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Organoselenides from *Nicotiana tabacum* genetically modified to accumulate selenium

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ABSTRACT

Nicotiana tabacum L. (tobacco) plants were transformed to overexpress a selenocysteine methyltransferase gene from the selenium hyperaccumulator *Astragalus bisulcatus* (Hook.) A. Gray (two-grooved milkvetch), and an ATP-sulfurylase gene from *Brassica oleracea* L. var. *italica* (broccoli). Solvent extraction of leaves harvested from plants treated with selenate revealed five selenium-containing compounds, of which four were identified by chemical synthesis as 2-(methylseleno)acetaldehyde, 2,2-bis(methylseleno)acetaldehyde, 4-(methylseleno)-(2*E*)-nonenal, and 4-(methylseleno)-(2*E*,6*Z*)-nonadienal. These four compounds have not previously been reported in nature.

1. Introduction

Selenium (Se) and sulfur (S) are chemically similar and hence flowering plants do not differentiate in their uptake of these two elements from the soil (White et al., 2007). Thus selenium (as selenate) is taken up by the plant's roots and processed via the sulfur assimilation pathway to ultimately form the selenium analogues of cysteine and methionine; selenocysteine (SeCys) and selenomethionine (SeMet) (Brown and Shrift, 1981). In plants not adapted to higher levels of selenium in the soil (non-accumulating plants), these seleno-amino acids are mis-incorporated into proteins resulting in Se toxicity with symptoms such as stunting, necrotic lesions on the leaves, and reduced root growth.

Selenium accumulators or hyperaccumulators are plants that tolerate high levels of selenium uptake and assimilation. They are able to take up and metabolise inorganic Se to organoselenides that are not harmful to the plant and which may in part be removed from the plant by volatilisation (Sors et al., 2005; Terry et al., 2000). This Se tolerance is created by the selenocysteine methyltransferase (SMT) enzyme which methylates selenocysteine (SeCys) to the well-tolerated methylselenocysteine (MeSeCys) (Neuhierl et al., 1999; Sors et al., 2005; Terry et al., 2000), which in turn is catabolised to produce the volatile, dimethyl diselenide ($MeSe_2Me$). Dimethyl selenide (MeSeMe) is also produced in large quantities, but from methylselenomethionine, which does not require the SMT enzyme. An exchange reaction between MeS_2Me and $MeSe_2Me$ produces dimethylselenosulfide (MeSeSMe) (Kubachka et al., 2007; McKenzie et al., 2009; Meija et al., 2002).

Se-accumulating (de Souza et al., 1998, 2002) and non-accumulating plants, which have been genetically engineered for overexpression of a *SMT* transgene, have potential for the phytoremediation of Se-contaminated soils (Ellis et al., 2004; LeDuc et al., 2004, 2006). For phytoremediation, the discharge of volatile organoselenides from the plant is more important than their accumulation, since volatilisation removes Se from the local food chain and the plants also achieve a longer useful life of removing Se from the soil rather than needing to be harvested, removed, and then replanted (Tagmount et al., 2002). Interest in enhancing organoselenide concentrations in plants has also arisen from the importance of an appropriate selenium intake for human health (Rayman, 2008) and the apparent linkage of selenium intake to a reduced risk of cancer (Ellis et al., 2004; McKenzie et al., 2009).

In a previous study (McKenzie et al., 2009), we demonstrated the accumulation of MeSeCys and γ-glutamyl-MeSeCys (GluMeSeCys) in tobacco plants overexpressing a constitutively controlled *SMT* transgene from *Astragalus bisulcatus* (Hook.) A. Gray. Accumulation of MeSeCys and GluMeSeCys was greater again in tobacco plants overexpressing the *SMT* gene as well as an ATP-sulfurylase transgene (ATPS) from broccoli (*Brassica oleracea* L. var *italica*). In addition to seleno-amino acid production, the transformed tobacco plants produced the volatile organoselenides, MeSeMe, $MeSe_2Me$, and MeSSeMe, which were measured in the headspace above the plants.

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In the above study, solvent extracts of the tobacco leaves also contained organoselenides, which were not found in the headspace. We have not found reports of such 'non-volatile', non-amino acid organoselenides in Se-accumulating plants. This study employed chemical synthesis to identify four of the 'non-volatile' organoselenides detected in the two tobacco populations. The identification of 2-(methylseleno)acetaldehyde **1**, in particular, suggests the operation of a new biosynthetic pathway for selenium mobilisation occurring in these Se-accumulating plants.

2. Results and discussion

2.1. Chemical analysis of SMT and ATPS/SMT transformed tobacco plants

The first two organoselenides to elute from the GC–MS were MeSeSMe (RT 9.12 min) and $MeSe_2Me$ (RT 12.62 min), which were previously identified in the headspace above these plants (McKenzie et al., 2009). $MeSe_2Me$, derived from MeSeCys, was the major selenium compound found in the tobacco leaf extracts (Table 1), and in some extracts it was a significant contributor to the total volatile profile (Fig. 1). $MeSe_2Me$ is the most commonly reported organoselenide in selenium accumulating organisms (Kubachka et al., 2007; Meija et al., 2002, 2003; Shah et al., 2007; Zhang and Chasteen, 1994). MeSeSMe may arise from disproportionation of $MeSe_2Me$ and MeS_2Me (Chasteen, 1993). However, while $MeSe_2Me$ was present in all transgenic plant lines, only traces of MeS_2Me were detected in extracts of two of the four plant lines (SMT2 and ATPS/SMT24, data not shown). These two organoselenides are highly volatile, as is evidenced by their detection in the headspace above the live plants (McKenzie et al., 2009). Therefore the concentrations given in Table 1 are likely to be lower limit estimates as there will have been loss of these compounds during solvent extraction and concentration of their solvent extracts. MeSeMe was not found in the solvent extracts, presumably because of its volatility.

In addition to $MeSe_2Me$, and MeSeSMe (McKenzie et al., 2009), five organoselenium compounds ((**1**)–(**4**) and Unknown 1), that were not found in the headspace above the plants, were identified in the solvent extracts (Table 1) based on their distinctive Se isotope patterns. Only line ATPS/SMT24 produced all compounds, including the single occurrence of Unknown 1. As too little sample was available to allow isolation and identification by NMR, possible structures for these volatiles were deduced from the mass spectral data and candidate compounds were prepared by organic synthesis for direct comparison of GC retention times and MS fragmentation patterns with the compounds extracted from the tobacco plants.

Table 1
Semi-quantitative analysis of organoselenium compounds in solvent extracts of the leaves from tobacco plants overexpressing an *AbSMT* (SMT) transgene or *BoATPS* and *AbSMT* (ATPS/SMT) transgenes measured by GC–MS as μg equivalents of $MeSe_2Me$ kg^{-1} (FW). Leaves were harvested following treatment with 300 μM sodium selenate for 10 (SMT) and 15 (ATPS/SMT) days. Each value is the average of two samplings from the same plant. Selenium compounds were not found in selenate-treated wild type plants or in transgenic plants that were not treated with selenate (McKenzie et al., 2009).

