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GROWTH-INHIBITORY VOLATILE AROMATIC COMPOUNDS PRODUCED BY *SOLANUM TUBEROSUM* TUBERS*

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Abstract—Some of the aromatic compounds evolved by stored potato tubers have been identified by combined GLC–MS. Of the identified compounds, benzothiazole, 1,4-dimethylnaphthalene and 1,6-dimethylnaphthalene are comparatively potent inhibitors of sprout growth in the potato tuber. The growth suppressing activity of the two dimethylnaphthalenes is comparable with that of isopropyl-(*N*-3-chlorophenyl)-carbamate, which is used commercially in potato storage.

INTRODUCTION

DORMANT potato tubers evolve volatile substances, some of which contribute to the smell and flavour of the potato and some of which have a suppressing effect on the growth of sprouts from the tubers. Burton and Meigh[1] stored potatoes at 10° in a closed container in which respiratory CO_2 was absorbed by solid NaOH and the consumed oxygen continuously replenished. Under these conditions sprouting was suppressed; some 20–30 substances were found to accumulate in the surrounding air, but in rather low concentrations equivalent to their production by the potatoes at individual rates of the order of 1 ng kg^{-1} hr^{-1}.

Flavour chemists have studied raw and cooked potatoes; in a recent review[2] over 100 volatile substances were listed as being present, of which five were aromatic hydrocarbons. Interest has usually centred on the more odorous constituents and little attention has been paid to hydrocarbons. It has been noticed in a survey[3] that a wide range of different foodstuffs yield aromatic hydrocarbons. The authors suggested that these compounds are not contaminants, as was first thought, but are in fact naturally occurring substances. Their role in plants, however, is unknown and their origin uncertain.

The aim of our work has been to identify some of the substances that are produced by the potato and to measure their growth inhibitory activities.

RESULTS AND DISCUSSION

It was clear from our exploratory work[1] that the technique of extracting volatile compounds from the potato in suitable quantities for identification, and free from extraneous materials, would present as many problems as the identification itself. Initially an established technique[4] was used to obtain compounds evolved by potato skin when immersed in boiling

* Part II in the series "The Production of Growth Suppressing Volatile Substances by Stored Potato Tubers". For Part I see BURTON, W. G. and MEIGH, D. F. (1971) *Potato Res.* **14**, 96–101.

[1] BURTON, W. G. and MEIGH, D. F. (1971) *Potato Res.* **14**, 96.
[2] JOHNSON, A. E., NURSTEN, H. E. and WILLIAMS, A. A. (1971) *Chem. Ind.* (*London*) 1212.
[3] JOHNSON, A. E., NURSTEN, H. E. and SELF, R. (1969) *Chem. Ind.* (*London*) 10.
[4] SELF, R., ROLLEY, H. L. J. and JOYCE, A. E. (1963) *J. Sci. Food. Agr.* **14**, 8.

water. A number of the more volatile products were found to correspond to those identified in the vapour from cooked, peeled potatoes. Other components that were identified are listed in Table 1, but those which have previously been found in the potato[2] are omitted. In addition, 22 benzenoid hydrocarbons were detected and their MS obtained. They were alkyl-substituted benzenes with molecular weights of 106, 120, 134 or 148. They could be characterized, if so desired, by a detailed study of the MS and GLC retention indices of the relevant standards. In the present work, however, bioassays showed that same members of this class had little activity and our interest turned to bicyclic compounds.

TABLE 1. NOVEL VOLATILE COMPOUNDS FOUND* IN COOKED POTATO PEEL

n-Heptane	3-Methylfuran	Benzothiophene	*o*-Xylene
n-Octane	2-Butylfuran†	2-Methoxy-3-isopropylpyrazine†	*m*-Xylene
n-Decane	2-Hexylfuran†	Benzene	1-Methylnaphthalene
2-Methylfuran	2-Methylpropanal	Toluene	2-Methylnaphthalene
	Decanal		

* Identification based on GLC retention data and MS.
† Reference compound not available. Tentative identification from the MS.

Attempts were then made to collect volatile substances evolved by respiring potatoes. The tubers were enclosed in a jar in such a way that the respired CO_2 was absorbed and the consumed oxygen continuously replenished. After 2–3 weeks, the air from the jar containing the accumulated volatile compounds was swept through a refrigerated trap, the volatiles freed from water, and analysed. The yield from this type of experiment was disappointingly small. An extract of ether-soluble lipid from the surface of the potato also yielded insufficient material.

