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TUBERONIC (12-OH-JASMONIC) ACID GLUCOSIDE AND ITS METHYL ESTER IN POTATO

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; determination, 3-oxo-2(5′-β-D-glucopyranosyloxy-2′-*z*-pentenyl)-cyclopentane-1-acetic acid; 3-oxo-2(5′-β-D-glucopyranosyloxy-2′-*z*-pentenyl)-cyclopentane-1-acetic acid methyl ester.

Abstract—Tuberonic acid glucoside and tuberonic acid glucoside Me ester were detected in the leaves of *Solanum tuberosum*. This is the first report of the isolation of the latter from potatoes. Higher concentrations of both substances were detected in leaves of plants grown under long than under short days. Methylation of tuberonic acid glucoside prior to GC-mass spectrometry increased the threshold for detection 12–50-fold, depending upon whether Ac or TMSi derivatives were made. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The biochemical nature of the stimulus that induces potato tuberization has been studied extensively [1]. The stimulus is graft transmissible and is widely presumed to be hormonal in nature. Many hormones have been proposed as components of the stimulus [1], the most recent of which is a compound related to jasmonic acid [2]. The active compound was identified as 3-oxo-2(5′-β-D-glucopyranosyloxy-2′-*z*-pentenyl)-cyclopentane-1-acetic acid, the glucoside of 12-OH-jasmonic acid [2]. The aglycone was named 'tuberonic acid'. The glucoside of tuberonic acid (**1**) in concentrations as low as 3×10^{-8} M added to the agar medium stimulates potato tuberization *in vitro* [3].

We report here the isolation of tuberonic acid glucoside Me ester (**2**) and describe methods for the detection of **1** and **2** in potato leaves. We also compare the GC-mass spectra of **3**, **4**, **5**, and **6**, four derivatives of **1**. The resulting information was used to measure **1** and **2** levels in leaves of tuberizing and non-tuberizing potato plants. This is the first report of **2** in potatoes.

RESULTS AND DIFFUSSION

Preliminary purification

Partial purification of leaf extracts prior to HPLC and GC-mass spectra was based initially upon a published procedure [4] that called for the use of charcoal columns and C_{18} reversed-phase cartridge-columns. Inconsistency in results led us to examine recovery of an authentic sample of **1** from the charcoal and C_{18} columns. The variability in recovery of **1** from the charcoal column was very high, whereas the C_{18} column gave recovery ranging from 89 to 95%. In limited testing we recovered no **2** from the charcoal column, and 93% of an authentic sample of **2** from the C_{18} column. Because there was no problem in detecting either compound in plant samples when the charcoal column was omitted, we concluded that it is better to use only the C_{18} column.

GC-mass spectrometry with standards

When authentic samples of **3–6** were compared by GC-mass spectrometry, the highest total ion current peak was detected for compound **6**, which was 4-fold higher than that of **4**. Compounds **3** and **5** gave peaks 50-fold lower than **6** (Table 1).

A problem in detecting derivatives of **1** and **2** by GC-mass spectra was that the most abundant ion fragments came from the Glc moiety. These fragments included those at *m/z* 98, 109, 115, 127, 169, 271, and 331 [5] from **3** and **4**; and ion fragments at *m/z* 103, 117, 129, 133, 147, 191, 204, 205, and 217 [6] from **5** and **6** (Table 1). The HPLC fractions of **1** and **2** from potato tissue extracts contain other glucosides, which may interfere with the detection on GC-mass spectra of ion fragments from **3**, **4**, **5**, and **6**. Therefore more specific ion fragments are needed for compound confirmation.