Compound	Ret. time (min)	Plant line			
		SMT37	SMT2	ATPS/SMT24	ATPS/SMT47
MeSeSMe	9.12	5	9	11	41
$MeSe_2Me$	12.62	121	58	860	750
$MeSeCH_2CHO$ (**1**)	17.36	31	39	49	97
Unknown 1	19.34	0	0	9	0
$(MeSe)_2CHCHO$ (**2**)	27.5	0	2	10	12
4-(Methylseleno)-(2*E*)-nonenal (**3**)	29.7	0	0	18	47
4-(Methylseleno)-(2*E*,6*Z*)-nonadienal (**4**)	30.4	5	0	40	119

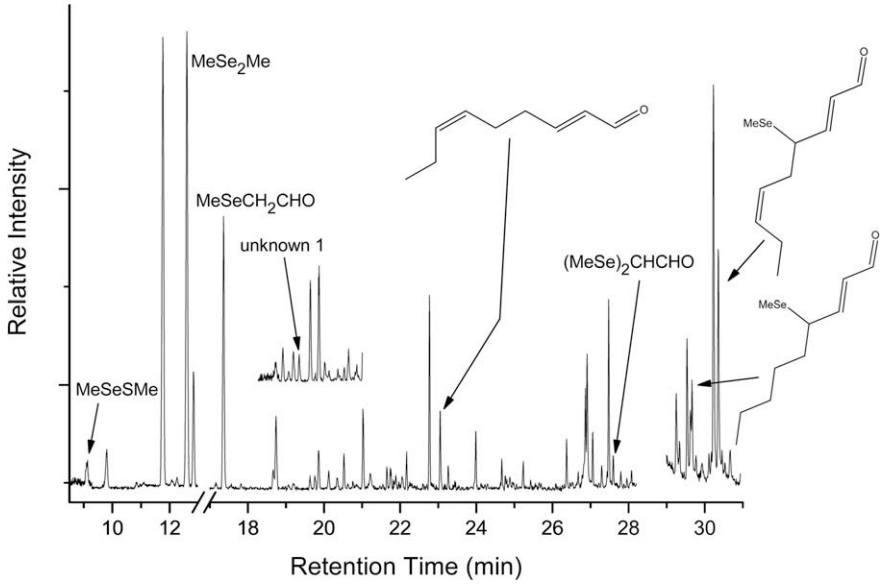

Fig. 1. TIC GC–MS traces showing the selenium metabolites found in solvent extracts of tobacco plants overexpressing both *BoATPS* and *AbSMT*, and treated with 300 μM sodium selenate. Not all metabolites were found in each sample. Sections of the GC–MS traces of extracts of different plants are shown to the same vertical and horizontal scale.

The third eluting compound, 2-(methylseleno)acetaldehyde (**1** in Fig. 2, RT 17.36 min), was the second most prevalent organoselenide in the solvent extracts (Table 1 and Fig. 1). The molecular formula was determined as C_3H_6OSe by high resolution EI-GC–MS, with the fragment ion *m*/*z* 108.9554 ($C_2H_5Se^+$) indicating loss of an aldehydic group (Fig. 3). A dominant feature of the fragmentation of alkylselenides is the loss of the alkyl group as an alkene, such as the loss of C_2H_4 from an ethyl selenide or C_3H_6 from a propyl selenide (Kubachka et al., 2007; Meija et al., 2002; Shah et al., 2007). No such fragments were observed (Fig. 3) and so the compound was unlikely to have an ethyl or propyl moiety. The mass spectrum did not match that reported for acetylselenomethane (Tsai et al., 1998), which has a very simple mass spectrum with prominent ions centred around *m*/*z* 138 (M^+) and *m*/*z* 93 ($SeCH^+$), and a base peak at *m*/*z* 43, but lacks the prominent ion cluster found around *m*/*z* 109 ($CH_3SeCH_2^+$) (Fig. 3). The reported high mass ions for aldehyde **1** (Adlington and Barrett, 1981), *m*/*z* 138, 136, 109, 107, 95, and 93, were in good agreement with the clusters of ions observed for the tobacco compound, although the lower mass ions (*m*/*z* 83, 57, and 43) did not match convincingly. Nevertheless, we synthesised aldehyde **1** and its GC retention time and mass spectrum (Fig. 3) agreed with those of the tobacco leaf compound. While **1** is a known synthetic compound (Adlington and Barrett, 1981), we have not found this compound reported as a natural product, and so **1** represents a new compound in the metabolism of selenium-accumulating plants.

Biosynthetically, aldehyde **1** may arise from the catabolism of SeMet in a manner analogous to that of methionine by *Lactococcus lactis* (Bonnarme et al., 2004). The first step would be transamination of SeMet to the 2-oxo acid, 4-(methylseleno)-2-oxobutanoic acid, which is then oxidatively cleaved, with the removal of two C-atoms as oxalic acid (HOOCCOOH), to give **1** (2-(methylseleno)acetaldehyde). Aldehyde **1** might also be formed from MeSeCys by a mechanism analogous to that for the catabolism of branched-chain amino acids, such as isoleucine, by yeast (Dickinson et al., 2000) as proposed in Fig. 3. Thus, transamination produces a 2-oxo carboxylic acid which can be decarboxylated directly to **1**. Alternatively, the 2-oxo acid may undergo decarbonylation, oxidation and CoA formation, and finally reduction to **1** (Fig. 3). A similar pathway has been proposed for the catabolism of methionine to 3-(methylthio)propanal in *Saccharomyces cerevisiae* (Perpète et al., 2006).

The formation of aldehyde **1** in the transformed tobacco plants is probably a consequence of introducing a new substrate into a pre-existing metabolic pathway. This new substrate might be either MeSeCys or SeMet, however the nature of the pathway is presently unknown. Certainly, a methionine catabolism pathway to dimethyl sulfide appears to exist in tobacco, as wild-type tobacco plants produce dimethylselenide when treated with selenate (McKenzie et al., 2009). There is no evidence for catabolism of MeSeCys by a naturally-occurring MeCys-specific catabolic pathway and the existence of such a pathway might be disputed since MeCys was only detected in the SMT-transformed plants (McKenzie et al., 2009).

The fourth organoselenide (Unknown 1, RT 19.34 min) to elute from the GC–MS system was present at less than 1% of the organoselenides in a single solvent extract (Table 1). The molecular formula was $C_4H_6O_2Se$ (*m*/*z* 165.9533), with the base peak at *m*/*z* 43.0188 ($C_2H_3O^+$) (Fig. 4). Significantly, Unknown 1 showed *m*/*z* 137.9589 ($C_3H_6OSe^+$), which resulted from loss of CO rather than C_2H_4 (Kubachka et al., 2007; Meija et al., 2002). Therefore, this compound does not have an alkyl moiety larger than CH_3. The mass

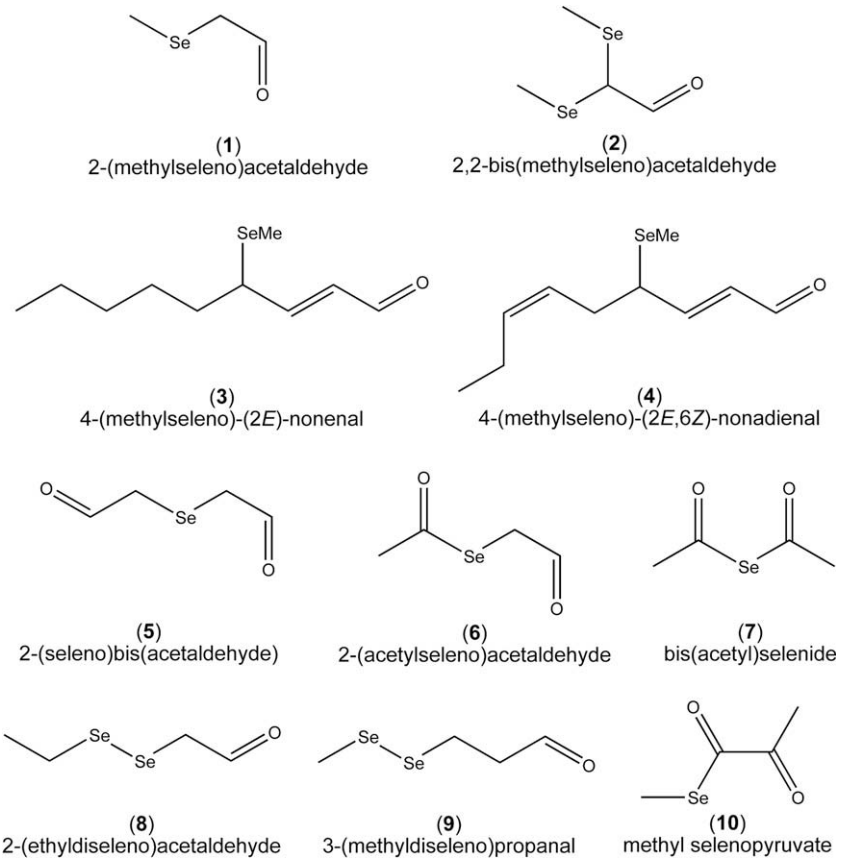

Fig. 2. Structures of selenium-containing volatiles identified in solvent extracts of leaves from transgenic tobacco plants, and of synthetic candidate compounds.

