INTERACTIONS OF THE FUNGICIDE 2-AMINOBUTANE WITH POTATO TUBERS

by

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To my wife

Barbara

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Declaration

I declare that this thesis is my own composition, that the work, of which it is a record, was carried out by myself, and that it has not been submitted in any previous application for a Higher degree.

The thesis describes results of research carried out in the Department of Chemistry, University of Edinburgh, under the supervision of Dr. A.G. Rowley since 1st October 1983, the date of my admission as a research student.

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The following post-graduate courses were attended in the last three years.

Edinburgh University Organic Chemistry Seminars	
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Industrial Chemistry	Dr. R.S. Sinclair *et al.*
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Abstract

A synthetic route to 2-aminobutane (2-AB) was developed, which enabled the synthesis of specifically and uniformly ^{14}C labelled 2-AB to be carried out. ^{14}C labelling was employed as a means of detecting 2-aminobutane in tuber tissue samples. An analytical technique, based on acid extraction and scintillation counting, was developed for the determination of 2-AB residues in potato tubers. This allowed studies to be carried out on various factors influencing the efficiency of fumigation of potato tubers with 2-aminobutane.

Using ^{14}C 2-AB and a specially constructed small scale fumigation chamber, an investigation into the absorption and penetration of 2-AB into tubers was undertaken. The rate and extent of 2-AB absorption were studied, and also the effect of initial fumigant concentration and length of exposure period, on the final 2-AB residues in tubers. The depth of penetration was shown to be limited to the first 1 cm of tissue indicating that after initial absorption, the amine is firmly bound at or near the tuber surface. Thin layer chromatography of extracts from fumigated tubers, provided evidence which suggested that 2-AB is not metabolised by the tubers during the storage period.

Damaged tubers were shown to absorb higher levels of 2-AB, and the effect of wound healing at different temperatures, prior to fumigation, was demonstrated. The preferential uptake of 2-aminobutane through tuber lenticels was highlighted as a possible cause of tuber to tuber variation in 2-AB residues. Studies showed that there was no appreciable loss of 2-AB from fumigated tubers during the storage period, and that transfer of 2-AB from tuber to tuber during air recirculation was very limited.

The absorption of 2-AB by soil, straw and wood, was examined and identified as a likely sink for the amine fumigant, and this could contribute to the low 2-AB residues recorded for some commercial fumigations.

A comparison of the antifungal activity of the optical isomers of 2-aminobutane, confirmed that the R-(-)- enantiomer was more active than the corresponding S-(+)- isomer, or racemic mixture, against the fungi responsible for gangrene and skin spot in potato tubers.

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Introduction.

A. Use of 2-aminobutane as a fungicide for the control of post-harvest disease of fruit.

A.1 Background.

By the early 1960's the fungistatic properties of quaternary ammonium salts and higher alkyl amines were already well documented.[1] Also, gaseous ammonia was reported to control Penicillium digitatum on citrus fruit[2], but little information was available on the activity of simple aliphatic amines or their salts. Eckert and Kolbezen[3] sought to remedy this by screening more than 40 amines, representing a variety of chemical structures, to determine their effectiveness as vapour phase fungicides for the control of P.digitatum on oranges. Preliminary trials[4] revealed a general correlation of effectiveness with boiling point of the amines, but some anomalies were present (Table 1).

Among the C_2-C_6 amines only sec-butylamine (2-aminobutane) and isopropylamine showed appreciable in vitro fungistatic activity. The inherent fungistatic properties of sec-butylamine are clearly reflected in the degree of decay control provided by this compound.

Table 1 Relation of vapour pressure and in vitro fungitoxicity of aliphatic amines to control of decay in oranges caused by P.digitatum.[a]

Amines	Vapour pressure mmHg at 20 °C	ED50[b] ($x10^{4}$ M)	% decay reduction[c]	Fruit injury index[d]
Ammonia	-	300	-	-
Ethylamine	873	300	88.9	2
Isopropylamine	455	35	82.7	0
tert-Butylamine	290	300	62.9	0
n-Propylamine	245	300	67.9	2
sec-Butylamine	135	3	100.0	0
Isobutylamine	110	260	56.8	1
n-Butylamine	71	300	47.0	2
1-Ethylpropylamine	44	300	45.7	2
1-Methylbutylamine	45	300	50.6	2
Isopentylamine	32	300	25.9	2
n-Pentylamine	24	300	14.8	2
1,3-Dimethylbutylamine	25	300	33.3	2
n-Hexylamine	8	240	1.2	3
1,1,3,3-Tetramethyl butylamine	8	135	11.1	2
Cyclohexylamine	7	300	30.9	2
n-Heptylamine	3	100	1.2	3
2-Ethylhexylamine	2	65	0	4
Isooctylamine	1	60	12.3	4
n-Octylamine	1	65	12.3	4
n-Nonylamine	<1	30	0	4
n-Decylamine	<1	7	3.7	4

a. Table after Eckert and Kolbezen.[4]

b. Evaluated as the chloride salts at pH 5.5 .

c. $\frac{\text{(\% Decay in controls - \% decay in treatment)}}{\text{\% decay in controls}} \times 100$
mean of 3 replicate cartons of 50 inoculated fruit each.

d. Fruit rind injury; 0=none; 1=very slight; 2=slight; 3=moderate; 4=severe.

These early encouraging results prompted Eckert and Kolbezen to undertake further experimental work to determine the possible usefulness of 2-aminobutane, and its salts, for control of post-harvest decay of citrus, apple, pear, peach and banana fruits.[5,6]

The _in vitro_ activity of 2-aminobutane against selected micro-organisms was determined in experiments involving a nutrient medium, to which was added portions of sec-butylammonium hydrogen phosphate stock solution and small quantities of inoculum (spores or hyphae) of the test organism. The reduction in growth due to presence of the 2-aminobutane was estimated visually after 3 or 5 days, depending on the organism being investigated. Table 2 shows the anti-microbial spectrum of 2-aminobutane and the relative sensitivities of all the micro-organisms tested. Although 2-aminobutane possesses a rather narrow spectrum of anti-microbial activity, it is nevertheless important, because it inhibits some of the fungi which are responsible for the most devastating fruit decays. These include _Penicillium digitatum_, _P.italicum_, _P.expansum_, _Monilinia fructicola_, _Gloesporium musarum_, _Glomerella cingulata_, _Thielaviopsis paradoxa_ and _Phomopsis citri_.

Table 2 Antimicrobial spectrum of 2-aminobutane (sec-butylammonium phosphate) in liquid medium at pH 6.[a]

micro-organism	ED50 - Mycelial growth (g cm^{-3})[b]
Alternaria citri	250
A.solani	250-500
Aspergillus niger	1000
Botryosphaeria ribis	500
Botrytis cinerea	750
Cladosporium cucumerinum	1000
Diplodia natalensis	1000
Erwinia carotovora	>1000
Fusarium oxysporum f. lycopersici	>1000
Geotrichum candidum	>1000
Gloesporium musarum	100-250
Glomerella cingulata	50
Monilinia fructicola	50-75
Monilinia laxa	75-100
Myrothecium verrucaria	>1000
Nevrospora sitophila	100
Penicillium digitatum	75
P. expansum	250
P. italicum	100-250
P. vermoeseni	750
Phomopsis citri	50
Pleospora lycopersici	100-250
Phytoplithora citrophthora	1000
Pythium debaryanum	>1000
Rhizoctonia solani	>1000
Rhizopus stolonifer	>1000
Saccharomyces pastorianus	>1000
Sclerotinia sclertiorum	250-500
Sclerotium rolfsi	500
Thielaviopsis paradoxa	75-100
Trichoderma viride	>1000

a. Table after Eckert and Kolbezen.[5]

b. ED_{50} values estimated at 3 days for all organisms except: Botryosphaeria, Cladosporium, Glomerella, Monilinia laxa, Phomopsis, Phytophthora, and Sclerotinia, which were evaluated at 5 days.

Further experimental work by Eckert and Kolbezen was aimed at establishing the effectiveness of 2-aminobutane for the control of post-harvest decay of citrus and pome fruits, incited by the above micro-organisms. In some cases the effectiveness of 2-aminobutane treatment was compared with other possible or existing treatments.[5,7] The results of this work are summarised below.

A.2 Comparison of 2-aminobutane with other aliphatic amines for the control of Penicillium decay of lemons.

In spore germination tests with P.digitatum, 2-aminobutane, n-decylamine, n-nonylamine and isopropylamine had the lowest ED_{50} values of 21 amines tested at pH 5.5 .[4] To determine if the same effectiveness would be shown in preventing fruit decay, lemons inoculated with P.digitatum were immersed for 3 minutes in 0.1M solutions of amine hydrochlorides at pH 6 and 23°C. The fruit were not rinsed after treatment. Three replications of 15 lemons and 2 inoculations per lemon were treated with each amine solution. In 2 experiments, lemons treated with the amines had the following decay after 7 days storage at 20°C: 2-aminobutane, 0 and 2%; nonylamine, 95% and 63%; and decylamine, 67% and 51%. Fruit receiving all other

amine treatments showed over 95% decay in both tests.

To determine if the anion in a 2-aminobutane salt solution had any influence on the effectiveness of the sec-butylammonium cation, inoculated lemons were treated with solutions of 2-aminobutane at pH 9 containing an equivalent quantity of HCl, H_2SO_4, H_3PO_4, acetic, propionic or citric acids. The lemons were rinsed after treatment and stored at 20°C for 14 days. All 2-aminobutane salt solutions reduced decay below 10% and there was no detectable difference in the effectiveness of the different salts.[5]

A.3 Effectiveness of 2-aminobutane, 2-aminopyridine, pyrrolidine and sodium o-phenylphenate in the control of *Penicillium digitatum* on Valencia oranges.

Table 3 shows that 2-aminobutane was more effective than either 2-aminopyridine or pyrrolidine, in controlling decay of oranges artificially inoculated with *P.digitatum*. Although 2-aminobutane and sodium o-phenylphenate gave equivalent protection when both treatments were followed by a water rinse, the unrinsed 2-aminobutane treatment was superior to the rinsed o-phenyl treatment (c.f. treatments 9 and 10). Many fungicides, such as sodium o-phenylphenate which are phytotoxic, must be rinsed from the fruit after treatment to prevent chemical damage.[12] 2-Aminobutane

Table 3 Effectiveness of 2-aminobutane, pyrrolidine, 2-aminopyridine and sodium o-phenylphenate in control of P.digitatum on Valencia oranges.[a]

	Treatment[b]	pH	rinsed[c]	% Decay at 14 days
1.	water	10	-	91.5
2.	0.63% 2-aminopyridine	6	No	40.9
3.	0.63% 2-aminopyridine	10	No	19.7
4.	0.48% pyrrolidine	6	No	25.7
5.	0.48% pyrrolidine	10	No	17.2
6.	0.49% 2-aminobutane	6	Yes	18.1
7.	0.49% 2-aminobutane	10	Yes	11.5
8.	0.49% 2-aminobutane	6	No	3.8
9.	0.49% 2-aminobutane	10	No	6.7
10.	0.5% sodium o-phenylphenate	11.8	Yes	14.4

a. Table after Eckert and Kolbezen.[5]

b. Weight concentrations for the amines correspond to 0.067M. Oranges submerged for 3 minutes in solutions at 20°C.

c. Where specified, oranges were rinsed by 2 second submersion in tap water.

is not phytotoxic and does not have to be rinsed from the fruit, therefore substantial residues remain to prevent growth of sensitive fungi, and problems resulting from wetting the fruit can be avoided.

A.4 Evaluation of 2-aminobutane treatment for control of post-harvest disease caused by *Penicillium expansum* on apples and pears.

Table 4 shows that 2-aminobutane was considerably more active than sodium o-phenylphenate in reducing decay of apples inoculated with *P.expansum*. Five different isolates of *P.expansum* were compared in inoculation tests and the control of each was essentially the same as the results presented in Table 4, confirming the superiority of 2-aminobutane over sodium o-phenylphenate.

Both sodium o-phenylphenate and 2-aminobutane were less effective in controlling decay of pears artificially inoculated with *P.expansum*, although 2-aminobutane offers some degree of protection on Winter Nellis pears treated when firm ripe (Table 4).

Table 4 Effectiveness of 2-aminobutane and sodium o-phenylphenate in control of P.expansum on apples and pears.[a]

Fruit	Treatment[b]	pH	Temp °C	% Decay[c] at 9 days	15 days
Apple	Water	11.5	2	77.8	95.5
			18	89.3	100
	0.5% 2-aminobutane	10.5	2	9.3	15.1
			18	2.2	2.7
	0.5% Na o-phenyl-phenate	11.5	2	56.0	80.5
			18	49.3	66.2
Pear	Water	11.5	2	97.4	
	1.0% 2-aminobutane	10.0	2	37.8	
	1.0% Na o-phenyl-phenate	11.5	2	80.0	

a. Table after Eckert and Kolbezen.[5]

b. Fruit submerged for 1 minute in solution and then rinsed in tap water for 3 seconds.

c. Percentage of artificial inoculations developing decay lesions; 3 replications of 15 fruit per treatment; each fruit inoculated in 5 locations.

A.5 Control of *Monilinia fructicola* on peaches.

Firm ripe Elberta peaches, inoculated with *Monilinia fructicola* spores, were dipped in fungicide solutions at 23°C for 1 minute. After storage for 3 days at 10°C and 3 days at 25°C the peaches were evaluated as decayed or not decayed. Of 75 fruit receiving each treatment the following numbers of fruit were decayed after storage:

water control	pH 11.5	75 decayed
0.5% sodium o-phenylphenate	pH 11.5	55 decayed
0.5% 2-aminobutane	pH 6.5	15 decayed
0.5% 2-aminobutane	pH 10.5	11 decayed

Treatment of peaches with 2-aminobutane thus provides greater protection against *Monilinia fructicola* than the alternative sodium o-phenylphenate treatment.[5]

A.6 Control of *Gloesporium musarum* on bananas.

In 2 tests, bananas inoculated with *Gloesporium musarum* and subsequently dipped for 1 minute in 1% 2-aminobutane at pH 10.5 and 23°C, showed 15% and 29% decay respectively after 14 days storage at 20°C. All control fruit (dipped in water) were decayed after the same storage period.[5]

The above work clearly illustrates how 2-aminobutane salt solutions can be used to control decay of several fruits. The absence of phytotoxicity associated with this treatment, which is probably due to the impermeability of the surface cells of the fruit to amine cations[8], gives it an advantage over other commercial treatments.

Other effective methods of applying 2-aminobutane to citrus fruits, which have achieved good disease control, include bulk fumigation and application of 2-aminobutane as a spray formulation. Fumigation of citrus fruits with 100ppm (vol/vol) 2-aminobutane in air is highly effective for controlling Penicillium decay of oranges. Application of 1 per cent 2-aminobutane (phosphate) from an overhead spray, after the fruit has been cleaned, has proved to be effective for disease control in citrus fruits.[8]

A.7 Fungistatic properties of optical isomers of 2-aminobutane.

Due to the presence of a chiral centre, 2-aminobutane exists as 2 optical isomers, although the commercially available products are racemic mixtures. In neutral aqueous solution, the hydrochloride salts of enantiomers of 2-aminobutane exhibited striking

differences in their fungistatic action against species of fungi that were inhibited by the racemic mixture.[9,10] The R-(-)-isomer or the racemic mixture at 0.1-0.3 micromole cm^{-3}, prevented germination of spores of Penicillium digitatum and Phomopsis citri, but 10-30 micromole cm^{-3} of the S-(+)-isomer were required to produce the same level of inhibition. Dry weights of mycelial mats of P.digitatum, Monilinia fructicola and Gloemerella cingulata, produced in 7 days on liquid medium containing 1 micromole cm^{-3} of R-(-)-2-aminobutane hydrochloride, averaged 25-50% of control weights, whereas cultures containing 10 micromole cm^{-3} of the S-(+)-isomer yielded 84-100% of the controls.In experiments with oranges, a drop of solution containing 10 micromole cm^{-3} of 2-AB hydrochloride, deposited at the site of inoculation with P.digitatum spores reduced subsequent lesion development from 79% in the control fruit to 67%, 12% and 5% in oranges treated with the S-(+)-isomer, the racemic mixture, and the R-(-)-isomer respectively. It was concluded that the R-(-)-isomer is responsible for most of the effectiveness of 2-aminobutane in controlling Penicillium decay of oranges.[9]

A.8 Effect of amine structure on fungistatic activity.

In the early investigations of Eckert and Kolbezen, on the relationship between the structure of aliphatic monoamines and fungistatic activity[4], it was found that 2-aminobutane was unique among primary amines of low molecular weight, in that it was very inhibitory to the germination of Penicillium digitatum spores in a medium at pH 5.5 . Further work, already described above, showed that 2-aminobutane was also effective in practice for the control of Penicillium decay of citrus fruits. Nonylamine and decylamine which exhibited the same degree of control in vitro as 2-aminobutane, were ineffective in preventing fruit decay. This unique fungistatic activity of the sec-butylammonium cation, prompted a systematic evaluation of the antifungal properties of a large number of aliphatic amines, in an effort to find other active compounds and to establish a relationship between structure and activity in this group.[10]

A comparison of the antifungal activity of 21 aliphatic monoamines revealed that sec-butylamine, isopropylamine and 1-methyl-2-propenylamine, were most effective in preventing P.digitatum spore germination. Table 5 shows the results for inhibition of germination of P.digitatum spores by the amine cations tested.

Table 5 Inhibition of germination of P.digitatum spores by amine cations.[a]

$$R_2-\overset{\displaystyle R_1}{\underset{\displaystyle R_3}{\overset{|}{\underset{|}{C}}}}-NH_3^{+}$$

Amine	R_1	R_2	R_3	ED50 $(M \times 10^4)$[b]
1. Ammonia				>1000
2. Methylamine	H	H	H	296
3. Ethylamine	H	CH_3	H	247
4. Propylamine	H	C_2H_5	H	135
5. n-Butylamine	H	n-C_3H_7	H	227
6. iso-Butylamine	H	iso-C_3H_7	H	94.9
7. 2-Methylbutylamine	H	sec-C_4H_9	H	189
8. Isopropylamine	CH_3	CH_3	H	18.4
9. sec-Butylamine	CH_3	C_2H_5	H	0.989
10. (+)-sec-Butylamine	CH_3	C_2H_5	H	23.5
11. (-)-sec-Butylamine	CH_3	C_2H_5	H	0.356
12. 1-Methyl-2-propenylamine	CH_3	$CH_2=CH-$	H	6.43
13. 1-Methyl-2-propynylamine	CH_3	$CH\equiv C-$	H	>1000
14. 1,2-Dimethylpropylamine	CH_3	iso-C_3H_7	H	407
15. 1-Methylbutylamine	CH_3	n-C_3H_7	H	377
16. 1-Methylpentylamine	CH_3	n-C_4H_9	H	206
17. 1-Methylhexylamine	CH_3	n-C_5H_{11}	H	62.3
18. 1,4-Dimethylpentylamine	CH_3	iso-C_5H_{11}	H	75.0
19. 1-Ethylpropylamine	C_2H_5	C_2H_5	H	637
20. tert-Butylamine	CH_3	CH_3	CH_3	165
21. tert-Pentylamine	CH_3	C_2H_5	CH_3	314

a. Table after Eckert and Kolbezen.[10]

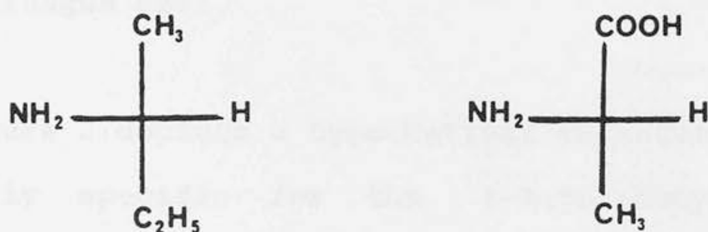
b. The solution of the amine hydrochloride was added to the assay mixture and the pH adjusted to 6.0 before addition of spores.

(-)-sec-Butylamine was about 2.8 times more active than racemic sec-butylamine, and approximately 65 times more active than its enantiomer. Modification of any substituents bonded to the chiral centre of sec-butylamine (R_1, R_2 or R_3 in Table 5), resulted in loss of fungistatic activity. A wide range of other substituted primary monoamines were also tested, and this showed replacement of the C-1 or C-4 methyl group of sec-butylamine with CF_3, CCl_3, COOH, OCH_3, CH_2OH, Cl, NH_2 or OH, gave compounds which were not active. These results showed that the structure of sec-butylamine (2-aminobutane), especially as spacially orientated in the (-) optical isomer, is crucial to the activity of the amine.

The absolute configuration of (-)-sec-butylamine, as elucidated by Kjaer and Hansen[11], shows its relationship to the natural amino acids (Figure 1).

The (-)-sec-butylamine cation is actively absorbed but not metabolised, in 8 hours by hyphal cells of P.digitatum.[10] Also sec-butylamine cations inhibit the uptake of several amino acids and their incorporation into the protein fraction of treated hyphae.[13] The fungistatic action of sec-butylamine against spores is strongly antagonised by many inorganic and organic acids including amino acids and homologs of

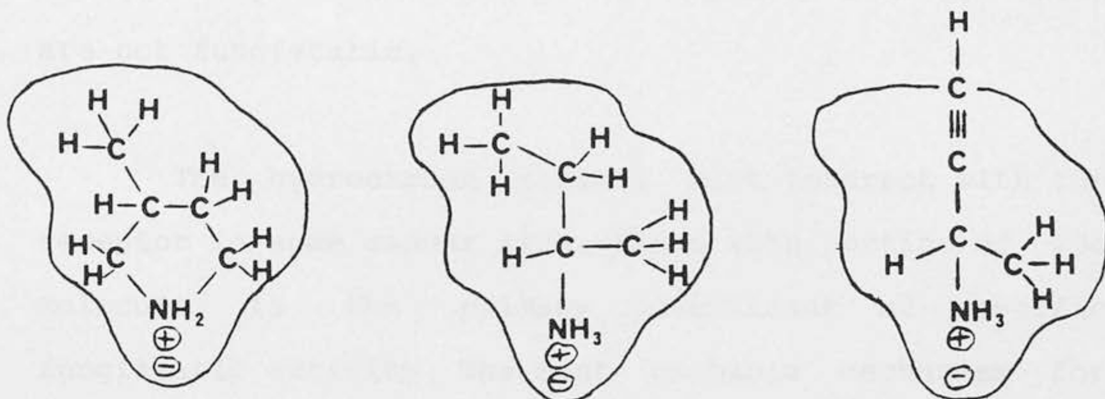
Figure 1 Configuration of (-)-sec-butylamine (Kjaer and Hansen, 1957) and L-(+)-alanine as Fischer projections.[a]



(-)-sec-butylamine L-(+)-alanine

a. Figure after Eckert *et al.*.[10]

Figure 2 Illustrations indicating one possible shape for the hypothetical receptor site on P.digitatum, showing why (-)-3-methyl-pyrrolidine and (-)-sec-butylamine would be recognised by the fungus whereas the unsaturated (+)-1-methyl-2-propynylamine would not.[a]



(-)-3-methyl-pyrrolidine (-)-sec-butylamine (+)-1-methyl-2-propynylamine

a. Figure after Eckert *et al.*.[10]

sec-butylamine which are not fungistatic. These observations suggest that the sec-butylammonium cation interacts reversibly with an immobile anion associated with the fungus cell.[15]

Figure 2 depicts a hypothetical receptor which is structurally specific for the (-)-sec-butylammonium cation and closely related compounds.[10] Stability of the amine-receptor complex would be conferred primarily by an ionic bond between the amine cation and an immobile anionic group (e.g. carboxyl or phosphate). This bond could be reinforced by one or more hydrogen bonds arising through the $-NH_3^+$ protons. The anionic component of the site must be freely accessible and relatively non-selective with regard to structures attached to the $-NH_3^+$ group in order to explain antagonism of the (-)-sec-butylammonium cation by organic cations which are not fungistatic.

The hydrocarbon radical must interact with the receptor in some manner also, since this portion of the molecule is the primary determinant of specific fungistatic activity. The most probable mechanism for this interaction is through hydrophobic bonding which has been shown by Inagami[14] to contribute substantially to the binding of the n-butylammonium cation to the enzyme trypsin.

The hypothetical receptor must also accomodate 3-methylpyrrolidine, pyrrolidine, 1-methyl-2-propenylamine and isopropylamine, all of which possess antifungal activity. The optical isomers of 3-methylpyrrolidine were equally active suggesting that the spatial orientation of H and CH_3 bonded to C-3 of this compound is less critical than the configuration of substituents adjacent to the chiral centre of sec-butylamine. Analogs of sec-butylamine with substituents of volume greater than CH_3 i.e. C_2H_5, CF_3 and CCl_3, would also be excluded from the receptor site and thus be inactive. Aliphatic amines of smaller molecular volume would be free to approach the receptor but would not be bound as tightly as sec-butylamine, because the former lack one or more groups which could participate in hydrophobic bonding.[10]

B. Development and use of 2-aminobutane fumigation for the control of post-harvest fungal diseases of potato tubers.

B.1 Background.

The potato is one of Scotlands main agricultural crops. All potatoes grown commercially are either classified as seed and sold to growers for replanting, or are marketed as ware tubers, for general consumption. The Scottish seed production figures for 1982/83 and 1983/84 were 375,000 and 329,000 tonnes respectively. As seed tubers sold for approximately £100 tonne^{-1} during those seasons, this gives an indication, in commercial terms of the value of the seed potato business in Scotland.

All seed potatoes marketed in the United Kingdom are required to have been classified under seed potato schemes, operated in the U.K., or under similar schemes in other countries.[32] Imports of seed tubers from outside Scotland are restricted under the classification scheme which operates in this country. Classification ensures that seed stocks are true to variety and have a specified tolerance to disease. This enables potato growers to buy seed potatoes of guaranteed quality and

goes some way towards helping to control disease, since badly infected stocks will not be classified.

Seed potatoes are normally harvested in the autumn, stored over the winter and replanted in the spring. During the long storage period the tubers are susceptible to attack by many fungal diseases, which can lead to considerable loss of the overall potato crop. Two of the most troublesome tuber diseases in Scotland are gangrene (Phoma exigua Desm. var. foveata (Foister) Boerema) and skin spot (Polyscytalum pustulans (Owen & Wakefield) Ellis). These are latent post-harvest diseases, present in infected tubers at harvest, which may develop during the storage period. Gangrene is the most damaging of the two, and is a rot of the tuber flesh which is encouraged by mechanical damage and cold, wet storage conditions. Skin spot appears as small pustules on the tuber surface and more importantly, eyes may be damaged affecting sprouting.[16] Other diseases include dry rot (Fusarium solani var. coerleum (Sacc.) Booth) and silver scurf, which disfigures tubers and causes flaccidity by accelerating water loss through the skin, and which is the result of attack by (Helminthosporium solani Dur. & Mont.).[17]

B.2 Recommended procedures for harvesting, handling and storage of seed potatoes.

In order to minimise the loss of crop through disease the following advice is offered to potato growers.[33]

B.2(a) Haulm destruction.

Before the potato crop is harvested, the haulm (i.e. that part of the potato plant which grows above the soil), must be destroyed. This is normally done chemically by burning down with a dessicant such as sulphuric acid or diquat. The haulm should be destroyed early to reduce the spread of bacterial, fungal and viral disease. The other main reason for haulm destruction is to prevent further tuber growth and ensure that the tubers are within the required size range for seed classification. Two or three weeks should be allowed after haulm death, before harvest, to provide time for the tuber skins to set, as this minimises mechanical damage during lifting. Lifting should not however be delayed beyond this period as tuber infections, including gangrene and skin spot, are likely to increase.

B.2(b) Lifting.

Growers are advised to avoid lifting in cold

weather, if possible, as this increases damage levels and thus susceptibility to disease. When temperatures are near freezing harvesting should be limited to the warmest part of the day. Lifting in wet conditions is also not recommended as this increases the risk of soft rotting during storage.

B.2(c) <u>Storage.</u>

When the tubers are transferred to the potato store as much soil as possible should be removed as it restricts proper ventilation. For dry, mature crops an initial curing period (10 to 14 days at 15°C) is recommended, as it helps to heal wounds and reduce disease development. For tubers lifted in wet conditions it is advised to ventilate the crop, to promote drying, during the early days of storage and follow this with a short curing period before lowering to the holding temperature. Seed tubers are best held over winter at a temperature of between 3 and 5°C to control sprouting and minimise disease development.

B.2(d) <u>Storage fungicides.</u>

Some storage diseases can be controlled by suitably timed application of fungicides. The time of application and the procedure involved, depends on the disease to be controlled and the fungicide being applied.

B.3 Development of 2-aminobutane as a storage fungicide.

In recent years enormous effort has been directed at developing chemical methods of controlling post-harvest fungal diseases. For some time the only effective method for treating tubers susceptible to gangrene and skin spot, was to dip them in a solution of an organomercury compound such as methoxyethylmercuric chloride (MEMC).[27] This treatment was introduced commercially as early as 1934, but possessed several major disadvantages, such as the toxic hazards associated with the mercurials and the fact that after treatment the tubers are hard to dry, leaving them susceptible to attack by bacteria which thrive in damp conditions. As a result of these problems the organomercury dip treatment was not universally adopted by the seed potato trade. Attempts to find suitable alternative fungicides were unsuccessful until the early 1970's, when Hide et al.[19] discovered that dusts and dips of the benzimidazole compounds benomyl (methyl 1-(butylcarbomyl)-benzimidazol-2-ylcarbamate) and thiabendazole (2- (thiazol-4-yl) benzimidazole) controlled gangrene, and Graham and Hamilton[16], reported that fumigation with 2-aminobutane (2-AB) did likewise.

Having noted the use of 2-aminobutane fumigation

for disease control in fruit[5], Graham and Hamilton investigated the possibility of using a similar treatment for the control of tuber diseases. They were particularly attracted to the idea of applying the fungicide as a gas because it might enable the compound to be introduced into bulks of stored tubers fairly easily. In initial tests, they fumigated freshly lifted naturally infected tubers of several varieties, with different doses of 2-aminobutane, in a steel fumigation chamber fitted with an external circulatory system, to assist application and distribution of the gas. The potatoes were exposed to the gas for about 2 hours during which most of it was absorbed. Treated and untreated tubers were then stored over the winter. The tubers were examined periodically until the end of March for the appearance of gangrene lesions, and skin spot infection was assessed at the end of the storage period in April. These early experiments showed promising control of both gangrene and skin spot (Tables 6 and 7) with no evidence of tuber phytotoxicity i.e. chemical damage caused by the 2-AB fumigant.

These results encouraged Graham _et al._ to investigate the treatment further.[17] Experiments were thus carried out to confirm that fumigation of tubers with gaseous 2-aminobutane would be effective in controlling gangrene and skin spot. Attempts were also

Table 6 Effect of treatment with 2-aminobutane on the incidence of gangrene.[a]

Cultivar	Dosage($mgkg^{-1}$)	No. of tubers examined	% Gangrene[b]
Majestic 1	85	229	6.1
	140	227	4.8
	Nil	195	33.3
Majestic 2	140	172	4.7
	Nil	171	18.1
King Edward	140	278	2.5
	350	288	4.2
	Nil	259	25.9

a. Table after Graham and Hamilton.[16]

b. Percentage of tubers with at least one gangrene lesion.

Table 7 Effect of treatment with 2-aminobutane on the incidence of skin spot (cultivar King Edward).[a]

	Dose $mgkg^{-1}$	%age of tubers affected in each category				
		None	Trace	Slight	Moderate	Severe
Stock 1	70	68	30	2	0	0
	120	66	26	8	0	0
	Nil	10	28	58	4	0
Stock 2	140	92	6	2	0	0
	350	98	2	0	0	0
	Nil	30	36	20	14	0

a. Table after Graham and Hamilton.[16]

made to establish the optimum conditions for good disease control with particular reference to; efficient distribution of fungicide throughout large bulks of tubers, the dosage required for effective control and the best time to apply the treatment.[25,26]

B.4 <u>Studies on the distribution of 2-aminobutane in large bulks of tubers.</u>

When treating large bulks of tubers with gaseous 2-aminobutane it is necessary, for effective disease control, to ensure that the fungicide is well distributed throughout the whole bulk. By analysis of 2-aminobutane residues in tubers, Graham *et al.*[17] were able to determine the distribution of the fumigant throughout bulks of tubers, and by experiment establish the optimum flow rate and dosage required for even distribution. In this early work the 2-aminobutane residues in the fumigated tubers were determined by gas chromatography using a modified version of the method developed by Day *et al.*.[20] In these experiments considerable variation was found in residues of individual potatoes and even of adjacent tubers. Graham *et al.*[17] concluded that this was almost certainly due to the condition of the tuber skin since they found higher residues in immature tubers and others where the skin was damaged. To reduce this variation, sound quarters

from each of 4 tubers of fairly uniform seed size, were taken for each analysis.[17]

A prototype fumigation chamber, capable of holding up to 5000 kg of potatoes, was built to study the process of fumigation with bulks of tubers. The design was a simple gas-tight wooden box with a slatted, false bottom, onto which the tubers were stacked. The 2-aminobutane was introduced at the bottom of the chamber, and a fan recirculated the amine up through the tubers, out the top of the chamber and back to the base of the chamber.

Early experiments had shown that as 2-aminobutane was absorbed very rapidly by potatoes, most of the fumigant could be absorbed by the lower layers, resulting in inadequate distribution throughout the bulk. To overcome this difficulty a relatively fast air flow was required to blow the gas up through the bulk to the top, before it was all absorbed. This flow of 5.6 m^3 min^{-1} $tonne^{-1}$, was about 5 times the rate recommended for normal ventilation of potatoes. It is important to note that the optimum air flow required for efficient distribution of the 2-aminobutane fumigant, depends on the design of fumigation chamber being used and will vary from chamber to chamber.

Graham and Hamilton fumigated batches of tubers, 48 hours after lifting, at different dosages. Analysis of gas samples, drawn through tubes ending at different points in the bulk, showed that even distribution of the fumigant was achieved only slowly. However, residue analysis on tuber samples taken from various positions in the bulk indicated that good distribution of the fumigant was achieved after 2 hours forced air recirculation.

Typical residues in potatoes fumigated in the specially constructed 5 tonne fumigation chamber are shown in Table 8. The dose was introduced over a period of 30-40 minutes and the air inside the chamber recirculated for 2 hours. The results show that even distribution was achieved with a dosage of 200 $mgkg^{-1}$, but at 50 $mgkg^{-1}$ most of the residue was confined to the lower tubers, indicating that this dose was insufficient for even distribution to occur.[17]

B.5 <u>Results of disease control by fumigation.</u>

Over the years 1967-71, 29 fumigation treatments were carried out on bulks of tubers, mostly in the 5 tonne prototype chamber. The experiments were designed firstly to determine the phytotoxic dose, secondly to establish the dose required to obtain good disease control, and thirdly to determine the efficiency of the treatment at different times after lifting.[17]

Table 8 Residues of 2-aminobutane in potatoes fumigated within 2 days of harvest in the five tonne prototype fumigation chamber.[a]

Cultivar	Dose($mgkg^{-1}$)	Sample position in chamber	residue($mgkg^{-1}$)
Redskin 1	200	top	194
		bottom	182
Majestic 1	200	top	103
		bottom	113
Redskin 2	200	top	92
		bottom	160
King Edward 1	200	top	92
		bottom	90
King Edward 2	50	top	12
		middle	39
		bottom	32
Redskin 3	50	top	2
		middle	11
		bottom	23

a. Table after Graham *et al.*.[17]

B.6 Phytotoxicity of 2-aminobutane.

In their early experiments, Graham and Hamilton found that a dosage of 200 $mgkg^{-1}$ of 2-aminobutane had no phytotoxic effect on tubers, other than a slight browning of skinned areas.[16] To determine the phytotoxic level, three separate lots of about 300 tubers of c.v. Majestic were treated at dosages of 500, 1000 and 5000 $mgkg^{-1}$. More marked browning of skinned areas was observed at the 500 $mgkg^{-1}$ level, whereas at 1000 $mgkg^{-1}$ there was some lenticel pitting and very marked browning of damaged areas. At 5000 $mgkg^{-1}$ dosage, chemical damage was severe and all eyes on the tuber were killed.

Graham et al. also observed that immature and badly skinned tubers could be injured even at the 200 $mgkg^{-1}$ dose, and thus recommended that only mature tubers should be treated.[17]

B.7 Control of gangrene, skin spot and silver scurf, by fumigation at different dosages at different times after lifting.

Experiments with organomercury disinfectant solutions, showed that in general, the longer the delay between harvest and treatment, the poorer the degree of

disease control.[27] This probably results from changes in the skin, especially suberisation, making it increasingly impervious to the dipping solution.

As Graham and Hamilton[17] thought that the same principle would apply to 2-aminobutane, they carried out fumigation experiments at times varying from 1 to 30 days after lifting, at dosages from 50 $mgkg^{-1}$ to 200 $mgkg^{-1}$. In every case the fumigant was introduced over 30-40 minutes and air recirculation continued for a further 2 hours. After fumigation the tubers were stored over the winter at temperatures between 5-15°C. Gangrene assessments were made once in February and again before planting in April. The skin diseases skin spot and silver scurf were assessed on washed samples of 50-100 tubers in April. For controls, 0.5 tonne bulks of tubers were stored and handled similarity to the treated crop.

Results of the treatments on gangrene and skin spot are shown in Table 9. The overall conclusion from these experiments was that efficient control of the tuber diseases gangrene and skin spot could be achieved at a dosage of 200 $mgkg^{-1}$, in a simply made fumigation chamber fitted with a vaporiser and gas recirculation system. Results of treatments at 50 $mgkg^{-1}$ indicated that it was too low a dosage, and although only a few treatments were carried out at the 100 $mgkg^{-1}$ level, it

Table 9 Results of experiments to test efficiency of treatment at different dosages and different times of lifting, on the incidence of gangrene and skin spot.[a]

Cultivar	Dosage $mgkg^{-1}$	Days between harvest and treatment	number of tubers examined	% gangrene	skin spot S.I.I.[b]
Redskin 1	200	2	552	0.7	0
	Nil	-	567	88.6	0
Redskin 2	200	1	1844	0.2	0
	200	14	2258	0.2	0.03
	Nil	-	1957	5.0	5.60
King Edward	50	2	2057	1.8	0.03
	100	2	1986	1.1	0
	200	2	2057	0.2	0
	50	13	2275	3.3	0
	200	13	2177	0.5	0.06
	50	27	2163	4.9	0.10
	200	27	2113	1.0	0.16
	Nil	-	2657	8.1	9.15
Red Craigs Royal	50	5	880	36.8	0.19
	200	5	874	13.8	0.09
	Nil	-	334	79.6	3.63

a. Table after Graham *et al.*.[17]

b. S.I.I. - surface infection index.[77]

appeared to give satisfactory control. Bearing in mind that in commercial fumigations, equal distribution of gas throughout tuber bulks would be more difficult due to the presence of soil, Graham <u>et al.</u> recommended that the 200 $mgkg^{-1}$ dose be adopted for commercial treatments.[17]

The results also show that the best control of gangrene and skin spot was obtained if fumigation was carried out within 3 days of lifting, but even after 14 days the degree of control was good at the 200 $mgkg^{-1}$ dosage rate. After about one month the treatment was less effective but limited control of gangrene and skin spot was still possible.