The molecular ions $[M]^+$ of all compounds were small (≤1% in relative values, Table 1), but in terms of

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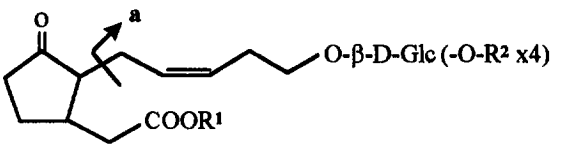

1 R^1=H, R^2=H (TAG)

2 R^1=Me, R^2=H (TAG-Me)

3 R^1=H, R^2=Ac (TAG-Ac)

4 R^1=Me, R^2=Ac (TAG-Me-Ac)

5 R^1=H, R^2=TMSi (TAG-TMSi)

6 R^1=Me, R^2=TMSi (TAG-Me-TMSi)

Fig. 1. Structure of tuberonic acid glucoside (TAG) and its derivatives.

absolute values they were 20- to 30-fold higher for methylated (**4**, **6**) than for non-methylated compounds (**3**, **5**). The $[M-H_2O]^+$ ion fragment was detected only for **3** and **4** (Table 1). Fragment a (Fig. 1) was observed in all four treatments (Table 1). This ion fragment was relatively high in **3**, but in absolute terms it was three times more abundant in **4**, so that sensitivity was greatly increased by the methylation of **1** prior to GC-mass spectral analysis. The total ion current peak in the scan mode for **6** was higher than for **4**, but the difference was associated only with the Glc moiety ion fragments; the detection of ion fragments not originating from the Glc moiety was unchanged (Table 1). Thus although we did not examine the detection limits of the derivatives, our data indicate that Ac (**4**) and TMSi (**6**) derivatives are equally effective for detection of **1** (after methylation) and **2** by GC-MS.

Tissue analyses

Based upon these results, potato leaves were collected for analysis from plants that represented various levels of induction to tuberize. One cultivar was Norchip, which tuberizes even under long days (LD), although tuberization is generally stronger under short days (SD); the other was LT-1, which requires SD for tuberization [7]. Each cultivar was grown under LD and SD. MeOH leaf extracts were passed over HPLC, and fractions containing **1** were methylated prior to making the Ac derivatives. (Fractions containing **2** were not analysed.)

In a second replication of the leaf analysis, EtOH rather than MeOH was used for extractions to be sure that **2** was not an artifact produced from **1** by spontaneous methylation. Even with this precaution **2** was detected, and there was no evidence that the amount of **1** was affected by the extraction procedure (Table 2); but MeOH had the advantage of extracting less pigment. Further experiments (not shown) in which authentic samples of **1** were stored in MeOH and then assayed for the presence of **2** indicated that if spontaneous methylation occurs, the conversion is far too slow to present any problem in terms of using MeOH for tissue extractions.

Table 1. Mass spectra, R_t, and relative peak size for compounds **3**–**6**. Fragment a is depicted in Fig. 1. Peak sizes are expressed relative to TAG-Me-TMSi, and represent the sum of all ion fragments detected by the scan mode in the range from 90 to 700 *m/z*

Compound	GC-MS (*m/z*, rel. int.)	R_t	Relative peak size
TAG-Ac (**3**)	169 (100), 109 (61), 331 (44), 127 (24), 98, (23), 415 (a, 19), 115 (17), 149 (16), 271 (12), 538 $[M-H_2O]^+$ (4), 556 $[M]^+$ (<1)	3287	2%
TAG-Me-Ac (**4**)	169 (100), 109 (39), 331 (38), 149 (22), 271 (11), 127 (10), 98 (5), 115 (5), 415 (a, 5), 552 $[M-H_2O]^+$ (1), 570 $[M]^+$ (<1)	3275	24%
TAG-TMSi (**5**)	204 (100), 217 (22), 147 (21), 205 (18), 341 (18), 129 (11), 133 (7), 191 (7), 361 (3), 117 (2), 535 (a, 1)	3193	2%
TAG-Me-TMSi (**6**)	204 (100), 205 (21), 217 (16), 341 (16), 147 (14), 129 (7), 103 (6), 117 (5), 191 (5), 361 (5), 133 (4), 535 (a, 1), 690 $[M]^+$ (<1)	3202	100%

