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STUDIES ON γ-RADIOLYSIS OF SULPHUR CONTAINING AMINO ACIDS—II

VOLATILE PRODUCTS FROM S-*n*-PROPYL-L-CYSTEINE SULPHOXIDE AND S-ALLYL-L-CYSTEINE SULPHOXIDE IRRADIATED IN OXYGEN-FREE AQUEOUS SOLUTIONS

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Abstract—In connection with flavour deterioration accompanied by food irradiation, the volatile products from sulphoxide amino acids, S-n-propyl-L-cysteine sulphoxide (PCSO) and S-allyl-L-cysteine sulphoxide (ACSO) irradiated in oxygen-free aqueous solution were analysed. The major volatile products formed by radiolysis of PCSO were di-n-propyl sulphide and di-n-propyl disulphide, and those derived from ACSO were allyl alcohol, n-propyl allyl sulphide and diallyl sulphide, which were isolated by GLC and identified by using mass spectrometry. From the experiments with N_2O or KBr addition during irradiation, i.e., from the scavenger effects, the formation processes of volatiles were partly elucidated.

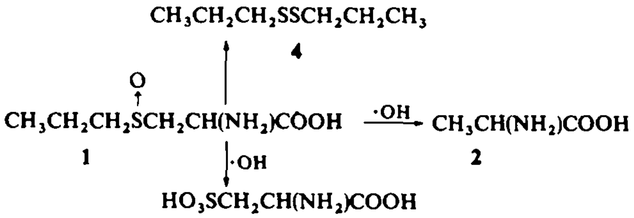
IN RECENT years, many works on the sprout inhibition of onion by γ-irradiation have been made in relation to its preservation.[1] Though it is generally said that the characteristic flavour of onion is made mild by γ-irradiation, the effect of irradiation on onion flavour has not yet been investigated chemically.

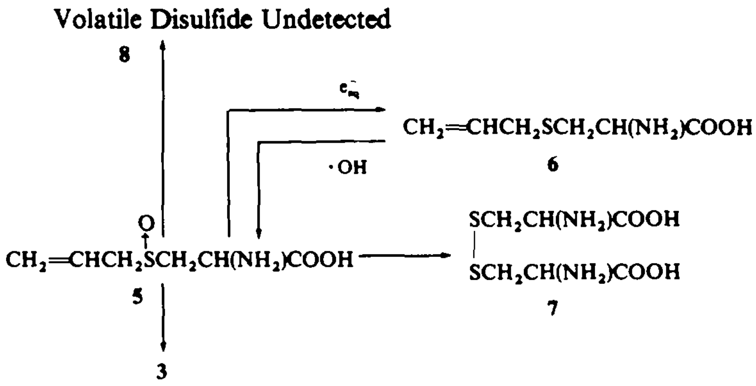
As sulphur containing amino acids are known to be highly sensitive to radiation,[2] it is of interest to investigate the radiolysis of sulphur containing amino acids.

The authors have investigated γ-radiolysis of characteristic sulphoxide amino acids in *Allium* species, e.g., PCSO (1) (flavour precursor of onion) and ACSO (5) (flavour precursor of garlic) as similar L-cysteine derivatives.

In the previous report,[3] alanine (2), cysteic acid (3) and di-n-propyl disulphide (4) as main products from irradiated PCSO in oxygen-free aqueous solution were identified by IR and mass spectrometry. Similarly, S-allyl-L-cysteine (6), cysteic acid (3) and cystine (7) as main products from irradiated ACSO in oxygen-free aqueous solution were identified. Moreover, irradiated PCSO solution exhibited onion-like odour and its putrid odour, and irradiated ACSO solution did not exhibit garlic odour but rust-like odour though ACSO is a precursor of garlic flavour. The fact that diallyl disulfide (characteristic garlic odour) has not been produced from ACSO by irradiation seems to be in agreement with the above-mentioned results.