In commercial practice, the Department of Agriculture and Fisheries for Scotland, recommend that fumigation be carried out within 2-4 weeks of lifting.[21] Earlier fumigation, although shown in the above work to give best disease control, is not advised, as it may lead to chemical damage of immature or mechanically damaged tubers.[25,31] The 2-4 week time limit allows ample time for harvesting, grading and handling of the crop and also adequate time for damaged tubers to heal sufficiently, to prevent chemical damage occuring as a result of the 2-aminobutane fumigation treatment.

Experiments run at the same time as the above, were designed to establish the degree of control of silver scurf, by fumigation with 2-aminobutane. As the results in Table 10 show, control of silver scurf is poorer than that seen for gangrene and skin spot in Table 9. However, limited control is still possible even if the treatment is carried out up to 29 days after harvest.[17]

B.8 Comparison of 2-aminobutane fumigation with other available treatments for control of fungal disease in potato tubers.

Over the years 1966-80, experiments have been carried out to compare the effectiveness of a number of fungicides and other chemical substances, for the control of the post harvest potato tuber diseases gangrene and skin spot.[24]

B.8(a) Comparison of 2-aminobutane fumigation with the organomercury dip treatment.

Experiments with organomercury dips have shown them to be effective only if the treatment is carried out within 3 days of harvest. This is a disadvantage since it allows the grower less time for handling the crop. By comparison, 2-aminobutane fumigation can be

Table 10 Control of silver scurf by fumigation with 2-aminobutane.[a]

Cultivar	Treatment		% Skin cover with silver scurf
	Dosage $mgkg^{-1}$	Days after harvest	
King Edward	200	2	13.1
	200	29	41.6
	Nil	-	71.6
Pentland Crown	200	2	18.4
	200	29	31.8
	Nil	-	47.1

a. Table after Graham et al..[17]

carried out up to 4 weeks after lifting and still give effective disease control.[26]

Table 11 shows the results for an experiment comparing the efficiency of 2-aminobutane fumigation at 200 $mgkg^{-1}$ dosage, with dipping in a solution of methoxyethylmercuric chloride (MEMC) containing 100 mg of mercury per kg. The results show that 2-aminobutane fumigation is more effective for disease control, and considering that it is a relatively simple process without any of the disadvantages associated with the organomercury dip treatment, it is easy to see why 2-AB fumigation has made a greater impact on seed potato growers.

B.8(b) Comparison of the fungicides 2-aminobutane and thiabendazole for the control of gangrene and skin spot on potatoes.

As already described above, 2-aminobutane was not the only alternative fungicide developed to replace the unsatisfactory organomercury dip treatment. Hide et al.[19] and Logan et al.[18,28], amongst others, have carried out work illustrating the effectiveness of thiabendazole dusts and dips for the control of gangrene and skin spot.

Table 11 Effectiveness of 2-aminobutane fumigation compared with methoxyethylmercuric chloride (MEMC) disinfectant solution, in controlling gangrene.[a]

Cultivar	Treatment	Number of tubers examined	% gangrene
Majestic	2-aminobutane	227	4.8
	MEMC	754	6.1
	Nil	195	33.3
Redskin	2-aminobutane	291	7.2
	MEMC	314	15.0
	Nil	329	43.4

a. Table after Graham *et al.*.[17]

Both 2-aminobutane fumigation and thiabendazole spray treatment show good disease control. Thiabendazole (TBZ) is marketed commercially as "Storite Flowable" which is a formulation containing about 45% active ingredient. The concentrated product is diluted with water before use and is applied as a 2% suspension using suitable spray equipment. One disadvantage of the TBZ treatment however, is that it must be carried out fairly soon after lifting, as with MEMC, so less time is available for handling the crop compared with when 2-AB fumigation is used. The thiabendazole is usually applied from spray equipment mounted on the harvester, on the elevator or on the roller table in farm stores. One advantage of the TBZ treatment over 2-AB fumigation is that, as well as controlling gangrene and skin spot it also gives control of dry rot, which is another major tuber disease.

Boyd _et_ _al._[22] carried out experiments to compare the effectiveness of 2-aminobutane fumigation with thiabendazole spray treatment and methoxyethylmercuric chloride dip treatment, for the control of several tuber diseases. The fungicides were applied to tubers of the King Edward and Redskin varieties during the 1974-75 and 1975-76 seasons, at the recommended dosages. 2-AB fumigation was carried out within a few days of lifting and the tubers stored in wooden sprouting trays.

Thiabendazole was applied using a Newforge Mister and at the same time an organomercury dip treatment was also carried out. The cultivar King Edward was chosen because of its susceptibility to gangrene and skin spot, and cultivar Redskin because of its susceptibility to dry rot and silver scurf. Untreated tubers of these stocks were also stored under normal conditions, and at intervals samples of about 1 cwt were removed, riddled and subjected to treatment by MEMC, 2AB and TBZ. Table 12 shows the disease development which occurred in the spring, in tubers treated at lifting and at various times thereafter. Gangrene was very well controlled by 2-AB and TBZ at the time of lifting but the action of MEMC was not so effective. There was a gradual loss of effectiveness of each compound when applied at intervals after lifting, but 2-AB retained its effect longer than TBZ. This pattern was also shown by skin spot control which was assessed by the "eye infection index" (E.I.I.).[29] Dry rot data were available for only one year and showed clearly that 2-AB was quite inactive against this disease. TBZ and MEMC gave satisfactory control of dry rot. All three treatments also showed some limited control of silver scurf but only if carried out close to the time of lifting the crop.

Further comparisons between the effectiveness of 2-aminobutane fumigation and thiabendazole spray have

Table 12 Disease development in spring, after various treatments at lifting and periodic removal from store.[a]

Date of treatment	Treatment	King Edward[1] % Gangrene		King Edward[1] Skin spot E.I.I.[b]		Redskin[2] % Dry rot	Redskin[2] Silver scurf S.I.I.[c]
		74-5	75-6	74-5	75-6	75-6	75-6
1 28 Oct.	None	8	6	55	67	9	14
2 26 Sept.	MEMC	2	3	11	4	2	9
	2-AB	0	1	6	0	22	15
	TBZ	1	1	16	36	1	10
1 14 Nov.	None	4	4	71	82	18	94
2 19 Nov.	MEMC	-	1	-	14	3	47
	2-AB	0	2	12	7	14	88
	TBZ	4	3	34	55	11	91
1 13 Dec.	None	4	9	67	80	17	90
2 15 Dec.	2-AB	1	4	36	24	22	92
	TBZ	4	8	67	81	5	79
1 20 Jan.	None	2	7	76	91	13	95
2 26 Jan.	2-AB	0	3	61	43	9	100
	TBZ	2	5	82	89	2	95

a. Table after Boyd *et al.*.[22]

b. E.I.I. - Eye infection index.[29]

c. S.I.I. - Surface infection index.[22]

been carried out in joint experiments between the Department of Agriculture and Fisheries for Scotland (DAFS) and the North of Scotland College of Agriculture (NOSCA).[23] In tests over several years, by DAFS and NOSCA, 2-aminobutane regularly gave good control of gangrene and skin spot, but thiabendazole (TBZ) was generally less effective when tested under commercial fumigation conditions. Table 13 shows the results of experiments carried out by DAFS and NOSCA on tubers from the North of Scotland (Grampian and Highland Regions) where gangrene and skin spot are particularly troublesome. The 2-AB fumigations were carried out in a commercial brick-built chamber designed for treating tubers in 1 tonne boxes, on a farm close to the experimental farms on which the test tubers were grown. Several different methods of TBZ application were investigated, using various combinations of sprayer type and location, and time of treatment.

Although a range of combinations of sprayer/applicators and lapse in time between harvest and treatment was represented by the fourteen stocks chosen, there was no combination which showed a significantly higher level of control of gangrene or skin spot by TBZ, compared to 2-AB.

In contrast to the work of Hide et al.[19],

Table 13 Effect of fungicide treatment and method of application on the incidence of gangrene on tubers.[a]

Cultivar	TBZ treatment Sprayer	TBZ treatment Location	% Tubers infected with gangrene TBZ	% Tubers infected with gangrene 2-AB	% Tubers infected with gangrene Untreated
DAFS supervised					
1 Record	Mantis	Harvester	2.2	0.9	4.0
2 Pentland Squire	Mantis	Harvester	0	0	0
3 Record	Mantis	Harvester	0.8	1.1	4.4
4 Desiree	Delevan	Harvester	5.3	3.9	15.0
5 Desiree	Delevan	Harvester	5.5	0.4	15.3
6 Pentland Hawk	Delevan	Elevator	0	0	0
7 Pentland Dell	Delevan	Elevator	0	0	0
8 Desiree	Mantis	Dresser	2.2	0.1	3.7
9 Up to Date	Mantis	Dresser	15.6	0.4	21.0
10 King Edward	Mantis	Dresser	7.9	0.5	17.6
NOSCA supervised					
11 Desiree	Delevan	Harvester	3	1	7
12 Desiree	Mantis	Harvester	2	0	0
13 Desiree	Mantis	Harvester	12	2	26
14 Desiree	Mantis	Harvester	31	0	27
Mean incidence of gangrene			6.3	0.7	10.1

a. Table after Hamilton *et al.*.[23]

Logan et al.[28] and Henriksen[30], the work carried out by Boyd[22] and jointly by DAFS and NOSCA, suggests that on typical Scottish seed tubers, best control of gangrene and skin spot is achieved with 2-AB fumigation rather than the TBZ spray treatment.

B.8(c) Combined 2-aminobutane (2-AB) and thiabendazole (TBZ) treatments.

Treatment with gaseous 2-aminobutane and thiabendazole, applied as a spray, have both been shown to be effective for control of post-harvest fungal disease.[24] In trials carried out in the North of Scotland however, thiabendazole appeared to be less effective in controlling gangrene and skin spot, than 2-AB fumigation. As some limitations in the efficiency of TBZ in controlling gangrene had also been found commercially in Northern Scotland, Merck, Sharp and Dohme Limited, the manufacturers of "Storite" (TBZ), developed a formulation of TBZ and a salt of 2-AB. Preliminary trials carried out by DAFS staff had indicated that such a mixture could reduce the incidence of gangrene, to levels similar to that achieved by fumigation with 2-AB.[23] Farm trials carried out by DAFS and NOSCA compared thiabendazole ("Storite") spray treatment with the mixed formulation of TBZ and 2-AB ("Storite Plus").[36] The results showed that control of

gangrene was better with the mixture of TBZ and 2-AB (83% control in 1982 test) than with TBZ alone (only 51% unaffected). Similarly the incidence of skin spot was reduced by 81% on tubers treated with the mixture, whereas the reduction using TBZ alone was only 29%. Results of the trials showed also that the mixture of TBZ and 2-AB gave effective disease control, even when applied to late lifted tubers. The "Storite Plus" spray treatment was introduced commercially in 1983.

B.9 <u>Commercial fumigation of seed and ware potatoes with 2-aminobutane.</u>

The research carried out by the Department of Agriculture and Fisheries for Scotland, Agricultural Scientific Services at East Craigs in Edinburgh, backed up by field trials run in conjunction with the North of Scotland College of Agriculture, has shown clearly that fumigation of potatoes with 2-aminobutane gas can result in effective control of gangrene and skin spot. Limited control of silver scurf has also been observed but unfortunately no control of the *Fusarium* rots.

The fumigation of potato tubers with 2-aminobutane is now a well established practice with many Scottish seed potato growers. The relative low cost of fumigation (approx. £1.85 tonne^{-1} in 1983), compared

to the financial gain that can result from the high level of disease control, makes it a cost effective treatment. Clearance for commercial treatment of seed grade tubers was obtained in 1972, under the British Pesticides Safety Precautions Scheme. Once commercial fumigation started it became clear that it might also be necessary to obtain clearance for treatment of ware tubers, otherwise farmers would have to separate seed tubers from the main crop prior to fumigation. Seed tubers are normally separated out on a grader, which selects tubers of appropriate size for seed (normally about 35-55 mm diameter tubers for high grade stocks, with possibly wider diameter range for lower grade stocks). Some farmers also wanted to fumigate high quality ware stocks, especially where they were susceptible to gangrene and skin spot, although normally ware tubers are not commercially valuable enough to merit fungicidal treatment. One area where ware tubers are fumigated is in the crisp industry. Cultivar Record tubers are popular for crisping but are susceptible to skin spot. If skin spot occurs more peel has to be removed in the preparation of tubers for crisping, and this leads to greater losses and increased costs. In this case therefore, the crisp manufacturers prefer 2-AB fumigated ware tubers, which will not be so susceptible to skin spot.

Graham and Hamilton carried out experiments to determine the residues of 2-aminobutane remaining after peeling, cooking and processing.[17] Fumigated tubers were peeled with an ordinary hand peeler which removed between 10-12% by weight of the tuber. The peelings and flesh were analysed separately. The results in Table 14 indicate that about 60-70% of the residue was in the peel. Results of 2-aminobutane residue analyses on fumigated tuber samples after boiling, crisping or processing (into potato flakes or granules) showed that significant levels of 2-AB were left in the tubers in each case. Initially these residues were thought to be too high and the fumigation of ware potatoes was not allowed. However increased pressure to allow the fumigation of ware tubers led to clearance being granted through the British Pesticides Safety Precautions Scheme in 1976, which allowed the treatment to be carried out on lots of up to 250 tonnes.[31] Larger bulks may also be treated but a separate individual limited clearance must be obtained first.

The procedure for commercial fumigation of seed or ware tubers with 2-aminobutane is straightforward and efficient treatments have been achieved in simple fumigation chambers.[17] Guidelines for safe and efficient fumigation practice have been issued by the Department of Agriculture and Fisheries for Scotland.[21] As

Table 14 Residues of 2-aminobutane in the peel and flesh of fumigated potatoes.[a]

Cultivar	Residue in flesh ($mgkg^{-1}$)	Residue in peel ($mgkg^{-1}$)	Calculated residue in whole tuber ($mgkg^{-1}$)	% Residue removed by peeling
Redskin 1	8	193	28	73
Majestic	13	294	48	75
Redskin 2	24	374	53	58
King Edward 1	28	508	66	60
King Edward 2	63	688	130	57
Redskin 3	16	365	45	66
King Edward 3	33	244	55	44
King Edward 4	22	192	40	50
Pentland Crown 1	24	471	62	64
Pentland Crown 2	23	420	56	70

a. Table after Graham *et al.*.[17]

2-aminobutane liquid is highly flammable (flash point -19°C) and the lower explosive limit in air is 2.1-2.5%, precautions must be taken to prevent leaks of gas from the chamber during fumigation.

Some commercial potato growers carry out large scale fumigations using all or part of their potato store as the fumigation chamber. Problems however can arise from inadequacies in the store ventilation equipment, which might not be able to distribute the 2-aminobutane fumigant evenly throughout the whole store, especially if the tubers are stored in loose bulks rather than in stacked 1 tonne wooden pallet boxes. Graham and Hamilton showed that for good disease control it is necessary to ensure efficient distribution of the amine fumigant, and this often means the store ventilation equipment must be capable of recirculating the air at a faster rate than that required for normal ventilation.[17]

To avoid problems associated with distribution of the 2-aminobutane most fumigations are carried out in smaller scale, purpose built fumigation chambers which appear to be more efficient. Several types of purpose built fumigation chamber are available commercially. Two of the most common are shown in Figures 3 and 4.

Figure 3 Twin, brick-built fumigation chamber.

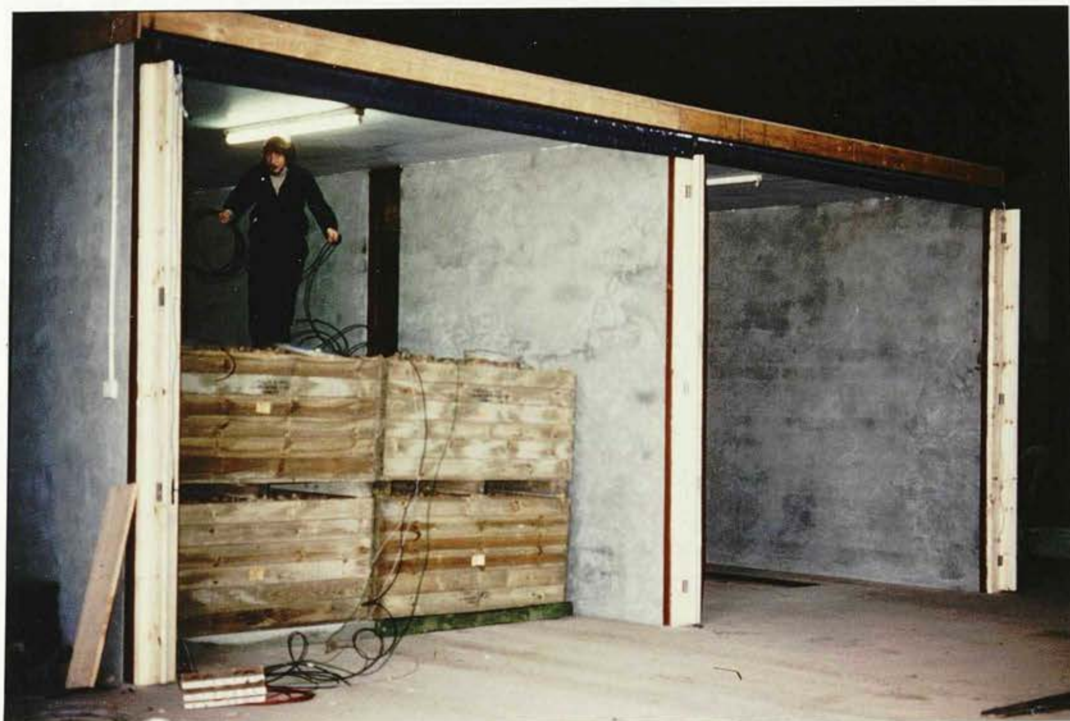


Figure 4 Portable 'tent' fumigation chamber.

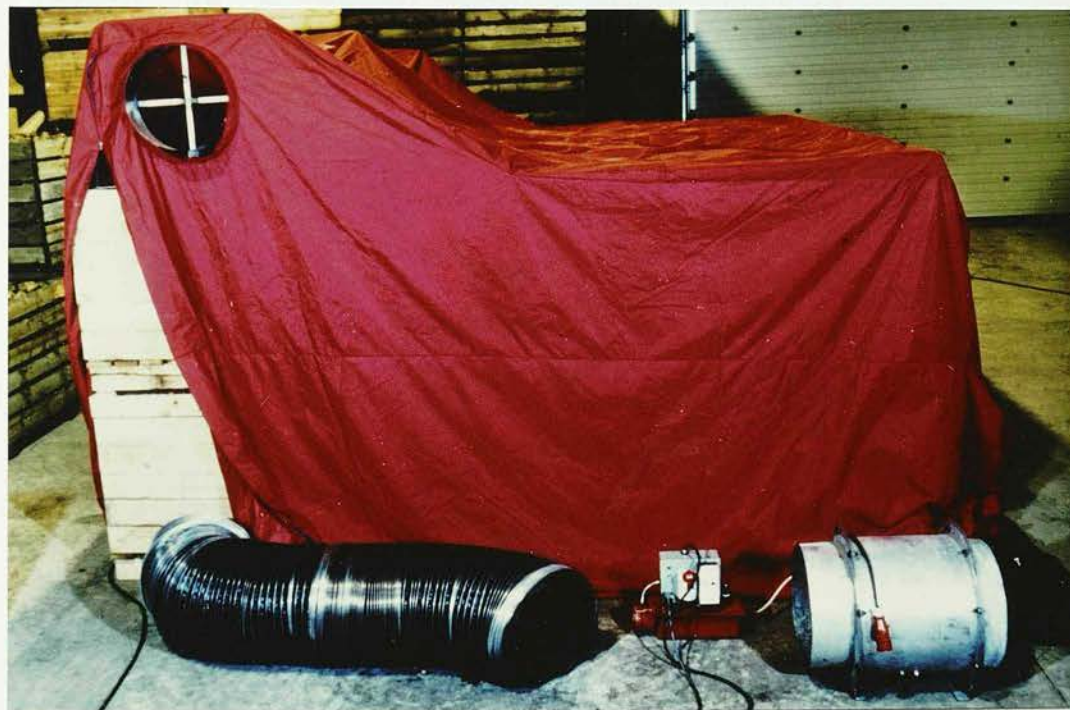


Figure 3 shows a twin, brick-built fumigation chamber, of the type used by the bigger seed potato growers. Tubers are loaded into the chamber in 0.5 or 1.0 tonne wooden pallet boxes. The chamber is sealed at the front, with a roller blind of gas proof fabric. The 2-AB fumigant is then introduced into the chamber's air recirculation system, through a heated vaporiser. The amine is pumped into the chamber through the slatted floor, passes up through the tubers and out through a vent in the roof. The air/amine mixture is then recirculated to the base of the chamber.

Figure 4 shows a cheaper, portable 'tent' fumigation chamber, composed of gas proof fabric, fitted with a vaporiser, and a fan for air recirculation. The 'tent' is slipped over stacked pallet boxes and the fumigation is carried out in the normal way. The 2-AB is added to the vaporiser and is pumped in at the base of the chamber. It then passes up through the tubers, out through a vent at the top and is recirculated to the bottom of the chamber.

With both designs, the chamber is flushed with air at the end of the fumigation, to vent any unabsorbed 2-AB. The tubers are then transferred to the potato store, prior to sale or replanting.

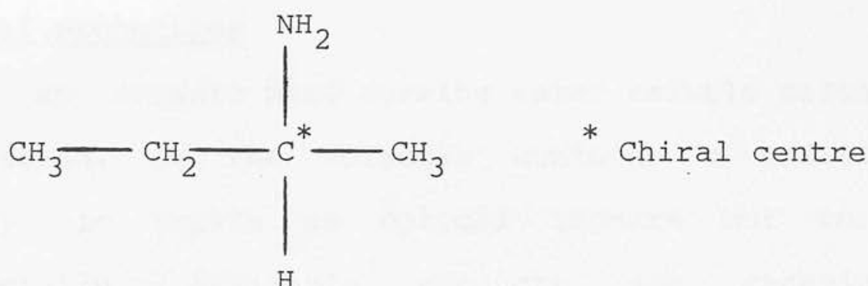
C. Summary data on 2-aminobutane.[37]

C.1 Identity.

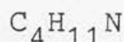
Chemical name

2-aminobutane also known as sec-butylamine or in "Chemical Abstracts" usage 2-butanamine (13952-84-6)

Structural formula



Empirical formula



$C_4H_{11}N$

Molecular weight

73.16

Products containing 2-aminobutane

The free base is marketed under the registered trademark "Tutane" by Eli Lilly and Co., and "Butafume" by BASF AG. Merck, Sharp and Dohme market a spray formulation containing 2-aminobutane as a salt, under the trade mark "Storite Plus".

C.2 Physical and chemical properties.

Physical properties

Colourless liquid with ammoniacal odour.

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Boiling point 63°C

Vapour pressure 135mmHg at 20°

Refractive index n^{20}_{D} 1.394

Density of liquid d^{20}_{4} 0.724

Solubility: miscible with water and most organic solvents.

Inflammable: Flash point -19°C; lower explosive limit in air is 2.1 - 2.5%.

Chemical properties

It is an organic base forming water soluble salts with acids. As the molecule contains a chiral centre, it exists as optical isomers but the commercially available products are racemic mixtures.

It is stable but corrosive to tin, aluminium, copper and its alloys and some steels.

C.3 Toxicity.

The acute oral LD50 for rats is 380 mg amine kg^{-1}, for dogs 225 $mgkg^{-1}$ and for hens 250 $mgkg^{-1}$.

It is a severe skin and eye irritant but the dermal toxicity for rabbits is more than 2500 $mgkg^{-1}$.

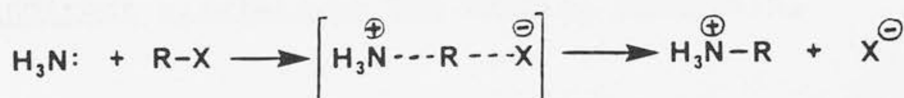
In two-year feeding tests, the "no-effect" level for rats and dogs is 2500 ppm of the diet.

D. General methods of preparing primary amines.

As the research project to be undertaken would inevitably involve synthesis of 2-aminobutane, a review of the general methods available for the synthesis of primary amines was carried out.

D.1 Ammonolysis of halides.

Many organic halogen compounds are converted into amines by treatment with aqueous or alcoholic solutions of ammonia.[38] The reaction is generally carried out either by allowing the reactants to stand at room temperature or by heating them under pressure. Displacement of halogen by NH_3 yields the amine salt from which the free amine can be liberated by treatment with hydroxide ion (Scheme 1).



Scheme 1

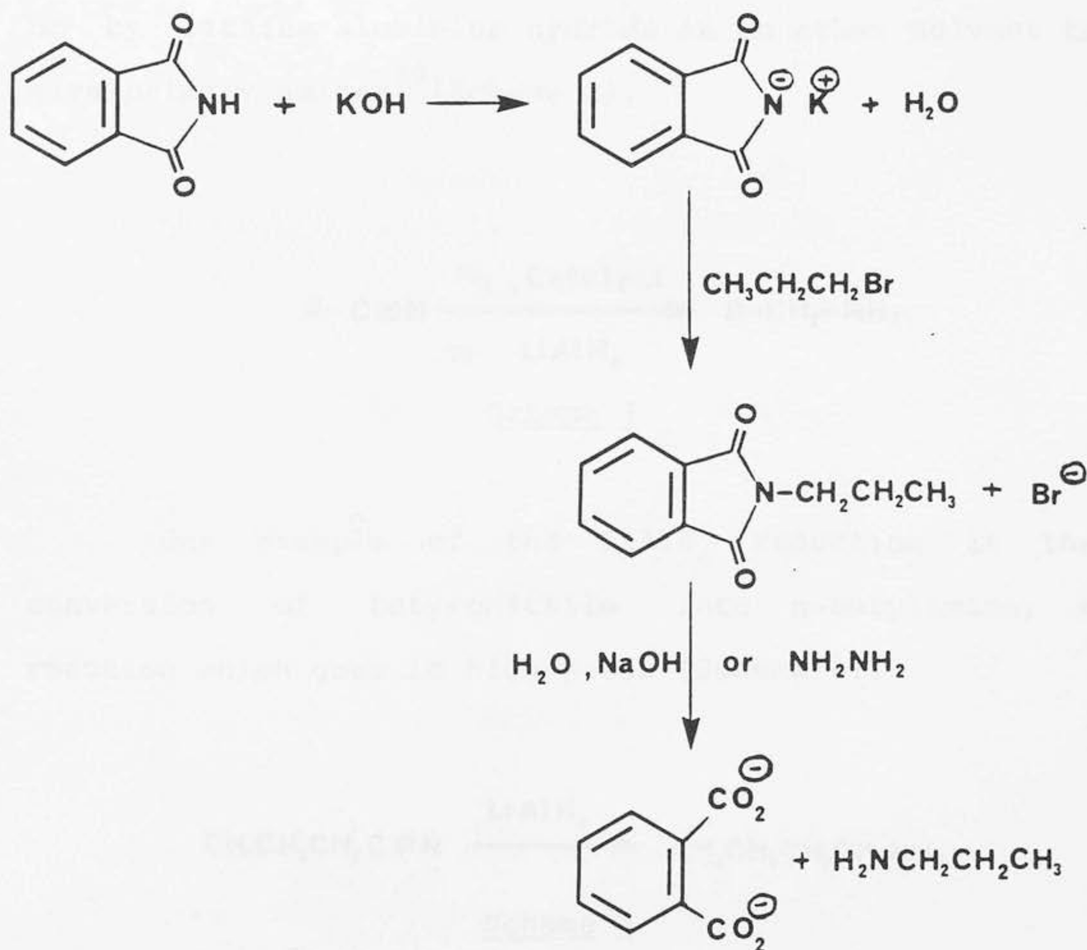
One problem associated with this type of nucleophilic substitution reaction is that there is a competing elimination process. Ammonolysis thus gives highest yields with primary halides (where substitution predominates) and is virtually worthless with tertiary halides (where elimination predominates).

Another, more serious, drawback to the synthesis of amines by ammonolysis is the formation of more than one class of amine. The primary amine, once formed, is also a nucleophilic reagent and it too can attack the alkyl halide to yield the salt of a secondary amine. Similarly, tertiary amines are formed by nucleophilic attack of the secondary amine on the alkyl halide. The presence of a large excess of ammonia suppresses this tendancy towards over-alkylation, but the yield of primary amines by this process still tends to be on the low side.

D.2 <u>Indirect alkylation: The Gabriel synthesis.</u>

Pure primary amines can be prepared in good yield by a method called the Gabriel synthesis.[38] The compound phthalamide is prepared from ammonia and the dicarboxylic acid, phthalic acid. In aqueous basic solution the compound is converted almost completely into the anion. The phthalamide anion has nucleophilic

properties and can enter into displacement reactions with alkyl halides. The product is an N-alkylphthalamide, and hydrolysis, or hydrozynolysis, gives the amine and phthalic acid. Scheme 2 illustrates the formation of the phthalamide anion and its reaction with 1-bromopropane to form n-propylamine.



Scheme 2

The absence of acidic protons in the N-alkylphthalamide means that no further alkylation can

take place, therefore this synthesis yields only pure primary amines. The best solvent for the alkylation appears to be dimethylformamide $HCON(CH_3)_2$.

D.3 Reduction of Nitriles.

Nitriles are reduced by hydrogen and a catalyst, or by lithium aluminium hydride in an ether solvent to give primary amines[39] (Scheme 3).

Scheme 3

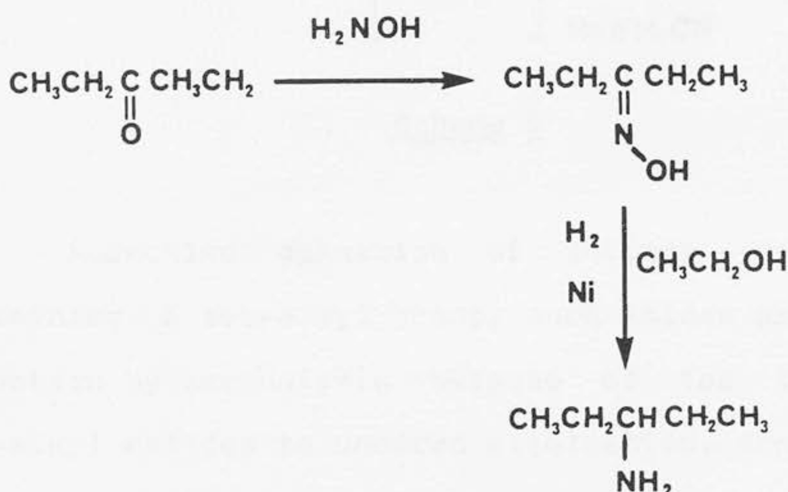
One example of the $LiAlH_4$ reduction is the conversion of butyronitrile into n-butylamine, a reaction which goes in high yield (Scheme 4).

Scheme 4

In the catalytic hydrogenation procedure, secondary amines are often produced as by-products. This side reaction may be suppressed by carrying out the hydrogenation in the presence of excess ammonia.

D.4 Reduction of Oximes.

Aldoximes and ketoximes, which may be prepared from aldehydes or ketones by reaction with hydroxylamine, are reduced by lithium aluminium hydride or hydrogen to primary amines.[38] Since oximes are easily generated in high yield, this method is a useful synthetic route to amines (Scheme 5).

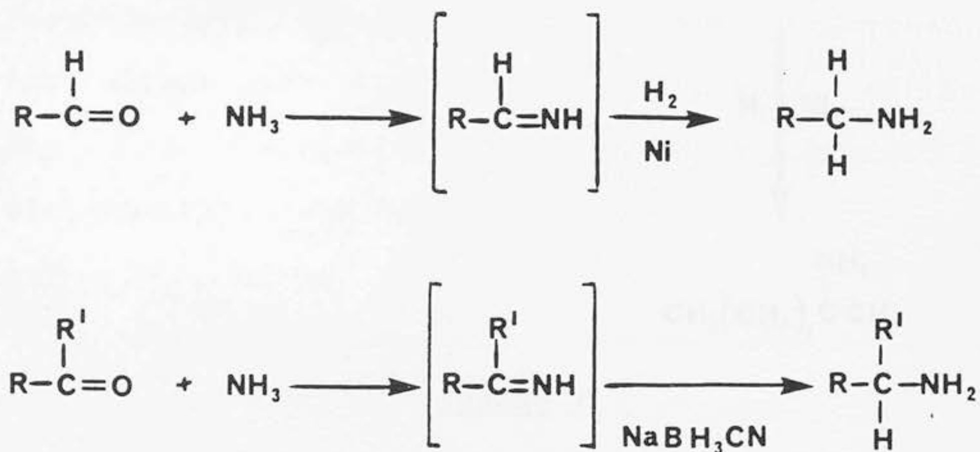


Scheme 5

D.5 Reduction of imines: Reductive Amination.

Many aldehydes (RCHO) and ketones (R_2CO) are converted into amines by reductive amination: reduction in the presence of ammonia.[39] Reduction can be accomplished catalytically or by use of sodium cyanohydrideborate, $NaBH_3CN$. Reaction involves reduction

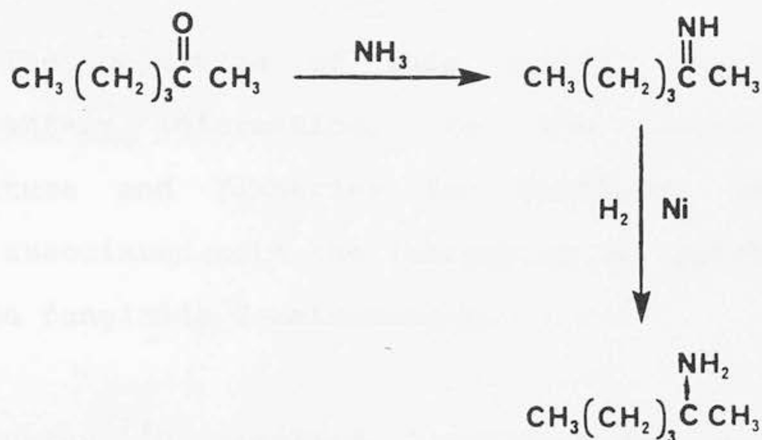
of an intermediate compound (an imine, RCH=NH or $R_2C=NH$) that contains a carbon-nitrogen double bond (Scheme 6).



Scheme 6

Reductive amination of ketones yields amines containing a sec-alkyl group; such amines are difficult to obtain by ammonolysis because of the tendancy of sec-alkyl halides to undergo elimination. Scheme 7 shows how 2-aminohexane can be prepared from reductive amination of the corresponding ketone, 2-hexanone.

During reductive amination the aldehyde or ketone can react not only with ammonia but with the primary amine that has already been formed, and thus yield a certain amount of the secondary amine. The tendancy for the reaction to go beyond the desired stage can be fairly well limited by the proportions of reactants employed and is seldom a serious handicap.[40]



Scheme 7

The above brief review, illustrates that the synthesis of primary amines can be approached from a wide range of precursors. It should be noted that the above list is far from exhaustive and many other methods of amine preparation, such as reduction of nitro compounds and the degredation of amides, are also well documented in the literature.

E. Program of Research.

The objective of this project was to provide supplementary information, to the Department of Agriculture and Fisheries for Scotland, on various topics associated with the fumigation of potato tubers with the fungicide 2-aminobutane.

Using ^{14}C labelled 2-aminobutane, as an easily identifiable tracer, it was hoped to study the uptake and penetration of the fungicide in some detail.

Other areas such as the loss of 2-AB from tubers; penetration of 2-AB into damaged and healed tubers; and the uptake of 2-AB on to soil, wood and straw, would also be investigated, to provide useful background information for DAFS in their on-going research into the bulk fumigation of potato tubers.

The main areas of research therefore were:

a) To develop a synthetic route to ^{14}C labelled 2-aminobutane.

b) To develop a simple analytical technique for determining 2-AB residues in tuber samples, utilising the ^{14}C label.

c) To use the ^{14}C labelled 2-aminobutane to study the fumigation process in more detail, looking at uptake, penetration and loss of 2-AB.

Experimental.

A. Symbols and abbreviations.

b.p.	boiling point
t.l.c.	thin layer chromatography
g.l.c.	gas liquid chromatography
h.p.l.c.	high pressure liquid chromatography
n.m.r.	nuclear magnetic resonance
i.r.	infra-red
l.s.	liquid scintillation
h, min, s	hours, minutes, seconds
p.p.m.	parts per million - used to express residues in tubers equivalent to $mgkg^{-1}$.
mmol, mol	millimoles, moles
M	molar
c.p.m.	counts per minute
l (litre)	dm^3
lit.	literature

B. Instrumentation.

Liquid scintillation counting.

A Beckman LS7000 liquid scintillation counter was used to measure the activity of liquid samples containing ^{14}C or ^{3}H labelled compounds. Aqueous samples were counted in general purpose NE265 liquid scintillator solution, supplied by Nuclear Enterprises.

Gas-liquid chromatography.

A Pye series 104 chromatograph fitted with a flame ionisation detector was used in analytical investigations. The column was 2 m by 4 mm i.d. and the carrier gas was nitrogen. The stationary phase was 20% PEG1500 on Chromosorb P-AW (80-100 mesh).

High performance liquid chromatography.

Several 2-AB residue analyses were carried out using a Waters liquid chromatograph, fitted with a Perkin-Elmer LC1000 fluorescence detector and SP4100 computing integrator. A 382 nm excitation filter tuned at 5° from normal was used and the resulting emission monitored at 487.5 nm. A spherisorb ODS column was used with a methanol-tris buffer (64:36) mobile phase.

Thin layer chromatography.

Chromatograms were developed on 0.25 mm layers of neutral alumina. Components of the chromatogram were detected using one of two methods. Either a ninhydrin (3g) in ethanol/acetic acid ($95cm^3/5cm^3$) spray reagent was applied, or bands of alumina were scraped from the plate, into scintillation vials, mixed with liquid scintillator solution and counted (see page 192).

Nuclear magnetic resonance spectroscopy.

Routine ^1H n.m.r. spectra were recorded on a Varian EM360 spectrometer. Chemical shifts were measured in parts per million relative to tetramethylsilane (T.M.S.) as internal standard ($\delta = 0.0$).