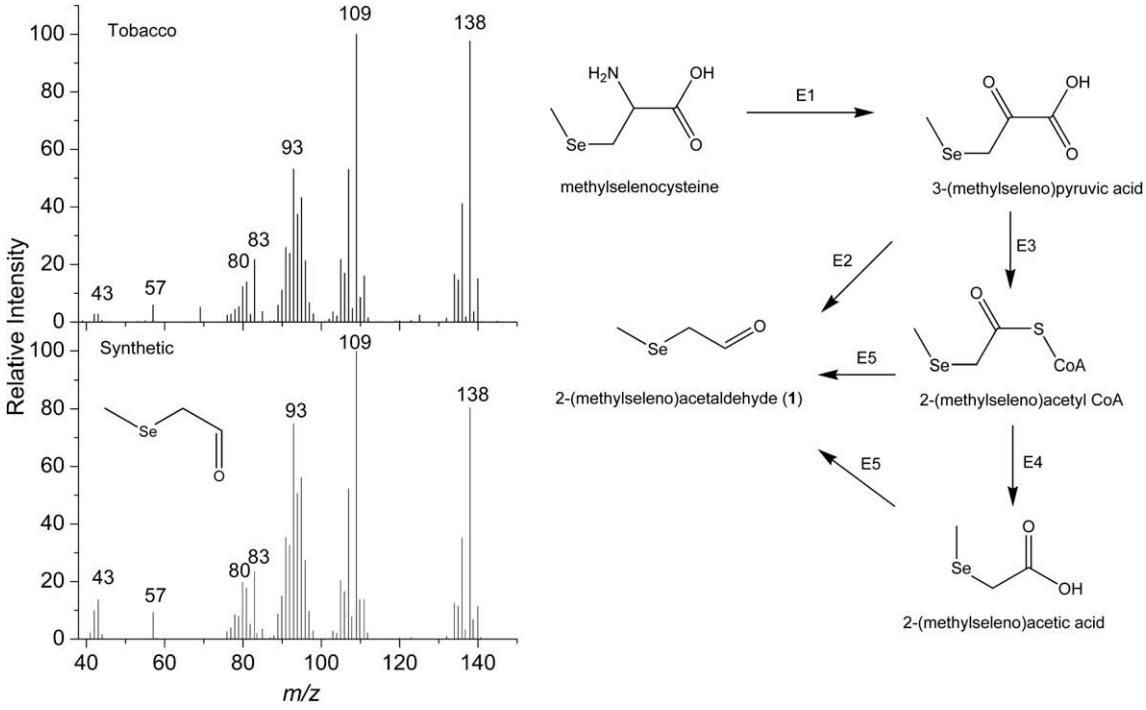

Fig. 3. MS fragmentation pattern of 2-(methylseleno)acetaldehyde (**1**) found in solvent extracts of the tobacco plants overexpressing both *BoATPS* and *AbSMT*, and treated with 300 μM sodium selenate and possible pathways in transgenic tobacco leaves for the metabolism of MeSeCys to **1**. E1, branched-chain amino acid amino transferase; E2, 2-oxoacid decarboxylase (cf. pyruvate decarboxylase); E3, 2-oxoacid dehydrogenase; E4, acyl-CoA hydrolase (Dickinson et al., 2000); E5, carboxylic acid and acyl-CoA reductases.

spectrum of Unknown 1 (Fig. 4) also had features in common with acetylselenomethane (Tsai et al., 1998). Therefore, the candidate compounds, 2-(seleno)bis(acetaldehyde) (**5**) (Blount and Robinson, 1932; Ficken et al., 1968), 2-(acetylseleno)acetaldehyde (**6**), and bis(acetyl)selenide (**7**) (Kageyama et al., 1989) were synthesised. The mass spectra of **5** and **7** are not available, and (**6**) has not been reported in the literature. Neither the GC retention times nor the mass spectra of these compounds matched that of Unknown 1 (Fig. 4). Other candidate compounds included methylseleno pyruvate (**10**), and 2-(methylseleno)malonaldehyde. If Unknown 1 is methylseleno pyruvate, rearrangement is required to produce *m/z* 138 (M^+-CO). Rearrangement of α-keto esters via decarbonylation is known (March, 1985), but we are not aware of a precedent in seleno-carbonyl compounds.

The fifth eluting Se-compound (**2**, RT 27.5 min) was the diselenide $C_4H_8OSe_2$ (*m/z* 231.8935, Fig. 5). The fragments *m/z* 202.8878 ($C_3H_7Se_2^+$) and *m/z* 175 ($CH_3Se_2^+$) indicated the loss of CHO and of C_2H_4. $C_2H_5Se^+$ (*m/z* 109) corresponded to M-SeC_2H_3O (M^+-123). Thus there appeared to be a terminal carbonyl and an ethyl group. 2-(Ethyldiseleno)acetaldehyde (**8**) and 3-(methyldiseleno)propanal (**9**) were synthesised as candidate compounds, but their mass spectra and RT did not match those of the tobacco compound (**2**). Most notably, their *m/z* 43 (CH_2CHO^+) and 160 (Se_2^+) fragments were not present for **2**, which itself had a cluster of ions at around *m/z* 203 ($C_3H_7Se_2^+$, Fig. 5) that was not present in the mass spectrum of **8** and **9**. The absence of *m/z* 160 from the mass spectrum of **2** suggests that this diselenide does not in fact contain a Se–Se unit.

A by-product (13%) from the synthesis of aldehyde **1**, by acid hydrolysis of 2,2-dimethoxyethylselenomethane, had a similar fragmentation to that of 2,2-bis(methylseleno)acetaldehyde (**2**). This by-product of the acid hydrolysis of (2,2-diethoxyethyl)(methyl)selane was characterised by NMR and GC–MS (Adlington and Barrett, 1981). This compound does not have a Se–Se bond, and therefore the above-mentioned *m/z* 175 ($MeSe_2^+$) fragment probably arose from rearrangement of $(MeSe)_2CH^+$ (*m/z* 203) to $MeSeSe^+CHMe$ prior to loss of 28 (C_2H_4). Similarly, our hydrolysis of (2,2-dimethoxyethyl)(methyl)selane produced **2** identical with the compound found in tobacco leaves. Aldehyde **2** would seem to arise from an acid-catalysed reaction between the keto and enol forms of 2-(methylseleno)acetaldehyde (Fig. 5). As with **1**, **2** has been prepared synthetically (Adlington and Barrett, 1981), but has not been reported from natural sources.

The sixth (**3**, $C_{10}H_{18}OSe$, RT 29.7 min) and seventh (**4**, $C_{10}H_{16}OSe$, RT 30.4 min) eluting organoselenides were found particularly in leaves overexpressing both the *ATPS* and *SMT* genes (Table 1). Compounds **3** and **4** both showed losses of $MeSe^·$ and MeSeH to give fragment ions $C_9H_{15}O^+$ and $C_9H_{14}O^+$ (3 DBE) at *m/z* 139 and 138, and $C_9H_{13}O^+$ and $C_9H_{12}O^+$ (4 DBE) at *m/z* 137 and 136, respectively. This, together with the 2 amu difference in the masses at *m/z* <110, suggested that these compounds differed by one double bond. The shorter retention time of the more saturated **3** on the polar GC column indicated it was not the alcohol derivative of **4**. Structures related to the reaction of methylselenol with unsaturated nonenals were indicated and this was supported by the identification of a number of aldehydes, including (2*E*,6*Z*)-nonadienal (Fig. 1) and (2*E*)-nonenal in the tobacco leaf extracts.