In this paper the authors deal with the identification and the formation mechanisms of volatile product (p_1) from irradiated PCSO and main volatile products (A_1, A_2, A_3) from irradiated ACSO, by using GLC and MS.





RESULTS AND DISCUSSION

The gas chromatograms of volatile products from irradiated PCSO and ACSO are shown in Fig 1. As the experiments were carried out at the rather high sensitivity condition of the gas chromatographic response, the chromatograms shown in Fig 1

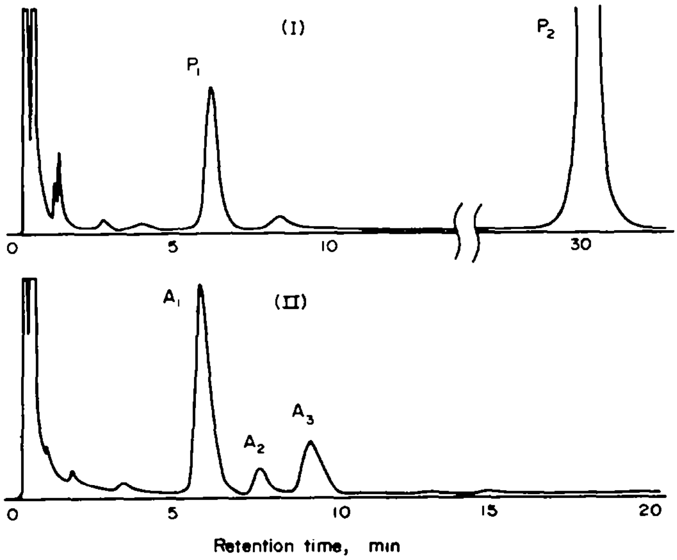


FIG. 1

is slightly different from the previous gas chromatographic data in the minor products (A_2 and A_3).[3]

To collect the fractions separated by gas chromatdgraphy, a FID gas chromatograph inserted with a splitter was also used under the same condition, and the fractions (peaks P_1, A_1, A_2 and A_3) were trapped in glass capillary tubes cooled with dry ice–ethanol. The mass spectrum of each fraction was obtained.

Mass spectra of the fractions P_1, A_1, A_2 and A_3 are shown in Figs 2, 3, 4 and 5, respectively. Moreover, these volatile products were identified by comparing with mass spectra of the authentic compounds.

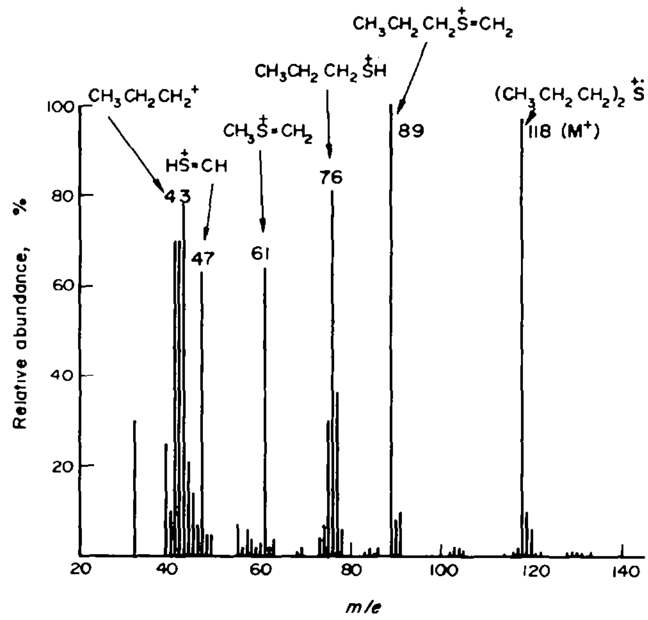


FIG. 2

From these results, volatile products P_1, A_1, A_2 and A_3 were identical with di-n-propyl sulphide (M^+ 118), allyl alcohol (M^+ 58), n-propyl allyl sulphide (M^+ 116) and diallyl sulphide (M^+ 114), respectively.