More successful results were obtained by removing the peel from potatoes, immediately freezing it in liquid nitrogen and then drying the peel under high vacuum at room temperature. This process yielded an aqueous distillate from which a volatile concentrate was obtained without heating. When this was analysed, it was found that the yield of compounds with boiling points higher than methylnaphthalene began to diminish. However, the dry peel yielded an ethereal extract from which a good yield of higher boiling components was obtained. The identified compounds are listed in Table 2. Their characterization was based, (a) on GLC retention times when run on columns with polar and non-polar stationary phases, and (b) on comparison of MS with reference spectra obtained with the same spectrometer. 2-Methoxy-3-ethylpyrazine was identified only by its GLC behaviour and its potato-like odour. There was insufficient material to determine a MS. Buttery *et al.*[5] have previously found benzothiazole, biphenyl, naphthalene and 2-methylnaphthalene in potatoes, steam distilled at 45–50°. They also obtained some unspecified evidence for the presence of 2-methoxy-3-ethylpyrazine.

The ether which was used to extract the dry peel was found to contain a number of impurities in concentrations comparable with those of the compounds derived from the potato. A control experiment was therefore carried out in order to examine an ether concentrate with GLC–MS. The results showed that negligible quantities of the compounds

[5] BUTTERY, R. G., SEIFERT, R. M. and LING, L. C. (1970) *J. Agr. Food Chem.* **18**, 538.

ascribed to the potato were present in the ether, with the exception of 1- and 2-methylnaphthalene. Since these compounds were also present in the distillate from the potato, the possible interference of the solvent can be ignored.

TABLE 2. VOLATILE COMPOUNDS FOUND* IN POTATO PEEL AFTER FREEZE-DRYING

Compounds identified	Found in distillate	Found in ethereal extract of dry peel	Approximate proportions found in distillate	Approximate proportions found in ethereal extract of dry peel
Benzothiazole	+		4	
2-Methoxy-3-ethylpyrazine†	+		<1	
Biphenyl	+	+	3	1
Naphthalene	+	+	4	10
1-Methylnaphthalene	+	+	1	2
2-Methylnaphthalene	+	+	1	2
1,4-Dimethylnaphthalene		+		2
1,6-Dimethylnaphthalene		+		2
2,6- or 2,7-Dimethylnaphthalene‡		+		1
Trimethylnaphthalene§ (configuration unknown)		+		4

* Identification based on GLC retention data and MS.

† Identification based on GLC retention data and odour only. Reference compound synthesized by the method of Siefert *et al.*[6]

‡ The authentic 2,7 isomer was not available. The two isomers have similar MS (see Hamming[7]) and identical retention times on three stationary phases (see Mosteky *et al.*[8]).

§ Retention data and MS were similar to those of the 2,3,5 isomer, but the characterization cannot be confirmed without GLC data and MS for all 14 isomers.

Burton and Meigh[1] showed that the natural volatile growth suppressant of the potato was stable in the vapour phase in air, (a) in the presence of solid NaOH, and (b) when passed through 96% H_2SO_4. There was a comparatively slight (15%) loss of activity when air from a jar containing potatoes was recirculated through 96% H_2SO_4. All the compounds in Table 2, except 2-methoxy-3-ethylpyrazine, satisfied these criteria; those which were commercially available were therefore tested for growth suppressing activity on samples of 20 unsprouted tubers. Other compounds which incorporate simple chemical modifications of the potato volatiles were also tested in the same way. The results of these bioassays are recorded in Table 3 and details of the storage times and sprouting behaviour of the control tubers, with which the treated ones were compared, in Table 4. The potency of each volatile compound was measured by its effect both on the total weight of sprouts produced by the tubers, and on the length of the longest sprout on each tuber. In general, the results obtained by using these two criteria are in fair agreement. There was not time exhaustively to test each substance at all the desirable concentrations. In addition, some substances were not sufficiently volatile to provide the desired concentration for complete inhibition of sprouting.

[6] SEIFERT, R. M., BUTTERY, R. G., GUADAGNI, D. G., BLACK, D. R. and HARRIS, J. G. (1970) *J. Agr. Food Chem.* **18**, 246.

[7] HAMMING, M. C. (1970) *Arch. Mass Spectral Data* **1**, 228.

[8] MOSTECKÝ, J., POPL, M. and KŘIŽ, J. (1970) *Anal. Chem.* **42**, 1132.

However, benzothiazole was tested at three different concentrations, which enables rough comparisons to be made for most of the compounds listed.