The fragmentation pattern of aldehyde **4** ($C_{10}H_{16}OSe$) included two groups of Se-containing fragment ions centred at *m/z* 163 (C_5H_7SeO), corresponding to loss of C_5H_9, and at *m/z* 135 (C_4H_7Se), corresponding to an additional loss of CO. These MS fragmentations suggest that this compound has a 4-(methylseleno)-2,6-nonadienal type structure. To confirm this, (2*E*,6*Z*)-nonadienal was added to (2,2-dimethoxyethyl)(methyl)selane prior to its acid hydrolysis, with the expectation that during the hydrolysis, a mechanism akin to that shown in Fig. 5 for the production of $(MeSe)_2CH_2CHO$ (**2**), would produce a MeSe adduct of (2*E*,6*Z*)-nonadienal. Examination of the ^1H NMR spectrum of the reaction product

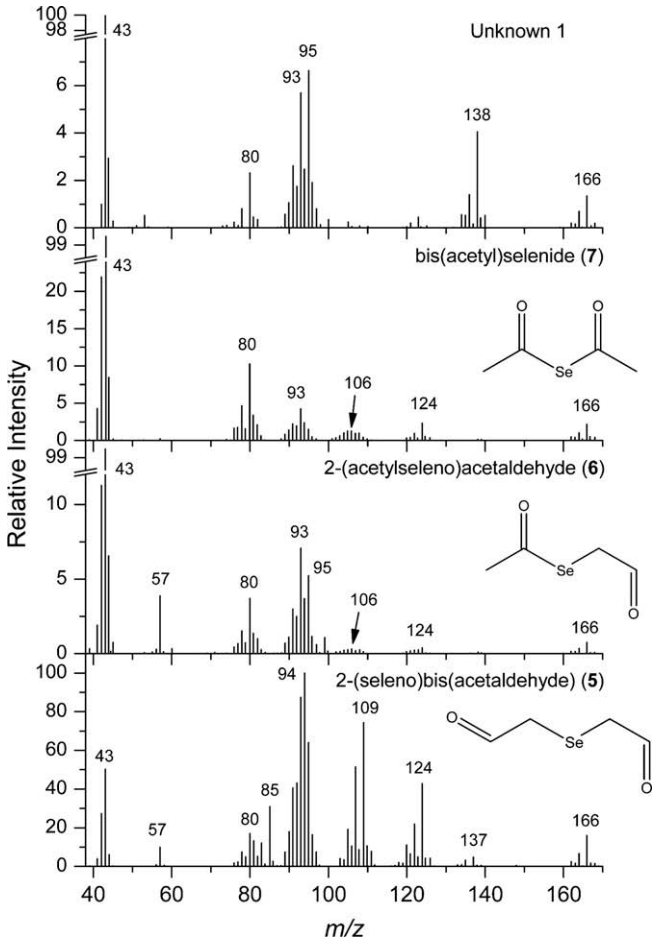

Fig. 4. Mass spectral fragmentation patterns of Unknown 1 from a tobacco plant overexpressing both *BoATPS* and *AbSMT*, and treated with 300 μM sodium selenate, and the three candidate synthetic compounds.

showed that this had occurred, with resonances for H2 and H3 of **4** fully resolved from those of the excess of (2*E*,6*Z*)-nonadienal also present. Resonances for **4** were fully assigned using COSY and selective TOCSY experiments. The signal for H3 appeared as a doublet of doublets (J = 9.6 and 15.4 Hz), rather than as a doublet of triplets (J = 6.4 and 15.7 Hz) as seen in (2*E*,6*Z*)-nonadienal, thus indicating the presence of a substituent at C4. This was confirmed by the reduced multiplicity observed for H2 of **4** (*ddd*, J = 0.7, 7.7, and 15.4 Hz) compared with H-2 of (2*E*,6*Z*)-nonadienal (*ddt*, J = 1.3, 6.4, and 15.7 Hz). The resonance assigned to H4 now appeared as a complex quartet at δ 3.08 for **4** rather than at δ 1.90 for (2*E*,6*Z*)-nonadienal. The mass spectrum (Fig. 6) and GC–MS retention time of the synthetic compound matched those of the compound in tobacco leaves.

The mass spectrum of **3** was less definitive, including only one set of weak Se-containing fragmentation ions centred at m/z 133 ($C_4H_7Se^{78}$) and m/z 135 ($C_4H_7Se^{80}$). However, given the presence of (2*E*)-nonenal in the tobacco leaf extracts, it was proposed that **3** might result from reaction of (2*E*)-nonenal with aldehyde **1**. Thus (2*E*)-nonenal was added to (2,2-dimethoxyethyl)(methyl)selane prior to its acid hydrolysis to give a product with the same GC retention time and MS (Fig. 6) as aldehyde **3** found in the plant extracts. Examination of the ^{1}H NMR of the synthetic sample showed resonances for H2, but not H3, of **3** were fully resolved from those of the excess of (2*E*)-nonenal present in the sample. Further resonances for **3** were identified using COSY and selective TOCSY experiments and used to confirm the structure of **3**. The signal for H2 appeared as a doublet of doublet of doublets (*ddd*, J = 0.7, 7.7, and 15.4 Hz), rather than as a doublet of doublet of triplets (*ddt*, J = 1.4, 7.7, and 15.6 Hz) as seen in 2*E*-nonenal, thus indicating the presence of a substituent at C4. The multiplicity of H3 was similarly reduced to a doublet of doublets (J = 9.7 and 15.3 Hz). H4 appeared as a complex quartet at δ 3.05. Compounds **3** and **4** have not previously been reported.

Given the ready conversion of aldehyde **1** to aldehyde **2** and the reactivity of **1** with (2*E*)-nonenal and (2*E*,6*Z*)-nonadienal, under acid conditions *in vitro*, we cannot be certain whether **2**–**4** are of biosynthetic origin or arise as a result of spontaneous chemical reaction in the plants or in the solvent extracts. Aldehydes **1**–**4** were not found using headspace SPME, but given their lower volatility with respect to $MeSe_2Me$, this was not unexpected.

Generally, the tobacco plant lines expressing both the *AbSMT* and *BoATPS* transgenes had higher levels of organoselenium compounds than those expressing the *AbSMT* gene alone (Table 1). In particular, the ATPS/SMT plants had around 10-fold more $MeSe_2Me$ than the SMT-expressing plants (Table 1). Also, the doubly transformed plants had more of the aldehydic organoselenides. However, as leaves were harvested from the doubly transformed plants after 15 days of watering with selenate fertiliser, whereas the harvest from the singly transformed plants was after 10 days, it is possible that these differences relate more to the amount of selenium accumulated in the plants.

Transformation with the *AbSMT* transgene not only converts tobacco from a Se non-accumulating species into an accumulator of MeSeCys (McKenzie et al., 2009, and data presented here), it has also resulted in the formation of new Se-containing metabolites. These metabolites may arise both through the catabolism of Se-amino acids, and by chemical reactions occurring *in planta* or in the plant extracts. The identification of aldehyde **1** as 2-(methylseleno)acetaldehyde, in particular, suggests the operation of a new pathway for Se mobilisation in plants with enhanced Se metabolism. The observation of such novel metabolites leads to a caution regarding the consequences of increasing the accumulation of organoselenium compounds in plants.

3. Experimental

3.1. Plant material

The tobacco plants (*Nicotiana tabacum* L. 'Samsun'), overexpressing the *SMT-A* gene from *A. bisulcatus* (*AbSMTA*, GenBank accession number AJ13143) under the control of the 35S promoter, either alone or in combination with the constitutively expressed broccoli *BoATPS1* (GenBank accession number U05218) gene were prepared as described previously (McKenzie et al., 2009). Three clonal plants of each line were ex-flasked into pots of medium grade vermiculite (Nuplex, Auckland, New Zealand) and acclimatized in a mist tent for 1–2 weeks before moving to a glasshouse, where they were placed in trays and watered from the bottom with half strength liquid Hoaglands solution (Hoagland and Arnon, 1950). After a further 2 weeks, when the plants were at the 5–6 leaf stage, sodium selenate (0 or 300 μM) was added to the watering liquid. Plants were watered from below with Se for 10 days (SMT plants) or 15 days (ATPS/SMT plants) before young (the two top fully expanded leaves below the meristem leaf cluster) and old leaves (the second and third fully expanded leaves at the bottom of the plant) were harvested.