Infra-red spectroscopy.

I.r. spectra were recorded on a Perkin-Elmer 781 Infra-red spectrophotometer. Liquid samples were recorded as thin films.

Optical rotation.

Optical rotation was measured on a Perkin-Elmer 141 automatic polarimeter and the specific rotation was calculated using:

$$[\alpha]_D^t = \frac{\alpha}{l.d}$$

α = angular (optical) rotation ($^\circ$)

l = cell length (cm)

d = density (gcm^{-3}) at temperature t ($^\circ C$).

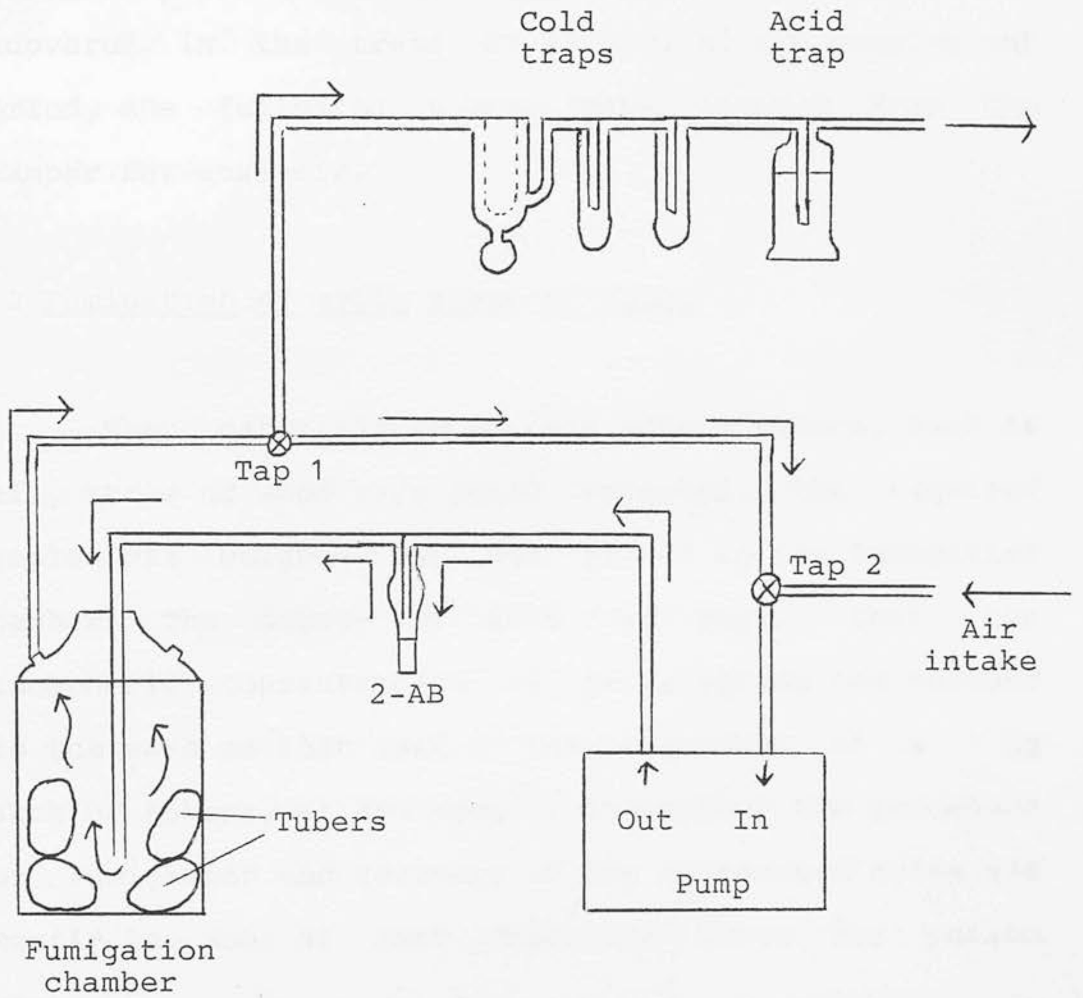
D = sodium D line (589.6 nm)

C. Procedure for fumigation with 2-aminobutane (2-AB), in the small scale fumigation chamber.

C.1 Fumigation of potato tubers.

In a specially constructed small scale fumigation apparatus (Figure 5), batches of tubers were fumigated with 2-AB, using the following procedure. Tubers (10-24) weighing approximately 1-2kg in total, were placed in the sealed fumigation chamber. 2-aminobutane, at the desired dose (normally 200 $mgkg^{-1}$ tubers), was then weighed into the small sample bottle, attached to the fumigation apparatus between the pump and fumigation chamber. Both taps on the apparatus were then set so that air would circulate around the closed system without being vented. The pump was switched on and the resulting air flow carried the volatile amine into the fumigation chamber. The air/amine mixture was recirculated around the closed system for 2 h (or any other desired length of time), to distribute the amine fumigant evenly throughout the batch of tubers. At the end of the fumigation period, tap 2 was opened to draw air into the fumigation apparatus, and tap 1 was turned to vent the air from the fumigation chamber, carrying any unabsorbed 2-AB, out through a series of traps. The first three traps were cold traps, cooled with an acetone/solid carbon dioxide (CO_2) mixture. The fourth

Figure 5 Small scale fumigation apparatus.



Arrows represent the direction of gas flow

and final trap was filled with 0.2 M hydrochloric acid (HCl) solution, through which was bubbled the vented air. The apparatus was normally flushed out with air for 1-2 hours, so that any unabsorbed 2-AB could be recovered in the traps. At the end of the pumping out period, the fumigated tubers were removed from the chamber for analysis.

C.2 Fumigation of soil, straw or wood.

When materials other than potato tubers, such as soil, straw or wood were being fumigated, the required sample was weighed out and placed in the fumigation chamber. The dosage of 2-AB was such, that the atmospheric concentration of amine within the chamber was the same as that used in the treatment of a 1 kg batch of tubers, at 200 $mgkg^{-1}$. Thereafter the procedure for fumigation and recovery of any unabsorbed amine was exactly the same as that described above for potato tubers.

D. Liquid scintillation counting.

D.1 Basic principles.

For the purposes of scintillation counting, the radiolabelled sample is mixed with a liquid scintillator (l.s.) solution. This l.s. solution normally comprises a scintillation solvent (e.g. toluene or pseudo-cumene), a fluor (e.g. 2,5-Diphenyloxazole (PPO)) and occasionally an emulsifier (to solubilise water samples into the overall scintillation 'cocktail'). As the radionuclide decays naturally, it emits energy, and for ^{14}C radionuclides this takes the form of beta particles. The prime function of the scintillation solution is to convert this energy into light, which is more easily detected and measured. Any beta particles emitted from the radioactive sample, will interact with the scintillation solvent to produce excited solvent molecules. This excitation energy is passed between solvent molecules until eventually it is transferred to, and trapped by, a fluor molecule. When the excited fluor molecule relaxes, it emits its excitation energy in the form of blue light (approx. 420 nm), which is easy to detect with a simple photomultiplier tube. The overall effect of the scintillation process, is that each decay from a radionuclide produces one flash of light, and each flash is recorded as one count. The number of

counts recorded per minute gives a measure of the amount of a particular radionuclide (e.g. ^{14}C) present, since each radionuclide has a fixed number of disintegrations (decays) per minute.

D.2 Quenching and quench correction.

In a 100% efficient scintillation system, the number of counts per minute (cpm) recorded for a sample, will correspond directly to the number of radioactive disintegrations per minute (dpm), for that same sample. One problem often encountered in liquid scintillation counting however, is quenching. This is a general term applied to several different processes, each of which affects the counting efficiency, by preventing some of the energy produced by the radioactive sample from reaching the detector, as light. The main types of quenching include chemical and colour quenching. In chemical quenching, a molecule other than the fluor absorbs the energy emitted by the radionuclide but does not release it again as light. Colour quenching occurs when red, yellow or green coloured samples are counted. Each absorbs the blue light, produced by relaxation of the excited fluor molecule, before it can be detected by the photomultiplier tube. Both types of quenching result in a decrease in the recorded number of counts per minute.

Several methods have been developed for measuring the amount of quenching that occurs in a sample and this enables a correction to be made to the recorded cpm value. Each method involves the preparation of a quench correction curve, as follows:-

a) At least six sample vials, containing an equal volume of scintillator solution and an equivalent aliquot of a ^{14}C standard of known activity (dpm), are prepared.

b) Each sample is counted on the liquid scintillation counter and at this stage should have the same cpm.

c) Sample 1 is left unaltered, but to each of the others is added an increasing amount of quenching agent, such as acetone (e.g. add 100 microlitres acetone to sample 2, 200 microlitres to sample 3, 400 microlitres to sample 4, e.t.c.).

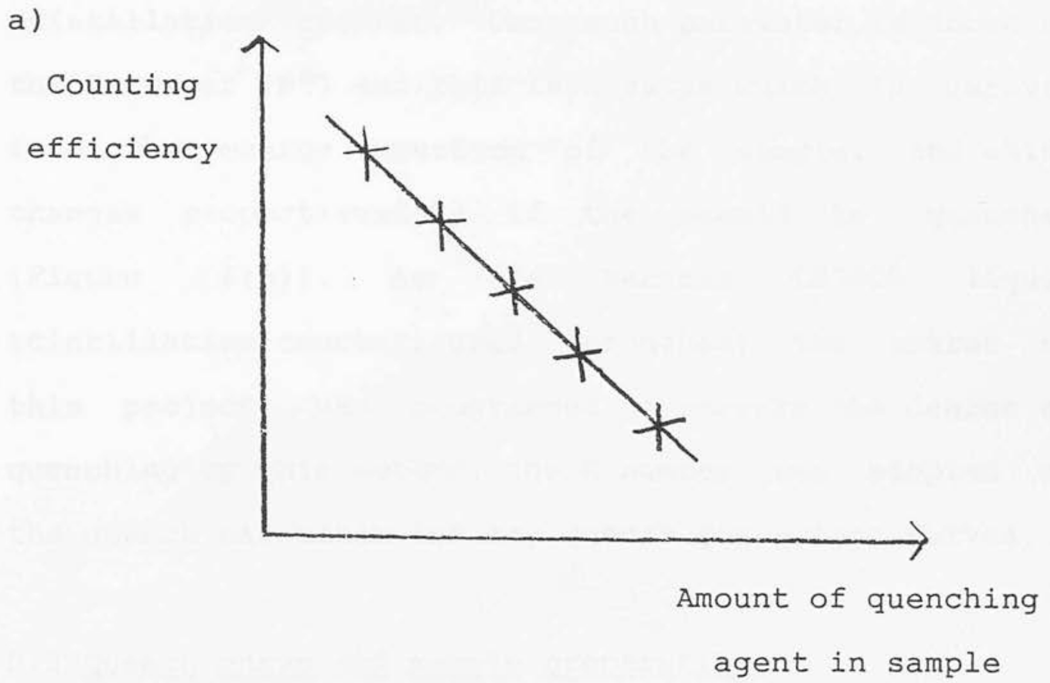
d) Each sample is then recounted, the new cpm value is recorded, and for each sample a counting efficiency is determined.

$$\text{Counting efficiency} = \frac{\text{cpm}}{\text{dpm}} \times 100\%$$

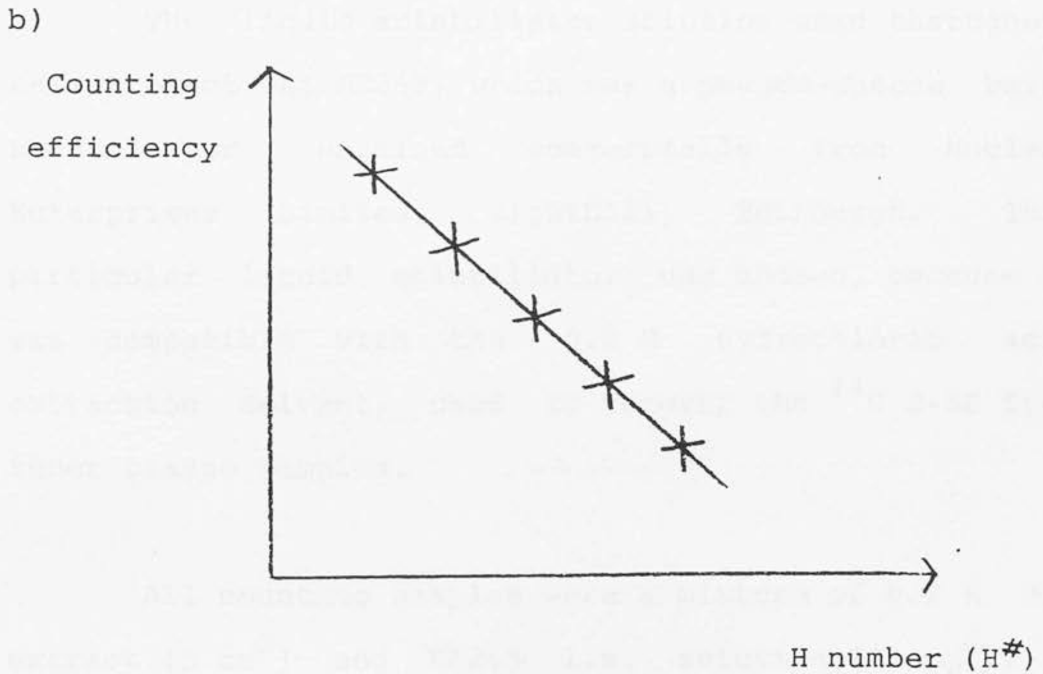
A plot of counting efficiency against some parameter which varies with quenching, gives the quench correction curve. One possible quench parameter is simply the amount of quenching agent present in each sample (Figure 6(a)). This is not particularly appropriate when samples of uncertain composition are counted, since the amount and type of quenching agent present, is not

Figure 6 Quench correction curves.

a)



b)



always known. It is more common therefore to use a quench parameter which is measured directly by the scintillation counter. One such parameter is known as the H number ($H^{\#}$) and this is a value which is derived from the energy spectrum of the sample, and which changes proportionately if the sample is quenched (Figure 6(b)). As the Beckman LS7000 liquid scintillation counter, used throughout the course of this project, was programmed to measure the degree of quenching by this method, the H number was adopted as the quench parameter for any quench correction curves.

D.3 <u>Quench curve and sample preparation.</u>

The liquid scintillator solution used throughout this project was NE265, which was a pseudo-cumene based scintillator obtained commercially from Nuclear Enterprises Limited, Sighthill, Edinburgh. This particular liquid scintillator was chosen, because it was compatible with the 0.2 M hydrochloric acid extraction solvent, used to recover the ^{14}C 2-AB from tuber tissue samples.

All counting samples were a mixture of 0.2 M HCl extract (5 cm^3) and NE265 l.s. solution (15 cm^3). A quench correction curve was prepared for this particular scintillation cocktail, using the procedure outlined

above. A ^{14}C toluene standard (of known activity) and acetone (quenching agent), were used in the preparation of the quench correction curve. The final graph was a plot of ^{14}C counting efficiency against H number (measured by the l.s. counter).

E. 2-aminobutane analysis.

E.1 Analysis of ^{14}C 2-AB in air samples, taken from the fumigation chamber during the fumigation period.

In several experiments, the concentration of 2-AB inside the fumigation chamber was monitored by taking air samples from inside the chamber, at regular time intervals. For these experiments a Suba-seal was fitted to the top of the fumigation chamber. Several flasks, containing 0.2 M HCl (50 cm^3), were also fitted with Suba-seals. The fumigation was carried out as normal, except that at regular time intervals a gas-tight syringe was used to take air samples (10 cm^3), from inside the chamber, through the Suba-seal. Each air sample was transferred directly to one of the sealed flasks, containing acid, to dissolve any 2-AB which had been removed from the chamber. The quantity of 2-AB in each air sample was determined by scintillation counting of an aliquot (5 cm^3) of the corresponding acid solution, after mixing with NE265 liquid scintillator solution (15 cm^3), and the resulting count was used to determine the concentration of unabsorbed 2-AB, inside the chamber at the time of sampling.

E.2 H.p.l.c. determination of 2-AB residues in fumigated tubers.

Analysis for unlabelled 2-AB in tuber samples, was carried out under the supervision of the Chemistry Section, Agricultural Sientific Services, DAFS, at East Craigs, Edinburgh, using the method developed by Hunter and Lindsay.[57] This involved extraction of the amine, derivatisation and detection by h.p.l.c.. The procedure for the analysis was as follows:-

a) Extraction of 2-AB from tuber samples.

Four tubers of similar size were taken at random from the fumigation batch and each was cut into quarters. Opposite quarters from each tuber were taken, diced into small pieces and macerated to a smooth pulp, in a Waring blender. A sub-sample of the pulp (25 g) was then weighed into a Kjeldahl flask, and 1 M calcium chloride solution (75 cm^3), magnesium oxide (10 g) and water (150 cm^3), were added. A few drops of BDH silicone antifoam and a few glass beads, to prevent foaming and bumping respectively, were also added to the flask. The mixture was then distilled, collecting distillate (80-90 cm^3), in a 150 cm^3 beaker containing 0.3 N H_2SO_4 (10 cm^3). The distillate was then made up to 100 cm^3 in a standard flask.

b) Derivatisation and h.p.l.c..

A reverse phase column (Spherisorb ODS) was set-up. A methanol-tris buffer (64:36) mobile phase was prepared, and degassed using an ultrasonic bath and vacuum line. The mobile phase flow rate was set at 2.0 $cm^3 min^{-1}$. Aliquots (0.5 cm^3) of standards and sample solutions were taken and mixed with borate buffer (0.2 cm^3). Fluorescamine (0.25 cm^3) was then added and mixed in immediately. An aliquot (20 microlitre) of the reaction mixture was injected into the liquid chromatograph, and the results from the fluorimeter monitored using an SP4100 computing integrator. 2-AB residues were obtained directly as mg 2-AB kg^{-1} tuber tissue (ppm).

E.3 Determination of ^{14}C 2-AB residues in tuber samples by liquid scintillation counting.

a) 2-AB residues in whole tubers.

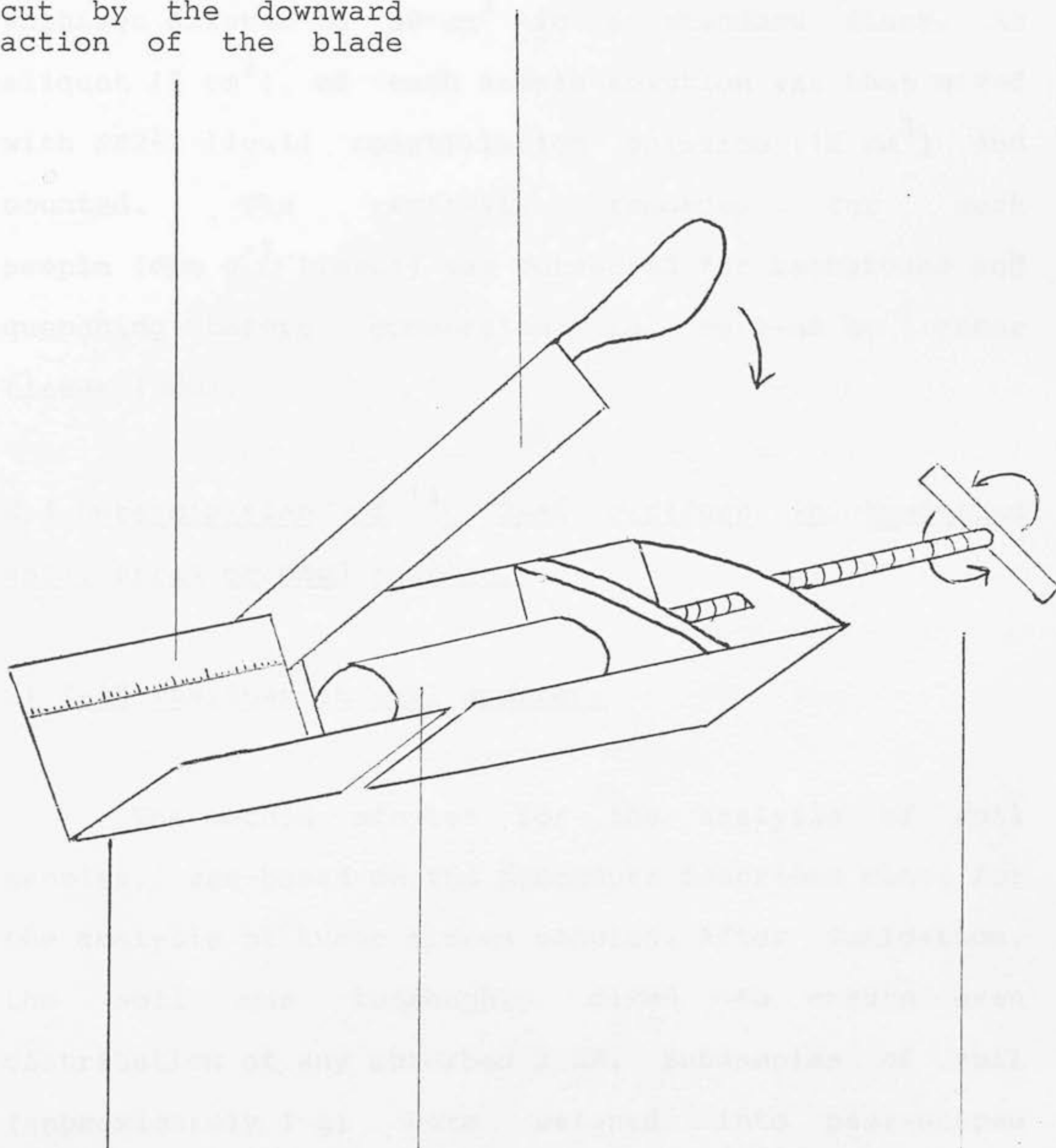
Tubers for analysis were sampled and pulped using the subsampling procedure, proposed by Hunter and Lindsay[57], described above. Two subsamples of pulp (3-5 g) were then weighed into 50 cm^3 pear-shaped Quickfit flasks, and 0.2 M hydrochloric acid (25 cm^3) was added to each. The tuber tissue samples were left to

soak overnight in the acid solution. A condenser was then fitted to each flask, and the acid/pulp mixture was heated on a boiling water bath for 25 minutes. After cooling, each sample was filtered to remove the residual tuber tissue, and the combined filtrate and washings were diluted with 0.2 M hydrochloric acid, to 50 cm^3 in a standard flask. An aliquot (5 cm^3) of each sample solution was then mixed with NE265 l.s. solution (15 cm^3), and the mixture counted. Since the activity (cpm mg^{-1}) of the ^{14}C 2-AB was known, the recorded cpm value for each sample, was easily converted after correction for background radiation and quenching, to a value corresponding to mg 2-AB kg^{-1} tuber tissue (ppm).

b) <u>2-AB residues in sample cores through tubers.</u>

In the 2-AB penetration experiments, instead of sampling the whole potato, a 2.5 cm (diameter) cork borer was used to take a core through the tuber. The surface skin from either end of the core was removed, and the core itself was sliced into 5 mm thick slices using a specially constructed mini-guillotine (Figure 7). Each skin sample, or 5 mm slice of tuber flesh, being too small to pulp, was then chopped finely and weighed into individual flasks. 0.2 M hydrochloric acid (25 cm^3) was added to each flask, and the samples

Figure 7 Mini-guillotine for slicing tuber cores.



were left to soak overnight. A condenser was then fitted to each flask and the samples were heated for 25 min on a boiling water bath. After cooling, the skin or tuber tissue was filtered off and the combined filtrate and washings diluted to 50 cm^3 in a standard flask. An aliquot (5 cm^3) of each sample solution was then mixed with NE265 liquid scintillation solution (15 cm^3) and counted. The activity recorded for each sample (cpm g^{-1} tissue) was corrected for background and quenching before conversion to mg 2-AB kg^{-1} tuber tissue (ppm).

E.4 <u>Determination of ^{14}C 2-AB residues in fumigated soil, straw or wood samples.</u>

a) <u>2-AB residues in soil samples.</u>

The method adopted for the analysis of soil samples, was based on the procedure described above for the analysis of tuber tissue samples. After fumigation, the soil was thoroughly mixed to ensure even distribution of any absorbed 2-AB. Subsamples of soil (approximately 1 g) were weighed into pear-shaped Quickfit flasks and 0.2 M hydrochloric acid (25 cm^3) was added to each. The samples were left to soak overnight and then, without any heating, were filtered to remove the residual soil particles. The combined filtrate and

washings were made up to 50 cm^3 in a standard flask. An aliquot (5 cm^3) of each final acid solution, was mixed with NE265 liquid scintillator solution (15 cm^3) and counted on the l.s. counter. As before the recorded cpm value for the known weight of sample could be converted into mg 2-AB kg^{-1} soil.

b) <u>2-AB residues in straw and wood shavings.</u>

When fumigated straw and wood shavings were being analysed for 2-AB, the procedure was exactly the same as that for the tuber tissue samples. Each fumigated sample was soaked overnight in 0.2 M hydrochloric acid (25 cm^3), heated on a boiling water bath for 25 minutes, and filtered. Scintillation counting of aliquots of the final acid solutions gave final residues of mg 2-AB kg^{-1} straw or wood shavings.

c) <u>2-AB residues in wooden blocks.</u>

In one experiment, wooden blocks of known weight and surface area, were fumigated with 2-AB. After fumigation, each block was soaked in 0.2 M hydrochloric acid (25 cm^3) for a period of three weeks, to ensure any absorbed 2-AB was extracted. Aliquots (5 cm^3) of each extract were mixed with NE265 l.s. solution (15 cm^3) and counted, to determine the amount of amine absorbed by

each block. The 2-AB residue was remeasured after a further period of soaking to ensure that the residue had not changed, and thus confirm that the initial extraction was efficient. The final calculated 2-AB residue was quoted as both mg 2-AB kg^{-1} wood, and mg 2-AB cm^{-2} wood surface area.

F. Materials.

F.1 Non-chemical materials.

a) Potato tubers.

All tubers used throughout the course of this project were provided by DAFS, East Craigs, Edinburgh. The variety and quality of the tubers depended on current availability, at the time each experiment was carried out. Attempts were made to maintain the quality of tubers, prior to use, by storing them in dark, cool surroundings. The tubers used were as far as possible of uniform size and shape, falling within the general size range for seed tubers (32-55 mm).

b) Soil samples.

All soil samples were provided pre-dried, by Dr. R. Spiers, of the East of Scotland College of Agriculture. Each sample was stored in an air-tight container prior to use, and the soils were wetted as required, before fumigation. Soil pH measurements were made, on soil-water slurries, using a Kent Eil 705 pH meter. Adjustments to the pH of soil samples were made possible by the addition of calculated amounts of acid (concentrated HCl) or alkali ($Ca(OH)_2$).

c) Straw and wood shavings.

Straw and wood shavings were obtained commercially as required.

d) Pallet box wood.

For one experiment, wood from a typical 1 tonne pallet box, used for the storage of potatoes, was obtained through DAFS.

F.2 Dry solvents.

"Dry ether" was prepared by treating commercially available diethylether with sodium wire.

"Super dry" ethanol was prepared by distillation from activated magnesium and stored over molecular sieve.

G. Preparation of 2-aminobutane (2-AB).

G.1 Preparation of 2-butanone.

a) Using an organocadmium reagent.

Magnesium (6 g, 0.25 mol) was placed in a 1 litre 3-necked flask, with sodium dried diethylether (50 cm^3). Approximately 1/20 of a total of 0.25 mol (27.25 g) of ethyl bromide, was added with stirring under an atmosphere of nitrogen, to initiate the Grignard reaction. The start of the reaction was shown by the occurrence of a slight turbidity and a rise in the temperature of the ether. The remaining ethyl bromide was dissolved in dry ether (60 cm^3) and added dropwise with further stirring, in such a way that the ether refluxed gently. Towards the end of the addition the mixture was boiled gently, on a water bath, until almost all the magnesium had dissolved (about 30 min). The mixture was then cooled to room temperature and cadmium bromide (35.25 g, 0.125 mol) was added in small portions, to form the corresponding organocadmium reagent (diethylcadmium). Acetyl chloride (19.63 g, 0.25 mol) was dissolved in dry ether (30 cm^3) and added dropwise with stirring to the reaction flask. After stirring for a further 8 h, crushed ice was added to the flask to hydrolyse the reaction mixture, followed by

sufficient 50% aqueous ammonium sulphate to dissolve the precipitate which had formed. The ether layer of the reaction mixture was separated and dried over magnesium sulphate. The dried ether solution was concentrated on a rotary evaporator and analysed by g.l.c.. Comparison of the peaks produced for the reaction mixture with the retention time for authentic 2-butanone showed that only small amounts of the desired ketone had been recovered.

b) Using a Grignard reagent.[41]

Magnesium (6 g, 0.25 mol) was placed in a 1 litre flask with sodium dried diethylether (50 cm^3). The flask was then flushed out with nitrogen. Approximately 1/20 of a total of 0.25 mol (27.25 g) of ethyl bromide, was added with stirring to initiate the formation of the Grignard reagent. The remaining ethyl bromide, dissolved in dry ether (60 cm^3), was added dropwise, with stirring at such a rate that the ether refluxed gently. Towards the end of the addition the mixture was boiled gently on a water bath, until almost all of the magnesium had dissolved (about 30 min). The ethylmagnesium bromide in ether solution, was then transferred under an atmosphere of nitrogen, to a dropping funnel. Acetyl chloride (19.63 g, 0.25 mol) was dissolved in dry ether (30 cm^3), in a separate flask. The flask was flushed out with nitrogen and the Grignard reagent, in

ether, added dropwise with stirring. Once addition was complete, the mixture was stirred and refluxed for a further 2 h. The reaction mixture was then hydrolysed by the addition of crushed ice, followed by sufficient 2 M hydrochloric acid, to dissolve the precipitate which had formed during the reaction. The ether layer was separated and the aqueous layer extracted with two aliquots (100 cm^3) of ether. The ether extract was dried over magnesium sulphate before concentration on a rotary evaporator and analysis by g.l.c.. Only small quantities of the desired 2-butanone were detected in the reaction mixture.

c) <u>Using a Grignard reagent in the presence of an iron(III) chloride catalyst.</u>

The preparation of 2-butanone from the reaction of ethylmagnesium bromide and acetyl chloride, in the presence of an iron(III) chloride catalyst, was attempted using the procedure outlined by Percival *et al.*.[42] The Grignard reagent was prepared, under an atmosphere of nitrogen, by adding ethyl bromide (27.25 g, 0.25 mol) to a mixture of magnesium (6 g, 0.25 mol) in dry ether (30 cm^3). Once prepared the ethylmagnesium bromide was transferred to a dropping funnel. A 2 litre flask was charged with acetyl chloride (58.9 g, 0.75 mol), in dry ether (80 cm^3). The

flask was flushed out with nitrogen and the contents cooled to -65°C, using an acetone/solid CO_2 bath. Iron(III) chloride (0.96 g, 5.9 mmol) was then added. The ethylmagnesium bromide, in ether, solution was added dropwise, with stirring, to the reaction flask. Once the addition was complete, the mixture was stirred for a further 10 min and then it was hydrolysed by the addition of ice. The yellow solid which had formed during the reaction dissolved without difficulty, on agitation of the reaction mixture. The solution was neutralised by the addition of sodium carbonate. The ether layer was separated and the aqueous portion extracted twice with aliquots (100 cm^3) of ether. The ether extract was dried over magnesium sulphate and then concentrated on a rotary evaporator for analysis by g.l.c.. The yield of 2-butanone by this method was higher than previously recorded but was still less than 10%. The aqueous layer of the reaction mixture was subjected to continuous extraction with ether, for 3 days, but this failed to significantly increase the recovery of the ketone product.

d) <u>Using an organocopper reagent.</u>[50]

Copper(II) sulphate (250 g, 1 mol) was dissolved in water (500 cm^3) and the solution saturated with sulphur dioxide. Potassium iodide (166 g, 1 mol) was

dissolved in water (150 cm^3) and again the solution was saturated with sulphur dioxide. The two solutions were then mixed and the white precipitate which formed was filtered off. The solid copper(I) iodide was washed with water (saturated with sulphur dioxide), and then with absolute ethanol. The precipitate was then dried in a vacuum oven at 110°C. Yield of copper(I) iodide, 185.4 g (0.97 mol, 97%).

Lithium metal (3.5 g, 0.50 mol) was placed in a 1 litre reaction flask with sodium dried ether (30 cm^3). The flask was flushed out with nitrogen to exclude moisture, and ethyl bromide (27.25 g, 0.25 mol) was added dropwise, with stirring, at such a rate that the ether refluxed gently. The mixture was heated at reflux for a further 2 h, to ensure complete reaction of the lithium metal, and was then cooled to 0°C. Copper(I) iodide (47.8 g, 0.25 mol) was then added, in portions, to prepare the lithium diethylcopper reagent. The mixture was cooled to -78°C and acetyl chloride (19.63 g, 0.25 mol) in dry ether (40 cm^3) added dropwise, with stirring. At the end of the addition, methanol (30 cm^3) was added to quench the reaction mixture, and the solution was allowed to warm up to room temperature before neutralising by addition of saturated ammonium chloride solution. Separation and analysis of the ether fraction of the reaction mixture, showed only

small quantities of the desired 2-butanone to be present.

G.2 <u>Preparation of 2-butanone oxime from 2-butanone.</u>

Using the procedure outlined by Marvel and Noyes[41], the preparation of 2-butanone oxime was carried out.

Hydroxylamine hydrochloride (50 g, 0.72 mol) was dissolved in water (300 cm^3). 2-butanone (40 g, 0.56 mol) and sodium carbonate (35 g, 0.33 mol) were added, and the mixture stirred for 24 h. At the end of this time the aqueous reaction mixture was extracted with three aliquots (100 cm^3) of diethylether, and the combined extracts dried over anhydrous calcium chloride. After drying, the mixture was distilled to remove the ether. The crude oxime which was left, was then also distilled and a fraction boiling between 150-152°C collected (lit.[51] 151°C). The clear liquid was confirmed as 2-butanone oxime by comparison of its n.m.r. and i.r. spectra with those for the authentic oxime, in the literature. The overall yield was 38.7 g (80.2%).

G.3 Preparation of 2-butanone and isolation as the oxime derivative.

2-butanone preparation was attempted using the procedure of Percival et al.[42], and prior to isolation, the ketone was converted into 2-butanone oxime following the procedure of Marvel and Noyes.[51]

Magnesium (14.4 g, 0.60 mol) was placed in a 1 litre flask with sodium dried ether (100 cm^3), and the flask flushed with nitrogen to exclude moisture. Approximately 1/20 of a total of 0.60 mol ethyl bromide (65.4 g), was added to the flask to initiate the formation of the Grignard reagent. The remaining alkyl halide, dissolved in dry ether (40 cm^3), was added dropwise to the reaction flask. Once the addition was completed, the mixture was boiled gently, on a water bath, until most of the magnesium had dissolved. The ether solution containing the ethylmagnesium bromide, was then allowed to cool before transferring it, under nitrogen, to a dropping funnel.

Acetyl chloride (114.3 g, 1.8 mol) was placed in a 2 litre flask with dry ether (100 cm^3). The flask was flushed out with nitrogen and then iron (III) chloride (2.7 g, 16.6 mmol) added. The flask and contents were cooled to -65°C on an acetone/solid CO_2

bath. The ethylmagnesium bromide, in ether, was then added dropwise, with strring. Near the end of the addition the formation of precipitate made it difficult to stir the reaction mixture, and therefore an extra portion (50 cm^3) of dry ether was added. Once addition of the Grignard reagent was complete, the reaction mixture was stirred for a further 10 min, and then hydrolysed by the addition of ice. The yellow precipitate which formed during the reaction, dissolved on hydrolysis. The mixture was then neutralised by addition of sodium carbonate, and steam distilled to give a clean ether/water mixture, which should have contained any 2-butanone formed during the reaction. The distillate from the steam distillation had a total volume of approximately 350 cm^3. To this was added hydroxylamine hydrochloride (50 g, 0.72 mol) and sodium carbonate (35 g, 0.33 mol), and the mixture was stirred for 24 h. The ether layer was separated and the aqueous layer extracted with three portions (100 cm^3) of ether. The combined ether extract was dried over calcium chloride, and then the solvent distilled off to leave the crude oxime. Distillation of the oxime at 150-152°C (lit.[51] 151°C) yielded 29.2 g of product. The clear liquid was confirmed as 2-butanone oxime from its n.m.r. and i.r. spectra, and the overall yield of product from ethyl bromide was 56%.

G.4 Reduction of 2-butanone oxime to 2-aminobutane.

a) Reduction with sodium metal.[51]

2-butanone oxime (9 g, 0.103 mol) was dissolved in "super-dry" ethanol (100 cm^3). To the solution was added sodium metal (15 g, 0.65 mol), in small portions over 20-30 min. The solution was refluxed gently, and more absolute ethanol (30 cm^3) was added, until all the sodium had dissolved. The mixture was then steam distilled and the distillate acidified by the addition of concentrated hydrochloric acid. The distillate was then evaporated to dryness on a rotary evaporator, to yield the solid amine hydrochloride. Saturated sodium hydroxide solution was then added to liberate the free amine. The amine layer which formed was separated, dried over solid sodium hydroxide pellets and distilled at 62-63°C. A total of 1.78 g of 2-aminobutane (lit.[51] b.p. 63°C) was recovered, and this corresponded to a yield of 25%. The identity of the amine was confirmed from its n.m.r. and i.r. spectra.

b) Reduction with lithium aluminium hydride.[52,56]

2-butanone oxime (11.3 g, 0.13 mol) was dissolved in sodium dried ether (130 cm^3). The solution was added dropwise, with stirring to lithium aluminium

hydride (10 g, 0.26 mol) in dry ether (300 cm^3). The mixture was refluxed with stirring, for 4 h, once the addition was complete. After cooling, the $LiAlH_4$ complex and any excess hydride, were decomposed using the method proposed by Micovic and Mihalovic.[56] This involved the addition of water (1 cm^3), 15% sodium hydroxide solution (1 cm^3) and water (3 cm^3), for each initial gram of lithium aluminium hydride. This produces a dry granular precipitate which absorbs very little substance and is easy to filter and wash. As 10 g of hydride had been used, the excess hydride and any product complex, were decomposed by the careful addition of water (10 cm^3), 15% sodium hydroxide solution (10 cm^3) and water (30 cm^3). The white precipitate which formed was filtered off and washed with three aliquots (100 cm^3) of ether. The combined ether filtrate was dried over magnesium sulphate. Distillation of the ether solution left only unreacted oxime, and no 2-aminobutane was recovered.

c) <u>Hydrogenation of 2-butanone oxime.</u>

An attempt at the hydrogenation of 2-butanone oxime, following the procedure of Reeve and Christian[53], was undertaken. The Raney nickel catalyst was prepared from stabilised BDH Raney nickel. This involved dissolving the required number of catalyst cylinders (cylindrical pellets of Raney nickel suspended in

resin), in dry ethanol at 30°C. This dissolved the resin and when the finely divided catalyst settled out of solution, the ethanol was decanted. The nickel residue was washed with three aliquots (25 cm^3) of ethanol and each time the washings were decanted.