The radiation-induced decomposition of water in the absence of oxygen is

$$H_2O \xrightarrow{\gamma\text{-ray}} H_2, H_2O_2, \cdot OH, e_{aq}^-, H_3O^+$$

Of these chemical species, the roles of hydroxy radical ($\cdot$OH) and hydrated electron (e_{aq}^-) are most important in the radiation chemical reactions.

In order to elucidate the degradation mechanisms of deoxygenated volatile products (di-n-propyl) sulphide and di-n-propyl disulphide) from irradiated PCSO, 20 mM oxygen-free aqueous solution of PCSO was irradiated after N_2O (specific scavenger for e_{aq}^-)[4] or KBr (specific scavenger for $\cdot$OH)[5] of the various concentrations had been added. The yield of di-n-propyl sulphide increased with increasing concentration of N_2O and decreased with increasing concentration of KBr, while that of di-n-propyl disulphide decreased with increasing concentration of either N_2O or KBr. Therefore it is considered that PCSO reacts with $\cdot$OH radical to produce

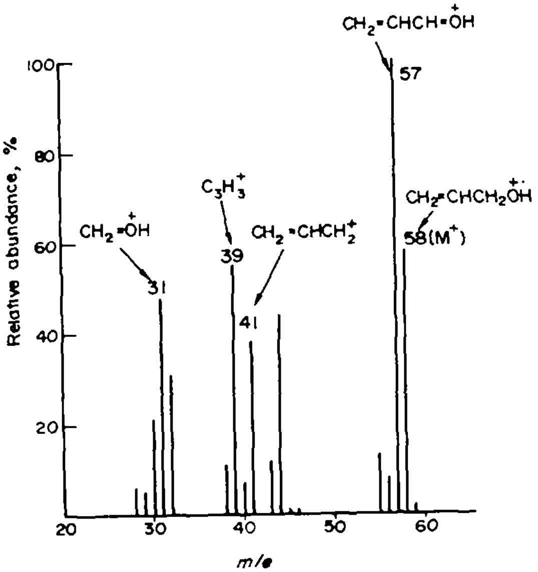

CH2=CHCH=OH+
57
100
80
60
40
20
Relative abundance, %
C3H3+
39
CH2=CHCH2+
41
CH2=OH+
31
CH2=CHCH2OH+·
58(M+)
20
30
40
50
60
m/e

FIG. 3

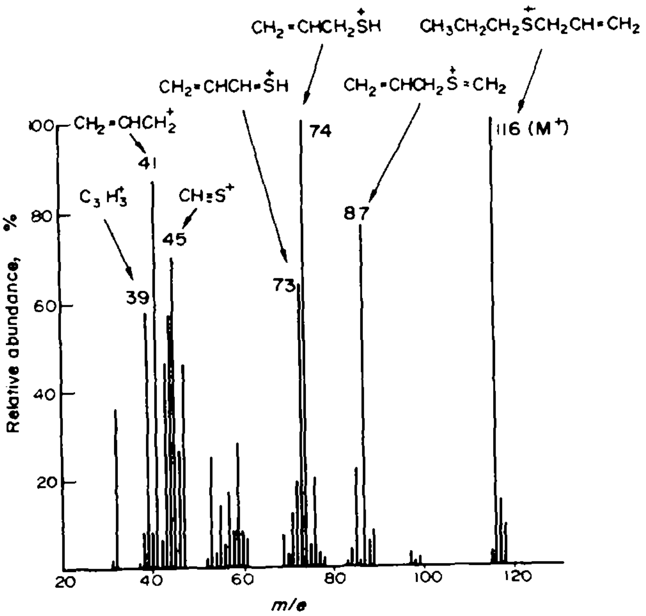

CH2=CHCH2SH+
CH3CH2CH2SCH2CH=CH2+
CH2=CHCH=SH+
CH2=CHCH2S=CH2+
74
116 (M+)
100
CH2=CHCH2+
41
C3H3+
CH≡S+
45
80
87
73
39
60
40
20
Relative abundance, %
20
40
60
80
100
120
m/e