3.2. Solvent extraction of tobacco leaves

Leaf material was removed from the plant, frozen immediately in liquid nitrogen and stored at −80 °C until extraction. Frozen

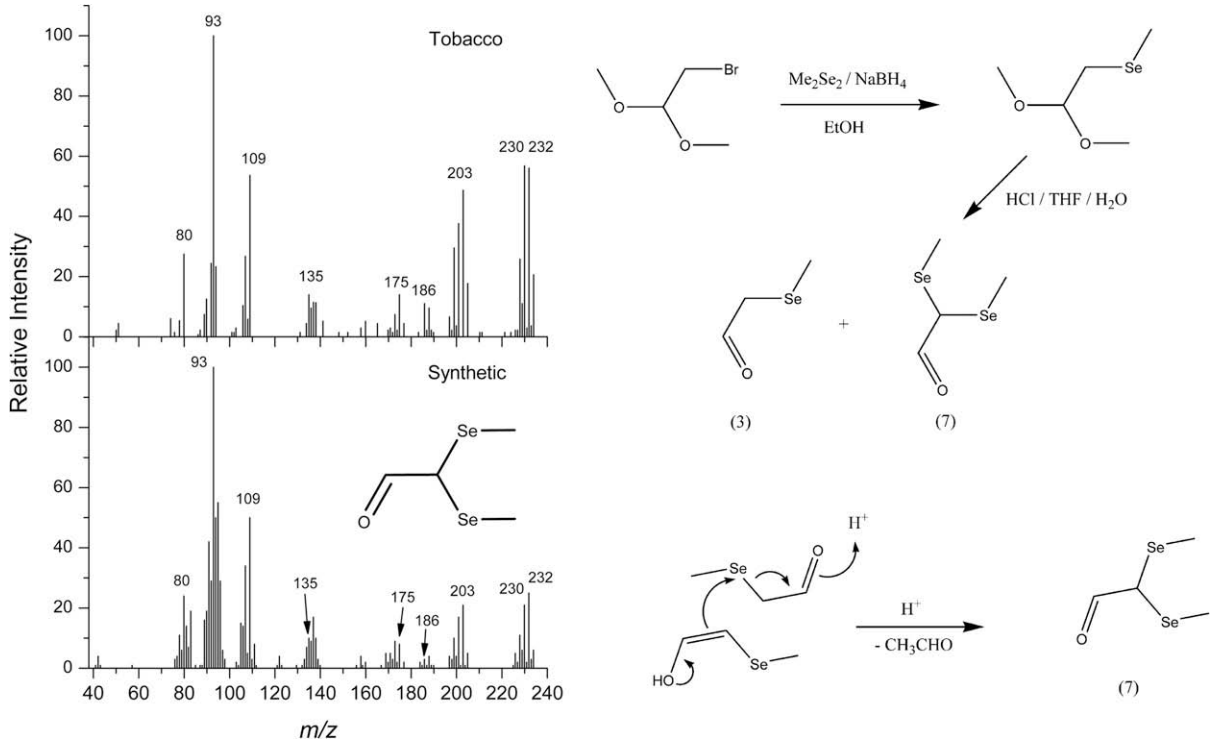

Fig. 5. MS fragmentation patterns of 2,2-bis(methylseleno)acetaldehyde (**2**) extracted from tobacco plants overexpressing *BoATPS* and *AbSMT* treated with 300 μM sodium selenate, and produced synthetically. Synthesis of 2-(methylseleno)acetaldehyde (**1**) and a possible mechanism for the acid-catalysed conversion of **1**–**2** during acetal hydrolysis.

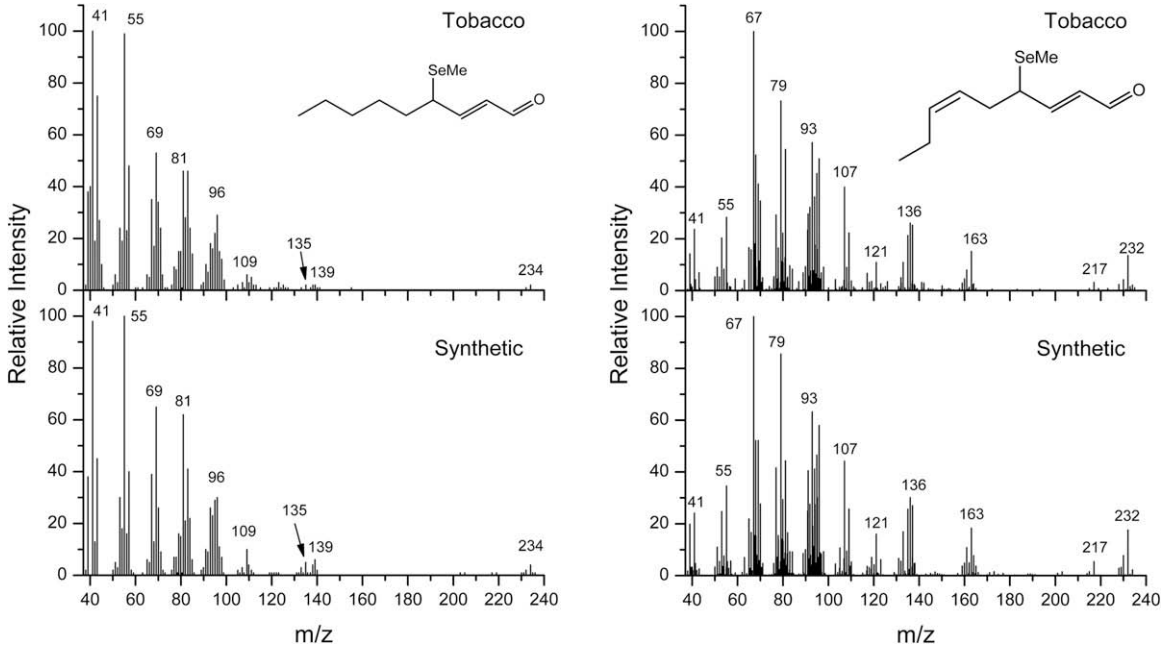

Fig. 6. MS fragmentation patterns of **3**, 4-(methylseleno)-(2*E*)-nonenal and **4**, 4-(methylseleno)-(2*E*,6*Z*)-nonadienal, which were identified in solvent extracts of tobacco plants overexpressing *BoATPS* and *AbSMT*, and treated with 300 μM sodium selenate, and of synthetic compounds.

tissue (10 g) was ground in a liquid nitrogen-cooled mortar and pestle and the cold powder mixed to a slurry in pentane:Et_2O (1:1, v/v, 20 ml). The slurry was stored at 4 °C for 6 days in a Schott bottle and frozen at −20 °C before the organic phase was decanted off. Prior to GC–MS analysis the solvent extracts were dried by passage through anhydrous $MgSO_4$, and their volumes reduced to 2.0 ml under a gentle stream of nitrogen.

3.3. GC–MS analysis of solvent extracts

GC–MS separations were carried out on an Agilent 6890 N GC using a 20 m × 0.18 mm i.d. × 0.18 μm film thickness DB-Wax (Agilent) capillary column with a He flow of 1 ml min^{-1} coupled to a Waters GCT time of flight (TOF) mass spectrometer with EI of 70 eV, a scan time of 0.4 s, and for HRMS lock masses of

121.0014 and 265.9964 (2,4,6-tris(trifluoromethyl)1,3,5-triazine). The oven temperature program was 2 min at 30 °C, 2 °C min^{-1} to 50 °C, 5 °C min^{-1} to 90 °C, 10 °C min^{-1} to 180 °C, hold 5 min. Injections were made into a PTV (Gerstel) injection port at −40 °C, which was held splitless for 30 s. Three seconds after the injection, the injection port temperature was ramped up to 260 °C at 700 °C s^{-1}. This injection port temperature and splitless regime improved the peak shape of the earlier eluting peaks. For the comparisons between the tobacco and synthetic compounds, 1-min splitless injections were made at 220 °C onto a 30 m × 0.25 mm i.d. × 0.25 μm film thickness HP-5MS (Agilent) capillary column. The oven temperature programme was 1 min at 35 °C, 5 °C min^{-1} to 240 °C, hold 10 min.