TABLE 3. SUPPRESSION OF SPROUTING IN STORED POTATOES (CV. KING EDWARD) AT 10° BY THE VAPOUR OF VARIOUS AROMATIC COMPOUNDS IN AIR

Compound	Expt. No.*	Concentration of compound ($\mu g\ l^{-1}$ air)	Sprout wt† (% control)	Sprout length‡ (% control)	Compound	Expt. No.*	Concentration of compound ($\mu g\ l^{-1}$ air)	Sprout wt† (% control)	Sprout length‡ (% control)
Found in potato					*Structurally related*				
Benzothiazole	4	4	67	68	2-Methylbenzothiazole	8	10	41	36
	4	8	23	28	3,3'-Dimethylbiphenyl	5	6	24	42
	2	28	0	0	4,4'-Dimethylbiphenyl	6	1	90	91
Biphenyl	1	16	28	42	1,2-Dimethylnaphthalene	7	13	9	28
Naphthalene	2	54	24	20	1,3-Dimethylnaphthalene	6	19	1	<1
1-Methylnaphthalene	9	10	48	56	1,5-Dimethylnaphthalene	8	6	5	9
2-Methylnaphthalene	1	66	2	8	2,3-Dimethylnaphthalene	3	7	38	36
1,4-Dimethylnaphthalene	7	14	0	0	2,6-Dimethylnaphthalene	8	5	70	64
1,6-Dimethylnaphthalene	7	16	0	0	2,3,5-Trimethylnaphthalene	7	5	6	20
					2,3,6-Trimethylnaphthalene	4	3	105	133

* Experimental data are shown in Table 4. Each sample consisted of 20 tubers and was treated for about 40 days.

† Total weight of sprouts, expressed as % of control.

‡ Average length of longest sprout on each tuber expressed as % of control.

It will be seen that the 1,4- and 1,6-dimethylnaphthalenes were the most potent aromatic growth inhibitors found in the potato. None of the related compounds (which were absent from the potato) appeared to be more potent than these two. Substitution of methyl groups appeared to enhance sprout inhibiting activity in the biphenyl and naphthalene series in a manner comparable to that found with methyl substitution in the cyclohexanol, cyclohexanone, cyclohexenone and acetophenone series;[9] but the position of the groups on the aromatic ring seems to be irrelevant. This effect is perhaps attributable to increasing solubility in lipids, since the potency of the volatile compounds is dependent on at least two factors, (1) the intrinsic activity at the point of action in the cell, and (2) the rate of transport from the vapour phase (in air) to the (presumed) lipid phase in the cell.

TABLE 4. DATA ON POTATOES (CV. KING EDWARD) STORED AT 10° AND TREATED WITH VARIOUS VOLATILE AROMATIC COMPOUNDS IN AIR

Expt. No.	Storage date	Storage period (days)	Av. tuber wt (g)	Control tubers: Av. sprout wt per tuber (g)	Control tubers: Av. length of longest sprout (cm)
1	1.3.72	42	120·0	1·9	4·4
2	24.4.72	42	131·8	2·0	5·5
3	10.5.72	35	153·5	2·0	5·1
4	31.5.72	42	107·1	2·0	5·1
5	21.6.72	40	122·5	2·1	6·2
6	20.7.72	40	112·8	1·7	4·9
7	25.8.72	45	132·3	2·2	3·0
8	11.9.72	42	106·7	1·2	4·1
9	18.9.72	42	111·6	1·0	3·7

[9] MEIGH, D. F. (1969) *J. Sci. Food Agr.* **20**, 159.

When flasks of potatoes, which had been treated with 1,4- or 1,6-dimethylnaphthalene, were opened to the air, there remained a lingering smell of the naphthalene. This observation, and the fact that less of each of the naphthalenes was detected in the potato than was necessary artificially to stop sprouting, suggest that neither compound is primarily responsible for the effects described by Burton and Meigh.[1] However, the possibility remains that some synergistic effect, between two or more of the potato products, may account for the phenomena. A single experiment was performed in which air containing both benzothiazole (6 $\mu g\, l^{-1}$ air) and naphthalene (12 $\mu g\, l^{-1}$ air) was passed over potatoes (Conditions, Table 4, as in Experiment 5). When the potatoes were examined, sprout weight was 58% and sprout length 52% of the control. In this instance no synergism was apparent but it is possible that by testing all possible combinations of compounds found in the potato some positive effect might be revealed.