FIG. 4

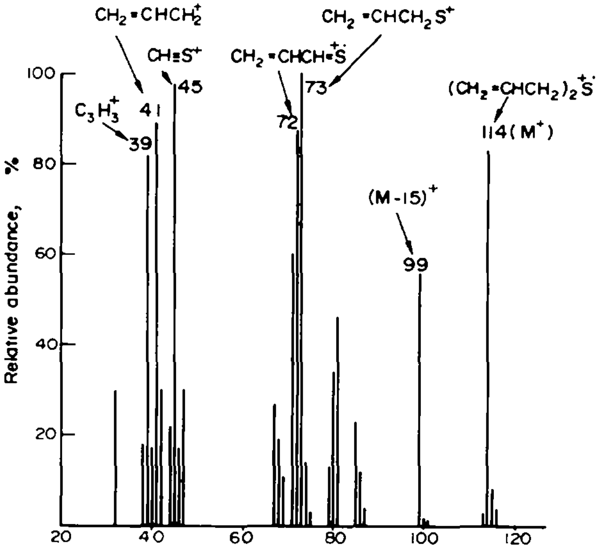


FIG. 5

di-n-propyl sulphide (**10**), and with both e_{aq}^- and ·OH to produce di-n-propyl disulphide. Though it seems that deoxygenated volatile products from irradiated PCSO are yielded *via* s-n-propyl-L-cysteine(**9**), it is also considered that those are directly produced from irradiated PCSO by reason of a little yield of S-n-propyl-L-cysteine.

On the other hand, the degradation mechanisms of volatile products from irradiated ACSO would not be elucidated completely, because the adequate information couldn't be obtained from our present study. However, the yield of allyl alcohol (**11**) from irradiated ACSO is considerably greater than that from irradiated S-allyl-L-cysteine (**6**), and it is considered that this is due to existence of oxygen in sulphoxide.

Moreover, in contrast with the formation of allyl alcohol from irradiated ACSO, the evidence that n-propyl alcohol isn't produced from irradiated PCSO means that the bond of S—C (allyl moiety) in ACSO is more easily cleaved than that of S—C (n-propyl moiety) in PCSO.

Proposed schemes of the radiolysis processes of PCSO and ACSO are shown in Figs 6 and 7 respectively. Main processes are represented with a thick line.

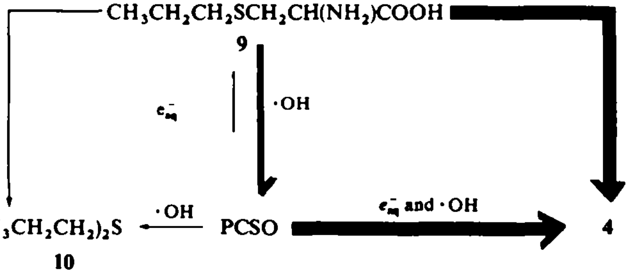


FIG 6. Formation of main volatile components from PCSO irradiated in oxygen-free solution