Raney nickel (1 g) was placed in the glass hydrogenation vessel with dry ethanol (50 cm^3). 2-Butanone oxime (15 g, 0.17 mol) was added and the solution was saturated with ammonia, by bubbling the gas through the solution for about 2 h. The hydrogenation vessel, containing the reaction mixture was then placed inside the high pressure autoclave, and the chamber was flushed out with hydrogen. The autoclave was then pressurised up to 160 atmospheres with hydrogen, and the reaction mixture was left under this pressure for 11 days. The reaction time was extended from hours to days as the autoclave had no facility for shaking or stirring the reaction mixture, and was not capable of reaching the high pressures (220 atmospheres) of hydrogen, suggested by Reeve and Christian.[53] At the end of the reaction time the chamber was depressurised and the nickel catalyst filtered off. The ethanolic filtrate was acidified to convert any 2-aminobutane into the corresponding hydrochloride salt. The ethanol was then removed on a rotary evaporator to leave a lime coloured solid. Saturated sodium hydroxide solution was added to

liberate the free amine, but only small amounts of 2-aminobutane were recovered. The hydrogenation was repeated with longer reaction times, but there was no increase in yield of the desired product, possibly due to the failure to recreate the exact reaction conditions of Reeve and Christian.[53]

d) <u>High temperature diborane reduction.</u>

2-Butanone oxime (8.7 g, 0.1 mol) was dissolved in diglyme (diethylene glycol methyl ether) (230 cm^3), and the solution cooled to $0^{o}C$. Diborane (B_2H_6) (4.14 g, 0.15 mol) dissolved in tetrahydrofuran (150 cm^3), was added dropwise by syringe, at such a rate that the temperature of the reaction mixture did not exceed $10^{o}C$. After the addition was completed, the mixture was heated at 105-110°C for 20 h. The reaction mixture was allowed to cool to room temperature, and then was cooled further by immersion of the flask in an ice/salt bath. The excess diborane was destroyed by the careful, dropwise addition, of 50% potassium hydroxide solution. The mixture was then acidified by the addition of concentrated hydrochloric acid, to convert any amine present, into the hydrochloride salt. The tetrahydrofuran, diglyme and water were distilled off to leave a brown solid (mixture of 2-AB hydrochloride and boron salts). The solid was treated with saturated

sodium hydroxide solution to liberate the free amine, but despite a strong ammoniacal odour, no 2-aminobutane was recovered from the mixture.

e) Reduction with Raney alloy in alkaline solution.

Using the procedure outlined by Staskun and Van Es[55], an attempt was made to reduce 2-butanone oxime with Raney alloy to prepare 2-aminobutane. Raney alloy (30 g), was added in small portions, to a stirred solution of 2-butanone oxime (10 g, 0.11 mol) in ethanol (200 cm^3) and 2 M sodium hydroxide solution (200 cm^3). During the addition the flask was cooled with an ice/water bath to prevent overheating and loss of amine product. Once the addition of the Raney alloy was completed the reaction mixture was stirred for a further 1 h. The nickel was then filtered off and washed with three aliquots (10 cm^3) of water. The combined filtrate and washings were acidified by addition of concentrated hydrochloric acid before removing the water and ethanol on a rotary evaporator. The residual amine salt was treated with saturated sodium hydroxide solution to liberate the free 2-aminobutane. The amine layer which formed was separated, dried over solid sodium hydroxide and distilled. A fraction boiling at 61-63°C was collected (lit.[51] 63°C), and was confirmed as 2-aminobutane by

comparison of its n.m.r. and i.r. spectra with those for the commercially obtained, authentic amine. The total yield of product was 2.8 g (34.9%).

G.5 Preparation of 2-aminobutane from ethyl iodide and acetyl chloride.

a) Using unlabelled ethyl iodide and acetyl chloride.

As a check on the whole synthesis, a quantity of 2-aminobutane was prepared from ethyl iodide and acetyl chloride.

Preparation of ethylmagnesium iodide.

Magnesium (9.12 g, 0.38 mol) was placed in a 1 litre flask with sodium dried diethyl ether (60 cm^3). The flask was then flushed out with nitrogen. Approximately 1/20 of a total of 60 g (0.38 mol) of ethyl iodide, was added to initiate the formation of the Grignard reagent. The remaining ethyl iodide, dissolved in dry ether (100 cm^3), was added dropwise to the mixture at such a rate that the ether refluxed gently. At the end of the addition the mixture was refluxed on a water bath, until most of the magnesium had dissolved (about 30 min).

Reaction of ethylmagnesium iodide and acetyl chloride.

A 2 litre, 3-necked flask was charged with acetyl chloride (89.5 g, 1.14 mol), dry ether (250 cm^3) and iron(III) chloride (1.7 g, 10.5 mmol). The flask was flushed with nitrogen, and then the flask and contents cooled to -65°C on an acetone/solid CO_2 bath. The ethylmagnesium iodide in ether, prepared above, was transferred under an atmosphere of nitrogen, to a dropping funnel. The Grignard reagent was then added dropwise with stirring, to the acetyl chloride in ether solution. Once the addition was complete the mixture was stirred for a further 5 min at -65°C, before it was allowed to warm up to room temperature. The reaction mixture was then hydrolysed by the addition of ice and neutralised with solid sodium carbonate. The reaction mixture was then steam distilled to produce a clean water/2-butanone/ether solution. A total of about 350 cm^3 of distillate was collected.

Conversion of 2-butanone to 2-butanone oxime.

Hydroxylamine hydrochloride (50 g, 0.72 mol) and sodium carbonate (35 g, 0.33 mol) were added to the distilled water/2-butanone/ether solution (350 cm^3). The mixture was stirred for 24 h and then the ether layer, containing the oxime product, was separated off. The

aqueous layer was extracted with three aliquots of ether and the combined ether extracts dried over calcium chloride. The ether was removed by distillation to leave the crude oxime which was then distilled, and a fraction boiling at 150-152°C collected. The yield of oxime was 18.0 g (53.8% from ethyl bromide).

<u>Reduction of 2-butanone oxime to 2-aminobutane.</u>

2-Butanone oxime (18.0 g, 0.21 mol) was dissolved in ethanol (365 cm^3) and 2 M sodium hydroxide solution (365 cm^3). The mixture was cooled on an ice-water bath, and Raney alloy (54 g) added in portions with stirring. The mixture was stirred for 1 h and then the nickel alloy was filtered off. The water/ethanol filtrate was acidified by addition of concentrated hydrochloric acid, to convert the amine product into the corresponding hydrochloride salt. The water and ethanol solvents were then stripped off on a rotary evaporator. The solid amine hydrochloride salt was treated with saturated sodium hydroxide solution to liberate the free 2-aminobutane. The amine layer which formed, was separated and dried over solid sodium hydroxide pellets. The amine was then distilled (b.p. 61-63°C) and a total weight of 4.60g of 2-aminobutane was recovered. This corresponds to a yield of 32%, based on the oxime.

b) Using 1-^{14}C labelled ethyl iodide and unlabelled acetyl chloride.

The synthesis of 1-^{14}C 2-aminobutane was carried out using 1-^{14}C ethyl iodide and unlabelled acetyl chloride. Four batches of ^{14}C labelled amine were prepared by this method. In each case the small quantity of ^{14}C labelled material was dissolved in unlabelled ethyl iodide, and the synthesis was carried out following the procedure outlined above for the preparation of unlabelled 2-AB. Table 15 shows the quantities and activities of 2-aminobutane prepared by this method.

Table 15 Details of ^{14}C labelled 2-AB synthesis.

Synthesis	Weight of EtI (g)	Activity of EtI (MBq)	Weight of 2-AB (g)	Total Activity of 2-AB (MBq)	Specific Activity of 2-AB ($MBqg^{-1}$)
1	60	1.85	4.74	0.42	0.11
2	60	3.58	3.85	0.75	0.16
3	60	9.25	5.88	1.07	0.18
4	60	9.25	4.64	0.88	0.19

G.6 Preparation of ethyl bromide and acetyl chloride from acetic acid.

a) Preparation of acetyl chloride from acetic acid.[58]

A quantity of acetic acid was dried by refluxing with P_2O_5. The acid was then distilled (b.p. 118^oC) and 60 g (1.0 mol) was weighed into a 1 litre flask. Thionyl chloride (300 cm^3) was added dropwise to the acid, at such a rate that the reaction was controllable. At the end of the addition the mixture was refluxed for 30 min. The mixture was then allowed to cool, and the excess thionyl chloride was destroyed by the addition of formic acid (144.5 g, 118.5 cm^3). The destruction of thionyl chloride by this method liberates CO, SO_2 and HCl, and it was therefore conducted in a well ventilated fume cupboard. The acetyl chloride was then distilled up a fractionating column, and a fraction boiling at 49-52^oC was collected (lit.[39] 51^oC). A total of 54.3 g of acetyl chloride was recovered (69% yield). The identity of the product was confirmed from its i.r. spectra.

b) Reduction of acetyl chloride to ethanol.[59]

Lithium aluminium hydride (10 g, 0.26 mol) was placed in a flask with sodium dried ether (300 cm^3), and the mixture stirred for 15 min. A solution of acetyl

chloride (34.0 g, 0.43 mol) in dry ether (75 cm^3) was added dropwise, at such a rate that the ether refluxed gently. Once the addition was complete the mixture was refluxed on a water bath for 1 h. The flask was cooled in an ice/water bath and the excess hydride destroyed by the careful addition of water (10 cm^3), 15% sodium hydroxide solution (10 cm^3) and water (30 cm^3), using the method of Micovic and Mihalovic.[56] A grey coloured precipitate formed, and this was filtered off and washed with ether. The combined ether filtrate and washings were collected and dried over magnesium sulphate. The ether was removed by fractional distillation to leave the crude alcohol. Distillation of the crude product at 76-78°C (lit.[39] 78°C) yielded 6.72 g (33.9%) of ethanol. N.m.r. and i.r. spectra confirmed the identity of the material.

c) Preparation of ethyl bromide from ethanol.[60]

48% Hydrobromic acid (109.9 g, 1.36 mol) was placed in a 1 litre flask, and concentrated sulphuric acid (55.2 g, 0.56 mol) added in small portions with stirring. The mixture was then allowed to cool and ethanol (46 g, 1 mol) added. A reflux condenser was attached to the flask and a further portion of concentrated sulphuric acid (92.0 g, 0.94 mol) was added slowly from a dropping funnel. The mixture was then

heated on a water bath for 1 h. The apparatus was then rearranged for distillation, and the ethyl bromide which had formed, was distilled from the reaction mixture. The crude ethyl bromide was washed with an equal volume of concentrated hydrochloric acid, with water and finally 3% sodium hydrogen carbonate solution (10 cm^3). The ethyl bromide was then dried over magnesium sulphate and distilled at 36-39°C (lit.[39] 38°C). A total of 78.9 g of product was recovered and this corresponded to a yield of 72.4%.

G.7 <u>Preparation of U-^{14}C labelled 2-aminobutane.</u>

a) <u>Preparation of U-^{14}C acetyl chloride from U-^{14}C acetic acid.</u>

11.1 MBq of U-^{14}C acetic acid (sodium salt) was dissolved in unlabelled acetic acid (60 g, 1 mol). The acid was then placed in a 1 litre flask and thionyl chloride (300 cm^3) added dropwise, at such a rate that the mixture refluxed gently. Once the addition was complete the mixture was refluxed on a water bath for 1 h. The excess thionyl chloride was destroyed by the addition of formic acid (144 g, 118 cm^3). The U-^{14}C acetyl chloride was then distilled from the reaction mixture and collected; yielding 60.94 g of acid halide, containing 8.4 MBq of U-^{14}C acetyl chloride.

b) Preparation of U-^{14}C ethanol from U-^{14}C acetyl chloride.

Lithium aluminium hydride (10 g, 0.26 mol) was placed in a 1 litre flask with sodium dried ether (300 cm^3). The mixture was stirred for 15 min and then a solution of acetyl chloride (34.0 g, 0.43 mol), containing 4.9 MBq of U-^{14}C acetyl chloride, in dry ether (75 cm^3), was added dropwise. Once the addition was complete the mixture was refluxed for 1 h. The flask was then cooled and the excess hydride destroyed by the careful addition of water (10 cm^3), 15% sodium hydroxide solution (10 cm^3) and water (30 cm^3). The grey precipitate which formed was filtered off and the combined filtrate and washings were dried over magnesium sulphate. The drying agent was then filtered off and the ether removed by distillation to leave the crude ethanol. This was distilled (b.p. 76-78°C) and 9.22 g of ethanol (containing 2.3 MBq of U-^{14}C ethanol) were recovered.

c) Preparation of U-^{14}C ethyl bromide from U-^{14}C ethanol.

48% Hydrobromic acid (96.7 g, 1.19 mol) was placed in a 500 cm^3 flask and concentrated sulphuric acid (27.9 g, 0.28 mol) added in small portions. The

mixture was allowed to cool and ethanol (23.3 g, 0.51 mol), containing 2.3 MBq of U-^{14}C ethanol, was added. A condenser was fitted and a further portion of concentrated sulphuric acid (46.6 g, 0.47 mol) was added slowly. After the addition was complete the mixture was refluxed for 2 h. The ethyl bromide product was then distilled from the reaction mixture. The crude alkyl halide was washed with an equal volume of concentrated hydrochloric acid, water and finally with 3% sodium hydrogen carbonate solution (5 cm^3), before drying over magnesium sulphate. Distillation of the dried ethyl bromide yielded 37.2 g of product, containing 1.51 MBq of U-^{14}C ethyl bromide.

d) <u>Preparation of U-^{14}C ethylmagnesium bromide.</u>

Magnesium (9.12 g, 0.38 mol) was placed in a 1 litre flask with sodium dried ether (60 cm^3). The flask was then flushed with nitrogen. Approximately 1/20 of a total of 60 g (0.38 mol) of ethyl bromide, (containing 0.76 MBq of U-^{14}C ethyl bromide) was added to initiate reaction. The remaining ethyl bromide, dissolved in dry ether (50 cm^3), was added dropwise with stirring, at such a rate that the ether refluxed gently. Once the addition was complete the mixture was refluxed on a water bath until all the magnesium had dissolved (about 30 min).

e) Preparation of U-^{14}C 2-butanone and isolation as U-^{14}C 2-butanone oxime.

Acetyl chloride (81.7 g, 1.04 mol), containing 2.28 MBq U-^{14}C acetyl chloride, was dissolved in dry ether (250 cm^3) in a 2 litre flask. The flask was flushed with nitrogen, and iron(III) chloride (1.67 g, 10.3 mmol) was added to the mixture. The flask and contents were then cooled to -65°C on an acetone/solid CO_2 bath. The ethylmagnesium bromide in ether solution, prepared above, was then added dropwise with stirring. 5 min after the addition was complete, the flask and contents were allowed to warm up to room temperature. The reaction mixture was then hydrolysed by the addition of ice and neutralised with solid sodium carbonate. The mixture was steam distilled to produce a clean water/2-butanone/ether solution. To this was added hydroxylamine hydrochloride (45 g, 0.65 mol) and sodium carbonate (35 g, 0.33 mol). The ether layer was separated and the aqueous layer extracted with three portions (100 cm^3) of ether. The combined ether extracts were dried over calcium chloride. The ether was removed by distillation to yield the crude oxime. This was then distilled and 15.97 g (0.18 mol) of 2-butanone oxime, containing 0.60 MBq of U-^{14}C oxime, was recovered.

f) Preparation of U-^{14}C 2-aminobutane from U-^{14}C oxime.

Raney alloy (47.8 g), was added in portions to a mixture of 2-butanone oxime (15.93 g, 0.18 mol), containing 0.60 MBq of U-^{14}C oxime, in ethanol (320 cm^3) and 2 M sodium hydroxide solution (320 cm^3). During the addition the flask was cooled on an ice/water bath. Once the addition was complete the mixture was stirred for 1 h. The alloy was then filtered off and the water/ethanol filtrate was collected. The combined filtrate and washings were acidified with concentrated hydrochloric acid, to convert the amine product to the hydrochloride salt. The water and ethanol were removed on a rotary evaporator to leave the solid amine salt. This was then treated with saturated sodium hydroxide solution and the liberated amine was collected and distilled. 0.40 g of 2-aminobutane, containing 0.02 MBq of U-^{14}C 2-aminobutane, was recovered.

H. Preparation of "Storite Plus" spiked with ^{14}C labelled 2-AB glycollate salt.

H.1 Preparation of "Storite Plus" spiked with 2-AB glycollate.

2-Aminobutane (6.36 g, 0.087 mol) was placed in a 50 cm^3 flask, fitted with a condenser. The amine was cooled to $0^{\circ}C$ on an ice bath. Glycollic acid (10 g, 0.13 mol) was added dropwise, with stirring, and the temperature maintained at less than $30^{\circ}C$.

2 g of the resulting liquid 2-AB glycollate mixture was added to "Storite Plus" concentrate (20 g). This increased the initial concentration of 2-AB glycollate in the concentrate from 40.0% to 45.5%, but the extra salt dissolved readily in the commercial formulation.

H.2 Preparation of "Storite Plus" spiked with ^{14}C labelled 2-AB glycollate.

2-Aminobutane (0.20 g, 2.74 mmol), containing 0.036 MBq of ^{14}C labelled 2-AB, was placed in a 25 cm^3 Quickfit flask. The amine was cooled to $0^{\circ}C$ on an ice bath, and glycollic acid (0.30 g, 3.9mmol) was added with swirling, and the temperature of the mixture was

kept below 30°C by cooling on the ice bath. "Storite Plus" concentrate (5.5 g) was added to the flask, and this easily dissolved the ^{14}C 2-AB glycollate mixture. The resulting solution was "Storite Plus" containing 45.5% 2-AB glycollate. The specific activity of the spiked formulation was 0.006 MBq g^{-1}. Prior to use the spiked "Storite Plus" concentrate was diluted with water (1 cm^3 of the spiked formulation was diluted to 5 cm^3 with water).

I. Attempted resolution of racemic 2-aminobutane.

I.1 Resolution of racemic 2-AB with (+)-tartaric acid in methanol.

Methanol (450 cm^3) was added to a flask containing (+)-tartaric acid (31.5 g, 0.21 mol). The mixture was heated almost to boiling on a water bath, and then racemic 2-AB (14.6 g, 0.20 mol) was added cautiously with swirling. The mixture was allowed to cool to room temperature and was then left to stand for 24 h. The crop of crystals which formed during this time was filtered off and dried. The methanolic filtrate was retained. The crystals of amine (+)-tartate salt were treated with saturated sodium hydroxide solution to liberate the resolved amine enantiomer. The amine layer was separated, dried over solid sodium hydroxide and distilled; 6.6 g of 2-AB were collected. The optical rotation of the amine was measured and the specific rotation calculated as $+0.96^{\circ}$ (neat) (lit.[74] $+7.4^{\circ}$ (neat)), indicating that the racemic 2-AB had only been partially resolved. The methanolic filtrate was evaporated to dryness and the amine liberated from the residual salt, by treatment with saturated sodium hydroxide solution. The specific rotation for the purified amine was -1.11° (neat) (lit.[74] -7.7° (neat)). Attempts to further resolve the two batches of partially

resolved amine using the same method, were unsuccessful, as very little crystallisation occurred in the initial methanol mixtures.

I.2 Resolution of racemic 2-AB with (+)-tartaric acid in water.

Following the procedure of Fleury-Larsonneau, a second attempt at the resolution of racemic 2-AB with (+)-tartaric acid was undertaken.[75] Racemic 2-aminobutane (24 g, 0.33 mol) was added to a flask and diluted with water (25 cm^3). Then, with cooling, a 30% aqueous (+)-tartaric acid solution was added slowly from a burette, until the mixture in the flask was neutral to litmus. A second quantity of the (+)-tartaric acid solution, equal to the first, was added to form the bitartrate salt. The mixture was then transferred to a rotary evaporator flask, concentrated by removing some of the water and left to sit for 6 h. The crystals which formed were filtered off and recrystallised from water; 4 g of bitartrate salt were removed from the recrystallisation, dissolved in a minimum quantity of water and treated with solid sodium hydrogen carbonate (hydrated) to liberate the free amine, which was then distilled. The specific optical rotation of the purified 2-AB was $+1.30^{o}$ (neat) (lit.[74] $+7.4^{o}$ (neat)). Again only partial resolution had been achieved.

I.3 Resolution of racemic 2-AB with (-)-malic acid.

2-Aminobutane (12 g, 0.16 mol) was mixed with water (12.5 cm^3). 30% Aqueous (-)-malic acid solution was added from a burette until the mixture was neutral to litmus. A second quantity of the acid solution was then added to form the bi-malate salt. The mixture was then concentrated on a rotary evaporator and left to crystallise. No crystals of diastereometric salt were recovered, and it was thus concluded that (-)-malic acid was not suitable for the resolution of racemic 2-AB.

I.4 Resolution of racemic 2-AB with (+)-mandelic acid.

Racemic 2-aminobutane (5.0 g, 0.068 mol) was mixed in a flask with water (5.2 cm^3). The mixture was cooled on an ice bath and 20% aqueous (+)-mandelic acid solution was added from a burette, until the mixture was neutral to litmus. The solution was concentrated and left to crystallise. The salt (2.0 g) which crystallised out was collected, dissolved in a minimal amount of water and treated with hydrated sodium hydrogen carbonate. The 2-aminobutane which was liberated was collected and distilled; 0.32 g of amine was recovered and this had a specific optical rotation of $+1.77^{o}$ (c ca. 25, H_2O) (lit.[11] $+7.4^{o}$ (c ca. 25, H_2O)). Again, only partial resolution had been achieved.

Results and Discussion.

Foreword

At the outset of this research project, the fumigation of potato tubers with 2-aminobutane was already a well established practice, for the control of the post-harvest diseases gangrene and skin spot. Despite the widespread use of the treatment, however, very little detailed information was available on topics such as the uptake and transport of 2-AB in individual tubers. Previous workers had concentrated mainly on determining total residues in tubers, and although Graham and Hamilton[17] had shown that approximately 60-70% of residual 2-AB was in the peel, nothing was known about the depth of penetration or mobility of 2-AB in individual potato tubers. The object of this project was to develop a method for studying in detail, the initial uptake, extent of penetration and ultimate fate of 2-AB in potatoes. This would obviously involve 2-AB residue analysis on whole tubers but also on small subsamples of single tubers, especially when studying the penetration or transport of 2-AB. Although methods were already available for determining 2-AB residues in tuber tissue[17,57], they did not readily lend themselves to the analysis of very small tuber samples. What was

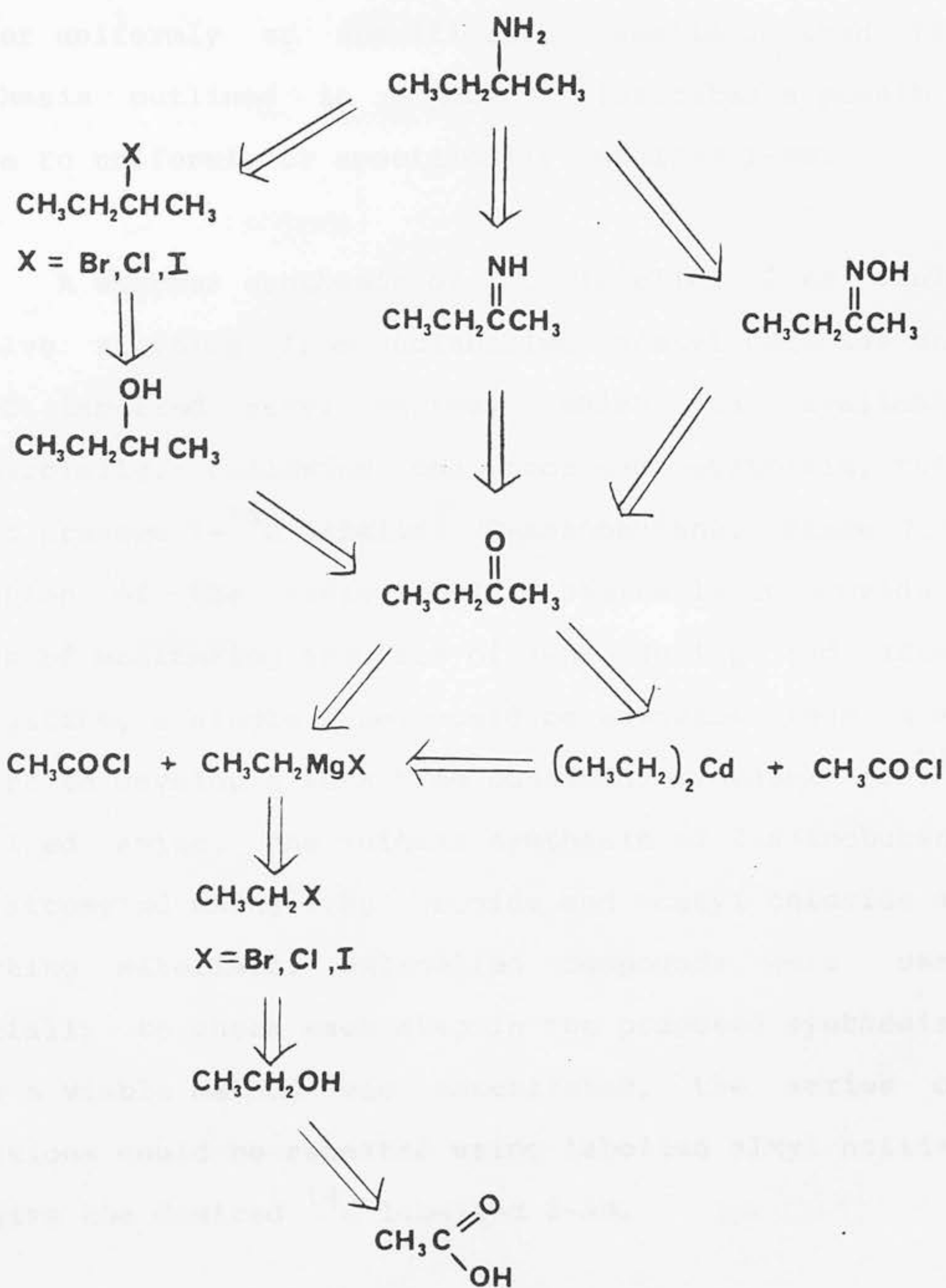
required therefore, was a simple method for detecting low levels of 2-AB in small samples of tuber tissue, which would allow easy handling of large numbers of samples. One possible method was the use of ^{14}C labelled 2-AB, as this would provide a means of tracking the amine fumigant into, and through, individual tubers. Use of a radio label would also aid detection of low levels of 2-AB in small samples. Another advantage would be that, unlike other available analytical methods, there would be no problems at low 2-AB levels with interfering naturally occurring amines, since the detection method would identify only the ^{14}C labelled 2-AB.

As ^{14}C labelled 2-aminobutane appeared to offer a number of advantages over existing analytical techniques, for the study of 2-AB in tubers, it was decided that it should be used in this project as the basis of an analytical method.

A. Synthesis of ^{14}C labelled 2-aminobutane(2-AB).

The general aim of this project was to use ^{14}C labelled 2-aminobutane, to study some of the physical and chemical processes, underlying the fumigation of potato tubers, with the fungicide 2-aminobutane. As the ^{14}C labelled compound is not readily available commercially, and since significant quantities of it would be required during the course of the intended research, the primary objective was to develop a synthesis of the ^{14}C labelled amine. Ideally the synthetic route chosen, would allow 2-aminobutane to be prepared either uniformly or specifically labelled.

Approaching the problem retro-synthetically it is clear that a number of compounds could be used as an immediate precursor to 2-aminobutane (Scheme 8). Each of these precursors could in turn be prepared, using established methods, from 2-butanone. The ketone can be formed from the reaction of acetyl chloride and an organometallic reagent, such as a Grignard or organocadmium compound. The acid chloride is readily obtained from acetic acid by treatment with thionyl chloride. The acetyl chloride can also be used to prepare the organometallic reagents following a series of steps. Firstly the acid chloride is reduced to ethanol, then converted into corresponding alkyl halide



Scheme 8

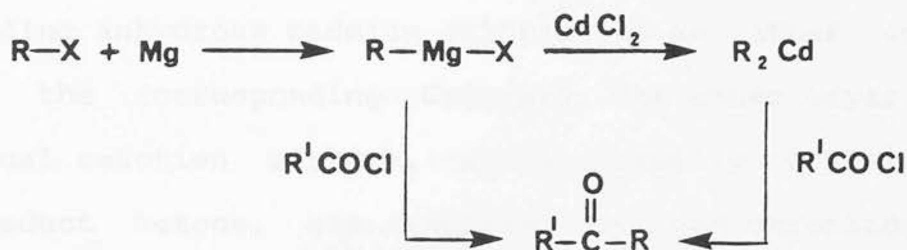
and finally, reaction with magnesium gives the Grignard reagent (which can be used to give other organometallic compounds). Since acetic acid is available commercially, either uniformly or specifically labelled, then the synthesis outlined in Scheme 8 illustrates a possible route to uniformly or specifically labelled 2-AB.

A shorter synthesis of ^{14}C labelled 2-AB would involve starting from unlabelled acetyl chloride and 1-^{14}C labelled ethyl halide, which is available commercially. Following the proposed synthesis, this would produce 1-^{14}C labelled 2-aminobutane. Since the function of the radio label is basically to provide a means of monitoring the fate of 2-AB, during and after fumigation, a single label would be adequate. Thus in an effort to develop a less time consuming synthesis of ^{14}C labelled amine, the initial synthesis of 2-aminobutane was attempted using ethyl bromide and acetyl chloride as starting materials. Unlabelled compounds were used initially to check each step in the proposed synthesis. Once a viable method was established, the series of reactions could be repeated using labelled alkyl halide, to give the desired ^{14}C labelled 2-AB.

A.1 <u>Synthesis of 2-butanone.</u>

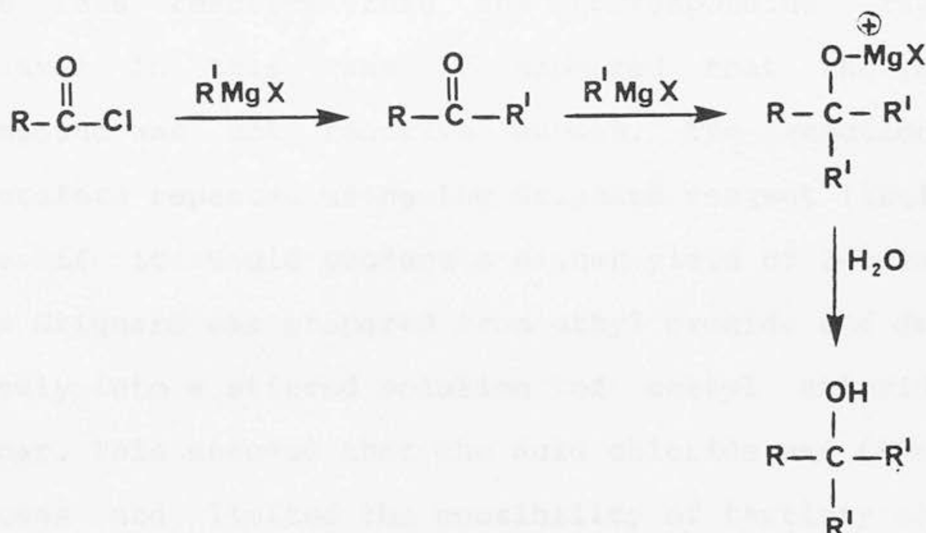
The first step in the proposed synthesis of

2-aminobutane is the preparation of 2-butanone. The synthesis of ketones from the reaction of acyl halides and organometallic reagents (prepared from alkyl halides), is a well established method[38,39] (Scheme 9).



Scheme 9

Grignard reagents (RMgX) are reputedly very reactive and have the potential to react further with the product ketone to form a tertiary alcohol, after hydrolysis (Scheme 10).

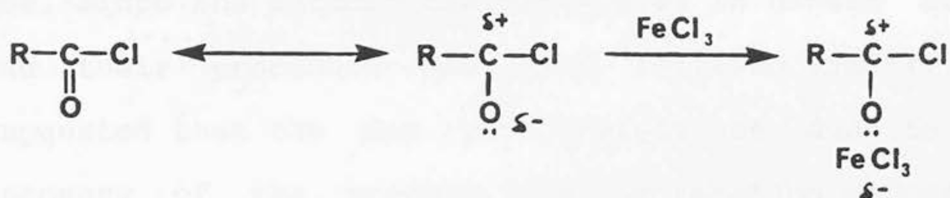


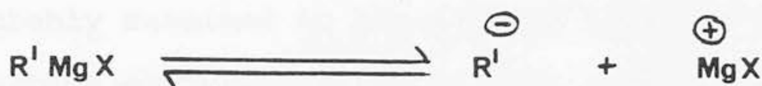
Scheme 10

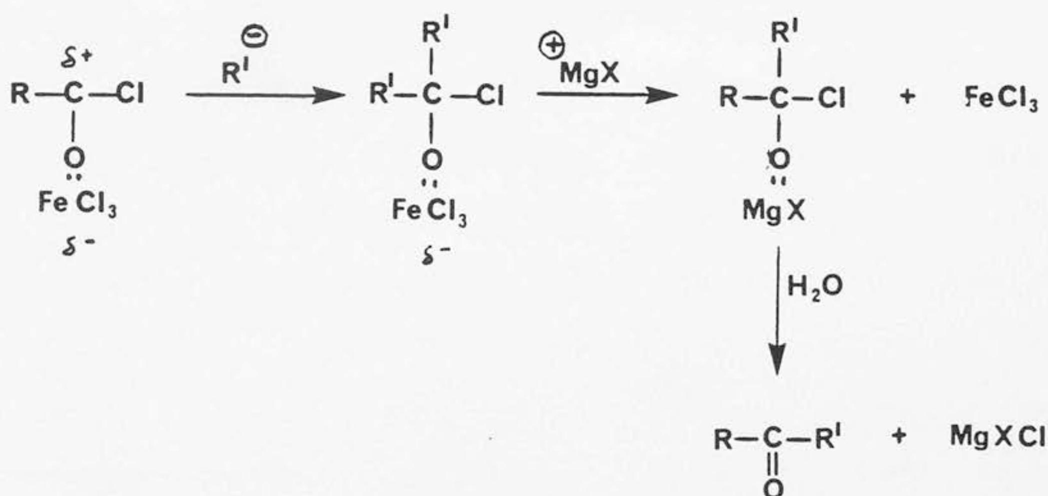
To avoid any problems associated with the activity of Grignard reagents, the initial attempts at synthesising 2-butanone involved reaction of acetyl chloride with di-ethyl cadmium. This was prepared by adding anhydrous cadmium chloride to an ether solution of the corresponding Grignard. The ether layer of the final reaction mixture, which normally contains the product ketone, was analysed by gas chromatography, using a "PEG-1500" on Chromosorb P-AW, column. Only very low levels of 2-butanone were detected. Repeated attempts at synthesising the desired ketone, from reaction of the organocadmium compound and acetyl chloride, were equally unsuccessful.

The organocadmium reagent was chosen because it was less reactive than the corresponding Grignard, however in this case it appeared that the cadmium compound was not reactive enough. The reaction was therefore repeated using the Grignard reagent itself, to see if it would produce a higher yield of 2-butanone. The Grignard was prepared from ethyl bromide and dripped slowly into a stirred solution of acetyl chloride in ether. This ensured that the acid chloride was always in excess and limited the possibility of tertiary alcohol formation. Again, after hydrolysis of the reaction mixture, the ether extract was analysed by gas chromatography. The products identified still only included small amounts of 2-butanone.

The low yield of 2-butanone in the above reactions, suggested that the synthesis of ketones by this method was not as straightforward as many text books claimed, and this was confirmed by a review of the relevant literature. Several workers had found that high yields of ketones in these types of reactions could only be obtained at very low temperatures in the presence of anhydrous metal halides which act as catalysts.[42,43,44] Percival, Wagner and Cook[42], state that the optimum conditions for the production of straight chain ketones, appears to be the addition of 1 mole of Grignard reagent, to one or more moles of the acyl chloride, at -65°C in the presence of an iron(III) chloride catalyst (1.5g/mole acid chloride). A free radical mechanism has been proposed by Kharasch[45] for similar aromatic reactions in the presence of cobalt(II) chloride and other metallic halides including iron(III) chloride. However, a thorough search by Percival et al. of the products of their reactions, performed under the aforementioned optimum conditions, showed no trace of disproportionation or coupling products, thus ruling out the possibility of a free radical process. Percival et al. therfore proposed a mechanism in which iron(III) chloride serves as a Lewis acid in an ionic reaction (Scheme 11).







Scheme 11

The reaction of EtMgBr and CH_3COCl was repeated according to the procedure outlined by Percival et al.[42], and the hydrolysed reaction mixture was extracted with ether. The extract was examined by gas chromatography, but although the yield of 2-butanone was

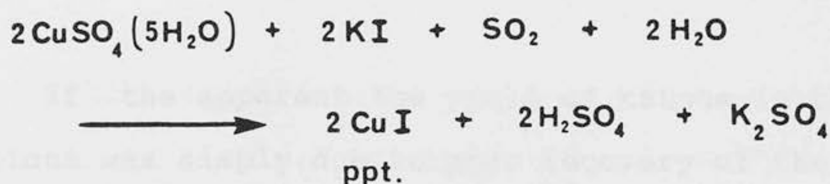
higher than in previous reactions it was still less than 10%. Since the authors claimed yields in excess of 70% and their procedure had been followed exactly, this suggested that the low yield might be due to poor recovery of the product from the reaction mixture. On further investigation it was discovered that 2-butanone was very water soluble, thus most of the product was probably retained in the aqueous layer of the hydrolysed reaction mixture. Attempts at continuous extraction, even over several days, failed to increase the recovery appreciably.

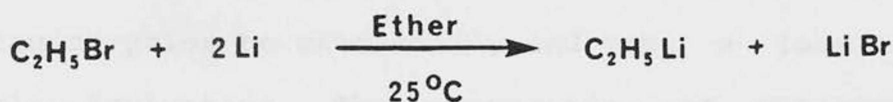
In another attempt to prepare 2-butanone from the reaction of acetyl chloride and an organometallic reagent, the use of lithium diorganocopper reagents (LiR_2Cu) was considered. Posner _et al._[46,47] reported that lithium di-methyl and di-n-alkyl copper reagents react cleanly and under mild conditions with a wide range of carboxylic acid chlorides, forming the corresponding methyl and n-alkyl ketones in good yield. The organo copper reagents are normally prepared at $0^{o}C$, under nitrogen, from the reaction of methyl-, ethyl- or n-butyl lithium with copper(I) iodide.