TABLE 5. A COMPARISON OF THE POTENCY OF SOME COMPOUNDS USED TO SUPPRESS SPROUT GROWTH IN POTATOES

Sprout suppressant	Approximate concentration required for complete suppression ($\mu g\, l^{-1}$ air)	($mg\, kg^{-1}$ potatoes)	Ref.
Isopropyl-(*N*-3-chlorophenyl)-carbamate (CIPC or chlorpropham)	10–20	10–20	10
1-Chloronaphthalene	—	100	11
Methyl 1-naphthaleneacetate	—	17–67	11
1-Methyl-4-isopropyl-cyclohex-1-en-8-ol (α-terpineol)	125	100	9, 11
2,3,5,6-Tetrachloronitrobenzene (TCNB)	—	100	12
3,5,5-Trimethylhexanol-1(nonyl alcohol)	60	—	9
1,4-Dimethylnaphthalene	14	—	This paper

Although both biphenyl and naphthalene have previously been found to suppress the sprouting of potatoes to some extent (R. G. Tomkins, personal communication) there is more interest in a comparison of the potency of the dimethylnaphthalenes with that of compounds which have been used as commercial sprout suppressants. A number of these are listed in Table 5. Comparison is not easy since the majority of them are applied to the potato in a solid carrier, rather than as a vapour in air. Maleic hydrazide is not listed since it is applied to the foliage at the growing stage. Of the others, there are reliable vapour phase figures for α-terpineol,[9] nonyl alcohol[9] and CIPC.[10] Since figures are quoted for CIPC and α-terpineol in terms both of concentration in air and of weight applied per kg potato, a rough comparison can be made with the other compounds. Since the potency of 1,4-dimethylnaphthalene is apparently comparable with that of the most potent commercial substance now in use, it might be worth studying the problems of using 1,4-dimethylnaphthalene on a large scale, and its possible toxicity. A choice would have to be made between the two available modes of application, (a) as a liquid in a solid carrier, and (b) as

[10] VAN VLIET, W. F. and SPARENBERG, H. (1970) *Potato Res.* **13**, 223.
[11] FINDLEN, H. (1955) *Am. Potato J.* **32**, 159.
[12] BROWN, W. and REAVILL, M. J. (1954) *Ann. Appl. Biol.* **41**, 435.

a vapour in air. The latter method involves a number of physical problems which, in the case of nonyl alcohol, have to some extent been investigated.[13]

The analysis of other volatile compounds produced by stored potato tubers is proceeding.

EXPERIMENTAL

Gas chromatograph. Pye Series 104, silanized glass column 1·5 m × 4 mm, packed with CQ support (85–100 mesh, JJ's (Chromatography) Ltd.) coated with silicone OV 17 (5%) or Carbowax 20M (5%). Packing prepared by the method of Parcher and Urone.[14] Oven programmed from 50 to 200° at 4° min^{-1} (or isothermal at 100°). F.I.D. Carrier gas, argon 45 ml min^{-1}.

GLC–MS assembly. Pye Series 104, SCOT stainless steel column 17 m long, coated with Carbowax 20M and programmed from 50 to 200° at 3° min^{-1}. Carrier gas, helium 5 ml min^{-1}. Attached to GEC–AEI MS902 spectrometer. MS results processed with an IBM 1130 computer.

Extraction of volatile products. (1) *Headspace analysis.* A dish filled with KOH pellets was placed in the bottom of a 10-l. flange-mouthed glass jar. Sound, washed potato tubers (5 kg) were packed inside and the jar was closed with a flanged glass cap fitted with a tubular inlet. O_2 was admitted to the jar under slight positive pressure obtained by allowing the gas to flow out slowly through a branch tube, the open end of which discharged a few cm under H_2O in a beaker. After storing the potatoes in this way at 10° for 2–3 weeks the jar was transferred to the laboratory. N_2 (purified by passing through a tube immersed in liquid N_2 and packed with pellets of molecular sieve type 5A) was used to sweep the accumulated volatiles from the jar and carry them first through a tube (200 × 4 mm) packed with dry gelatine powder to remove H_2O vapour[15] and then into a stainless steel trap[16] immersed in liquid O_2. The gelatine powder (40–60 mesh) was dried before use by flushing with N_2 for 16 hr at about 90°. The contents of the trap were liberated by ohmic heating and delivered through heated stainless steel tubing to the gas chromatograph.

(2) *Extraction of whole tubers.* 10 tubers were each washed by fixing on a spike and rotating for 30 sec in a dish of Et_2O. The combined Et_2O washings were concentrated to about 5 ml by fractional distillation. The concentrate was frozen and the volatile components distilled into a glass trap, cooled in liquid N_2, under high vacuum. A sample of this condensate (10 μl) was injected into the gas chromatograph.