Table 2. Levels of TAG (**1**) and TAG-Me (**2**) in potato leaves as affected by cultivar and photoperiod (SD and LD, 10 hr and 16 hr photoperiod, respectively). Values of **1** were determined after extraction with MeOH and EtOH; values for **2** were determined only after EtOH extraction

Cultivar	Photoperiod	TAG (**1**) MeOH extraction (ng g^{-1} fr. wt)	TAG (**1**) EtOH extraction (ng g^{-1} fr. wt)	TAG-Me (**2**) EtOH extraction (ng g^{-1} fr. wt)
LT-1	LD	503	531	6
LT-1	SD	206	150	2
Norchip	LD	529	432	26
Norchip	SD	289	203	10

In as much as 11-OH-JA was found in *Solanum demissum* leaves [8], it may be asked whether the glucoside of this compound might be confused with **1**. This is difficult to predict, since an authentic sample of the glucoside of 11-OH-JA is not available to us. However, we think it unlikely that the two compounds would be identical in terms of HPLC retention time (R_t) and ion fragmentation. If the two compounds are different in respect to any one of these, then the presence of the glucoside of 11-OH-JA should not cause a problem in the detection or quantification of **1** by our method.

Levels of **1** were 2- to 3-fold higher in LD than in SD leaves (Table 2). No differences were observed between the two cultivars. The concentrations of **2** were 15- to 100-fold lower than **1** (Table 2), with the highest level of **2** detected in Norchip growing under LD. As was found for **1**, SD conditions appeared to reduce the level of **2** in both cultivars (Table 2).

The difference between LD and SD in **1** and **2** concentrations was the reverse of what was anticipated based upon evidence that both compounds promote *in vitro* tuberization [3, 9]. We can only speculate as to the explanation for this apparent anomaly. It has been suggested [10] that the effect of SD is to increase the transport of the tuberization stimulus out of the leaves, conceivably lowering the amount of the active compound remaining there. Or perhaps the active form of the hormone is the aglycone, and the glucoside is only a storage product or the transport form. In this connection we are preparing to examine levels of the aglycones as affected by photoperiod. In *Solanum demissum* free 11-OH-jasmonic acid and 12-OH-jasmonic acid were found only in SD, not in LD, treatments [8].

Although **2** was detected in Jerusalem artichoke through the use of potato bioassays [4, 9], ours is the first report of its occurrence in potatoes. Confirmation of its presence in potato was obtained by FD-mass spectrometry and FAB-mass spectrometry. After preliminary purification and repeated HPLC, *ca* 4 μg of oily pure compound was obtained from 184 g (fresh weight) of 'Norchip' leaves. FD-mass spectra of this compound gave ion peaks of *m/z* 403, 425, and 441, respectively, for $(M+H)^+$, $(M+Na)^+$, and $(M+K)^+$. FAB-mass spectra of the compound gave $(M+H)^+$ *m/z* 403 in addition to 387, 371, 345, 329, 241, 223, 193, 177, and 155. These mass spectral data are in agreement with data for **2** isolated from Jerusalem artichoke [11].

Our preliminary results (unpublished) also show the presence of **1** in potato tubers. If the ratio of **1** to **2** concentrations in tubers was similar to what we observed in leaves, the level of **2** would have been too low to detect in the sample size examined (5 g fresh weight).

EXPERIMENTAL

Plant growing conditions. Plantlets of *S. tuberosum* L. cvs Norchip and LT-1 were transplanted from *in vitro* culture to the glasshouse on 9 June and harvested 6 weeks later. For 10 days before harvest, black shade cloth was drawn on all treatments to give 10 hr of full light. The SD treatment did not receive supplemental light; the LD treatment received dim incandescent light, 3 hr before and 3 hr after the 10 hr of full light.