The synthesis of 2-butanone by the above method involves reaction of acetyl chloride and lithium diethyl copper reagent (Scheme 12).

Scheme 12

The required amount of lithium di-ethylcopper reagent was produced from the reaction of CuI and EtLi, both of which were freshly prepared using the methods shown in Scheme 13.[48,49]





Scheme 13

Reaction of acetyl chloride with the Et_2CuLi reagent followed, using the procedure outlined by Katzenellenbogen et al.[50] This involved adding the acetyl chloride, as an ether solution, dropwise to the freshly prepared organometallic reagent at -78°C. Analysis of the ether layer of the hydrolysed reaction mixture, again showed only low quantities of 2-butanone. As with the Grignard reagent the desired product was probably retained in the aqueous fraction of the reaction mixture, due to its water solubility.

It was clear that, if 2-butanone was to be formed by reaction of acetyl chloride and an organometallic reagent, which requires hydrolysis of the final reaction mixture, then a way round the problem of product water solubility had to be found.

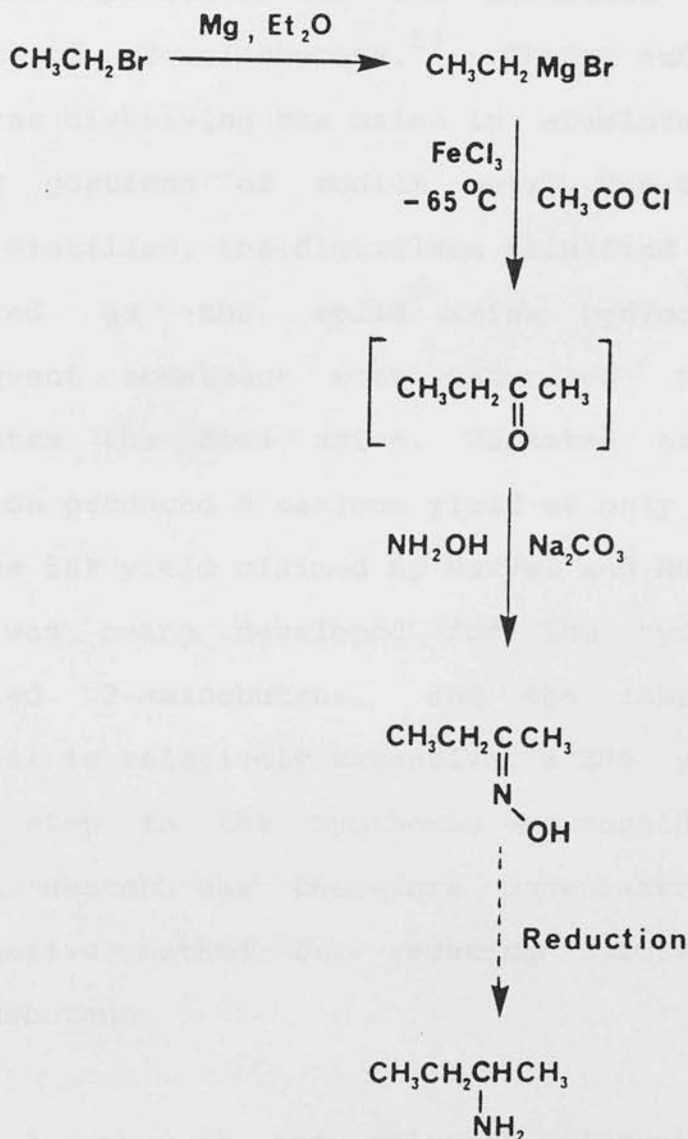
A.2 Synthesis of 2-butanone and isolation as the corresponding oxime derivative.

If the apparent low yield of ketone in the above reactions was simply due to poor recovery of the desired product from the hydrolysed reaction mixtures, then one way to overcome the problem would be to derivatise the 2-butanone prior to extraction, and make a less water soluble derivative. The preparation of the oxime of 2-butanone is one possibility outlined by Marvel and Noyes.[51] Use of this particular derivative is attractive for several reasons. Firstly, the ketone to oxime reaction is carried out in high yield, in water, so derivatisation could take place *in situ* avoiding isolation of 2-butanone and the problems associated with it. Also, Marvel and Noyes[51], claimed that the oxime of 2-butanone was easily extracted from the aqueous reaction mixture with ether, so good recovery of the oxime derivative would be possible. Finally, several methods have been reported in the literature for the reduction of oximes to the corresponding amines[51-55], which would neatly complete the desired 2-AB synthesis.

The method of oxime preparation proposed by Marvel and Noyes[51] was checked, and the claimed 80% yield confirmed. The procedure is simple and involves stirring the ketone, in water for 24 hours with a corresponding weight of hydroxylamine hydrochloride and sodium carbonate. The oxime is then extracted with ether, and since it has a boiling point of 152°C it is easily separated by distillation from the ether solvent.

With an efficient method of derivatisation and extraction available, the synthesis of 2-butanone was attempted again. The ketone was prepared from EtMgBr and CH_3COCl using the method of Percival <u>et al.</u>[42], as this had produced the highest yields of 2-butanone in earlier reactions. No attempt was made to isolate the ketone, which was converted to the corresponding oxime using the method of Marvel and Noyes.[51] The yield of 2-butanone oxime produced, based on the amount of ethyl bromide starting material, was about 56%. This fits in with the expected yields of about 100%, 70% and 80% for the three steps in the synthesis from ethyl bromide to oxime. The good yield of 2-butanone oxime, confirms that 2-butanone is produced in high yield by reaction of the Grignard reagent and acetyl chloride, and that the previously recorded low yields were due to poor recovery of the water soluble ketone from the hydrolysed reaction mixture.

Scheme 14 shows the steps involved in converting ethyl bromide and acetyl chloride into 2-butanone oxime. The only remaining step in the synthesis of 2-aminobutane therefore, is the reduction of the oxime.



Scheme 14

A.3 Reduction of 2-butanone oxime to 2-aminobutane.

A.3(a) Reduction with sodium metal.

Marvel and Noyes, whose method of oxime preparation was adopted into the synthesis of 2-AB, also outlined a procedure for the reduction of 2-butanone oxime to 2-aminobutane.[51] Their reduction method involves dissolving the oxime in absolute ethanol and adding portions of sodium metal. The mixture is then steam distilled, the distillate acidified and the amine isolated as the solid amine hydrochloride salt. Subsequent treatment with saturated NaOH solution liberates the free amine. Repeated attempts at this reaction produced a maximum yield of only 20% compared to the 56% yield claimed by Marvel and Noyes. Since the route was being developed for the synthesis of ^{14}C labelled 2-aminobutane, and the labelled starting material is relatively expensive, a 20% yield for the final step in the synthesis was considered to be too low. A search was therefore undertaken to find an alternative method for reducing 2-butanone oxime to 2-aminobutane.

A survey of the relevant literature uncovered several alternative methods of oxime reduction, each of which was investigated in an effort to improve on the yield of amine.

A.3(b) Reduction with lithium aluminium hydride.

Smith et al.[52] utilised the reducing power of lithium aluminium hydride ($LiAlH_4$) to prepare amines from the corresponding oximes. Following their procedure several attempts were made at reducing 2-butanone oxime. In each case the excess $LiAlH_4$ was destroyed in a controlled manner, using the method of Micovic and Mihalovic[56] (i.e. addition of just sufficient water and 15% NaOH to decompose the reduction complex and excess $LiAlH_4$). Efforts to isolate 2-aminobutane from either the aqueous or ether layers of the reaction mixture were fruitless and it was concluded that the $LiAlH_4$ reduction would not be suitable as a final step in the 2-AB synthesis.

A.3(c) Hydrogenation of 2-butanone oxime

Reeve and Christian[53] outlined the use of Raney nickel catalysts for the hydrogenation of oximes. At pressures of 200-220 atmospheres of hydrogen and temperatures of between 80 and 125°C, the authors claimed high yields of amine. Using the only available high pressure autoclave, and freshly prepared Raney nickel catalyst, several attempts were made at hydrogenation of 2-butanone oxime. The oxime was added to a mixture of dry ethanol and nickel catalyst. Ammonia

gas was bubbled through the mixture to saturate the ethanol, as Reeve and Christian claimed this aided amine formation. The reaction tube was then placed in the autoclave and the chamber pressurised up to 160 atmospheres with hydrogen gas. This was the maximum pressure attainable and was substantially less than the 220 atmospheres used by the previous authors. Since the autoclave had no facility for shaking or stirring the mixture, the reaction time was extended from hours to days. Several attempts at hydrogenation were made with increasing reaction times. At the end of each reaction the chamber was depressurised, the catalyst filtered off and the reaction mixture analysed. No amine was isolated from any of the mixtures. The apparent failure of the hydrogenation, was attributed to the inability to recreate the reaction conditions used by Reeve and Christian, for their successful reductions.

A.3(d) Reduction with diborane.

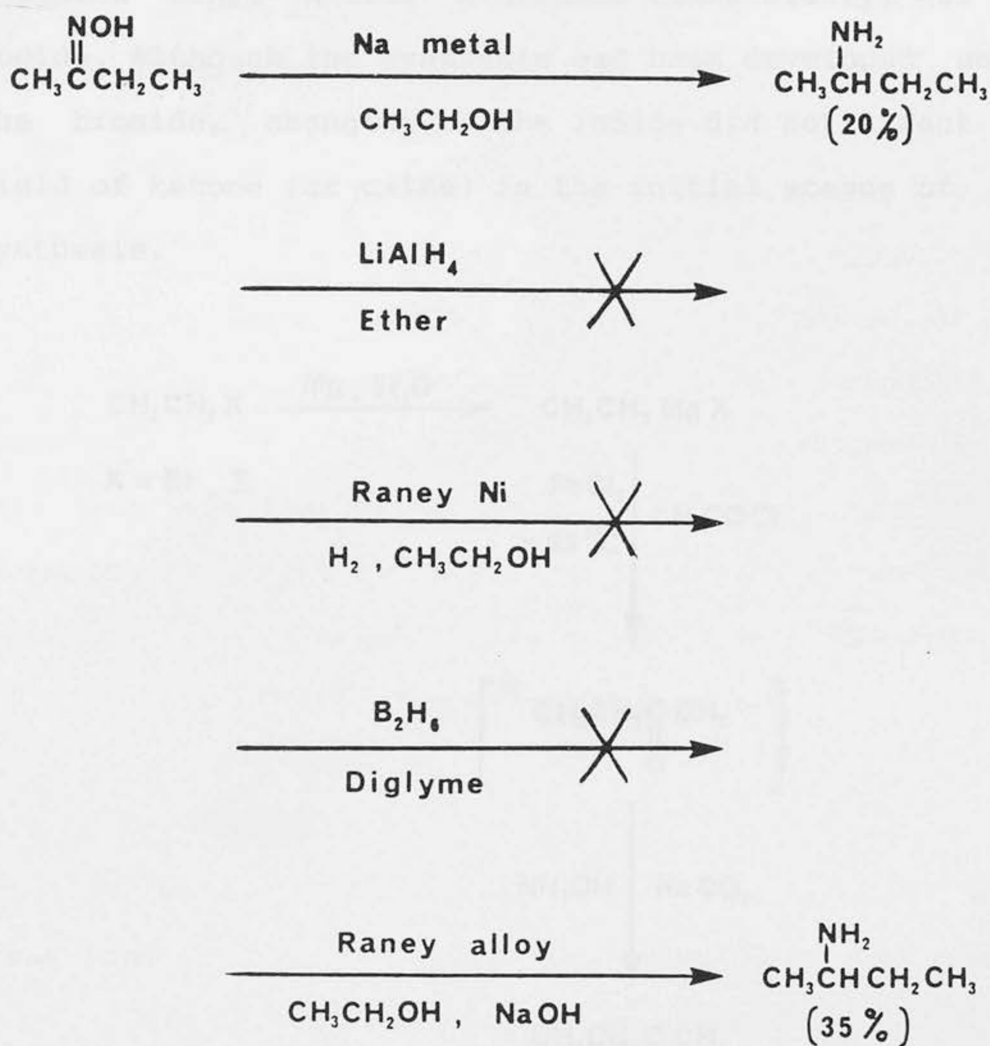
Diborane (B_2H_6) has been used by several workers as a reducing agent in the reduction of aldoximes and ketoximes. Feuer and Braunstein[54] reported the reduction of oximes with diborane as a synthetic route to amines. Using the procedure which they described, a high temperature diborane reduction of 2-butanone oxime was attempted. At the end of the reaction, the excess

diborane was destroyed by the addition of 20% KOH solution. Again, attempts to isolate the desired amine from the reaction mixture were unsuccessful and this method was abandoned.

A.3(e) Reduction with Raney alloy in alkaline solution.

The continued search in the literature for a viable method of reducing 2-butanone oxime to 2-aminobutane, uncovered a second method involving Raney nickel. Staskun and Van Es[55] outlined the reduction of oximes and nitriles with Raney alloy in alkaline solution. The procedure was straightforward and involved the gradual addition of Raney alloy (Ni/Al alloy containing 50% Ni) with stirring to a mixture of the oxime in ethanol and NaOH solution. The mixture is stirred for 1 hour, the Nickel filtered off and the amine recovered from the filtrate. Attempts at this preparation led to recoverable yields of amine of about 30-35%. I.r. and n.m.r. spectra confirmed that the product was 2-aminobutane.

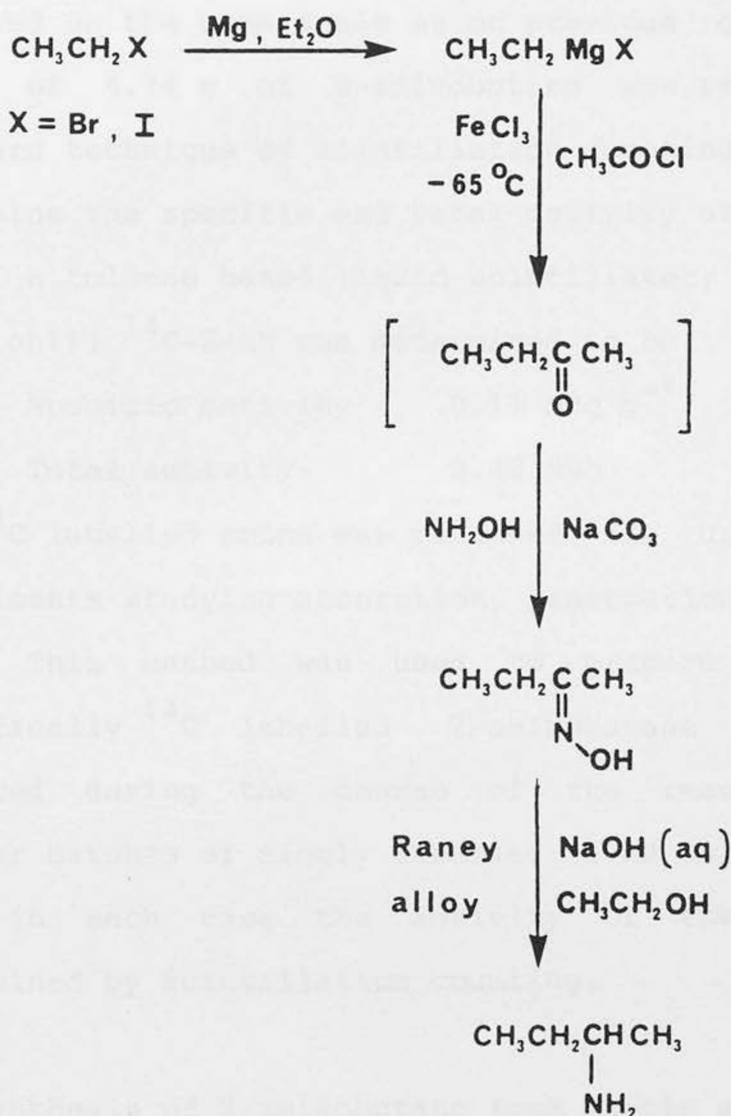
Scheme 15 summarises the various methods which were investigated as possible ways of reducing 2-butanone oxime to 2-aminobutane. Since enough time had been spent searching for a suitable reduction method, the Raney alloy reduction, which had given the



Scheme 15

highest yield of amine, was adopted as the last step in the synthesis. Scheme 16 shows the overall synthesis of 2-AB from EtBr and CH_3COCl incorporating the Raney alloy reduction step. As a final check, the whole synthesis was repeated using unlabelled ethyl iodide and acetyl chloride, and the efficiency of each step confirmed. The switch from ethyl bromide was made because the only ^{14}C

labelled ethyl halide available commercially, was the iodide. Although the synthesis had been developed using the bromide, changing to the iodide did not affect the yield of ketone (or oxime) in the initial stages of the synthesis.



Scheme 16

A.4 Synthesis of 1-^{14}C 2-aminobutane.

A quantity of 1-^{14}C ethyl iodide (1.85 MBq) was purchased. This was diluted with a known quantity of unlabelled ethyl iodide, and the synthesis of 2-AB repeated on the same scale as on previous occasions. A total of 4.74 g of 2-aminobutane was recovered. The standard technique of scintillation counting was used to determine the specific and total activity of the amine. Using a toluene based liquid scintillator, the activity of Batch(1) ^{14}C-2-AB was determined to be:

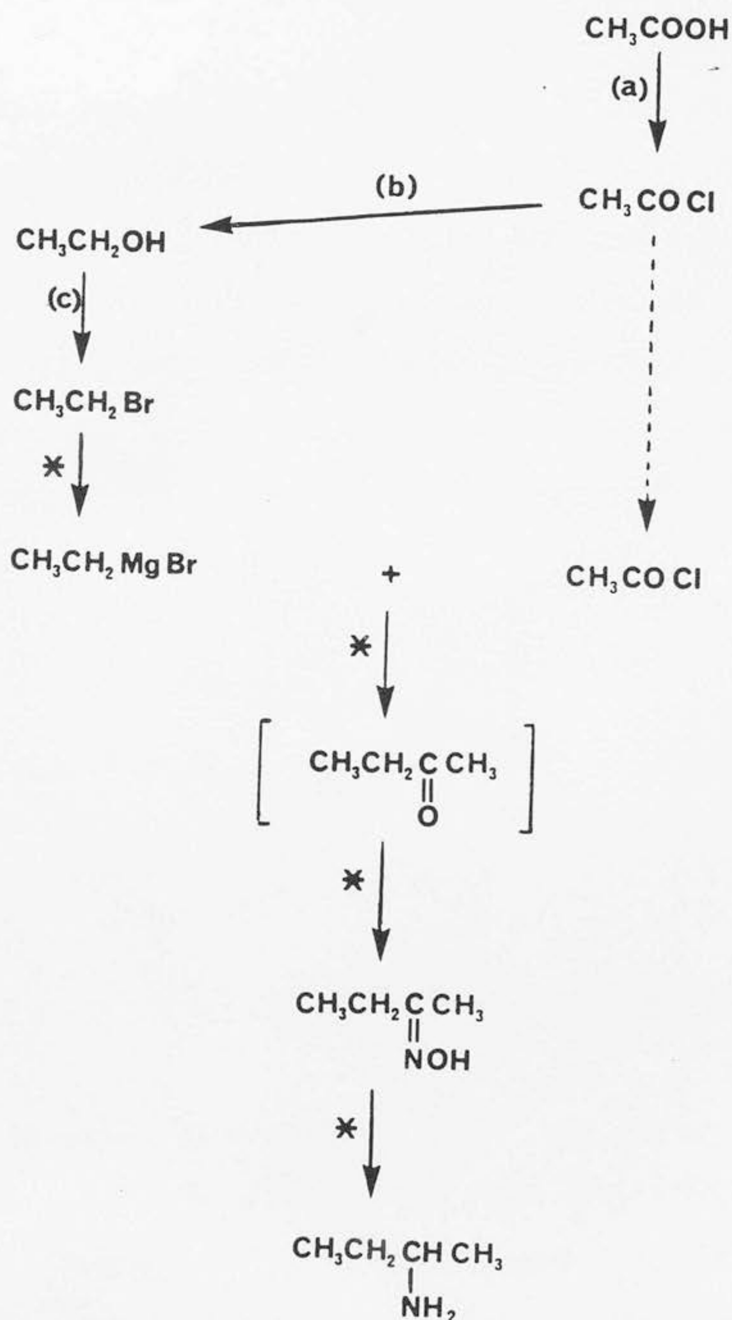
Specific activity	0.11 MBq g^{-1}
Total activity	0.42 MBq

The ^{14}C labelled amine was retained for use in tuber experiments studying absorption, penetration and loss of 2-AB. This method was used to prepare all of the specifically ^{14}C labelled 2-aminobutane which was required during the course of the research. Three further batches of singly labelled 2-AB were prepared and in each case the activity of the amine was determined by scintillation counting.

A.5 Synthesis of 2-aminobutane from acetic acid.

Although most of the ^{14}C labelled amine used throughout the course of this project had only a single

radio-label and was prepared from $1\text{-}^{14}C$ EtI and unlabelled CH_3COCl, some $U\text{-}^{14}C$ 2-aminobutane was also synthesised. As described previously, uniformly labelled amine could be obtained from $U\text{-}^{14}C$ acetic acid. Scheme 17 shows the most likely route from acid to amine.



Scheme 17

The latter part of the synthesis had already been developed for the preparation of 1-^{14}C 2-AB. Each step marked "*", in Scheme 17, had been checked previously, and only steps (a), (b) and (c) required confirmation.

A.5(a) Conversion of acetic acid into acetyl chloride.

The reaction of a carboxylic acid with thionyl chloride to produce the corresponding acid chloride, is well documented. This method was employed for the conversion of acetic acid to acetyl chloride and, using the procedure outlined by Vogel[58], the efficiency of the reaction was confirmed. Excess thionyl chloride is destroyed, after the reaction is complete, by dropwise addition of the calculated amount of formic acid (Scheme 18).

Scheme 18

A.5(b) Reduction of acetyl chloride to ethanol.

Several attempts at the reduction of acetyl chloride with $LiAlH_4$ confirmed that this method could be used to prepare ethanol. The experimental procedure followed was outlined by Skoog and Woodburn[59], and involved dropwise addition of the acetyl chloride to a

flask containing $LiAlH_4$ in dry ether.

A.5(c) Conversion of ethanol into ethyl bromide.

Using the procedure proposed by Vogel[60], the conversion of ethanol into the corresponding alkyl bromide, by treatment with HBr and H_2SO_4 was investigated. High yields of ethyl bromide were obtained by this method and it was adopted into the synthesis.

The above reactions confirmed that it was possible to prepare ethyl bromide and acetyl chloride from acetic acid. As before the alkyl halide and acid chloride can be used to prepare 2-aminobutane, using the synthesis outlined in Scheme 16. Thus, the synthesis proposed in Scheme 17 for the conversion of acetic acid into 2-aminobutane was indeed a viable one, and it was used to prepare U-^{14}C 2-AB from U-^{14}C acetic acid.

A.6 Synthesis of U-^{14}C labelled 2-aminobutane.

Using the synthesis outlined in Scheme 17, a quantity of U-^{14}C labelled 2-aminobutane was prepared as follows. 9.25 MBq of U-^{14}C labelled acetic acid was obtained commercially and dissolved in a known quantity of unlabelled acid. All of the compound was then converted into U-^{14}C acetyl chloride and this was divided into two portions. The first, smaller, portion

was further converted into ethanol, then ethyl bromide and finally into the Grignard reagent (ethylmagnesium bromide), using the procedures outlined above. The organometallic reagent was then reacted with the second portion of original acetyl chloride to produce U-^{14}C 2-butanone. The ketone was converted through U-^{14}C 2-butanone oxime, to give the desired U-^{14}C labelled 2-aminobutane. Due to the many steps in the conversion of acetic acid into 2-AB, the overall yield was only 3%, but this still provided 0.39 g of amine, which would allow some work with the U-^{14}C compound to be carried out, if required. More time could have been spent improving the above synthesis, but since for most of the intended research 1-^{14}C 2-AB would be adequate and as it could be produced in much higher yield, the search for a better synthesis of U-^{14}C amine was not pursued.

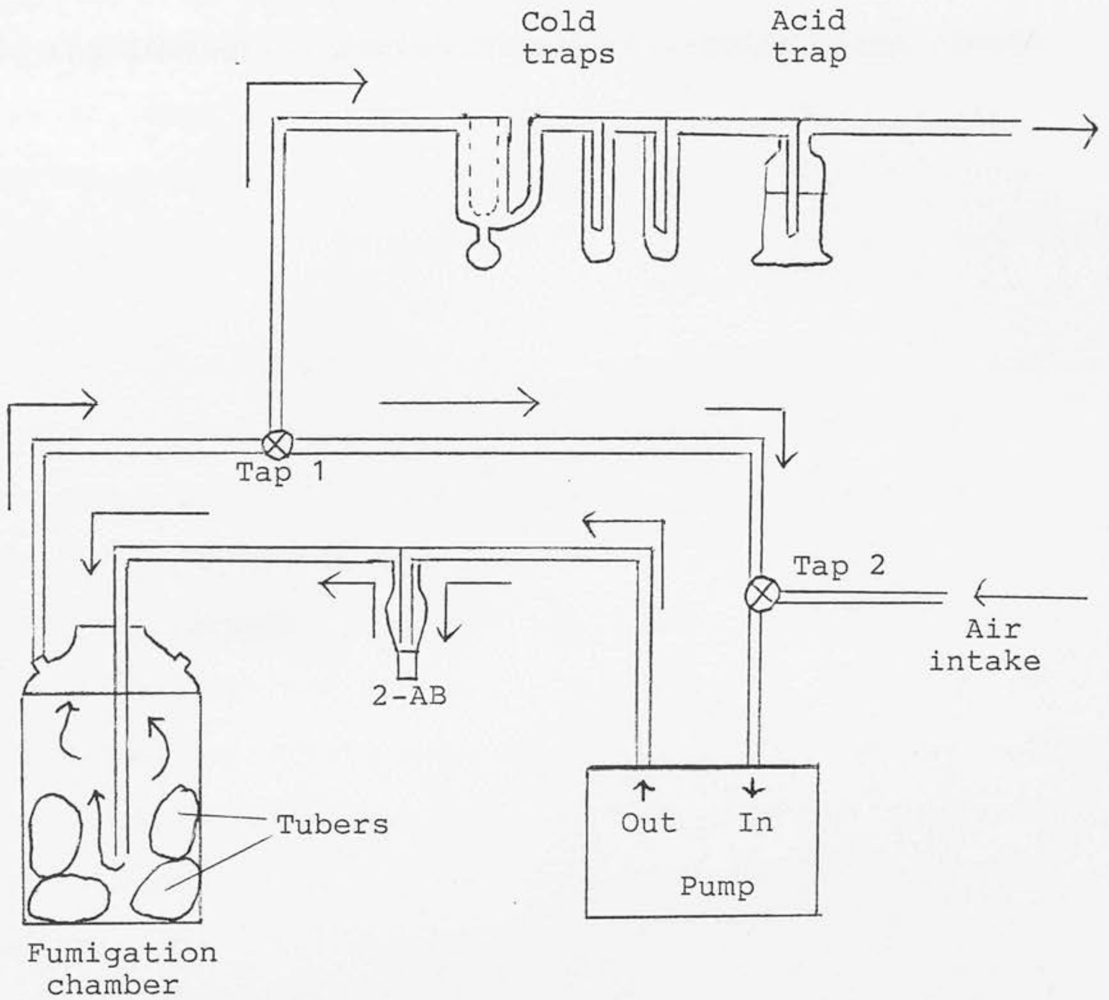
The first stage of the project was thus complete. A synthesis of 2-aminobutane had been developed and used successfully to prepare quantities of 1-^{14}C and U-^{14}C labelled 2-AB. Before this material could be used however, to study absorption, penetration and loss of 2-AB in tubers, two further tasks had to be carried out. Firstly, it was necessary to construct a small scale fumigation apparatus for the treatment of tubers with the ^{14}C labelled amine. Secondly, an efficient method of extraction and detection of ^{14}C 2-AB in fumigated tuber samples, had to be developed.

B. Construction of fumigation apparatus and development of an analytical technique for determining ^{14}C 2-AB residues in tuber samples.

B.1 Construction and testing of the small scale fumigation chamber.

Basically what was required, was an apparatus in which potato tubers could be subjected to an atmosphere containing a controlled amount of 2-AB, incorporating some form of forced air recirculation, to give good distribution of the amine fumigant. Since radio-labelled material would eventually be used, the chamber, unlike commercial fumigation chambers, had to have a facility for recovering any amine not absorbed by the potatoes. Figure 8 shows the fumigation apparatus which was constructed, as far as possible using all glass components. The fumigation chamber was a 5 litre glass jar and this was connected with glass tubing, in a closed circuit, to a Genevac (DPC5/LV) corrosion resistant vacuum compressor pump (flow rate 5000 $cm^3 min^{-1}$). All joints were sealed with inert p.v.c. tubing, and where two pieces of glass tubing met, the ends were butted up against one another. A Dreschel head and sample bottle, fitted between the pump outlet and fumigation chamber, provided a means of introducing 2-AB into the system. When the pump is switched on, the air

Figure 8 Small scale fumigation apparatus.



⟶ Arrows represent the direction of gas flow

flow is sufficient to vaporise the entire dose of the volatile amine, at ambient temperature, carrying it into the fumigation chamber. The completed circuit allows the amine to be recirculated around the system. At the end of the fumigation period 2 taps, fitted into the closed circuit, can be opened to draw fresh air into the pump and fumigation chamber, and to vent the unabsorbed amine out through a series of traps.

Once construction of the fumigation apparatus was complete, the next step was to check that it functioned properly, and that satisfactory levels of treatment could be achieved. Two 1 kg batches of 'Cyprus' tubers were therefore fumigated with unlabelled 2-aminobutane. The dosage was set at 200 mg of amine per kg tubers and the amine recirculated for 2 hours, as recommended for commercial fumigation treatments.[21] Once the two trial fumigations were completed, the tubers were taken for analysis to the chemistry section of the Agricultural Scientific Services, DAFS, at East Craigs in Edinburgh. The analytical method used to determine the residues of 2-AB, was developed by Hunter and Lindsay[57] for the analysis of tubers from trial commercial fumigations, and involved extraction and h.p.l.c. of the amine fumigant. It should be noted that in previous fumigations by DAFS at a dosage of 200 $mgkg^{-1}$, the actual residues recorded in tubers were often significantly lower than this. In fact, average residues

for successful commercial fumigations carried out by DAFS, vary from 40-70 $mgkg^{-1}$.[57]

The levels of 2-AB recorded for samples from the two batches of 'Cyprus' tubers, fumigated in the small scale fumigation apparatus, are shown in Table 16. The results show clearly that satisfactory levels of treatment are achieved in the simple small scale fumigation chamber. Although there is some variation between tuber samples from the same fumigation batch, the difference is no greater than that observed in commercial fumigations, or in other fumigations throughout this project. The question of the origin of this variation, is dealt with later in Section C.1 (Results and Discussion), of this Thesis.

Table 16 2-AB residues in 'Cyprus' tubers from trial fumigations at 200 $mgkg^{-1}$.

Fumigation Batch	Sample	Residue of 2-AB ppm ($mgkg^{-1}$)
1	A	125.8
1	B	88.1
1	C	102.2
2	A	126.6
2	B	119.7
2	C	107.8

B.2 Development of an analytical method for measuring 2-AB residues in tubers, fumigated with ^{14}C labelled 2-AB.

The proposed method for determining 2-AB residues in tuber samples fumigated with ^{14}C labelled 2-AB involved two steps. Firstly, solvent extraction of the ^{14}C amine from the tuber sample, and secondly, scintillation counting of an aliquot of the extract after mixing with a suitable liquid scintillation solution. Since the activity of the ^{14}C 2-AB (cpm mg^{-1}) is known, then the number of counts per minute (cpm) recorded for the extract solution can be used to determine the weight of amine in the original tuber sample.

In developing the above analytical method there were two important points to remember. First of all, the extraction efficiency had to be reproducable and ideally as close to 100% as possible. Also, the solvent used for extracting the ^{14}C 2-AB from the tuber tissue should not interfere with the final scintillation counting process. Some compounds if present in a scintillation 'cocktail' cause an effect called quenching, which leads to a decrease in the recorded number of counts per minute for the sample. Small amounts of quenching can be corrected for using a quench correction graph which shows how the activity of the ^{14}C labelled material varies in the

presence of increasing amounts of quenching agent. It should be noted that if the solvent used for the extraction is itself capable of quenching, then the effect of its presence in the scintillation cocktail could be to cause complete suppression of the scintillation process, thus preventing detection of the ^{14}C labelled 2-AB.

Some tubers of the 'Arran Pilot' variety were pulped and subsamples of the macerate spiked with known quantities of ^{14}C labelled 2-aminobutane. Attempts were then made to extract the amine quantitatively from the tuber pulp, using a variety of solvents.

Extraction with organic solvents was attempted first. The spiked pulp samples were soaked in ether, chloroform or methylene chloride, for varying lengths of time with and without heating. The solvents were then filtered off and the extracts diluted to standard volumes with fresh solvent. Counting of aliquots of the extract solutions after mixing with the liquid scintillator solution, led to measured residues far below the actual values. This was attributed to a mixture of excessive quenching and poor extraction.

Since 2-AB and its salts are water soluble, extraction of spiked tuber samples with water was attempted. Again, the samples were soaked for varying

lengths of time and the effect of heating the samples as part of the extraction, was investigated. Although some quenching occurred when aliquots of the final extract solutions were counted, a correction could be made for this. Recovery levels of ^{14}C 2-AB were higher in those samples which had been heated, but the measured residues were still significantly less than the actual weights of amine in the pulp samples. This again pointed to inefficient extraction of 2-AB from the tuber tissue.

As the liquid scintillator solution being used was readily miscible with 0.2 Molar hydrochloric acid solution, the latter was also investigated as a possible extraction solvent. Initial attempts at extraction and counting indicated that good recovery of ^{14}C 2-AB with acid was possible. Further tests with various proportions of acid/tuber pulp, showed that quantitative recovery of 2-AB was possible using 25 cm^3 of 0.2 M HCl mixed with up to 5 g of pulp. Extraction was most efficient if the acid/pulp mixture was heated on a boiling water bath for about 25 minutes. Table 17 shows typical values for overall recovery of ^{14}C 2-AB from spiked samples of 'Arran Pilot' tuber pulp, using the 0.2 M HCl extraction procedure.

Table 17 Recovery of 2-aminobutane from potato macerates (Arran Pilot tubers).

Sample	Recovery(%) of 2-AB
1	99.6
2	97.8
3	101.8
4	98.7

As the above results showed that the acid extraction was efficient, it was adopted as the standard method for recovery of ^{14}C 2-AB from fumigated tuber samples. The procedure is straightforward and involves mixing the pulp with 0.2 M HCl, leaving the sample to soak overnight, then heating the mixture on a boiling water bath. The pulp material is then filtered off and the filtrate (extract) diluted to a standard volume. An aliquot of the extract solution is mixed with a portion of NE265 liquid scintillator solution and the mixture counted on the Beckman LS7000 liquid scintillation counter. Since the specific activity (cpm mg^{-1}) of the ^{14}C labelled 2-AB, when counted in a mixture of acid/scintillator is known, then the counts per minute obtained for the extract solution can be used to calculate the weight of 2-AB in the original sample. One advantage of this procedure is that it lends itself to the analysis of very small tuber samples, as any ^{14}C 2-AB present in the tuber tissue extract can be easily detected by scintillation counting. The background radiation for samples counted during this project was

typically 20 cpm. The detection limit for the analytical method was therefore taken as twice the background (i.e. 40 cpm), which for a 5 g sample of pulp corresponds to a ^{14}C 2-AB residue of 0.5-1.0 $mgkg^{-1}$. Overall this method is simpler and less time consuming than the method of Hunter and Lindsay[57], and demonstrates the advantage of using ^{14}C labelled 2-aminobutane, in this study.

As a final check, the acid extraction and scintillation counting method of analysis, was compared directly with the H.P.L.C. method of Hunter and Lindsay.[57] A batch of 'Cyprus' tubers was fumigated at 200 $mgkg^{-1}$ using the standard procedure. Samples of the fumigated and unfumigated tubers were then pulped and analysed by both methods. Table 18 shows the results which confirm the efficiency of the scintillation counting method of 2-AB residue determination.

Table 18 Comparison of scintillation counting and HPLC methods of 2-AB residue determination in tuber samples.

Sample	Dosage $mgkg^{-1}$	Method of analysis	2-AB residue $mgkg^{-1}$
1	200	HPLC	86.6
2	200	HPLC	93.9
3	200	Scintillation Counting	89.4
4	200	Scintillation Counting	85.6

C. Absorption and penetration studies.

Using the ^{14}C labelled 2-aminobutane, the small scale fumigation apparatus and the scintillation counting method of analysis developed above, experiments were carried out to study aspects of the absorption and penetration of 2-AB in tubers.

C.1 Absorption of 2-aminobutane by potato tubers.

In work carried out by Graham et al.[17,25], where they looked at levels of 2-AB in tubers from bulk fumigations, it was clear that there was often significant variation in the 2-AB residues of individual tubers, even when they were adjacent. Variation also occurred between different fumigation batches. In order to establish the degree of variation which existed in tubers fumigated in the small scale fumigation chamber, a reproducibility study was carried out.

Two batches of 12, cultivar Foxton, seed tubers were fumigated using the standard procedure, in the small scale fumigator at a dosage of 200 $mgkg^{-1}$ with ^{14}C labelled 2-AB. Each tuber was then analysed separately, to determine the 2-AB residue in each individual potato. Table 19 shows the results of the residue analyses, with

mean residues and standard deviations. In both batches there is a spread of residues, showing that each tuber does not absorb the same level of 2-AB per unit weight of tissue. Statistical analysis of the residue data shows that at 95% confidence the mean residues for the two batches are 135.0 $\pm$ 26.1 $mgkg^{-1}$ and 105.1 $\pm$ 9.1 $mgkg^{-1}$. As the confidence intervals overlap, it is clear that there is no significant difference in 2-AB residues between the two batches. This was important, as it implied that despite the tuber to tuber variation within each batch, successive fumigations would achieve comparable levels of treatment.

Table 19 Residues of 2-AB in individual tubers from reproducibility study.