(3) *Distillate from freeze-dried potato skin.* Sound, washed tubers were peeled with a stainless steel household peeler and the peelings immediately dropped into liquid N_2. The frozen peel (75 g) was crushed into small pieces with a glass rod and transferred to 1 l. flasks. When the liquid N_2 had evaporated almost to dryness the flasks were connected to large U-tubes immersed in liquid N_2 and the peel allowed to dry for 24 hr under high vacuum. The flask and U-tube connections were made with Sovirel joints (SLV 30) to avoid the possibility of contamination by volatile impurities in grease. The aqueous distillate collected in the U-tubes was allowed to melt at 2°, filtered through glass paper (Whatman type GF/C) and transferred in 200 ml portions to wash bottles fitted with sintered glass gas distributors. Enough anhyd. Na_2SO_4 was added to these to saturate the solution and provide an excess. Volatiles were then swept out of the aqueous medium for 8 hr with N_2 (purified as before). At first, N_2 was allowed to flow at a rate of 600 ml hr^{-1}, the H_2O vapour removed with gelatine (as before) and the volatiles collected in a stainless steel trap. Later it was found that better recoveries were obtained by introducing an additional trapping stage. Nitrogen was passed at a higher rate (20 l. hr^{-1}); both the H_2O vapour and the volatiles were trapped in a U-tube with a sintered glass plate fitted near the lower end of the inlet arm (30 mm bore) and immersed in powdered solid CO_2. Sodium sulphate (10 g) was spread on the sintered plate before trapping began. Afterwards the U-tube was allowed to warm in the room. The melted ice was absorbed into the Na_2SO_4 layer and purified N_2 (10 ml min^{-1} for 4 hr) was used to sweep the volatiles through the gelatine column into the stainless steel trap. The contents of the trap, containing the products from 1·15 kg potato skin, were injected into the gas chromatograph.

(4) *Extract of freeze-dried potato skin.* Freeze-dried skin (150 g) was extracted with Et_2O (250 ml) at 2° for 16 hr. After filtration the Et_2O extract was concentrated to 5 ml by fractional distillation. This concentrate was frozen and redistilled under high vacuum to remove non-volatile impurities. Finally the volume was reduced to about 0·5 ml under a gentle stream of N_2 and a sample (10 μl) injected into the gas chromatograph. To provide a check on the impurities present in the Et_2O, a concentrate from a similar vol. of Et_2O was prepared in the same manner.

Assay of sprout suppression. Potatoes, cv. King Edward, were stored at 2° or 4° to inhibit sprouting and transferred to a room at 10° about 1 week before use. Sound, washed tubers were placed in 5 l. flat flange flasks (20 tubers per flask). Each lid was fitted with a B34 taper socket which formed the point of attachment for the vapour dispensing apparatus. The one used for assaying liquids has been described.[17] It consisted

[13] CURRAH, I. E. and MEIGH, D. F. (1968) *J. Sci. Food Agr.* **19**, 409.
[14] PARCHER, J. F. and URONE, P. (1964) *J. Gas Chromatog.* **2**, 184.
[15] SZYMANSKI, H. A. and AMABILE, T. (1969) *J. Chromatog. Sci.* **7**, 575.
[16] SWOBODA, P. A. T. and LEA, C. H. (1965) *J. Sci. Food Agr.* **16**, 680.
[17] MEIGH, D. F. (1967) *Chem. Ind. (London)* 1487.

essentially of a standard cone joint to which was attached a silicone rubber tube closed at one end to contain the liquid. The rate of vapourization was controlled by adjusting the length of the rubber tube. When very low rates were needed, the glass tube was closed with a plug made of silicone rubber paste (type SR300, Esco (Rubber) Ltd.). The rate of vapourization was then controlled by altering the width of the glass opening and thickness of the rubber plug. The vapour diffuser was housed in an outer glass tube through which the ventilating air passed to reach the flask. To assay solids, the air was allowed to flow through a tube or conical flask in which the solid was placed. Each flask of potatoes was wrapped in black polythene sheet to exclude light, placed in a room at 10° and ventilated with air drawn from outside the building by a diaphragm pump. The air was dried over lumps of $CaCl_2$ before flowing through each flask at a rate of 5 l. hr^{-1}. The compounds used for the tests were of the highest purity commercially available.

After about 6 weeks the potatoes were removed for examination. The sprouts were separated from the tubers, the length of the longest sprout in each tuber was measured and the sprouts were weighed.

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