3.3.1. Mass spectral data of selenium compounds in tobacco leaves

*3.3.1.1. 2-(Methylseleno)acetaldehyde (**1**).* Accurate Mass EI-MS, *m/z* (Δ) [fragment]: 137.9586 (0.2 mDa) [$C_3H_6O^{80}Se$, M^+], 108.9554 (−0.2 mDa) [$C_2H_5^{80}Se$, M^+-CHO], and 94.9395 (−0.2 mDa) [$CH_3^{80}Se$].

*3.3.1.2. 2,2-bis(Methylseleno)acetaldehyde (**2**).* Accurate Mass EI-MS, *m/z* (Δ) [fragment]: 231.8935 (2.9 mDa) [$C_4H_8O^{80}Se_2$, M^+], 202.8878 (0.0 mDa) [$C_3H_7^{80}Se_2$, M^+-CHO], and 108.9548 (−0.8 mDa) [$C_2H_5^{80}Se$].

*3.3.1.3. 4-(Methylseleno)-(2E)-nonenal (**3**).* Accurate Mass EI-MS, *m/z* (Δ) [fragment]: 234.0564 (4.1 mDa) [$C_{10}H_{18}O^{80}Se$, M^+], 219.0365 (7.7 mDa) [$C_9H_{15}O^{80}Se$, M^+-CH_3], and 139.1135 (1.2 mDa) [$C_9H_{15}O$].

*3.3.1.4. 4-(Methylseleno)-(2E,6Z)-nonadienal (**4**).* Accurate Mass EI-MS, *m/z* (Δ) [fragment]: 232.0366 (1.0 mDa) [$C_{10}H_{16}O^{80}Se$, M^+], 217.0112 (−2.0 mDa) [$C_9H_{13}O^{80}Se$, M^+-CH_3], 162.9693 (+3.1 mDa) [$C_5H_7Se^{80}O$], 137.0966 (−1.1 mDa) [$C_9H_{13}O$], 136.0878 (−1.0 mDa) [$C_9H_{12}O$], 134.9896 (+10.6 mDa) [$C_4H_7Se^{80}$], 108.9580 (2.4 mDa) [$C_2H_5^{80}Se$], and 67.0541 (−0.7 mDa) [C_5H_7].

3.3.1.5. Unknown 1. EI-MS, *m/z* (rel. int.): 166 (1.3), 138 (4), 136 (1.4), 96 (2), 95 (6.5), 94 (2.5), 93 (6), 91 (3), 80 (2.4, Se), and 43 (100). Accurate Mass EI-MS, *m/z* (Δ) [fragment]: 165.9533 (0.0 mDa) [$C_4H_6O_2^{80}Se$, M^+], 137.9589 (0.5 mDa) [$C_3H_6O^{80}Se$, M^+-CO], 94.9408 (0.8 mDa) $CH_3^{80}Se$, and 43.0188 (0.4 mDa) C_2H_3O.

3.4. Synthesis of candidate compounds

All Se-containing compounds are presumed to be highly toxic and should be handled with appropriate precautions. To minimise risk while handling these volatile and potentially toxic materials, chemical reactions were conducted on a small scale and designed to minimise handling, purification steps and the generation of chemical wastes.

*3.4.1. 2-(Methylseleno)acetaldehyde (**1**)*

$NaBH_4$ (60 mg) was added to a solution of $MeSe_2Me$ (100 mg, 530 μmol, Acros) in absolute EtOH (5 ml) under N_2. After the colour of the reaction mixture changed from yellow to white, bromoacetaldehyde dimethyl acetal (2,2-dimethoxyethylbromide, 127 mg, 750 μmol, Aldrich) was added. After 90 min of stirring under N_2, the colour reverted to yellow. Further $NaBH_4$ (20 mg) was added and the reaction stirred overnight. Water (5 ml) was added and the solution extracted with distilled pentane (4 × 25 ml) and dried with $MgSO_4$. The solvent was removed *in vacuo* and to give a yellow oil (108 mg), which by GC–MS consisted of $MeSe_2Me$ (65%) and (2,2-dimethoxyethyl)(methyl)selane (35%). EI-MS, *m/z* (rel. int.): 184 (25) M^+, 153 (35), 137 (3), 109 (15), 93 (13), 75 (100), 58 (17), 47 (15), and 43 (12). ^1H NMR (400 MHz, $CDCl_3$): δ 2.07 (*s*, MeSe), 2.71 (*d*, *J* = 5.6 Hz, CH_2Se), 4.57 (*t*, *J* = 5.6 Hz, CH). A portion of the above mixture (81 mg) was taken up in THF (3 ml), H_2O (200 μl) and 25 mg of HCl (conc.) and left to stand overnight. The acid was neutralized with satd. aq. $NaHCO_3$ (3 ml) and the product extracted with pentane (4 × 25 ml) and dried ($MgSO_4$) to give **1** (87%) EI-MS, *m/z* (rel. int.): 138 (80) M^+, 136 (35), 109 (100), 107 (51), 96 (26), 95 (55), 94 (50), 93 (74), 92 (31), 91 (34), 83 (23), and 80 (19). ^1H NMR (400 MHz, $CDCl_3$): δ 2.03 (3H, *s*, MeSe), 3.16 (2H, *d*, *J* = 4.2 Hz, CH_2Se), 9.37 (1H, *t*, *J* = 4.2 Hz, CHO) (Adlington and Barrett, 1981), and a by-product (13%) 2,2-bis(methylseleno)acetaldehyde (**2**) EI-MS, *m/z* (rel. int.): 232 (27) M^+, 230 (23), 203 (21), 137 (19), 109 (51), 107 (38), 96 (28), 95 (56), 94 (51), 93 (100), 92 (30), 91(32), and 80 (23). ^1H NMR (400 MHz, $CDCl_3$): δ 1.93 (6H, *s*, MeSe), 4.44 (1H, *d*, *J* = 4.0 Hz, CHSe), and 9.12 (1H, *d*, *J* = 3.9 Hz, CHO) (Adlington and Barrett, 1981).

*3.4.2. 4-(Methylseleno)-(2E)-nonenal (**3**) and 4-(methylseleno)-(2E,6Z)-nonadienal (**4**)*

Crude 2,2-(dimethoxyethyl)(methyl)selane was prepared as for the synthesis of aldehyde **1**, above. For the acid hydrolysis of this compound, 10 mg of either (2*E*,6*Z*)-nonadienal (Aldrich) or (2*E*)-nonenal (Aldrich) was added before adding 25 mg of HCl (conc.). The reaction mixtures were analysed by GC–MS. For the reaction containing (2*E*,6*Z*)-nonadienal, the products were $MeSe_2Me$ (82%), aldehydes **1** (11.5%) and **2** (1.5%), and 4-(methylseleno)-(2*E*,6*Z*)-nonadienal (**4**) (4.8%). EI-MS, *m/z* (rel. int.): 232 (14, M^+), 217 (3), 163 (15), 137 (29), 136 (27), 135 (23), 121 (14), 107 (41), 96 (52), 95 (63), 94 (56), 93 (64), 91 (53), 80 (45), 79 (76), 68 (75), 67 (100), 55 (31), and 41 (28). ^1H NMR (400 MHz, d_6-benzene) δ 9.42 (1H, *d*, *J* = 7.7 Hz, H1), 6.28 (1H, *dd*, *J* = 9.6 and 15.4 Hz, H3), 5.75 (1H, *ddd*, *J* = 0.7, 7.7, and 15.4 Hz, H2), 5.50 (1H, *m*, H7), 5.29 (1H, *m*, H6), 3.08 (1H, *m*, H4), 2.31 (*m*, H5), 1.96 (*m*, H8), and 0.96 (*t*, *J* = 7.6 Hz, H9). For the reaction obtained after addition of (2*E*)-nonenal, the products were $MeSe_2Me$ (31%), aldehydes **1** (29%) and **2** (10%), 4-(methylseleno)-(2*Z*)-nonenal (3%), and 4-(methylseleno)-(2*E*)-nonenal (**3**) (27%). EI-GC–MS, *m/z* (rel. int.): 234 (4, M^+), 232 (2), 219 (1), 139 (6), 135 (5), 108 (10), 97 (11), 96 (30), 95 (29), 94 (23), 93 (26), 92 (9), 84 (22), 83 (41), 81 (62), 69 (65), 55 (100), and 53 (30). ^1H NMR (400 mHz, d_6-benzene) δ 9.45 (1H, *d*, *J* = 7.7 Hz, H1), 6.25 (1H, *dd*, *J* = 9.7 and 15.3 Hz, H3), 5.75 (1H, *ddd*, *J* = 0.7, 7.7, and 15.4 Hz, H2), 3.05 (1H, *m*, H4), 1.45 (*m*, H5), 1.1–1.55 (*m*, H6–H8), and 0.98 (*t*, *J* = 7.1 Hz, H9).