Tuber	2-AB residue ($mgkg^{-1}$) Batch 1	Batch 2
1	182.9	115.1
2	204.3	126.5
3	86.5	85.6
4	135.9	98.4
5	145.9	109.4
6	140.9	104.7
7	194.5	119.3
8	116.5	79.9
9	91.7	105.6
10	83.9	117.5
11	123.8	109.6
12	113.6	89.2
Mean	135.0	105.1
Std. Dev.	$\pm$ 41.1	$\pm$ 14.4

The 2-AB residues achieved in the small scale fumigator were generally higher than those found by Graham *et al.*[17], in fumigations carried out in their 5 tonne chamber. This suggested that the small scale fumigation apparatus was capable of more efficient treatment than the larger pilot scale fumigation chamber.

The above experiment highlighted variation in levels of 2-AB absorption between different tubers within the same fumigation batch. The extent of this variation was investigated further, by looking at how 2-AB residues varied across the surface of individual tubers. Circles of skin (2.5 cm in diameter) were sampled over the surface of single tubers. The tubers used in these experiments were fumigated and sampled relatively soon after harvest, and this allowed the surface skin (periderm) to be easily removed.[61] Extraction and analysis of 2-AB residues in the tuber skin samples, revealed that absorption of 2-AB was not uniform across the surface of individual tubers (Table 20).

Table 20 2-AB residues in circular skin samples from different positions on the tuber surface.

Tuber	Time between fumigation and harvest	Dosage $mgkg^{-1}$	2-AB residues in skin samples $mgkg^{-1}$
1	1 day	200	2396.5
			1460.3
			2324.9
			1865.2
			1869.0
2	8 days	200	1286.9
			1267.0
			858.0
			1720.8
			1954.5

The residues of 2-AB recorded in the surface skin samples are very high. As the overall residues for tubers fumigated at 200 $mgkg^{-1}$ tend to be less than the dosage concentration, the above results imply uneven distribution of 2-AB throughout the tuber, with most of the absorbed amine being concentrated at the surface. This was suggested by Graham _et al._ when they showed that 60-70% of 2-AB residues can be removed by peeling[17], and it was hoped that later experiments, looking at the depth of penetration of 2-AB in individual tubers would confirm their observation.

There were several plausible explanations for across tuber, or tuber to tuber, variation in 2-AB residues. One possibility, especially in bulk fumigations, was tuber to tuber contact decreasing the tuber surface area exposed to the amine fumigant.

Another likely cause was the soil, which often adheres to tubers, also decreasing the area of tuber surface exposed to 2-AB fumigation or selectively absorbing 2-AB. However, the tubers which were treated with ^{14}C 2-AB in the small scale fumigation apparatus were free from soil and had only minimal contact with other tubers. Despite this, they too showed significant variation in 2-AB residue, indicating that some other factor was making a major contribution to the observed tuber to tuber variation in 2-AB absorption.

In an effort to establish another possible cause of the across tuber, or tuber to tuber variation in 2-AB residues, absorption through tuber lenticels was investigated. Several authors have reported that diffusion of oxygen into tubers, during respiration, occurs exclusively through the lenticels and not through the rest of the periderm.[62,63,64] It was shown by Wigginton[62], that the number of lenticels in cultivar King Edward tubers varied between 74 and 141 per tuber, and that the lenticels were scattered randomly across the tuber surface, with some patches clear of lenticels altogether. Similar patterns were also observed for other cultivars. If preferential absorption did occur through the lenticels, then their random distribution might account for some of the recorded variation in 2-AB residues.

Lenticels are known to be open during the early weeks after harvest[65], and for this reason freshly grown tubers were used for the lenticel experiments. Two batches of mixed, unnamed cultivars, were fumigated at 200 $mgkg^{-1}$ using the standard procedure. In the first batch, the tubers had been kept in an atmosphere of 100% relative humidity for 10 days prior to fumigation, to encourage the lenticels to open up. In the second batch, the lenticels were in their 'normal' state. After fumigation, tubers were selected at random from the two batches and samples of surface tissue taken for analysis. Each sample was composed of either only lenticel or non-lenticel tissue. Extraction and analysis of the ^{14}C residue, in each of the samples, provided the results shown in Table 21. It is clear that preferential absorption does take place through tuber lenticels and that this effect is more dramatic when most lenticels are fully open (batch 1). Unlike the uptake of oxygen into potato tubers, which only occurs through lenticels, 2-AB is also absorbed through areas of periderm devoid of lenticels, but to a much lesser extent. This result is very important as it indicates a very likely reason for uneven distribution of 2-AB between tubers, or across a tuber surface, since those tubers with more lenticels, or more open lenticels, will clearly absorb more 2-AB.

Table 21 2-AB residues in lenticel and non-lenticel surface tissue samples, after fumigation at 200 $mgkg^{-1}$.

Fumigation batch	Sample	'Open'[a] lenticels	Tissue type	2-AB residue $mgkg^{-1}$
1	A	-	Non-lenticel	58.4
1	B	-	Non-lenticel	90.0
1	C	-	Non-lenticel	110.4
1	A	Yes	Lenticel	3472.2
1	B	Yes	Lenticel	3737.6
1	C	Yes	Lenticel	2953.5
2	A	-	Non-lenticel	78.8
2	B	-	Non-lenticel	82.6
2	C	-	Non-lenticel	63.7
2	A	No	Lenticel	659.5
2	B	No	Lenticel	695.1
2	C	No	Lenticel	484.4

a. Tubers with 'open' lenticels had been kept in an atmosphere of 100% relative humidity for 10 days, prior to fumigation.

C.2 Absorption of 2-AB as a function of time (length of fumigation period) or concentration (dosage of 2-AB applied).

The following experiments were undertaken to determine the effects of varying length of fumigation period, and 2-AB dosage, on the overall residues of 2-AB in fumigated tubers. In one experiment, three 1 kg batches of 'Epicure' tubers were fumigated for the same length of time (2 hours), but at different doses (100 $mgkg^{-1}$, 200 $mgkg^{-1}$ and 500 $mgkg^{-1}$). In another experiment, three 1 kg batches of the same cultivar were fumigated at the normal 2-AB dosage (200 $mgkg^{-1}$), but

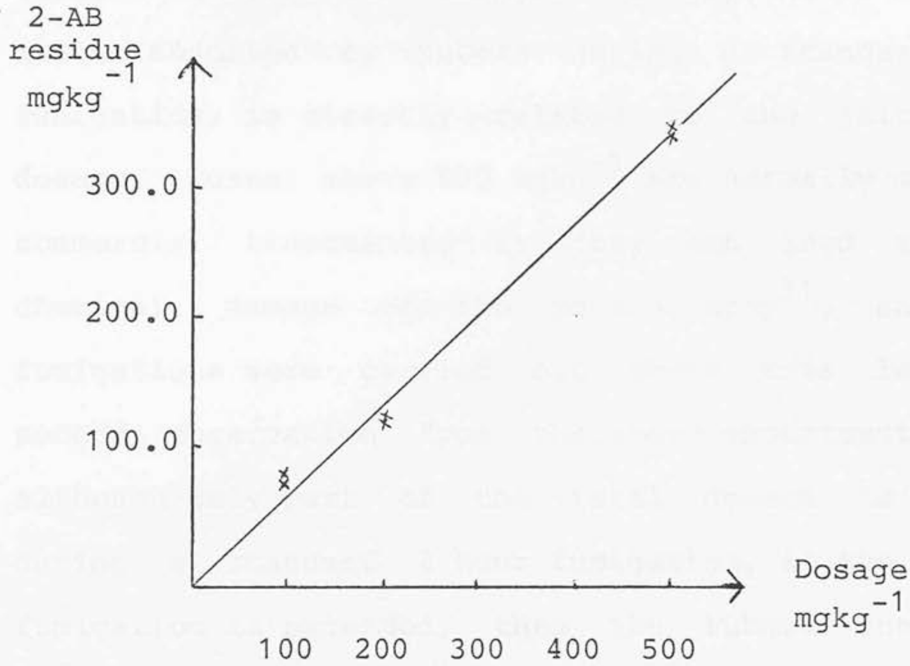
for different periods of time (2 hours, 7 hours and 24 hours). All the fumigations were carried out as close together in time as possible, in order to minimise any variations arising from differences in tuber age, between successive batches.

The overall 2-AB residue was determined twice for each fumigation batch. Each pulp sample was derived from 4 randomly selected tubers, using the subsampling technique proposed by Hunter and Lindsay[57] to reduce the possibility of variability, due to tuber to tuber variation in 2-AB levels. The results of all the analyses are shown in Table 22 and are represented graphically in Figure 9.

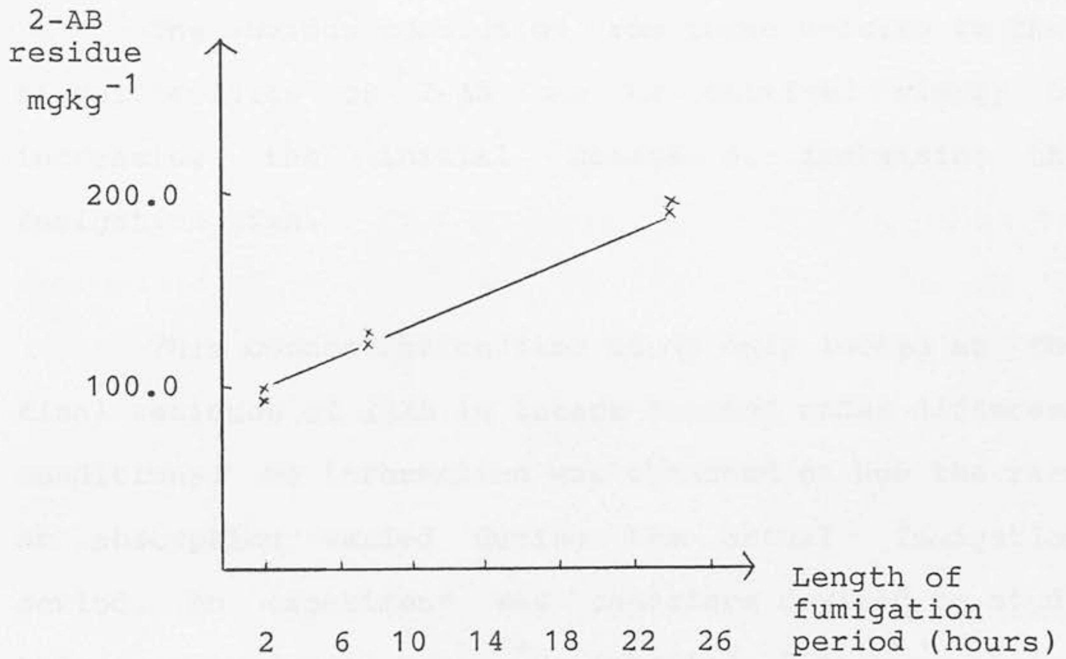
Table 22 2-AB residues in tubers from fumigations at different concentrations, and for different lengths of fumigation.

Fumigation batch	Sample	Length of fumigation period (h)	2-AB Dosage $mgkg^{-1}$	2-AB Residue $mgkg^{-1}$
C1	1	2	100	76.2
C1	2	2	100	78.3
C2	1	2	200	114.9
C2	2	2	200	116.4
C3	1	2	500	331.9
C3	2	2	500	334.6
T1	1	2	200	97.4
T1	2	2	200	93.0
T2	1	7	200	125.5
T2	2	7	200	129.7
T3	1	24	200	194.4
T3	2	24	200	188.8

Figure 9 Graphical representation of results of concentration/time studies.



A:- Varying concentration/constant time (2 h).



B:- Varying time/constant concentration (200 mgkg^{-1}).

The results of the above concentration/time studies indicate two things. Firstly, the amount of amine absorbed by tubers during a standard 2 hour fumigation, is directly related to the initial 2-AB dosage. Doses above 500 $mgkg^{-1}$ are normally avoided in commercial treatments, as they can lead to severe chemical damage of the potato crop[31], and so no fumigations were carried out above this level. The second observation from the above experiment is that, although only part of the total dosage is absorbed during a standard 2 hour fumigation, if the length of fumigation is extended, then the tubers continue to absorb any available 2-AB. After 24 hours virtually all of the fumigant has been absorbed by the tubers.

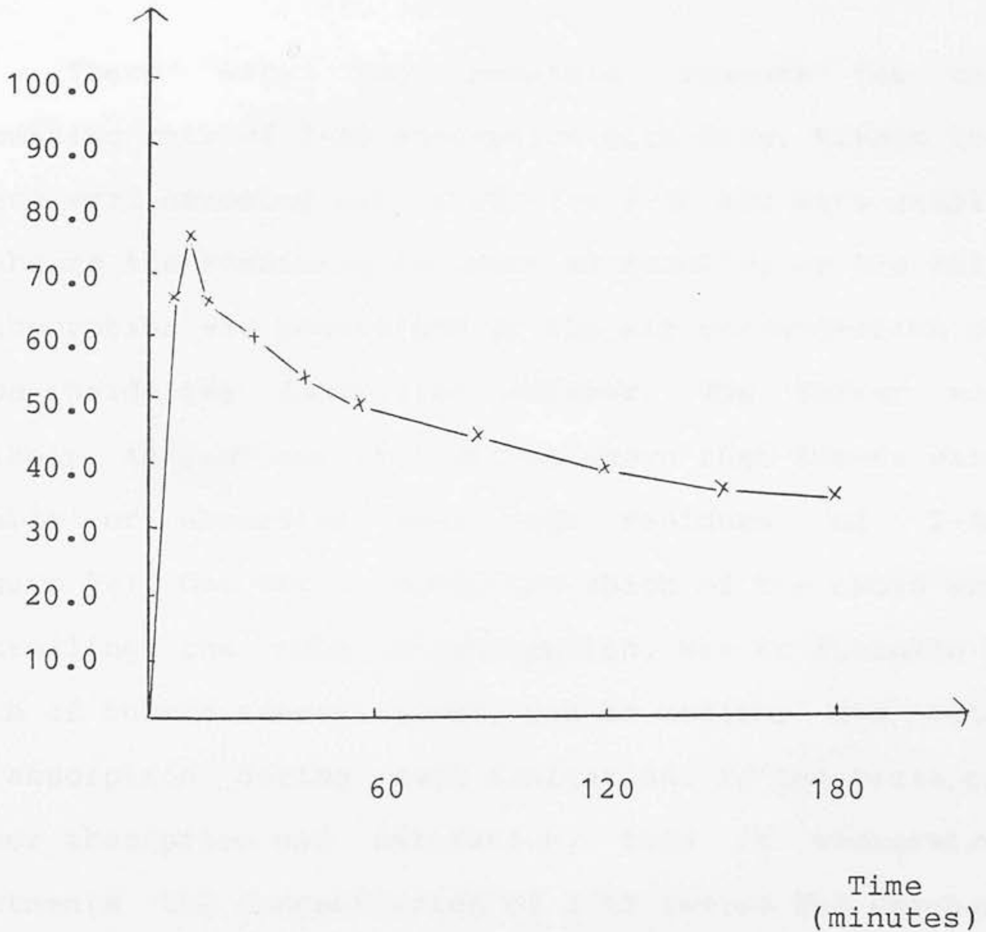
The obvious conclusion from these results is that higher residues of 2-AB can be obtained simply by increasing the initial dosage or increasing the fumigation time.

This concentration/time study only looked at the final residues of 2-AB in tubers treated under different conditions. No information was obtained on how the rate of absorption varied during the actual fumigation period. An experiment was therefore devised to study this in more detail. As ^{14}C labelled 2-AB was being used, it was not practical to study the uptake of 2-AB

by sampling the tubers themselves, as this would involve opening up the apparatus. Instead the rate of 2-AB absorption was monitored indirectly, by sampling the air inside the fumigation chamber, at intervals during the fumigation. Air samples, taken with a gas-tight syringe through a Suba-seal fitted to the top of the fumigation chamber, were bubbled through acid solutions which extracted any ^{14}C 2-AB. Scintillation counting of the resulting acid/amine solutions, gave a measure of the 2-AB present in the air samples and thus of the fumigant concentration inside the chamber at the time of sampling.

Several fumigations of 'Desiree' tubers were carried out, at a dosage of 200 $mgkg^{-1}$, and the above air sampling technique was used to monitor the rate of absorption. Figure 10 shows how the air concentration of 2-AB varied as a function of time over a 3 hour period, in a typical fumigation. As expected, the air concentration of 2-AB rises sharply at the beginning of the fumigation period, as the amine is pumped into the chamber. A peak in 2-AB concentration is normally reached after about 10 minutes. Although all the amine is vaporised within 10-15 minutes of starting the fumigation, the maximum air concentration of 2-AB never exceeds 70-75% of the initial dosage. This indicates that the tubers start absorbing 2-AB early on in the

Figure 10 Graph illustrating how 2-AB concentration inside the fumigation chamber, varies with time, during the fumiagtion period.

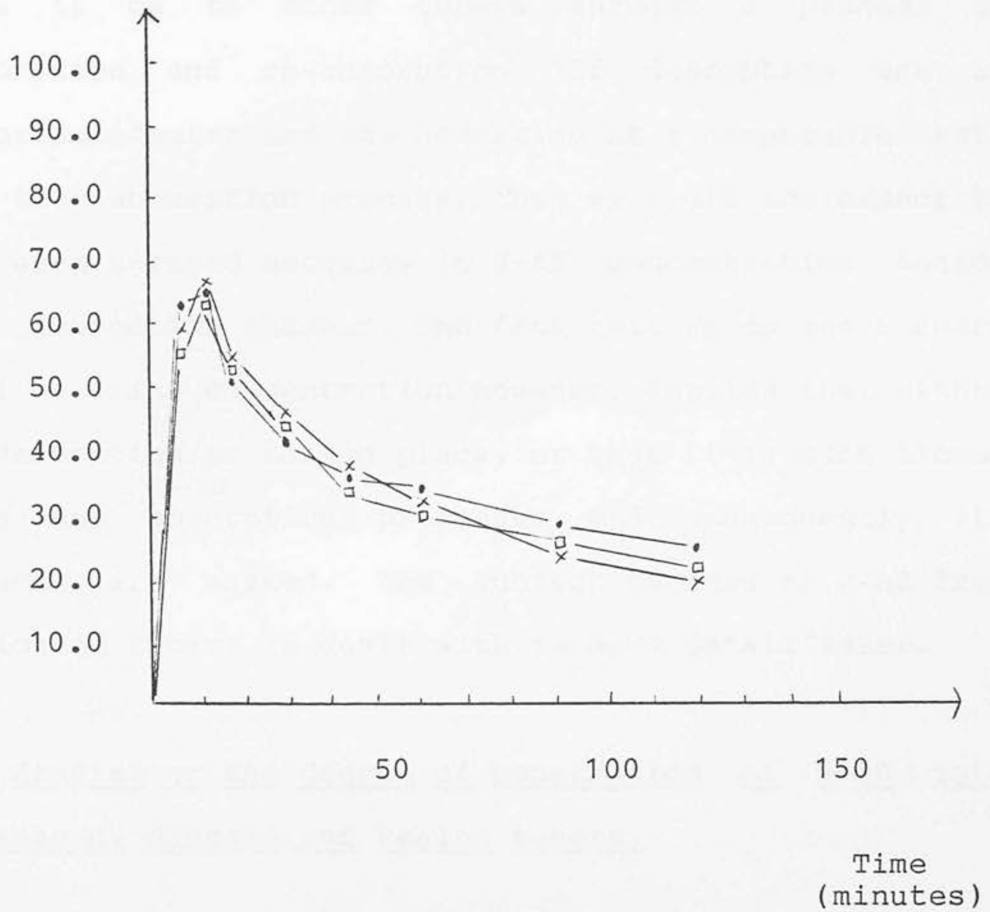


fumigation. Figure 10 also shows that near the start of the treatment, the tubers absorb the amine rapidly, and after the initial peak the 2-AB concentration inside the chamber drops quite quickly. Notably, the rate at which the tubers absorb 2-AB is not constant, but instead decreases with time during the fumigation.

There were two possible reasons for the decreasing rate of 2-AB absorption with time. Either the tubers were becoming saturated with 2-AB and were unable to absorb the remaining fumigant as quickly, or the rate of absorption was controlled by the air concentration of amine inside the fumigation chamber. The former was unlikely as previous studies had shown that tubers were capable of absorbing very high residues of 2-AB (Figure 9A). One way to establish which of the above was controlling the rate of absorption, was to fumigate a batch of tubers several times, and to monitor the rate of absorption during each fumigation. If the cause of slower absorption was saturation, then in successive treatments the concentration of 2-AB inside the chamber should still rise sharply but not fall off as quickly as in the first fumigation. If however, the pattern of absorption was the same in each fumigation, this would suggest that rapid absorption is caused by high amine concentrations and the decreasing rate of absorption merely corresponds to the drop in air concentration of 2-AB.

Multiple fumigation experiments were therefore carried out on batches of 'Pentland Crown' tubers. Each fumigation was at a dosage of 200 $mgkg^{-1}$ and the rate of absorption was monitored at regular time intervals, during each successive treatment, using the air sampling technique. An attempt was made to standardise conditions by carrying out the fumigations as close together in time, as possible. Figure 11 illustrates graphically the pattern of absorption recorded in 3 successive fumigations of a 1 kg batch of 'Pentland Crown' tubers. For each treatment we see the familiar pattern; a sharp increase in 2-AB concentration as the fumigant enters the chamber, followed by rapid initial absorption which decreases with time. From these results we can conclude, that the decrease in rate of absorption observed during a single fumigation is not due to saturation of the tubers, since in each successive fumigation they absorb more and more 2-AB. It does appear however, that the rate of absorption is controlled by fumigant concentration, since in each fumigation we see rapid absorption when the 2-AB concentration is high, and a decreasing rate of absorption as the level of 2-AB inside the fumigation chamber drops. These results therefore imply that absorption of 2-AB by tubers could be encouraged, and the required level of treatment achieved in a shorter time, if the amine concentration inside fumigation chambers was maintained at a high level.

Figure 11 Graph illustrating how 2-AB concentration inside the fumigation chamber, varies with time, during the fumigation period.



It is worth noting at this point, that in all of the experiments which measured the rate of absorption of 2-AB by tubers, there was no evidence of any subsequent desorption. Graham and Hamilton[17] suggested in their early work on the distribution of 2-AB in bulk fumigations, that tubers which had absorbed amine could pass it on to other tubers through a process of desorption and re-absorption. If desorption was an important factor and was occurring at a comparable rate to the absorption process, then we would not expect to see such a rapid decrease in 2-AB concentration inside the fumigation chamber. The fact that we do see a sharp fall in amine concentration however, implies that either no desorption is taking place, or that it is much slower than any absorption processes and consequently its effects are masked. The subject of loss of 2-AB from fumigated tubers is dealt with in more detail later.

C.3 <u>Studies on the degree of penetration of 2-AB into undamaged, damaged and healed tubers.</u>

Hamilton and Ruthven[67], had shown that methoxyethylmercuric chloride (MEMC) did not penetrate tubers to any great extent, with more than 90% of the organomercury compound being confined to the surface peel (i.e. the thin layer of surface periderm and tissue removed in conventional culinary peeling).

Graham *et al.*[17], showed that 2-aminobutane was more effective in penetrating tubers, with only 60-70% of the total residue being removed by peeling. The following work describes experiments designed to provide more information on the degree of penetration of 2-AB into tubers. Penetration into damaged and healed tubers was also investigated.

The penetration of 2-AB into tubers was studied by taking cylindrical cores through fumigated tubers, slicing the core and analysing each slice individually. This provided a profile of 2-AB concentration through the sampled tubers.

C.3(I) Penetration of 2-AB as a function of tuber maturity.

The efficiency of 2-AB fumigation, for satisfactory disease control, has been shown to diminish as the period between harvest and treatment lengthens to 30 days. Graham *et al.*[17] suggested that the decline in effectiveness resulted from the skin and damaged areas becoming increasingly impervious to the fumigant, as a consequence of physico-chemical changes in the skin after harvest. The penetration of 2-aminobutane into tubers fumigated at different times after harvest, was studied in order to establish whether the uptake and

degree of penetration was affected by tuber maturity. Batches of 'Arran Pilot' tubers were fumigated at 1, 8 and 21 days after harvest, at a dosage of 200 $mgkg^{-1}$. Analysis of sliced cores through tubers from each batch, provided a profile of 2-AB penetration as a function of tuber maturity. Table 23 shows the results obtained from the above experiment. The surprising observation was that 2-AB failed to penetrate the tubers to any great extent, even when the surface skin was relatively immature. Highest residues were recorded in the tuber skins, with the remaining amine being confined to the first 5 mm of tuber tissue. Penetration beyond this depth was rare. Considerable variation in the recorded residues were apparent in tubers from the same batch, and there was also marked variation between ends of a single core. This variation made it difficult to establish any significant difference in absorption and penetration due to tuber maturity, although there would appear to be slightly lower levels in the three week old tubers compared to those which were only one day old.

C.3(II) Penetration of 2-AB with time.

In most of the early penetration experiments, core samples were taken immediately after fumigation. In order to determine whether the 2-AB penetrated the tubers further with time, a batch of 'Arran Pilot'

Table 23 Typical profiles of 2-AB concentration in cores through 'Arran Pilot' tubers, fumigated at 1, 8 and 21 days after harvest.

Batch	1	2	3
Age of tubers at fumigation	1 day	8 days	21 days
Residue of 2-AB[a] in sample slice ($mgkg^{-1}$)			
1(skin)	1818.2	1953.6	610.8
2	292.0	90.3	19.7
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	44.3	25.3	30.2
9(skin)	795.4	590.9	944.0

a. Samples 1 and 9 were the surface skin from either end of the cylindrical tuber core. Samples 2-8 were 5 mm thick slices of the intervening tuber tissue.

tubers was fumigated at a dosage of 200 $mgkg^{-1}$, and core samples taken after 1 day, 4 weeks, 8 weeks and 12 weeks storage. Table 24 shows the results of the residue analyses on the sliced core samples. Again, considerable variation in 2-AB residues was apparent between the various tubers, which were all from the same fumigation batch, and also between opposite ends of the same sample cores. However, it is clear from the results that there was no further penetration during storage, after treatment, indicating that after the initial absorption the amine is held at or near the tuber surface.

This lack of 2-AB mobility within tubers was possibly due to the absorbed amine becoming firmly attached to some part of the tuber tissue structure. Alternatively, the water soluble 2-AB could simply be dissolving in aqueous tissue fluids, which were themselves not free to move around the tuber. An experiment was set up to determine which of the above factors was the most likely cause of the low degree of penetration exhibited by 2-AB. A small cut was made on one tuber and a 100 microlitre sample of water, containing 3.9 MBq of T_2O (^3H_2O), was injected onto the superficial wound. The tuber was left for 4 days and at the end of this time, a core taken through the damaged area was sliced and each sample slice analysed, using

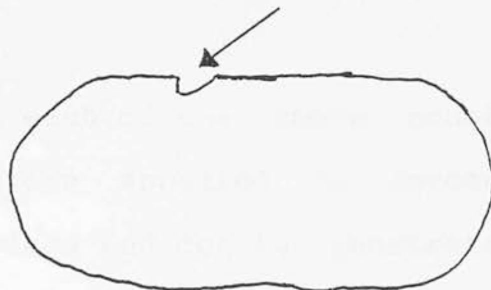
Table 24 Profiles of 2-AB concentration through 'Arran Pilot' tubers, at 1 day and 4, 8 and 12 weeks, after fumigation.

Tuber	1	2	3	4
Length of time after fumigation	1 day	4 weeks	8 weeks	12 weeks
Residue of 2-AB[a] in sample slice ($mgkg^{-1}$)				
1(skin)	1093.9	3191.5	2397.0	1829.7
2	9.1	118.9	10.8	36.3
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	29.0	-	-	-
8	144.2	36.3	3.6	118.9
9(skin)	6076.9	1829.7	964.9	2865.1

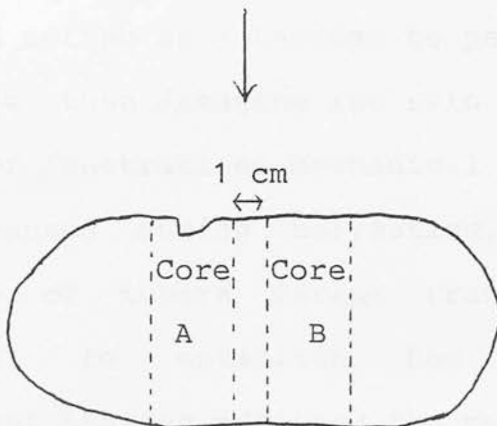
a. Samples 1 and 9 were the surface skin from either end of the cylindrical tuber core. Samples 2-8 were 5 mm thick slices of the intervening tuber tissue.

the scintillation counting method, to determine how far the radio-labelled T_2O had penetrated the tuber. A second core, parallel to the first but 1 cm further along the tuber, was taken and analysed in the same way, to check for any sideways transport of the T_2O. Figure 12 outlines the above experiment and the results obtained from it, which are expressed as cpm mg^{-1} rather than $mgkg^{-1}$. The raw scintillation counting data was used in this case because no quench correction graph for tritium (^3H) was available. Despite this, the spread of activity clearly illustrates that the T_2O had permeated the whole tuber, and this indicated that the aqueous tissue fluids with which it had mixed, were indeed mobile. Since 2-aminobutane does not show the same degree of mobility within tubers, it obviously does not simply dissolve in the tuber tissue fluid, after absorption. It would appear therefore that the lack of 2-AB penetration is possibly due to the amine becoming attached to some immobile part of the tuber tissue, which prevents it from moving throughout the tuber along with the aqueous interstitial fluids. If this is the case, it may also explain why 2-AB is not easily extracted from the tuber tissue with organic solvents or water.

Figure 12 T_2O - Tissue fluid mobility experiment.



T_2O/H_2O mixture syringed onto the cut in the tuber surface



After four days, two core samples were taken through the tuber

Sample slice[a]	Core A	Core B
1 (skin)	126458.6	66905.4
2	488695.5	173208.1
3	499651.8	161623.8
4	454295.8	157210.8
5	389742.8	157770.1
6	299886.7	142517.3
7	222353.6	126874.1
8	159438.7	109151.1
9 (skin + tissue)	68751.5	42439.7

Cpm g^{-1} recorded for each sample slice in the two tuber cores

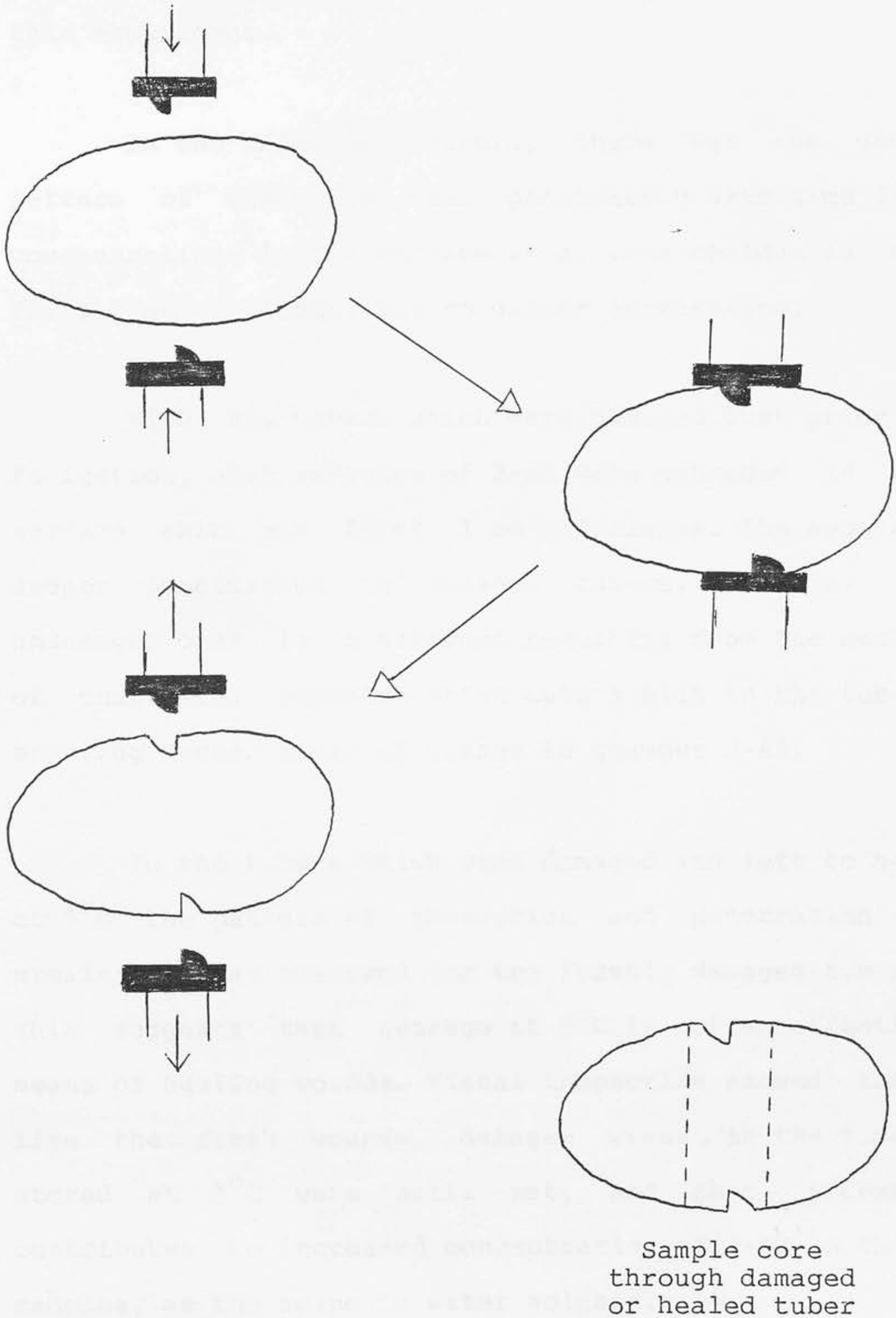
a. Sample 1 was the surface skin from the damaged end of the cylindrical tuber core. Samples 2-8 were 5 mm thick slices of the intervening tuber tissue. Sample 9 was skin + tissue from the bottom end of the core.

C.3(III) Penetration of 2-AB into damaged and healed tubers.

In each of the above penetration studies, the 2-aminobutane appeared to concentrate at or near the tuber surface and not to penetrate the tuber to any great extent. It was therefore possible that the surface skin was acting as a barrier to penetration. If this was the case then damaging the skin might lead to a higher degree of penetration. Mechanical damage to tubers is often caused during harvesting, grading or by rough handling of tubers during transport, and it was important to establish how this damage and any subsequent healing affected the penetration of 2-AB.

For the purpose of these experiments, tubers were damaged in a controlled manner using the method employed by Hide *et al.*[76], in their work on the prevalence of *Phoma exigua* in potatoes. Two standard depth wounds, on opposite sides of each tuber, were achieved using a modified drill stand fitted with two "cut and crush" brass teeth (Figure 13). These wounds were then allowed to heal in storage for 12 days, at either 5°C or 15-20°C. The healed tubers were then fumigated, under standard conditions, at 200 mgkg^{-1}, along with some freshly damaged and undamaged tubers. After fumigation cores were taken through each type of tuber. In the

Figure 13 Use of modified drill stand, fitted with 'cut and crush' brass teeth, to damage tubers in a controlled manner, and position of sample core in relation to wound.



damaged or healed tubers the core was through the damaged or healed areas (Figure 13). Table 25 shows typical 2-AB concentration profiles through tubers from this experiment.

In the undamaged tubers, there was the usual pattern of absorption and penetration with high 2-AB concentrations in the surface skin, some residue in the first 5 mm of tissue, but no deeper penetration.

With the tubers which were damaged just prior to fumigation, high residues of 2-AB were recorded in the surface skin and first 1 cm of tissue. The apparent deeper penetration in damaged tubers, compared to undamaged ones, is an artifact resulting from the method of controlled wounding which cuts a slit in the tuber, exposing deeper areas of tissue to gaseous 2-AB.

In the tubers which were damaged and left to heal at 5°C, the pattern of absorption and penetration is similar to that observed for the freshly damaged tubers. This suggests that storage at 5°C is not an effective means of healing wounds. Visual inspection showed that, like the fresh wounds, damaged areas in the tubers stored at 5°C were still wet, and this probably contributes to increased concentration of 2-AB in these samples, as the amine is water soluble.

Table 25 Profile of 2-AB concentration through 1) undamaged, 2) freshly damaged and 3) damaged and healed, 'Arran Pilot' tubers.

Residue of 2-AB in sample slice[a] ($mgkg^{-1}$)	(1) Undamaged tuber	(2) Tuber damaged just before fumigation	(3) Tuber damaged and allowed to heal
			Healed at 5 °C
1(skin)	1083.8	4573.9	3434.7
2	16.1	2991.3	2457.3
3	-	186.9	196.0
4	-	-	-
5	-	-	-
6	-	-	-
7	-	505.6	552.2
8	8.1	2063.5	3415.4
9(skin)	793.7	3955.7	6793.0
			Healed at 15-20 °C
1(skin)	2892.6	8644.0	2254.7
2	8.9	2019.9	140.5
3	-	67.0	8.5
4	-	-	-
5	-	-	-
6	-	-	-
7	-	13.6	13.9
8	3.7	1395.6	192.7
9(skin)	1645.2	6256.4	1872.9

a. Samples 1 and 9 were the surface skin from either end of the cylindrical tuber core. Samples 2-8 were 5 mm thick slices of the intervening tuber tissue.

In the damaged tubers which were allowed to heal at 15-20°C the healing process appears to have been more effective. The levels of uptake and penetration into the dried-out wounds were much lower than in the freshly damaged tubers. This confirms that the recommended curing period for dry, mature crops, of 10 to 14 days at 15°C, is sufficient for adequate healing of mechanical damage and will help prevent excessive residues of 2-AB, which could cause chemical damage, arising as a result of the fumigation treatment.

C.4 Comparison of the absorption of R-(-)- and S-(+)- 2-aminobutane by potato tubers.

Due to the presence of a chiral centre 2-aminobutane exists as two optical isomers. The commercial products used for the fumigation of potato tubers are a racemic mixture of the two enantiomers. The R-(-)- isomer was shown by Eckert and Kolbezen[10] to be more active as a fungicide, than the corresponding S-(+)- isomer, in the control of fruit diseases. The difference in the activity of the two isomers, against the fungi responsible for the potato diseases gangrene and skin spot, is dealt with later in Section H.2 (Results and Discussion), of this Thesis. A comparison of the absorption of R-(-)- and S-(+)- 2-AB was made at this point in order to determine whether

either isomer was selectively or preferentially absorbed by potato tubers. As attempts to resolve racemic 2-AB had been unsuccessful, the amine used for this experiment was obtained commercially. Small quantities of optically pure R-(-)- and S-(+)- 2-aminobutane were purchased from Jeanneret, Pousaz, Soerensen and Cie, a Swiss company specialising in the preparation of resolved compounds. Two batches of 'Epicure' tubers were fumigated at 200 $mgkg^{-1}$, using the standard method, one batch with the R-(-)- isomer and the other with the corresponding S-(+)- isomer. Since the resolved amine was not ^{14}C labelled, the fumigated tubers were analysed using the distillation and HPLC method of Hunter and Lindsay.[57] Six separate residue analyses were carried out for each enantiomer. The results are shown in Table 26. The mean residue for batch 2 was higher than that for batch 1, but statistical analysis shows that the error for the analytical method is greater than the difference between the two mean residues. Thus, within the error bounds of the analysis, there is no significant difference between the levels of absorption of the two optical isomers of 2-aminobutane. This was not an unexpected result since absorption and penetration of 2-AB, unlike the fungicidal activity, is likely to be a physical process independent of the optical configuration of the amine molecule.