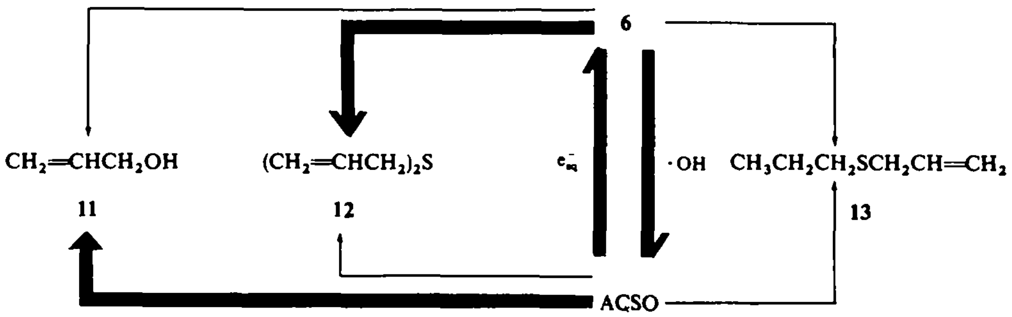


FIG 7. Formation of main volatile components from ACSO irradiated in oxygen-free solution.

EXPERIMENTAL

Irradiation. Irradiation was carried out by exposure to γ-rays from cobalt-60 of 3800 Ci at dose rate of $7{\cdot}28 \times 10^4$ rad/hr at room temp. The neutral solns of sulphoxide amino acids (20 mM) dissolved in triply distilled water were placed in stoppered Pyrex tubes and irradiated to the extent of 1×10^6 rad under oxygen-free condition by bubbling purified N_2 prior to irradiation.

Irradiations in the presence of N_2O or KBr were also done for investigations relating to the degradation mechanism.

Separation and identification of the irradiation products. Gas chromatography of volatile products by using Yanagimoto model GCG-500 PF gas chromatograph was undertaken with the following conditions: Column, 20% Reoplex 400 on 40–60 mesh acid washed C-22 (5mm × 1·5m); temp, 100° (injection port 170°); carrier gas (N_2), 22ml/min. Vapour samples for gas chromatographic analysis were obtained from head space of the glass tubes of the irradiated samples after standing at 40° for 5 min. To obtain the volatile products separated, a FID gas chromatograph in which a splitter was inserted was also used under the same condition, and each fraction was collected in a glass trap cooled with dry ice-EtOH.

In order to identify such the purified components, a Hitachi model RMU-6 mass spectrometer was used.

(±)*S-Alkyl-L-cysteine sulfoxide (PCSO and ACSO).* The synthetic procedure used has already been reported in the previous paper.[3] PCSO (1): m.p. 195–198° (dec); IR ν_{max}^{KBr} cm^{-1}; 2940–2820, 2620, 2110 (NH_3^+), 1580 (COO^-), 1012 (sulphoxide). (Found: C, 39·7; H, 7·21; N, 7·73. $C_6H_{13}NO_3S$ requires: C, 40·2; H, 7·26; N, 7·82%). ACSO (5); m.p. 165° (dec); IR ν_{max}^{KBr} cm^{-1}; 3020–2860, 2060 (NH_3^+), 1580 (COO^-), 1020 (sulphoxide), 990 and 915 (allyl double bond). (Found: C, 39·2; H, 6·13; N, 7·74. $C_6H_{11}NO_3S$ requires: C, 40·7; H, 6·23; N, 7·91%).

Di-n-propyl sulphide (**10**). Di-n-propyl sulphide was prepared from n-propyl bromide according to the method of Shriner *et al.*[6] in preparing dibenzyl sulphide from benzyl chloride with Na_2S, b.p. 142°; IR ν_{max} cm^{-1} 2990 and 2890 (methyl C—H stretching vibration), 2960 (—CH_2—), 1460 (C—H vas); MS *m/e*: 118 (M^+), 89 ($CH_3CH_2CH_2\overset{+}{S}{=}CH_2$), 76 ($CH_3CH_2CH_2\overset{+\cdot}{S}H$), 61 ($CH_3$—$\overset{+}{S}{=}CH_2$), 47 ($H\overset{+}{S}{=}CH_2$), 43 ($CH_3CH_2CH_2^+$). (Found: C, 61·10; H, 11·92. $C_6H_{14}S$ requires: C, 61·02; H, 11·86%).