*3.4.3. 2-(Seleno)bis(acetaldehyde) (**5**)*

Sodium chips (100 mg, 4.3 mmol) were added to Se powder (171 mg, 2.1 mmol) and naphthalene (55.3 mg, 0.43 mmol) in dry distilled THF (7 ml) under N_2. The mixture was stirred for 24 h to yield a pale pink soln. of NaSeNa (Thompson and Boudjouk, 1988). Bromoacetaldehyde dimethyl acetal (500 μl, 4.2 mmol) was added and the mixture refluxed for 3 h to produce a cream ppt. in a yellow soln. The reaction was filtered to give a soln. of unreacted bromoacetaldehyde dimethyl acetal (29%), bis(2,2-dimethoxyethylselenide) (26%), and bis(2,2-dimethoxyethyl)selenide (45%). EI-MS, *m/z* (rel. int.): 258 (3) M^+, 195 (8), 137 (12), 109 (7), 76 (13), 75 (100), 58 (16), 47 (11), and 43 (14). Solvent was removed and the residue taken up in THF (3 ml) and water (200 μl). HCl (25 mg, conc.) was added and the sample stirred under argon for 24 h at room temperature to produce **5**. $NaHCO_3$ (5 ml, satd. aq.) was added, the mixture extracted with 3 × 50 ml of pentane:Et_2O (50:50), and dried ($MgSO_4$). The mixture contained an unknown organoselenide (26%), the non-hydrolysed bis(2,2-dimethoxyethylselenide) (48%), and **5** (26%), reported (Blount and Robinson, 1932; Ficken et al., 1968) but MS and NMR data are not available. The MS was consistent with the target compound **5**. EI-GC–MS, *m/z* (rel. int.): 166 (16) M^+, 109 (74), 124 (43), 123

(21),107 (52), 95 (64), 94 (100), 93 (88), 92 (43), 91 (40), 85 (31), 43 (50), and 42 (27).

*3.4.4. 2-(Acetylseleno)acetaldehyde (**6**)*

To Se powder (30 mg, 375 μmol) in degassed water (2.5 ml) under argon was added a solution of $NaBH_4$ (30 mg, 790 μmol) in degassed water (2.5 ml). After stirring for 20 min a colourless soln. of NaSeH formed. Se powder (30 mg) was added and the flask placed in hot water (65 °C) to speed the formation of the red/brown $NaSe_2Na$ (Klayman and Griffin, 1973). The soln. was allowed to cool and bromoacetaldehyde dimethyl acetal (88 μl, 750 μmol) was added and the mixture stirred for 22 h under argon, after which the product was taken up in 100 ml of pentane, filtered and dried ($MgSO_4$). The mixture contained unreacted bromoacetaldehyde dimethyl acetal (81%), the by-product bis(2,2-dimethoxyethyl)selenide (6%), and bis(2,2-dimethoxyethylselenide) (13%). EI-MS, *m*/*z* (rel. int.): 338 (1) M^+, 275 (0.5), 217 (2), 191 (1), 160 (2), 138 (6), 107 (6), 93 (5), 75 (100), 58 (34), and 43 (27).

Solvent was removed and the sample was taken up in dry THF (4 ml) and stirred with of 1 M $Li(Et)_3BH$ (1.5 ml, 1.5 mmol, Acros) for 30 min under argon. Acetyl chloride (200 μl) was added and stirred for 30 min, when satd. aq. $NaHCO_3$ (10 ml) was added and the mixture extracted with Et_2O (4 × 25 ml), and dried ($MgSO_4$) to produce 1-acetylseleno-2,2-dimethoxyethane. EI-MS, *m*/*z* (rel. int.): 181 (2) M^+-OCH_3, 138 (18), 107 (10), 93 (5), 75 (85), 58 (29), 47 (21), and 43 (100). The above ethereal solution was stirred with *p*-toluenesulfonic acid (40 mg) under argon for 19 h to give **6** (96% hydrolysis). EI-GC–MS, *m*/*z* (rel. int.): 166 (1) M^+, 124 (0.5), 93 (8), 80 (4), 57 (4), and 43 (100).

*3.4.5. Bis(Acetyl)selenide (**7**)*

Sodium chips (150 mg) were added to Se powder (171 mg, 2.1 mmol) and naphthalene (55 mg) in dry THF (7 ml). Reflux for 2 h under N_2 gave a pale grey solution of NaSeNa. Acetyl chloride (400 μl) was added, the reaction flask placed in hot water (65 °C) and the mixture stirred for 30 min. After cooling to room temperature, satd. aq. $NaHCO_3$ (20 ml) was added and the mixture extracted with Et_2O (3 × 50 ml), and dried ($MgSO_4$) to give a mixture of bis(acetylselenide) (17%) and **7** (83%) (Kageyama et al., 1989). EI-MS, *m*/*z* (rel. int.): 166 (2) M^+, 124 (2), 106 (1), 93 (4), 80 (10), and 43 (100).

*3.4.6. 2-(Ethyldiseleno)acetaldehyde (**8**)*

Bromoethane (78 μl, 1.05 mmol) was added to a solution of $NaSe_2Na$ (0.5 mmol) prepared under argon from Se and Na metals as described previously. Reflux for 2 h gave complete reaction of the bromoethane and formation of $EtSe_2Et$ by GC–MS (Chatterjee et al., 2001). This solution was added to a solution of bis(2,2-dimethoxyethylselenide), produced as described above, and stirred under argon for 1 h to allow exchange between the two diselenides. The three products were $EtSe_2Et$ (4%), $(SeCH_2CH(OCH_3)_2$ (90%), and $EtSe_2CH_2CH(OCH_3)_2$ (6%) by GC–MS. $EtSe_2CH_2$-$CH(OCH_3)_2$ gave EI-MS, *m*/*z* (rel. int.): 278 (5) M^+, 276 (5), 274 (2), 247 (2), 189 (5), 187 (4), 160 (1), 137(1), 109 (2), 107 (2), 75 (100), 59 (5), 58 (13), 47 (19), and 43 (14). The above sample was filtered and stirred for 1 h under argon with *p*-toluenesulfonic acid (50 mg) to give **8** (5%). EI-MS, *m*/*z* (rel. int.): 232 (65), 230 (53), 228 (31), 189 (23), 188 (21), 187 (22), 160 (58), 159 (23), 158 (51), 157 (23), 156 (29), 109 (18), 107 (18), 93 (37), 44 (47), 43 (100), and 42 (46).

*3.4.7. 3-(Methyldiseleno)propanal (**9**)*

bis(2,2-Dimethoxypropylselenide) was prepared by addition of 3-bromopropionaldehyde dimethyl acetal (114 μl, 0.75 mmol, Aldrich) to aq. $NaSe_2Na$ prepared as described above by reduction of Se metal (60 mg) with $NaBH_4$. This solution was stirred under argon for 18 h at room temperature, during which time the dark red $NaSe_2Na$ soln. changed to a pale orange. The reaction mixture was extracted with Et_2O (3 × 25 ml) and dried ($MgSO_4$). $MeSe_2Me$ (30 μl) was added and the solution stirred under argon for 2 h. GC–MS showed the major constituents to be the unreacted bis(2,2-dimethoxypropylselenide), bis(2,2-dimethoxypropyl)selenide, and a poor yield (1%) of 1-(3,3-dimethoxypropyl)-2-methyldiselane. EI-MS, *m*/*z* (rel. int.): 278 (11), 276 (12), 151 (16), 103 (12), 75 (100), 73 (10), 71 (80), 57 (11), 55 (8), 47 (38), 45 (63), 41 (42), and 39 (10). The above sample was filtered and stirred for 1 h, under argon, with *p*-toluenesulfonic acid (10 mg) to give a sample of aldehyde **9** (26% of a mixture of organoselenides). EI-MS, *m*/*z* (rel. int.): 232 (100), 230 (85), 229 (32), 228 (54), 176 (54), 175 (33), 174 (56), 173 (40), 172 (43), 160 (48), 158 (47), 109 (25), 107 (22), 95 (31), 93 (60), and 57 (86).