TAG standard detection by GC-MS. Authentic sample of **1** was treated in four different ways prior to GC-MS analysis. Half of the sample was methylated to form **2**. Methylation was carried out by the addition of 1 ml freshly prepared CH_2N_2 in Et_2O to a soln of the sample in 200 μl of MeOH. Samples were left at room temp for 30 min, and the methylation was repeated. The solvent and the excess reagent were removed by a stream of N_2. Half of the **2** and half of the remaining **1** were acetylated at 100° for 3 hr with 5 μl Ac_2O and 5 μl dry pyridine, forming **3** and **4**, respectively. The other frs of **1** and **2** were dried overnight *in vacuo* and treated for 1 hr at 60° with 5 μl pyridine and 5 μl of a mixt. of bis(trimethylsilyl)trifluoroacetamide + 1% trimethyl chlorosilane to make TMSi derivatives (**5**, **6**). All four compounds (**3**, **4**, **5**, **6**) were separately injected without splitting onto a 25 m × 0.2 mm HP1 (methyl silicone) bonded phase silica capillary column. The GC-MS analyses were done with an HP 5890A gas chromatograph connected with a 5970B Mass Selective Detector. The first injection of each sample was analysed in the scan mode, and the second injection in the selected ion monitoring (SIM) mode to confirm ion fragmentation. Paraffin mixtures were run twice with the same temp program to estimate R_t. The following program was used: injection port, 250°; transfer line, 300°; He mean linear velocity, 30 cm sec^{-1}. The oven temp was kept at 105° for 1 min, and increased at 30° min^{-1} to 230°, then at 4° min^{-1} to 300° for 7 min.

Isolation of TAG from leaves. Samples (*ca* 5 g, fresh weight) of the first fully expanded leaves were frozen in liquid N_2 and stored at −80°. Tissue was homogenized in ice-cold 80% MeOH (25 ml). The homogenate was left overnight at 4° prior to vacuum filtration with a Büchner funnel that contained a celite bed. The filtrate was reduced *in vacuo* at 40° to the aq. phase, adjusted to pH 8.5 with NH_4OH, and extracted ×5 with hexane. The pH was adjusted to 3.0 with HCl. After additional vacuum filtration the aq. phase was partitioned ×3 with EtOAc, and the aq. phase was concd with a stream of N_2 for 1 hr. The sample (*ca* 4.5 ml, pH 3) was loaded onto a Bakerbond SPE C_{18} column and eluted with 3 ml 100% MeOH. MeOH was evapd with a stream of N_2, and the sample was re-suspended in 100 μl 100% MeOH and 1 ml H_2O (containing 2 ml l^{-1} glacial HOAc). This was loaded onto an analytical C_{18} (5 μm Spherisorb ODS-2) HPLC column (0.4 × 25 cm), and run at 1 ml min^{-1} in an H_2O (containing 2 ml l^{-1} glacial HOAc) (A) to 100% MeOH gradient (B). The gradient used was: 0% to 30% B over 4 min, 30% to 45% B over 30 min, 45% to 100% B over 10 min, with holding at 100% B for 10 min. The column eluate was passed through a UV detector (Lambda-Max Model 481) at λ_{190} nm. The two frs containing **1** and **2**,

respectively, were collected according to data obtained from the HPLC of their respective standards (provided by T. Yoshihara). The R_t of **2** standard was 24.2 min. The authentic sample of **1** was eluted at $R_t = 18.4$ min. HPLC frs containing **1** and **2**, respectively, were dried by a stream of N_2 and then dissolved in 200 μl of MeOH. Derivatization to give **4** was as described above. Detection of compounds was by R_f along with SIM. The concns of compounds were calcd by comparison with authentic standards and were not adjusted for percentage recovery. Six fragments were used for SIM detection and quantification: *m/z* 109, 169, 331, 415 (a), 552 $[M-H_2O]^+$, and 570 $[M]^+$. Within the margin of expected variation, the relative intensities of these six fragments were the same at the R_f for **4** derived from the authentic sample as from tissue samples. For this reason all six fragments were given equal weight in the calculations that compared the tissue samples to authentic standards; i.e. the six proportional values obtained by comparing the tissue sample with the authentic sample were averaged to obtain the overall value.

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