Table 26 Recorded residues in Epicure tubers fumigated with R-(-)- or S-(+)- 2-aminobutane, at a dosage of 200 $mgkg^{-1}$.

	2-AB residues ($mgkg^{-1}$)	
Batch	1	2
Isomer used in fumigation	S-(+)-2-AB	R-(-)-2-AB
Sample 1	69.49	83.26
2	86.06	77.11
3	70.28	89.25
4	49.07	73.45
5	79.62	84.45
6	69.48	106.33
Mean	70.66	85.64
Std. Dev.	±12.54	±11.57

D. Loss of 2-aminobutane from fumigated potato tubers.

Seed potatoes are normally stored over the winter months at a temperature of about 5°C (7°C for ware tubers). These low temperatures help prevent sprouting, softening, and the onset of bacterial soft-rotting. However, in stores kept at these temperatures, gangrene can be a problem since lesions may develop during the storage period on wounds which have not healed.[68] Skin spot can also become particularly severe in cool, humid stores. Its symptoms usually develop 2-3 months after lifting, killing buds in the 'eyes' of infected tubers. Fumigation of tubers with 2-aminobutane helps to control gangrene and skin spot, but if the amine were to be lost from the tubers during storage they might become more susceptible to disease development. Several workers have shown that fumigation of tubers with 2-aminobutane will provide good control of gangrene and skin spot throughout the whole storage period. Table 27 shows the results of some unpublished work by Carnegie.[66] This demonstrates that 2-AB can provide a long lasting protection against gangrene infection, if a reasonable residue of fungicide has been achieved during the fumigation treatment. This suggests indirectly that 2-AB residues persist in fumigated tubers throughout the storage period.

Table 27 Development of gangrene on untreated tubers and tubers fumigated with 2-aminobutane, when inoculated artificially at various intervals after fumigation.[a]

	Surface area covered by lesions (mm^2)			
	1977-78 (Record)		1978-79 (Redskin)	
	Untreated	Fumigated	Untreated	Fumigated
2-AB residue in fumigated tubers	-	19.5mgkg^{-1}	-	10mgkg^{-1}
Weeks after fumigation				
2	633	132 (21)[b]	2741	1949 (71)[b]
4	1308	260 (20)	-	- -
6	-	- -	2448	1829 (74)
8	1844	130 (7)	-	- -
12	1077	314 (29)	-	- -
16	1127	327 (29)	-	- -
18	-	- -	4458	3530 (79)
20	1029	217 (20)	-	- -
24	1098	211 (19)	844	922(109)

a. Table after Carnegie.[66]

b. Figures in parenthesis are for gangrene development on fumigated tubers as % of that on untreated tubers.

An attempt was made to establish, directly, whether loss of 2-AB from tubers was an important factor. Two types of loss were investigated. Firstly, by measuring 2-AB residues periodically during the storage period, an estimation of 'natural' loss was obtained. Secondly, experiments were carried out to determine whether loss of 2-AB could be encouraged as a result of forced air ventilation.

D.1 <u>'Natural' loss of 2-AB during the storage period.</u>

Two 'natural' loss studies were carried out over the 1984-85 and 1985-86 seasons. In each, batches of tubers were fumigated at a dosage of 200 $mgkg^{-1}$ soon after harvest, and then stored at about $5^{o}C$. 2-AB residues were measured immediately after fumigation and then again every month during the storage period, to assess whether any decrease in residue, as a result of loss of 2-AB had occurred. In loss study 1 (1984-85), tubers were also fumigated in February and March, 1985, and residues in these tubers were monitored until April.

Table 28 shows the results from loss study 1 which involved tubers of the cultivars Kerr's Pink and Maris Piper. The results for batches 1 to 4, which were fumigated within the recommended time after harvest, show satisfactory levels of 2-AB in the tubers analysed

Table 28 Results of 2-AB residue analysis on tubers from loss study 1 (1984/85), measured at intervals during the storage period.

Weeks after fumigation	2-AB residue $mgkg^{-1}$ 1	5	10	14	18
Batch 1[a]	76.4	70.5	63.0	64.4	78.6
	85.5	75.2	74.5	67.4	79.6
2[a]	102.8	103.9	89.0	76.7	81.8
	76.6	98.9	79.0	78.4	82.7
3[a]	91.0	68.0	70.1	74.6	74.0
	95.0	72.2	73.3	74.3	78.0
4[a]	75.4	96.4	67.3	58.8	75.8
	80.8	105.6	71.0	61.3	77.1
(Mean)	(85.4)				(78.5)
(Std. Dev.)	(±10.0)				(±2.9)
Batch 5[b]	56.8	49.5	59.5		
	58.7	48.7	60.4		
6[b]	65.7	42.7	57.7		
	63.7	42.7	56.5		
7[c]	53.1	36.9			
	53.9	36.6			
8[c]	53.9	44.8			
	56.8	44.8			

a. Kerrs Pink tubers - fumigated on 9/11/84.

b. Maris Piper tubers - fumigated on 1/2/85.

c. Maris Piper tubers - fumigated on 12/3/85.

immediately after fumigation. Despite occasional fluctuations in the levels of 2-AB, which can be accounted for by the normal tuber to tuber variation, the overall picture throughout the storage period is one of little or no loss of amine. Substantial residues of 2-AB were still present in the tubers which were analysed at the end of the storage period. Statistical analysis of the results showed no significant difference, at the 95% confidence level, between the 2-AB residues measured at 1 and 18 weeks after fumigation. In batches 5 to 8, which were fumigated in February and March, the initial residues of 2-AB, in tubers analysed immediately after treatment were lower than those recorded initially for batches 1 to 4. This was as expected since tubers fumigated late in the season, have been shown in the past to absorb less 2-AB than those treated close to harvest. Monitoring of the 2-AB residue in tubers from batches 5 to 8, again showed no signs of any significant loss, with substantial residues still apparent in April.

One problem encountered in loss study 1, was that the tubers in batches 1 to 4, which were stored in a cold room at about 5°C, showed some signs of shrivelling near the end of the storage period. This indicated possible loss of moisture and was a result of the artificial storage conditions. As a check on the results

of loss study 1, a second study was carried out over the 1985-86 season. In this second loss study, the tubers were stored in a ventilated potato store, and as a check on possible water loss from the stored tubers, the percentage moisture content of the tuber samples was measured each month, along with the 2-AB residue.

Table 29 shows the results for loss study 2 which involved 4 batches of 'Pentland Crown' tubers. Each batch was fumigated at 200 $mgkg^{-1}$ and then stored in a potato store at a maximum of $5^{o}C$. Samples of tubers were taken for analysis immediately after fumigation and then again every month during the storage period. Each time the 2-AB residue was measured, the percentage moisture content (% wt/wt) of the tubers was also determined, by drying a known weight of pulp to a constant weight. The results from loss study 2 again showed no evidence for loss of 2-AB from the tubers with time. Substantial residues of amine were still present at the end of the storage period. Statistical analysis of the results showed no significant difference, at the 95% confidence level, between the 2-AB residues measured at 1 and 23 weeks after fumigation. As the tubers in loss study 2 had been stored under more ideal conditions, they showed no outward signs of water loss, and this was confirmed by the results of the percentage moisture content analyses, which showed no significant loss of

Table 29 Results of 2-AB residue analyses on tubers from loss study 2 (1985/86), measured at intervals during the storage period.

Weeks after fumigation	2-AB residue $mgkg^{-1}$					
	1	5	9	15	19	23
Batch 1[a]	145.9	126.4	124.7	167.9	109.6	153.2
	152.4	129.4	119.6	164.4	137.7	145.7
2[a]	143.6	114.4	98.8	143.8	113.1	179.0
	195.0	115.5	99.9	149.1	108.4	178.4
3[a]	121.6	105.0	139.9	146.4	83.5	133.4
	120.6	114.4	139.0	146.9	78.8	127.4
4[a]	122.9	69.8	68.2	144.4	87.0	67.4
	125.0	67.9	74.7	138.4	87.5	67.5
(Mean)	(140.9)					(131.5)
(Std. Dev.)	(±25.2)					(±43.7)

a. Pentland Crown tubers - fumigated on 30/10/85.

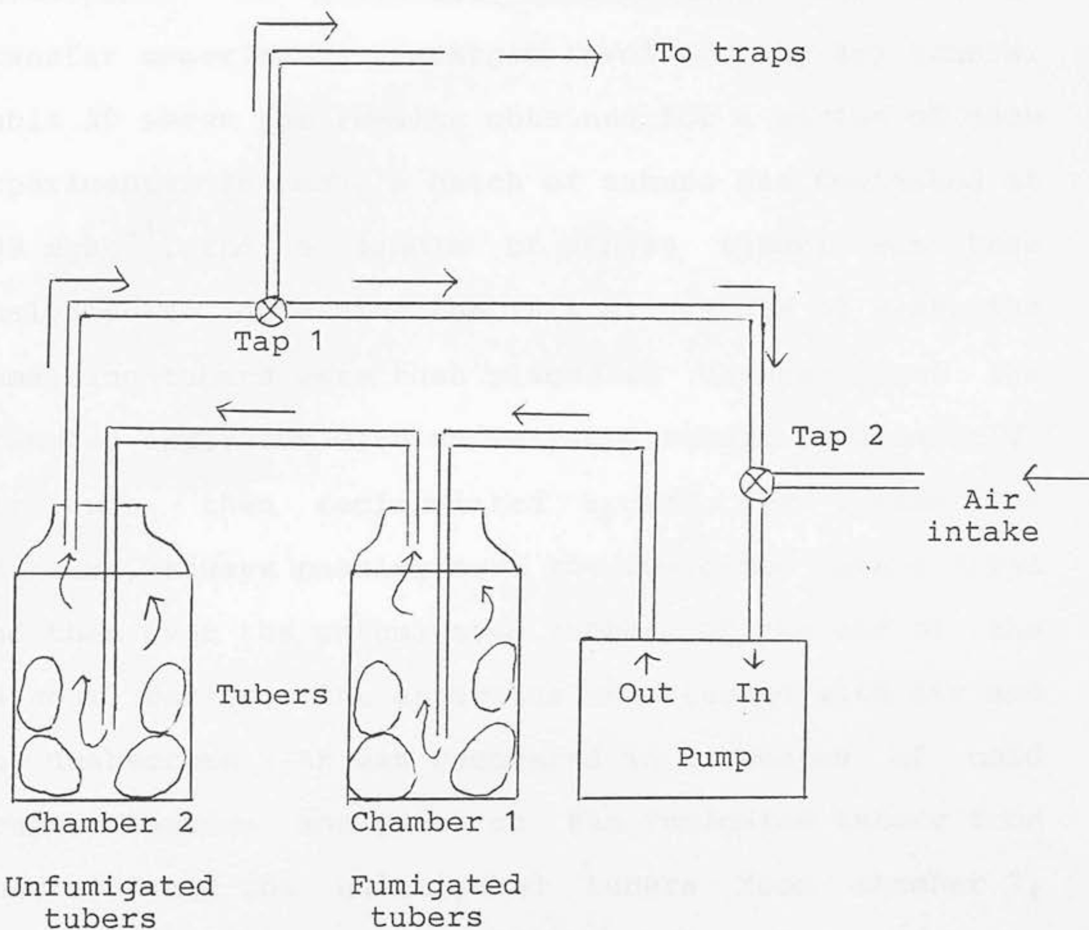
moisture during the six month storage period. After 1 week, the mean moisture content (% wt/wt) for the tuber samples was (78.1 ± 1.8)%, compared to a value of (77.9 ± 1.2)% after 23 weeks storage.

D.2 Loss of 2-AB from fumigated tubers through forced air ventilation.

Graham *et al.*[17] indicated that the absorption, desorption and re-absorption of 2-AB, was one of the processes involved in the distribution of fumigant throughout large bulks of tubers. They suggested that once 2-AB is absorbed by tubers, it can effectively be 'blown' out again during air recirculation, and be transferred on to other tubers.

A series of 'transfer' experiments was carried out to determine whether 2-AB, once absorbed by tubers, could be passed on to unfumigated tubers as a result of forced air ventilation. Figure 14 illustrates the apparatus which was constructed for these experiments. Basically it was a modification of the small scale fumigation equipment, fitted with a second chamber. The design allows air to be circulated around a sealed system, passing first over fumigated tubers and then over unfumigated ones. Any amine detected in the previously unfumigated material, after a prolonged air

Figure 14 Apparatus constructed for the study of 2-AB transfer from fumigated to unfumigated tubers, during air recirculation.



Arrows represent the direction of gas flow

recirculation, must therefore have been transferred from the fumigated tubers.

Tubers which are treated with 2-aminobutane are normally dry, since the storage of wet tubers is actively discouraged as it can lead to extensive development of bacterial soft-rotting. The initial transfer experiments therefore involved only dry tubers. Table 30 shows the results obtained for a series of such experiments. In each, a batch of tubers was fumigated at 200 $mgkg^{-1}$, and a sample of these tubers was then analysed to determine the initial residue of 2-AB. The remaining tubers were then placed in chamber 1 of the transfer apparatus with unfumigated tubers in chamber 2. Air was then recirculated around the system for 24 hours, always passing over the fumigated tubers first and then over the unfumigated tubers. At the end of the 24 hour period, the apparatus was flushed with air and any unabsorbed 2-AB was recovered in a series of cold traps. Residue analysis on the fumigated tubers from chamber 1 and the unfumigated tubers from chamber 2, indicated whether any transfer had taken place. In experiments 1 and 2, batch 1 was fumigated prior to the 24 hour transfer experiment, and following the normal procedure the apparatus was pumped out at the end of the fumigation. As this pumping out step would possibly strip off any 2-AB which was available for transfer, it was not included in later experiments.

Table 30 2-AB residues recorded in tubers before and after 24 hour transfer experiments.

Expt.	Batch (1) pumped out after fumig.	Wet or dry tubers	2-AB residue $mgkg^{-1}$ Batch (1) Before	Batch (1) After	Batch (2) Before	Batch (2) After	wt. of 2-AB in traps (mg)
1	Yes	Dry	178.0	139.1	-	12.0	0.5
			178.4	140.6	-	11.4	
2	Yes	Dry	105.3	114.0	-	10.2	1.1
			108.5	116.4	-	9.2	
3	No	Dry	151.6	95.8	-	18.7	1.1
			151.5	96.8	-	18.8	
4	No	Dry	85.3	85.4	-	16.2	2.0
			87.2	85.2	-	15.2	
5	No	Wet	94.3	67.1	-	25.7	1.0
			95.1	67.2	-	26.5	
6	No	Wet	94.4	94.5	-	29.1	1.5
			94.9	94.5	-	28.8	

In experiments 1 and 2, low residues of 2-AB were recorded in the unfumigated tubers indicating that a small amount of transfer had taken place. The low levels of transferred 2-AB and the variation in tuber to tuber residues made it difficult to establish an accurate final material balance. However, as the fumigated tubers were the only possible source of ^{14}C labelled 2-AB, it was indisputable that they provided the amine recorded in the unfumigated tubers, by some process of transfer. In experiments 3 and 4, where the pumping out step was excluded from the initial fumigation of batch 1 tubers, the transfer experiment produced higher residues in the unfumigated tubers, indicating that some 2-AB was being lost in the pumping out process. The overall conclusion from experiments 1 to 4, on dry tubers, was that transfer of 2-AB was possible but fairly limited.

The first transfer experiments involved only dry tubers, as potatoes should normally be dried out soon after entering a potato store. Sometimes however, when fumigation takes place, stored potatoes may still be damp. This could be due to moisture from respiration, condensation or wet soil, which has not been removed by correct ventilation of the potato store. Two further transfer experiments (5 and 6) were therefore carried out using wet tubers (these had been dipped in water and allowed to drain freely for 15 minutes). The results in

Table 30 show that when 'wet' tubers are fumigated and subjected to the transfer experiment, the level of transfer from fumigated to unfumigated tubers increases. The observation that higher transfer occurs from wet tubers is not difficult to explain, as any 2-AB dissolved in the surface moisture will be more loosely bound than amine absorbed into dry tubers. Ventilation of wet fumigated tubers, leads to evaporation of the surface moisture and assists the release of dissolved, rather than absorbed, 2-AB.

The overall conclusions from the above studies on the loss of 2-AB from tubers, are therefore;

a) 2-aminobutane persists in stored tubers throughout the whole of the storage period. This is important as it means that the 2-AB will be able to provide long term protection against development of gangrene and skin spot.

b) Only a small proportion of absorbed 2-AB, can be transferred from fumigated to unfumigated tubers as a result of forced air ventilation, but most is firmly held. The low degree of 2-AB transfer between tubers, means that the absorption, desorption and re-absorption processes discussed by Graham _et al._[17], cannot be relied on as an aid to effective distribution of 2-AB

throughout a potato store. The low levels of amine transfer recorded in these experiments underlines the importance of ensuring effective distribution of 2-aminobutane during the initial fumigation treatment.

c) Transfer of 2-AB from wet fumigated tubers is more pronounced, than that observed for dry tubers, but it still only occurs on a small scale. Wetting tubers prior to fumigation could possibly aid distribution, by providing a quantity of 2-AB dissolved in surface moisture which might be free for redistribution to untreated tubers, during air recirculation. However, the adverse effects of such a drastic action i.e. the possible increase in other diseases such as bacterial soft rots, could outweigh any likely benefits, thus making it an extremely unattractive proposition.

E. Metabolism of 2-aminobutane in fumigated tubers.

The evidence available from loss studies indicates that 2-aminobutane persists in potato tubers throughout the normal storage period. Results of disease control experiments have shown that 2-AB is still active against gangrene and skin spot many months after the initial fumigation treatment. This implies that the amine is not metabolised but is retained in its original form. An attempt was made to verify the view, that 2-AB was not changed chemically in tubers, by analysing extracts from fumigated potatoes by thin layer chromatography (t.l.c.). Uniformly labelled 2-aminobutane was used in these experiments as it would hopefully enable any breakdown products to be more easily detected.

Using unlabelled 2-aminobutane, an appropriate set of t.l.c. conditions was identified. Grasshof[70] outlined the analysis by t.l.c. of a variety of amines including 2-AB, on neutral alumina plates, in a 50:50 methanol:chloroform mixture. Experiments showed that an 80:20 methanol:chloroform mixture increased the r.f. value for 2-AB to a more acceptable level. A solution of ninhydrin in alcohol and acetic acid (95:5), was used as a spray reagent to detect the unlabelled 2-AB, and its behaviour under a variety of solvent conditions, on

neutral alumina plates was recorded.

A 1 kg batch of tubers was fumigated at 200 $mgkg^{-1}$ with U-^{14}C 2-AB. The tubers were then stored for 3 months at $5^{o}C$. After this prolonged period of storage, attempts were made to extract the labelled amine from samples of the fumigated tubers. Previous experimental work, during the development of an analytical method for ^{14}C 2-AB residue determination, had shown that extraction with organic solvent was difficult. However, if the tuber samples were ground with a mixture of sand and anhydrous Na_2SO_4, this helped to granulate the tuber tissue. Addition of solid KOH to liberate any free amine and prolonged (24 h) soxhlet extraction with ether, led to recovery of some of the absorbed 2-AB, although not on a quantitative scale.

The ether extract was analysed by t.l.c. using two methods. Firstly, neutral alumina plates were spotted with the ether extract and authentic 2-aminobutane, and developed in an 80:20 methanol:chloroform solvent mixture. A ninhydrin spray reagent was used to detect the components of the extract mixture and their resulting r.f. values were compared with that of authentic 2-AB. This method identified 2-aminobutane as one component of the mixture. Secondly, as a check that ^{14}C 2-AB had been recovered from the

fumigated tubers, t.l.c. plates with bands rather than spots of the extract solution, were run under the same solvent conditions. Instead of spraying the plates in the normal way, bands of alumina were scraped off into scintillation vials and mixed with 0.2 M HCl and NE265 liquid scintillator solution. When these samples were counted it provided an indication of the position of any ^{14}C labelled material, and it was found that the only sample band which contained any detectable radioactivity was that which would also have contained 2-aminobutane. Both of the above experiments were repeated using a variety of t.l.c. developing solvents i.e. methanol, chloroform or ethyl acetate, and similar results were observed. This all pointed to the fact that 2-aminobutane was recovered in the ether extract of fumigated tuber samples.

It should be noted that the above work was far from conclusive, since the same effects could have been achieved with a metabolite which had a similar r.f. value to 2-aminobutane. However, if the U-^{14}C 2-AB had been metabolised then labelled fragments of the molecule with chromatographic properties different to 2-AB might reasonably be expected. The absence of any evidence which suggests breakdown of 2-AB is occurring, combined with other corroborative evidence, such as the apparent persistence of 2-AB in stored tubers and the longterm

control against fungal disease, indicates that within the duration of the normal storage period, 2-aminobutane is not metabolised by potato tubers.

F. Application of 2-AB glycollate to tubers, in solution as a spray formulation.

All of the work discussed so far has been concerned with the application of 2-aminobutane to tubers as the free amine vapour. Recently however, there has been some interest in the use of salts of 2-aminobutane as fungicides. Merck, Sharp and Dohme Limited, currently market a fungicide containing 2-AB as a salt. "Storite Plus" is the trademark of a spray formulation containing 2-AB glycollate and another fungicide, thiabendazole (TBZ). Field trials carried out jointly by the Department of Agriculture and Fisheries for Scotland (DAFS) and the North of Scotland College of Agriculture (NOSCA), have shown this mixture to be more effective than TBZ alone, for control of gangrene and skin spot.[36] The manufacturers of "Storite Plus" claim, that unlike fumigation with 2-AB which involves waiting until the tubers are cured, this fungicide should be applied at harvest, using hydraulic spray equipment attached to the harvester. This eliminates the cost and inconvenience of double handling, and the added cost of specialised fumigation equipment.

As shown in previous work, there seems to be some physical or chemical barrier to extensive penetration of free 2-AB, which may involve the amine becoming attached

to acidic sites in the tuber tissue. Since in "Storite Plus" the 2-AB is applied as a salt, it was possible that the amine would stay dissolved in the tuber tissue fluids and therefore be free to penetrate the tuber to a greater extent than gaseous 2-AB. An attempt was made to assess whether the degree of penetration of 2-AB was greater when it was applied as a glycollate salt in the spray formulation "Storite Plus".

F.1 Preparation of "Storite Plus" spiked with ^{14}C labelled 2-AB glycollate.

Due to patent restrictions, the exact composition of "Storite Plus" was not available, although it was known that the active ingredient was the glycollate salt of 2-AB. The easiest way to obtain some "Storite Plus" containing labelled 2-AB glycollate was therefore to prepare some high activity salt, and use this to spike some of the commercial formulation. This would provide the desired ^{14}C label without drastically altering the composition of the commercial mixture. A quantity of ^{14}C labelled 2-AB glycollate was prepared by mixing glycollic acid and ^{14}C labelled 2-aminobutane. The resulting labelled amine salt was then spiked into aliquots of the "Storite Plus" concentrate.

F.2 Experiments on the penetration of 2-AB glycollate in tubers, when applied as a component of "Storite Plus" spray formulation.

"Storite Plus" is normally applied as a spray, at a rate of 400 cm^3 of the commercially available concentrate (diluted to 1 or 2 litres with water) per 1000 kg of tubers. Since, unlike commercial treatments, the experiments involving "Storite Plus" spiked with ^{14}C labelled 2-AB were to be carried out on a small scale, it was decided that spraying the tubers was impractical, as much of the limited quantity of labelled material would be used. Instead, the "Storite Plus" mixture was applied to the surface of two-day old tubers by syringe. 100 microlitre quantities of "Storite Plus", spiked with ^{14}C 2-AB glycollate and diluted in the correct proportions with water, were applied to several tubers. Half of the test tubers were undamaged. The remaining tubers were damaged in a controlled manner, at one point on their surface, and the spray formulation was applied to the area of the wound. Core samples were taken through the treated tubers after either 5 days or 6 weeks. Table 31 shows the results of the above experiments, indicating how far the 2-AB penetrated the tubers when applied as a glycollate salt. In the sample cores taken at 5 days, the degree of penetration is not significantly different from that observed earlier for

Table 31 Results of 2-AB glycollate penetration experiments on Maris Piper tubers.

Residue of 2-AB in sample slice[a] ($mgkg^{-1}$)	5 Days after treatment	6 Weeks after treatment
Damaged tubers		
1(skin)	1348.3	1161.7
2	258.6	236.8
3	19.6	73.6
4	-	30.8
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10(skin)	-	-
Undamaged tubers		
1(skin)	19910.7	21790.8
2	84.1	185.8
3	-	29.8
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10(skin)	-	-

a. Samples 1 and 10 were the surface skin from either end of the cylindrical tuber core. Samples 2-9 were 5 mm thick slices of the intervening tuber tissue.

free 2-AB. After 6 weeks, the amine appeared to have progressed slightly further into the tubers, although it was obviously still not free to permeate the whole tuber. Overall the glycollate salt of 2-AB did not appear to behave very differently to free gaseous 2-aminobutane.

G. Absorption of 2-aminobutane by soil, straw and wood.

When potato tubers are fumigated it is usually in the presence of other materials. Large quantities of soil, either loose or adhering to the tubers, are often present in the fumigation chamber. Straw bales are sometimes used by farmers to divide up different varieties and grades of tubers during the fumigation and subsequent storage. Also, if the tubers are stored in pallets rather than as a loose bulk, the wooden boxes will also be exposed to treatment with 2-AB. A study was therefore carried out to determine whether soil, straw and wood could absorb 2-AB, since it was a possible source of loss of fumigant, and might contribute to the low tuber residues recorded for some fumigation treatments.

G.1 Absorption of 2-AB by soil.

The amount of soil present during a fumigation will depend on the conditions under which a crop was harvested, and the degree of handling prior to fumigation. Tubers harvested under wet conditions will often have more soil adhering to them, than those harvested under dry conditions (where the weight of soil can often be as high as 10% of the total weight of a 1 tonne box of tubers). Soil in stored tubers is known

to impede ventilation, causing overheating and preventing efficient distribution of a variety of gaseous chemical treatments. Removal of excess soil at harvest is therefore recommended. If this is not possible, then often the tubers are stored along with any adhering soil, which will be removed at a later date, but which may be present during fumigation. Experiments were therefore carried out to determine the extent of any 2-AB absorption by soil, and whether the level of absorption was related to the soil type (composition) or pH.

Consultation with the Soil Science Department of the East of Scotland College of Agriculture (ESCA) provided information on "typical" potato growing soils, of which there are many types. ESCA were also able to provide 4 samples of different soils, which covered a range of pH values. Details of the composition of each soil, in terms of coarse sand, fine sand, silt, clay and % organic matter, were also supplied. Table 32 shows the details of the 4 soil types. All 4 soils are used for growing potatoes, but in general it is common for potato soils to be at the higher end of this pH range. It was hoped, that by studying absorption of ^{14}C 2-AB by samples of these soils, to determine the degree of any absorption, and whether a relationship existed between 2-AB uptake and soil pH. Any difference in 2-AB

Table 32 Details of the 4 soil samples used in experiments on the absorption of 2-AB by soil.

Soil sample	A	B	C	D
Name	Porter East Seaton	Glentyre No. 6	Glentyre No. 3	Wheat East Seaton
pH	4.4	5.4	5.7	6.3
% Organic matter	10.7	7.5	6.7	5.9
% Coarse sand[a]	27.6	29.6	25.1	32.5
% Fine sand[a]	31.9	28.4	29.4	32.0
% Silt[a]	18.0	28.0	30.0	16.5
% Clay[a]	22.5	14.0	15.5	19.0

a. % of total, determined by particle size.

absorption related to soil composition, would hopefully also become apparent.

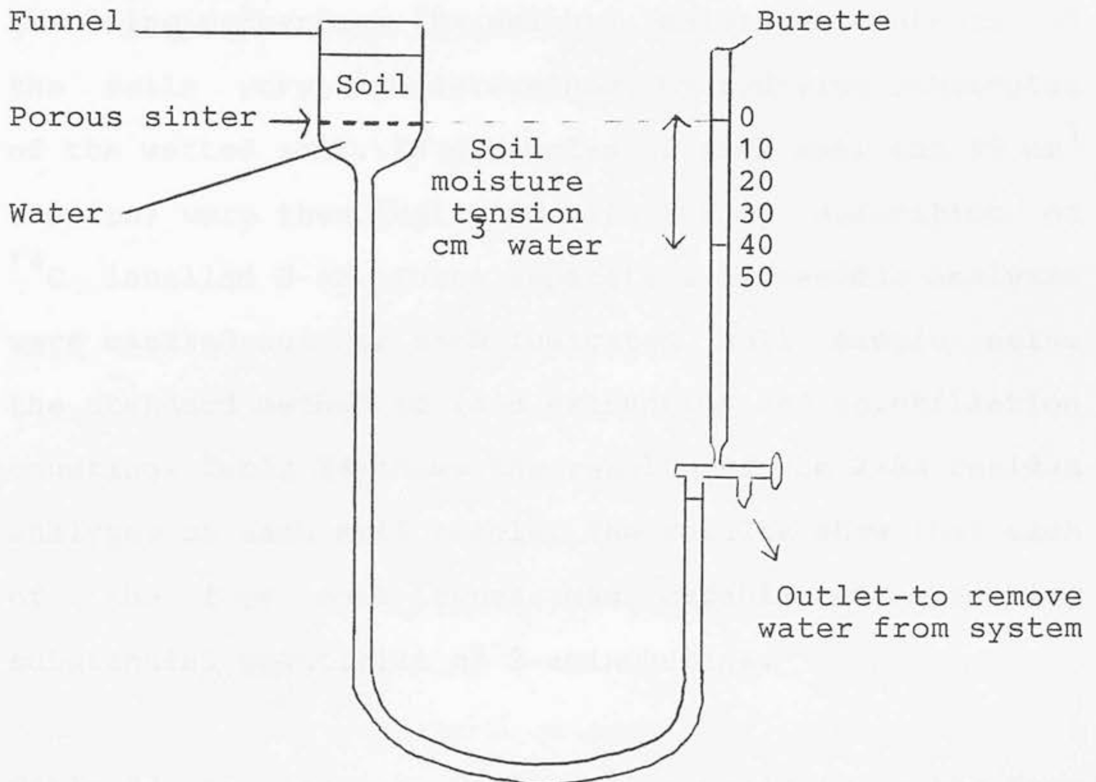
Before attempting to fumigate any samples of soil it was necessary to confirm that the analytical method developed for determining 2-AB residues in tuber samples, could be applied to analysis of soils. Samples of soil B (pH 5.4) and soil D (pH 6.3) were spiked with known quantities of ^{14}C labelled 2-AB and the samples extracted with 0.2 M HCl following the analytical method used on tuber samples. Table 33 shows typical values for recovery of ^{14}C 2-AB from the soils. This confirmed that the same analytical method was effective, and it was also noted that heating the soil samples during the acid extraction step, was not necessary for efficient recovery of 2-AB.

Table 33 Recovery of 2-AB from samples of soil.

Soil sample	Soil pH	% Recovery of 2-AB from spiked samples
B1	5.4	99.3
B2	5.4	95.3
B3	5.4	98.0
D1	6.3	101.8
D2	6.3	98.9
D3	6.3	100.6

The next step was to fumigate samples of each soil to establish whether any absorption of 2-AB actually took place. The soils had been supplied pre-dried (at 30°C), but since there was interest in the absorption of 2-AB by wet or damp soils, as well as by dry soil, a method for restoring water to the soil samples was required. Further consultation with ESCA suggested that to obtain soils of directly comparable soil moisture content, it was incorrect to compare soils with the same percentage weight of water, rather soils with the same soil moisture "tension" should be compared.[71] If different soils are thoroughly wetted and allowed to drain freely, each will drain to an equilibrium position. At the point of equilibrium each soil has the same soil moisture tension, although their percentage (by weight) moisture content will probably be different. The soil moisture tension achieved at the point of equilibrium can be easily altered by adding water to, or drawing more water from, the soil sample. Under the guidance of staff at ESCA, a simple apparatus, was constructed which enabled soil samples to be wetted to any desired soil moisture tension (Figure 15). Soil moisture tension is quoted in units of cm^3 water and a typical value for soils, at the time of the potato harvest is 40 cm^3 water. With a method available for wetting soils to comparable degrees, it was possible to go on and study the absorption of 2-AB by soils, under controlled conditions.

Figure 15 Suction tensiometer for measuring and varying the soil moisture tension of soil samples.[71]



a) If the water level in the burette is set at zero and the burette tap opened to the funnel, then the soil will absorb water, and the water level in the burette will drop. Once absorption of water by the soil stops and the system is at equilibrium, the soil moisture tension can be read off as the difference, in cm^3 water, between the soil and water level in the burette.

b) If the measured tension is lower than desired, some water is removed from the system through the outlet. Turning the tap back to the funnel will draw more water from the soil, and when equilibrium is again reached, the new higher soil moisture tension can be read off.

c) If the measured tension is higher than desired, some extra water can be added to the burette. Opening the tap to the funnel then causes the soil to absorb more water. Again, once equilibrium is established, the new soil moisture tension can be read off.

Samples of each of the four soils A to D (pH 4.4 to 6.3) were wetted using the method described above, to a soil moisture tension of 40 cm^3 water. The resulting percentage (by weight) moisture contents of the soils were also determined, by redrying subsamples of the wetted soil. 20 g samples of each soil (at 40 cm^3 tension) were then fumigated with 200 mg quantities of ^{14}C labelled 2-AB. Three separate 2-AB residue analyses were carried out for each fumigated soil sample using the standard method of acid extraction and scintillation counting. Table 34 shows the results of the 2-AB residue analyses on each soil sample. The results show that each of the four soil types was capable of absorbing substantial quantities of 2-aminobutane.

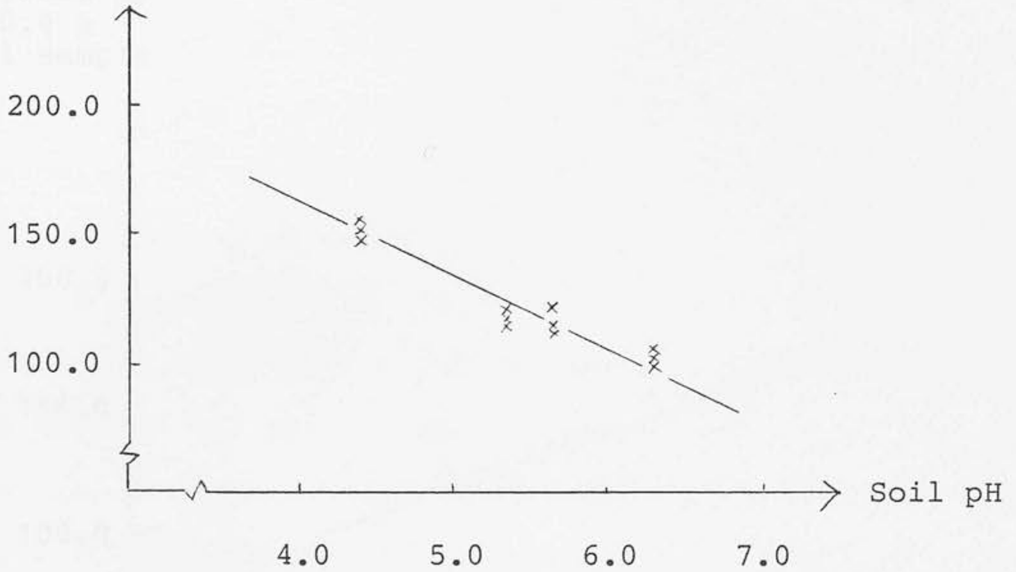
Table 34 Results of 2-AB residue analysis on the four fumigated soil samples (20 g), exposed to typical 2-AB vapour concentrations.

Soil	pH	% Water content (by weight) at 40 cm^3 tension	wt. of 2-AB applied (mg)	wt. of 2-AB (mg) absorbed by soil sample
A	4.4	33.9	200	149.3
				151.3
				159.3
B	5.4	37.7	200	120.1
				124.3
				118.9
C	5.7	33.9	200	117.9
				116.1
				125.3
D	6.3	25.8	200	105.8
				108.1
				110.1

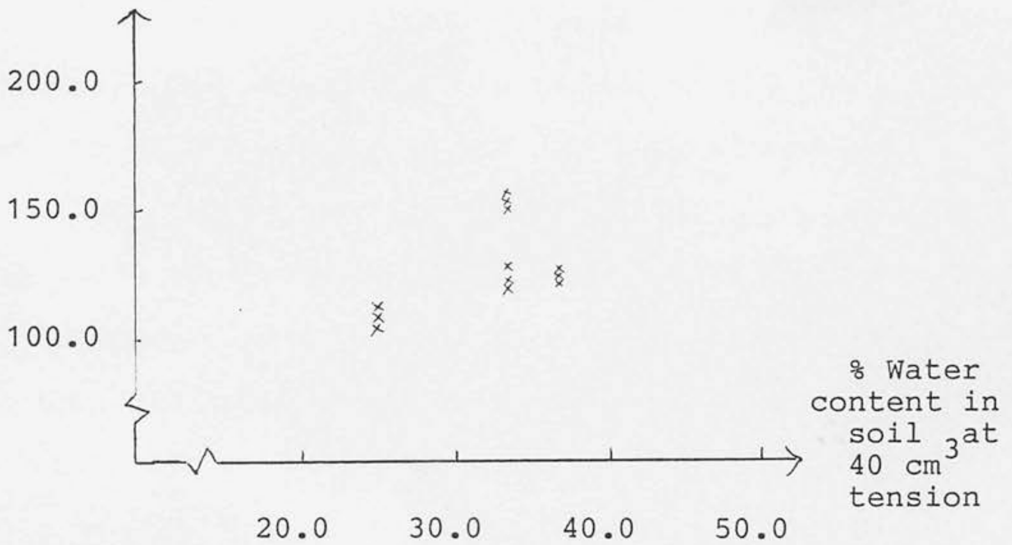
The values from Table 34 were plotted graphically, to see how the level of 2-AB absorption related to the soil pH or % moisture content (Figure 16). No relationship appeared to exist between soil water content and level of absorbed 2-AB, but there did seem to be a direct correlation between absorbed 2-AB and soil pH, with the more acidic soils absorbing proportionately more amine. As the composition of each soil was known, a comparison was also made between levels of absorption and the % coarse sand, fine sand, silt or clay, but no correlation existed. However, when the same comparison was made between absorbed 2-AB and % organic matter content, there was a direct correlation (Figure 17). Thus for soils A to D, there appeared to be a direct relationship between the level of absorbed 2-AB and the soil pH or % organic matter content. As organic matter is a major contributory factor in determining the eventual pH of a soil, it is not surprising that the two appear to be linked. In fact for soil samples A to D there was a direct correlation between soil pH and % organic matter content.

