasmonate (6) +
	Cucurbic acid (7) +
	-
	-

Fig. 2. Tuber-inducing Activity of the Related Compounds (racemic mixture, 10^{-5} M).

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