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References

Adlington, R.M., Barrett, A.G.M., 1981. Concise syntheses of 3-methylenetetrahydrofuran-2-one derivatives and related systems. J. Chem. Soc., Perkin Trans. 1 (11), 2848–2863.

Blount, B.K., Robinson, R., 1932. Some analogs of pseudopelletierine, namely, thiotropinone, selenotropinone and *N*-methylaztropinone. J. Chem. Soc. 248, 5–2487.

Bonnarme, P., Amarita, F., Chambellon, E., Semon, E., Spinnler, H.E., Yvon, M., 2004. Methylthioacetaldehyde, a possible intermediate metabolite for the production of volatile sulphur compounds from L-methionine by *Lactococcus lactis*. FEMS Microbiol. Lett. 236, 85–90.

Brown, T.A., Shrift, A., 1981. Exclusion of selenium from proteins of selenium-tolerant *Astragalus* species. Plant Physiol. 67, 1051–1053.

Chasteen, T.G., 1993. Confusion between dimethyl selenyl sulfide and dimethyl selenone released by bacteria. Appl. Organomet. Chem. 7, 335–342.

Chatterjee, A., Shibata, Y., Yoneda, M., Banerjee, R., Uchida, M., Kon, H., Morita, M., 2001. Identification of volatile selenium compounds produced in the hydride generation system from organoselenium compounds. Anal. Chem. 73, 3181–3186.

de Souza, M.P., Pickering, I.J., Walla, M., Terry, N., 2002. Selenium assimilation and volatilization from selenocyanate-treated Indian Mustard and Muskgrass. Plant Physiol. 128, 625–633.

de Souza, M.P., Pilon-Smits, E.A.H., Lytle, M., Hwang, S., Tai, J., Honma, T.S.U., Yeh, L., Terry, N., 1998. Rate-limiting steps in selenium assimilation and volatilization by Indian Mustard. Plant Physiol. 117, 1487–1494.

Dickinson, J.R., Harrison, S.J., Dickinson, J.A., Hewlins, M.J.E., 2000. An investigation of the metabolism of isoleucine to active amyl alcohol in *Saccharomyces cerevisiae*. J. Biol. Chem. 275, 10937–10942.

Ellis, D.R., Sors, T.G., Brunk, D.G., Albrecht, C., Orser, C., Lahner, B., Wood, K.V., Harris, H.H., Pickering, I.J., Salt, D.E., 2004. Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. BMC Plant Biol. 4, 1.

Ficken, G.E., Mason, L.F.A., Fry, D.J., 1968. Gelatin hardening agents and processes. Great Britain, p. 6.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ., vol. 347, Berkeley, CA.

Kageyama, H., Tsutsumi, H., Murai, T., Kato, S., 1989. Aliphatic bis(acyl) selenides – synthesis and characterisation. Z. Naturforsch. [B] 44, 1050–1052.

Klayman, D.L., Griffin, T.S., 1973. Reaction of selenium with sodium borohydride in protic solvents. A facile method for the introduction of selenium into organic molecules. J. Am. Chem. Soc. 95, 197–199.

Kubachka, K.M., Meija, J., LeDuc, D.L., Terry, N., Caruso, J., 2007. Selenium volatiles as proxy to the metabolic pathways of selenium in genetically modified *Brassica juncea*. Environ. Sci. Technol. 41, 1863–1869.

LeDuc, D.L., AbdelSamie, M., Montes-Bayon, M., Wu, C.P., Reisinger, S.J., Terry, N., 2006. Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. Environ. Pollut. 144, 70–76.

LeDuc, D.L., Tarun, A.S., Montes-Bayon, M., Meija, J., Malit, M.F., Wu, C.P., AbdelSamie, M., Chiang, C.-Y., Tagmount, A., deSouza, M., Neuhierl, B., Bock, A., Caruso, J., Terry, N., 2004. Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian Mustard increases selenium tolerance and accumulation. Plant Physiol. 135, 377–383.

March, J., 1985. Advanced Organic Chemistry. Wiley–Interscience, New York.

McKenzie, M.J., Hunter, D.A., Pathirana, R., Watson, L.M., Joyce, N., Rowan, D.D., Matich, A.J., Brummell, D.A., 2009. Accumulation of an organic anticancer selenium compound in a transgenic *Solanaceous* species shows wider applicability of the selenocysteine methyltransferase transgene from selenium hyperaccumulators. Transgenic Res. 18, 407–424.

Meija, J., Bryson, J.M., Vonderheide, A.P., Montes-Bayon, M., Caruso, J.A., 2003. Studies of selenium-containing volatiles in roasted coffee. J. Agric. Food Chem. 51, 5116–5122.

Meija, J., Montes-Bayon, M., Le Duc, D.L., Terry, N., Caruso, J.A., 2002. Simultaneous monitoring of volatile selenium and sulfur species from Se accumulating plants (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction. Anal. Chem. 74, 5837–5844.

Neuhierl, B., Thanbichler, M., Lottspeich, F., Böck, A., 1999. A family of *S*-methylmethionine-dependent thiol/selenol methyltransferases – role in selenium tolerance and evolutionary relation. J. Biol. Chem. 274, 5407–5414.

Perpète, P., Duthoit, O., De Maeyer, S., Imray, L., Lawton, A.I., Stavropoulos, K.E., Gitonga, V.W., Hewlins, M.J.E., Dickinson, J.R., 2006. Methionine catabolism in *Saccharomyces cerevisiae*. FEMS Yeast Res. 6, 48–56.

Rayman, M.P., 2008. Food-chain selenium and human health: emphasis on intake. Brit. J. Nutr. 100, 254–268.

Shah, M., Meija, J., Caruso, J.A., 2007. Relative mass defect filtering of high-resolution mass spectra for exploring minor selenium volatiles in selenium-enriched green onions. Anal. Chem. 79, 846–853.

Sors, T.G., Ellis, D.R., Salt, D.E., 2005. Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth. Res. 86, 373–389.

Tagmount, A., Berken, A., Terry, N., 2002. An essential role of *S*-adenosyl-L-methionine: L-methionine *S*-methyltransferase in selenium volatilization by plants. Methylation of selenomethionine to selenium-methyl-L-selenium-methionine, the precursor of volatile selenium. Plant Physiol. 130, 847–856.

Terry, N., Zayed, A.M., de Souza, M.P., Tarun, A.S., 2000. Selenium in higher plants. Annu. Rev. Plant Physiol.: Plant Mol. Biol. 51, 401–432.

Thompson, D.P., Boudjouk, P., 1988. A convenient synthesis of alkali metal selenides and diselenides in tetrahydrofuran and the reactivity differences exhibited by these salts toward organic bromides. Effect of ultrasound. J. Org. Chem. 53, 2109–2112.

Tsai, J.H., Hiserodt, R.D., Ho, C.-T., Hartman, T.G., Rosen, R.T., 1998. Determination of volatile organic selenium compounds from the Maillard reaction in a selenomethionine-glucose model system. J. Agric. Food Chem. 46, 2541–2545.

White, P.J., Bowen, H.C., Marshall, B., Broadley, M.R., 2007. Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants. Ann. Bot. 100, 111–118.

Zhang, L., Chasteen, T.G., 1994. Amending cultures of selenium-resistant bacteria with dimethyl selenone. Appl. Organomet. Chem. 8, 501–508.