Further experimental work was carried out to determine whether both soil pH and organic matter content had the same effect on 2-AB absorption, or if one had more effect than the other. Samples of soil A (pH 4.4) were treated with solid $Ca(OH)_2$ to raise the soil pH to 6.6 . This provided a sample with the

Figure 16 Graph of 2-AB residue (mg absorbed by a 20 g soil sample) against a) soil pH, and b) % moisture content of soil.

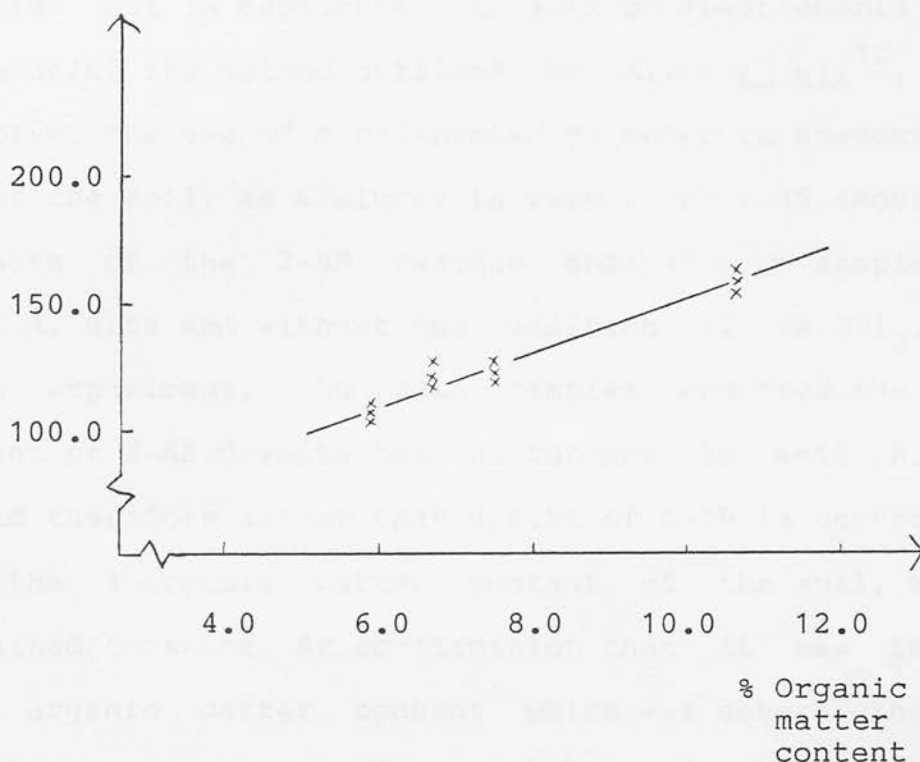


a) Absorbed 2-AB against soil pH.



b) Absorbed 2-AB against % water content.

Figure 17 Graph of 2-AB residue (mg absorbed by a 20 g soil sample) against % organic matter content of soil.



same % organic matter content as soil A, but with a higher pH. Samples of both the natural and the limed soils were then fumigated, after wetting to a soil moisture tension of 40 cm^3. After fumigation, each sample was then analysed for absorbed 2-AB. As a check on the accuracy of the results, this experiment was carried out in duplicate. All soil pH measurements were made using the method outlined by Allen <u>et al.</u>[72], and involved the use of a calibrated pH meter to measure the pH of the soil, as a slurry in water. Table 35 shows the results of the 2-AB residue analysis on samples of soil A, with and without the addition of $Ca(OH)_2$. In each experiment, the soil samples absorbed the same amount of 2-AB despite the difference in soil pH. It would therefore appear that uptake of 2-AB is controlled by the % organic matter content of the soil, which remained constant. As confirmation that it was indeed the organic matter content which was determining the level of absorption, two samples of soil D were fumigated. One was at its natural pH of 6.3, whilst the other was adjusted to pH 3.9 by the addition of a calculated amount of concentrated hydrochloric acid. Again the % moisture content for the two soil samples was constant. After fumigation both samples of soil D were analysed and again, despite the difference in soil pH, both had absorbed the same levels of 2-AB (Table 35).

Table 35 2-AB residues in soil samples (20 g) of different pH but the same % organic matter content (O.M.C.).

Fumigation[a]	Soil type	Soil pH	% O.M.C.	wt. of 2-AB absorbed (mg)
1[b]	A	4.4	10.73	118.4 , 117.2 , 118.6
2[c]	A	6.6	10.73	119.9 , 118.1 , 117.0
3[b]	A	4.4	10.73	123.6 , 125.7 , 127.6
4[c]	A	6.6	10.73	122.2 , 127.6 , 125.4
5[b]	D	6.3	5.91	115.8 , 113.9 , 113.9
6[d]	D	3.9	5.91	114.6 , 117.0 , 115.9

a. Each soil sample was wetted to 40 cm^3 tension prior to fumigation.

b. Soil sample fumigated in natural state.

c. Soil sample fumigated after addition of $Ca(OH)_2$ to raise the soil pH.

d. Soil sample fumigated after addition of HCl to lower the soil pH.

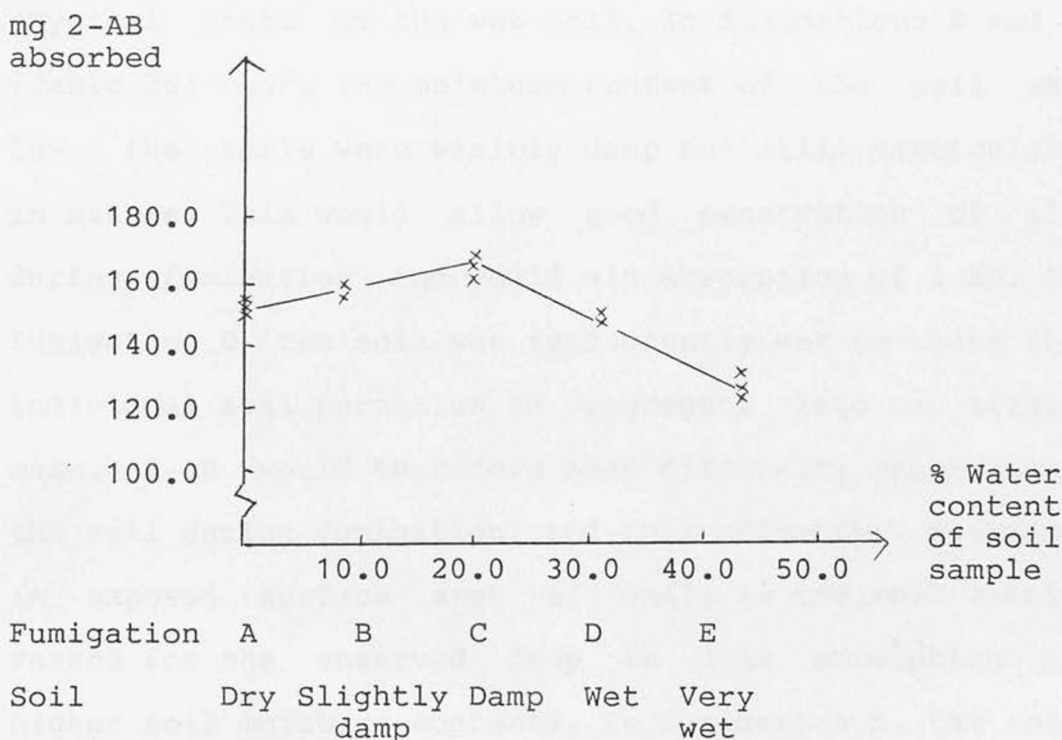
From the above experiment, it was apparent that the level of 2-AB absorption in soil is controlled by the % organic matter content of the soil sample, and not by soil pH. The correlation observed originally between absorbed 2-AB and soil pH, is due to the fact that the % organic matter content determines the level of absorbed amine, and is also a major contributory factor in determining the overall soil pH. The observed affinity between organic matter in soil and 2-AB is not surprising, considering the extent to which 2-AB is absorbed by potato tubers and many types of fruit, when it is applied as a fungicide.

Initial experiments had shown no relationship between % water content, of the four soil samples A to D, and the level of 2-AB absorption. However, as it was possible that any effects due to the differences in the % water content were being overshadowed by the effects of differences in % organic matter content, a separate experiment using one soil type was carried out. Soil C (pH 5.7) was used for this experiment and a series of samples, each of the same pH and % organic matter content but different % moisture content, were prepared. Each sample was fumigated with 200 mg quantities of ^{14}C labelled 2-aminobutane, and after each fumigation the residue of 2-AB in each soil sample was determined three times. Table 36 shows the results of all the residue analyses. Figure 18 is a graphic

Table 36 2-AB residues recorded in samples of soil C (pH 5.7), at different % water contents.

Fumigation	Wt (g) soil C (pH 5.7)	Wt(g) water added	% Water content	Absorbed 2-AB (mg)
A	20.0	-	-	149.1 , 152.8 , 154.7
B	20.0	2	9.1	157.3 , 156.2 , 156.0
C	20.0	5	20.0	167.4 , 165.1 , 161.7
D	20.0	9	31.0	147.4 , 147.2 , 148.7
E	20.0	15	42.9	124.2 , 122.7 , 130.0

Figure 18 Effect of soil moisture content on absorption of 2-AB by soil C (pH 5.7).



representation of the same results. The important conclusions from this experiment are as follows. Firstly, even when the soil is in an apparently dry state it still has an enormous capacity to absorb 2-AB. This means that any dry soil adhering to tubers during fumigation, has the potential to absorb large quantities of 2-AB. Secondly, as moisture is added to the soil its ability to absorb 2-AB appears to rise initially, up to a maximum at 20% moisture content. Since 2-AB is water soluble it is not unexpected that moisture will aid its absorption. However, at higher moisture contents (i.e. above 20%), the uptake of 2-AB decreases and continues to do so as the percentage water content is increased. This decrease in absorption of 2-AB at high % moisture content is most likely to be due to the physical state of the wet soil. In fumigations B and C (Table 36) where the moisture content of the soil was low, the soils were visibly damp but still particulate in nature. This would allow good penetration of air during fumigation and would aid absorption of 2-AB. In fumigation D, the soil was sufficiently wet to cause the individual soil particles to aggregate into a single mass. 2-AB would therefore have difficulty penetrating the soil during fumigation, and this effective decrease in exposed surface area of soil, is the most likely reason for the observed drop in 2-AB absorption at higher soil moisture contents. In fumigation E, the soil

was very wet, and during the fumigation soil particles settled out of the mixture, resulting in the formation of 2 layers, an upper aqueous layer and a lower soil layer. Again the air/amine mixture would have had difficulty reaching the soil, and this again would account for the drop in the level of absorbed 2-AB.

In conclusion, the results of the above experiments on the uptake of 2-AB by soils, have shown that absorption is highest in those soils with the highest % organic matter content. Also, soils have the capacity to absorb high levels of 2-AB even when in a dry state, and this increases as the soil moisture content rises, up to a maximum of 20% soil moisture content. Absorption decreases in soils of high moisture content (> 30%) but it is unlikely that tubers would be fumigated if covered with soil which was as wet as this. The storage of tubers with large quantities of loose or adhering soil is not recommended, for reasons discussed earlier (such as restricting effective store ventilation), but the results described above, provide further arguments for removing excess soil before attempting fumigation. On average, a 20 g sample of dry soil can absorb approximately 125 mg of 2-AB. As 2-AB is normally applied at a dosage of 200 g $tonne^{-1}$ tubers, the entire dose could in theory be absorbed by as little as 32 kg of soil, and many potato crops will have higher

proportions of adhering soil (up to 10% by weight i.e. 100 kg in a 1 tonne box). It is therefore clear that the presence of soil inside the fumigation chamber is a major sink for applied 2-AB and will contribute to lower residues in the treated tubers, resulting in less effective disease control.

G.2 <u>Absorption of 2-AB by straw.</u>

Straw bales are often used by farmers as divisions in potato stores, to separate different varieties or grades of tubers. Also, sometimes large quantities of loose straw are spread over the top of tuber bulks, to absorb moisture removed during respiration and to act as insulation against frost damage. Some fumigations are actually carried out in the store, rather than in a purpose built fumigation chamber. This means that sometimes large quantities of straw are exposed, along with the tubers, to treatment with gaseous 2-AB. An experiment was carried out to assess the capacity of straw to absorb 2-AB. The previous study on soil had shown that % organic matter was a controlling factor in the level of 2-AB absorption, and so it was expected that straw would also absorb substantial quantities of 2-AB.

A batch of cereal straw was obtained commercially. Two batches of straw were exposed to the

same atmospheric concentration of 2-AB as that used for the fumigation of potatoes. After fumigation, samples of the straw were taken for ^{14}C 2-AB residue analysis, which was carried out using the same procedure as that developed for the analysis of tuber tissue samples. Table 37 shows the high residues of 2-AB recorded in the straw samples. It would thus appear that the presence of straw inside a fumigation chamber is another sink for 2-AB, and may contribute to less effective fumigation treatment. It is therefore advisable to avoid extensive use of straw inside the fumigation chamber or potato store, until fumigation procedures are complete.

Table 37 Residues of 2-AB in straw samples exposed to typical 2-AB vapour concentrations.

Fumigation	Sample	2-AB residue ($mgkg^{-1}$)
1	a	1432.8
	b	1351.1
2	a	1099.4
	b	1092.5

G.3 Absorption of 2-AB by wood.

Many potato growers load tubers into wooden pallet boxes at harvest and also use them for long term storage, rather than storing the tubers loose in bulks. Tubers are normally loaded into the fumigation chamber in the same boxes, as it simplifies handling and reduces tuber damage. The slatted boxes, and the spaces left between stacked boxes, are also an effective aid to good amine distribution. Since the wooden boxes were likely to absorb 2-AB during the fumigation, an attempt was made to assess the level of amine absorption that could be expected.

As an initial experiment, on the absorption of 2-AB by wood, a batch of wood shavings were fumigated with ^{14}C 2-AB. Samples of the treated wood were then analysed to determine residues of 2-AB. Table 38 shows the results of the residue analyses on samples of fumigated wood shavings. The results show that wood appears to absorb more 2-AB, per unit weight, than straw. This experiment however, although it highlighted the potential of wood as a sink for 2-AB, was somewhat artificial, as wood shavings have a higher surface area than the same weight of wood in a single block.

Table 38 Residues of 2-AB in wood shavings samples exposed to typical 2-AB vapour concentrations.

Sample	2-AB residue ($mgkg^{-1}$)
1	8444.6
2	7721.0
3	8780.7
4	8906.1

A second experiment was therefore carried out to try to assess the level of 2-AB absorption, as a function of exposed wood surface area. A piece of wood from an actual farm potato pallet box was obtained and cut into blocks. Five of these blocks were weighed, and measured to determine overall surface area. The blocks were then fumigated using the standard procedure, with ^{14}C 2-AB. After fumigation, each block was soaked in 0.2 M HCl to extract any absorbed 2-AB. After soaking for two weeks, samples of each solution were analysed by scintillation counting to determine the residue of 2-AB recovered from the wood. After soaking for a further two weeks, each acid solution was sampled again, and analysed by scintillation counting. Table 39 shows the results of the residue analysis. The levels of absorbed 2-AB are lower than those observed for the wood shavings, but this was probably due to the lower overall

Table 39 Residues of 2-AB measured in wooden blocks.

Sample	Weight g	Total surface area cm^2	wt. 2-AB absorbed mg	2-AB residue $mg\ kg^{-1}$	2-AB residue $mg\ cm^{-2}$
1	17.95	76.66	8.78[a]	489.1	0.115
			9.47[b]	527.7	0.124
2	18.76	79.54	8.34[a]	444.5	0.105
			8.22[b]	438.2	0.103
3	20.70	92.70	10.38[a]	501.3	0.112
			10.77[b]	520.0	0.116
4	21.22	94.58	11.42[a]	538.3	0.121
			11.19[b]	527.1	0.118
5	18.72	68.26	7.21[a]	385.3	0.106
			7.56[b]	404.4	0.111
(Mean)				(447.6)	(0.113)

a. Results of first analysis, after soaking for 2 weeks.

b. Results of second analysis, after soaking for a further 2 weeks.

surface area. An average residue of 0.113 mg cm^{-2} was recorded. In a typical fumigation chamber, the area of exposed wood is approximately 80000 cm^2, for each pallet box holding 1 tonne of tubers. Thus in a commercial fumigation, each box could absorb up to 9 g of 2-AB. This corresponds to 4.5% of the normal applied dose of 200 g $tonne^{-1}$ (200 $mgkg^{-1}$). It is a reasonable assumption that absorption could be higher on new boxes. As the loss of 2-AB by absorption into wood is significant, it would be advisable to either avoid excessive use of wood in fumigation chambers, or to treat areas of exposed wood with some form of chemical resistant paint.

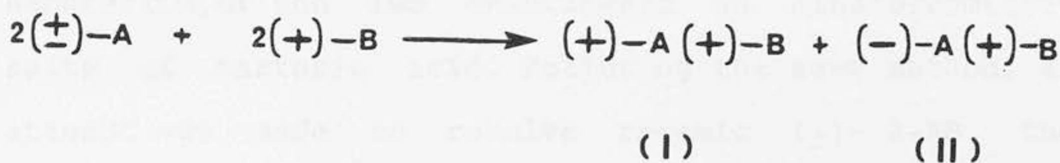
The results of the experiments on the absorption of 2-AB by soil, straw and wood, show that each has the capacity to absorb relatively large quantities of 2-AB. This fact, particularly when accompanied by inefficient fumigant distribution, may be a principal reason for the low 2-AB residues recorded in some bulk commercial tuber fumigations.

H. <u>Comparison of the antifungal activity of R-(-)- and S-(+)- 2-aminobutane.</u>

Racemic 2-aminobutane is used commercially to control gangrene and skin spot in potato tubers. However, Eckert <u>et al.</u>[10] showed in both <u>in vitro</u> and <u>in vivo</u> studies, that the R-(-)- isomer of 2-AB is more active than the corresponding S-(+)- enantiomer, for the control of fungi which cause decay in citrus fruit. An attempt was made to assess whether the same difference in activity was exhibited against the fungi which cause gangrene and skin spot in potatoes. Prior to carrying out experiments to compare the fungicidal activity of the two optical isomers, preparation of optically pure R-(-)- and S-(+)- 2-AB, was attempted, by resolution of the racemic amine.

H.1 <u>Attempted resolution of R-(-)- and S-(+)- 2-AB.</u>

The most common method for the resolution of a racemic mixture of two optical isomers, involves its conversion into a pair of diastereometric derivatives by reaction with an optically pure, chiral reagent e.g. the formation of a pair of diastereometric salts (I) and (II) (Scheme 19).



Scheme 19

Frequently the diastereomers have physical properties e.g. solubility, boiling points or chromatographic behaviour, which are sufficiently different to allow them to be separated. Resolution of the original racemic mixture can then be achieved if one of the diastereometric derivatives can be recovered in an optically pure state, and providing that regeneration of the pure enantiomer is not accompanied by any racemisation.

One chemical method of optical resolution, involves the formation of diastereometric salts from racemic acids and bases by neutralisation with optically pure bases or acids, respectively. The required optically pure reactants can be chosen from tartaric, malic or mandelic acids, and alkaloids such as brucine, strychnine, morphine and quinine, all of which are naturally occurring and thus readily available commercially.

For the resolution of (±)- 2-aminobutane Eckert[10] used the method of Bruck *et al.*[74], which involved separation of the two enantiomers as diastereometric salts of tartaric acid. Following the same method, an attempt was made to resolve racemic (±)- 2-AB. The procedure was straightforward and involved adding the racemic amine to a near boiling mixture of methanol and (+)- tartaric acid. On cooling, the less soluble (-)- amine (+)- tartate salt should have crystallised out, but although some crystallisation did occur the overall yield and optical purity of the recovered R-(-)- amine was poor. Attempts to isolate the (+)- isomer from the mother liquor were also unsuccessful. Repeated attempts at the above resolution failed to achieve the desired result.

In their original work, Bruck *et al.*[74] point out that the resolution of (±)- 2-aminobutane in alcohol is difficult and suggest that another possible solvent for the procedure is water. Fleury-Larsonneau[75] outlined in more detail the resolution of (±)- 2-AB, in water, with (+)- tartaric acid. Following this procedure another attempted resolution was carried out. Isolation of the crystals which were produced in the aqueous (±)- 2-AB/(+)- tartaric acid mixture, and regeneration from them of 2-AB, failed to produce the desired optically pure amine.

The most likely explanation for the poor efficiency of resolution, was that other workers who achieved successful enantiomeric separation, had used optically pure (-)- 2-AB (+)- tartate crystals, to seed the aqueous or alcoholic mixtures and encourage selective crystallisation. However, as no optically pure (-)- 2-AB (+)- tartate salt was available, it was not posible to reproduce the method exactly, and this may have led to both diastereometric salts crystallising out of the original mixture.

Other attempts at resolution, using the same aqueous method of Fleury-Larsonneau[75], but with (-)- malic acid or (+)- mandelic acid, were also unsuccessful.

Had more time been available, the resolution of (±)- 2-AB would have been explored further. However, as the main aim of this part of the study was to compare the activity of R-(-)- and S-(+)- 2-AB, the search for a suitable method of resolution was abandoned, and quantities of the two optically pure enantiomers were obtained commercially.

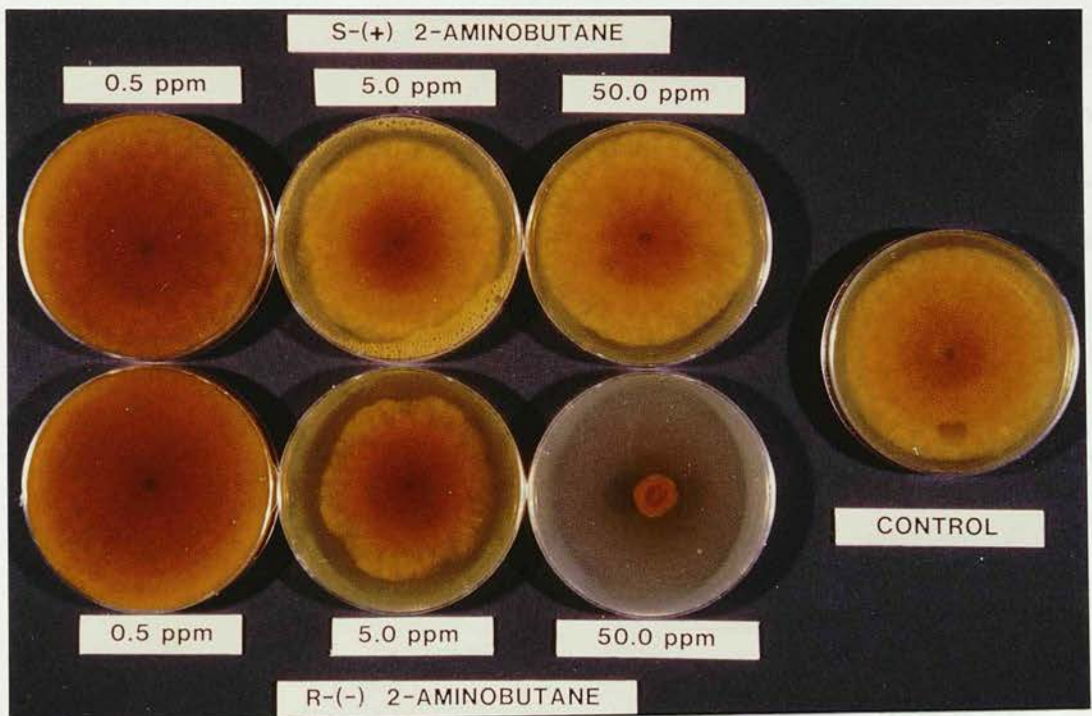
H.2 In vitro studies on the fungicidal activity of R-(-)- and S-(+)- 2-AB, against the fungi which cause gangrene and skin spot in potato tubers.

Using the commercially obtained, optically pure R-(-)- and S-(+)- 2-AB, three experiments were carried out to compare the fungicidal activity of the two enantiomers.

The first experiment compared the effect of different concentrations of the two isomers on the linear growth, on agar, of *Phoma exigua* var. *foveata*, the fungus responsible for gangrene in potato tubers. The basal medium was 2.5% malt extract agar, sterilised at 121°C for 15 minutes. One litre batches of agar were prepared and after cooling to 60°C the appropriate quantities of aqueous solutions of R-(-)- and S-(+)- 2-AB were added, to give the required concentrations of 0.5, 5 and 50 ppm. Each batch was then dispensed into petri dishes at the rate of 18 cm^3 per dish, and the agar left to set. The effect of the different concentrations of each enantiomer on the linear growth of *P. exigua* was compared with that of basal medium. To test growth, agar plugs 3 mm in diameter were cut from the edge of colonies, of 4 stock cultures of *P. exigua*, using a sterilised cork borer (Figure 19). From each stock culture, 4 agar plates at

Figure 19 P. exigua culture dish from which plugs of agar, containing a sample of the fungus have been taken for inoculation of the R-(-)- and S-(+)- 2-AB spiked, agar plates.

Figure 20 Typical plates illustrating the comparable antifungal activity of R-(-)- and S-(+)- 2-AB against P. exigua, and the degree of control of linear growth at each concentration.



each concentration, were inoculated by inserting a single plug in the centre of each plate. All the plates were then incubated at 18-20°C for 7 days, after which the amount of growth which had taken place from the initial plug in the centre of each dish, was assessed by measuring the colony diameter. Table 40 shows the results for linear growth of P. exigua on agar media, spiked with the various concentrations of each enantiomer. Figure 20 shows typical plates from the experiment. From Table 40, it is clear that R-(-)- 2-AB is more active against P. exigua than S-(+)- 2-AB, since for each stock culture (isolate), at each of the three S-(+)- amine concentrations, growth is uninhibited and corresponds to that observed for the control plates (basal medium). For the agar plates spiked with R-(-)- 2-AB; at 5.0 ppm there was a slight reduction in growth, and at 50.0 ppm very definite growth inhibition.

The above experiment identified R-(-)- 2-AB as the more active component of racemic 2-AB, but gave no indication of the relative activities of the optical isomers, since at the concentrations investigated no effect on fungal growth by S-(+)- 2-AB was observed. Further work was therefore carried out using a wider concentration range, in order to measure the activities of both isomers and to compare these with the activity of the racemic 2-AB mixture.

Table 40 Response of 4 isolates of *P. exigua* var. *foveata* to a series of concentrations of R-(-)- and S-(+)- 2-AB.

Amine concentration ($mg\ litre^{-1}$)	Isolate 1 Linear growth[b]	Isolate 2 Linear growth[b]	Isolate 3 Linear growth[b]	Isolate 4 Linear growth[b]
	% of basal agar (control)[a]			
Control	100	100	100	100
S-(+) 0.5	100	96	97	96
S-(+) 5.0	100	94	99	99
S-(+) 50.0	102	100	100	98
R-(-) 0.5	100	98	96	98
R-(-) 5.0	89	89	95	91
R-(-) 50.0	25	32	44	46

a. Each value is expressed as a percentage of the response observed for the control plates.

b. Linear growth was determined by measuring colony diameter (mm).

The second experiment compared the antifungal properties of R-(-)-, S-(+)- and racemic 2-AB against P. exigua. As before 1 litre sterile agar solutions were prepared, and to each was added sufficient amine to produce solutions of either R-(-)- , S-(+)- or racemic 2-AB, at 5, 50, 200 or 500 ppm. The spiked agar media was then poured into plates and left to set. The effect of each amine, at each concentration, on the linear growth and spore germination of P. exigua was investigated as follows:-

a) P. exigua - linear growth : Using the plug method of inoculation described above, agar plates containing one isomer or racemic 2-AB, were inoculated with one of 3 isolates of P. exigua. The plates were incubated for 7 days at 18-20°C, and then the growth was assessed by measuring the colony diameter on each plate.

b) P. exigua - spore germination : To assess the effect of R, S and racemic 2-AB on spore germination, serial dilutions of filtered spore suspensions were made, and aliquots (0.5 cm^3 - containing up to 100 spores cm^{-3}) were added to each test plate. The plates were incubated for 7 days at 18-20°C, and then the degree of control assessed by estimating the total % surface area covered by growth.

In both the linear growth and spore germination tests, a set of control plates containing no added amine, was inoculated and incubated following the same procedure outlined above. The results from these studies, comparing the response of 3 isolates of P. exigua with a series of concentrations of the two amine enantiomers and the commercial racemic 2-AB mixture, are shown in Table 41. The results clearly illustrate that R-(-)- 2-AB is more active than racemic 2-AB, which in turn provides better control than the S-(+)- isomer, both in preventing spore germination and limiting growth of P. exigua. The R-(-)- 2-AB appears to be at least 10 times more active than the corresponding S-(+)- 2-AB in controlling linear growth, but the difference in the degree of control of spore germination is less pronounced. Figure 21 shows typical plates from the above experiment and illustrates the difference in activity, against linear growth of P. exigua, exhibited by S-(+)- , R-(-)- and racemic 2-aminobutane.

A third experiment was set up to investigate the effect of both enantiomers and the racemic 2-AB mixture, on the linear growth and spore germination of Polyscytalum pustulans, the fungus responsible for skin spot in potatoes. The procedure for preparing the amine-spiked agar plates, was as described above and the

Table 41 Response of three isolates of *Phoma exigua* var. *foveata* to a series of concentrations of R-(-)-, S-(+)- or racemic (±) 2-AB.

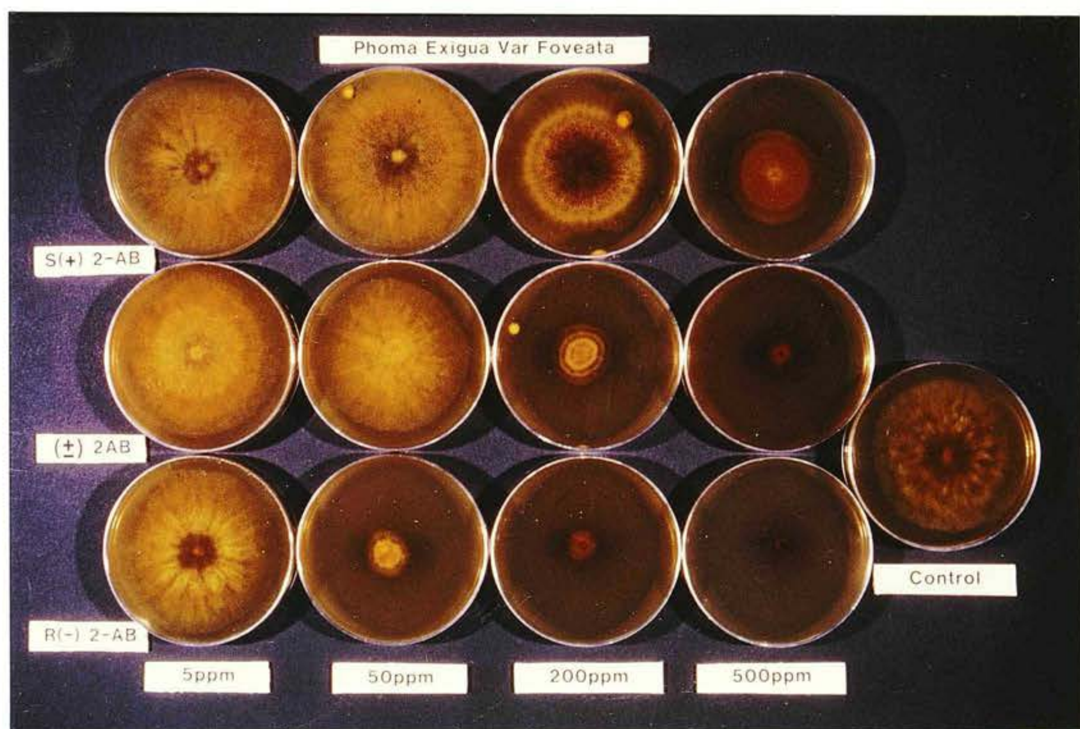
	% of basal agar (control)[a]				
	Isolate 1		Isolate 2		Isolate 3
Amine concn mg l^{-1}	Linear growth[b]	Spore germination[c]	Linear growth[b]	Spore germination[c]	Linear growth[b]
Control	100	100	100	100	100
S-(+) 5	107	103	101	100	106
S-(+) 50	116	101	107	100	112
S-(+) 200	115	100	107	98	101
S-(+) 500	64	12	49	0	58
R-(-) 5	106	97	101	100	99
R-(-) 50	27	89	92	94	39
R-(-) 200	20	1	0	3	23
R-(-) 500	0	0	0	0	0
± 2AB 5	103	98	100	101	100
± 2AB 50	86	97	101	101	105
± 2AB 200	39	26	48	15	47
± 2AB 500	18	0	0	0	21

a. Each value for linear growth or spore germination is expressed as a percentage of the response observed for the control plates.

b. Linear growth was determined by measuring colony diameter (mm).

c. Spore germination was assessed by estimating the percentage of total surface area covered by *P. exigua* colonies.

Figure 21 Typical plates illustrating the difference in linear growth of P. exigua, on agar plates containing R-(-)-, S-(+)- or racemic (±)2-AB.



experiment was set up as follows:-

a) P. pustulans - linear growth : Agar plates containing 5, 50, 200, or 500 ppm of either 2-AB enantiomer or the racemic mixture, were inoculated using the agar plug method described above, with one of two isolates of P. pustulans. The plates were incubated for 3 weeks at 20°C, and then the amount of linear growth was assessed by measuring the diameter (mm) of the colony.

b) P. pustulans - spore germination : Again, plates at each concentration of R-(-)- , S-(+)- or racemic 2-AB were inoculated with an aliquot (0.5 cm^3) of a solution containing spores of P. pustulans (approx. 100 spores cm^{-3}). The plates were incubated for 3 weeks at 18-20°C and the degree of spore germination was then assessed, by counting the number of colonies on each plate.

As in previous experiments, a series of control plates (basal medium only) were prepared, inoculated and incubated, following the same procedure outlined above. Table 42 shows the results for the P. pustulans experiment and again shows the same order of activity; R-(-)- 2-AB > racemic 2-AB > S-(+)- 2-AB.

Table 42 Response of two isolates of Polyscytalum pustulans to a series of concentrations of R-(-)-, S-(+)- or racemic (±) 2-AB.

Amine Concentration (mg litre^{-1})	% of basal agar (control)[a]		
	Isolate 1	Isolate 2	
	Linear growth[b]	Linear growth[b]	Spore germination[c]
Control	100	100	100
S-(+) 5	87	90	129
S-(+) 50	59	78	149
S-(+) 200	49	52	122
S-(+) 500	36	35	27
R-(-) 5	104	72	82
R-(-) 50	32	12	27
R-(-) 200	0	0	0
R-(-) 500	0	0	0
(±)2AB 5	104	86	122
(±)2AB 50	89	78	89
(±)2AB 200	4	0	0
(±)2AB 500	0	0	0

a. Each value for linear growth or spore germination is expressed as a percentage of the response observed for the control plates.

b. Linear growth was determined by measuring colony diameter (mm).

c. Spore germination was assessed by estimating the number of colonies on each plate.

The above experiments demonstrate that racemic 2-AB, which is normally used for commercial potato fumigation, can provide control of growth and spore germination of the fungi which cause gangrene and skin spot. Compared to other established fungicides however, quite high concentrations of 2-AB are required. For example, similar fungicidal activity experiments, using thiabendazole against P. exigua[69], showed 70% of control growth at 1 ppm and complete growth inhibition at 5 ppm (c.f. results for racemic 2-AB in Table 41). This difference in activity is reflected in the commercial doses required for effective disease control in potato tubers, namely 200 $mgkg^{-1}$ for 2-AB and only 40 $mgkg^{-1}$ for thiabendazole.

It is also clear that the R-(-)- enantiomer provides a higher degree of control than the S-(+)- isomer, or the racemic amine. This means that if commercial fumigations were carried out using only the R-(-)- enantiomer, the same level of disease control achieved with racemic 2-AB, could be obtained with a lower initial amine dosage. The resulting decrease in 2-AB residues in fumigated tubers would make the treatment of ware tubers more acceptable. However, the high cost of the resolved material means that, at present, this is not a commercially viable proposition.

I. Summary of conclusions.

1. The rate and extent of 2-AB absorption by tubers, is largely controlled by the amine vapour concentration inside the fumigation chamber.

2. Absorption of 2-AB by tubers occurs throughout the duration of the fumigation. The rate of absorption is greater at the start of the fumigation, when the amine vapour concentration is at a maximum. As the amine vapour concentration is reduced by absorption on tubers, the rate of absorption decreases accordingly.

3. Penetration of 2-AB into tubers is limited to the first 1 cm of tissue, with highest concentrations recorded in the surface skin (periderm).

4. Preferential uptake of 2-AB occurs through tuber lenticels, especially when the lenticels are open. Lower levels of absorption occur through areas of the periderm free of lenticels.

5. Levels of absorption are higher in damaged tubers, probably due to the moisture in fresh wounds aiding the uptake of water soluble 2-AB, but no deeper penetration takes place.

6. 2-AB residues in damaged tubers left to heal before fumigation, showed that wound healing is much more effective at 10-15°C, than at the normal long-term storage temperature of 5°C. This confirms the advisability of a suitable curing period, prior to fumigation, to avoid chemical damage occuring on wounded tubers.

7. Both optical isomers of 2-AB are absorbed, by tubers, to the same extent.

8. T.l.c. studies on extracts from fumigated tubers, indicated that 2-AB is not metabolised by tubers during the normal storage period.

9. Loss studies confirmed that absorbed 2-AB persists in tubers throughout the duration of the storage period.

10. Transfer of absorbed 2-AB from tuber to tuber during air recirculation is very limited.

11. Uptake and penetration of 2-AB, when applied as a glycollate salt, in the commercial "Storite Plus" formulation, is comparable to that observed for free 2-AB vapour.

12. Absorption of 2-AB by soil, straw and wood is appreciable and will contribute to reduced efficiency of treatment in bulk commercial fumigations.

13. The R-(-)- enantiomer of 2-aminobutane is more active than the corresponding S-(+)- isomer, or racemic mixture, in controlling the fungi responsible for gangrene and skin spot in potato tubers.

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