Diallyl sulphide (**12**). Diallyl sulphide was prepared by the above-mentioned method, b.p. 139°; IR ν_{max} cm^{-1}: 3125 and 2975 ($CH_2{=}$), 3020 ($=CH$—), 1645 (double bond), 990 and 913 (allyl double bond); MS *m/e*: 114 (M^+), 99 (M^+-15), 73 ($CH_2{=}CHCH_2S^+$), 72 ($CH_2{=}CH$—$CH{=}S^+$), 45 ($CH{\equiv}S^+$), 41 ($CH_2{=}CHCH_2^+$), 39 ($C_3H_3^+$). (Found: C, 63·30; H, 8·76. C_6H_{10} requires: C, 63·16; H, 8·77%).

n-Propyl allyl sulphide (**13**). The synthetic procedure used is a modification of the method of Kirner and Richter[7] in preparing α-furfuryl ethyl sulphide from furfuryl mercaptide with ethyl bromide, b.p. 140°; IR ν_{max} cm^{-1}: 3125 ($CH_2{=}$), 2990 and 2890 (methyl C—H stretching vibration), 2950 (—CH_2), 1645 (double bond), 989 and 912 (allyl double bond); MS *m/e*: 116 (M^+), 87 ($CH_2{=}CHCH_2\overset{+}{S}{=}CH_2$), 74 ($CH_2{=}CHCH_2\overset{+\cdot}{S}H$), 73 ($CH_2{=}CHCH{=}\overset{+}{S}H$), 41 ($CH_2{=}CH$—$CH_2^+$). (Found: C, 61·90; H, 10·39. $C_6H_{12}\overset{+}{S}$ requires: C, 62·07; H, 10·34%).

Di-n-propyl disulphide (**4**). This compound was prepared by the application of synthetic procedure of *p,p'*-dinitrophenyl disulphide[8] from *p*-nitrophenyl chloride with Na_2S_2, b.p. 195°; IR ν_{max} cm^{-1}: 2990 and 2890 (methyl C—H stretching vibration), 2950 (—CH_2—), 600–800 (C—S), MS *m/e*: 150 (M^+), 108 ($CH_3CH_2CH_2S\overset{+\cdot}{S}H$), 66 ($HS\overset{+\cdot}{S}H$), 43 ($CH_3CH_2CH_2^+$). (Found: C, 48·02; H, 9·29. $C_6H_{14}S_2$ requires: C, 48·00; H, 9·33%).

Diallyl disulphide (**8**). This compound was derived from allyl bromide by the above-mentioned method, b.p. 173°; IR ν_{max} cm^{-1}: 3125 and 2960 (CH_2=), 3020 (=CH—), 1645 (double bond), 989 and 915 (allyl double bond); MS *m/e*: 146 (M^+), 105 (CH_2=$CHCH_2SS^+$), 73 (CH_2=$CHCH_2S^+$), 41 (CH_2=$CHCH_2^+$). (Found: C, 49·43; H, 7·04. $C_6H_{10}S_2$ requires: C, 49·31; H, 6·85%).

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REFERENCES

[1] J. P. Skou, *Kernteknik* **8**, 510 (1966)
[2] J. Kolousek, J. Liebster and A. Babicky, *Nature, Lond.* **179**, 521 (1957)
[3] H. Nishimura, S. Kawakishi and M. Namiki, *Agr. Biol. Chem. Japan* **34**, 609 (1970)
[4] J. H. Baxendale, *Radiation Res.* **17**, 312 (1962)
[5] G. E. Adams and J. W. Boag, *Proc. Chem. Soc.* 1112 (1964)
[6] R. L. Shriner, H. C. Struck and W. J. Jorison, *J. Am. Chem. Soc.* **52**, 2060 (1930)
[7] W. R. Kirner and G. H. Richter, *Ibid.* **51**, 3131 (1929)
[8] M. T. Bogert and A. Stull, *Org. Syntheses* Coll. Vol. I, p. 220 (1941)