to $10^{-3}$ mm as indicated by the McLeod gauge. The gas sample was then measured and injected into the gas chromatograph.

Using this apparatus, it was found that volumes of gases between 0.04 ml and 1.0 ml at N.T.P. could be analyzed. The usual size of gas sample produced by the radiolysis of organic compounds was, in the present work, of the order of 0.1 ml. It was found that this could be separated and that each component could be analyzed quantitatively, the accuracy of the analysis being dependent on the size and composition of the sample. In a typical experiment where methyl cyanide was irradiated with gamma-rays, the only gaseous radiolysis products were shown to be methane and hydrogen: each component in a 0.1-ml sample was analyzed with an accuracy of $\pm$ 5%.

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## Paper chromatography of some 2,4-dinitrophenyl S-alkyl-(L)-cysteines and corresponding sulfoxides

Recently we reported on the isolation of (+)S-methyl- and (+)S-*n*-propyl-(L)-cysteine sulfoxides from the onion (*Allium cepa*) as 2,4-dinitrophenyl derivatives[1]. In the course of this work, derivatives of some other S-alkyl-(L)-cysteines were also synthesized and studied. Before these compounds could be isolated in pure form by silicic acid chromatography, it was necessary to determine their chromatographic behavior and the feasibility of separating them from neutral amino acids obtained coincidentally from onion extracts.

This report describes the paper chromatography of the N-2,4-dinitrophenyl derivatives of these amino acids. Chromatography of N-2,4-dinitrophenylamino acids[2] has been widely used in end-group determinations of proteins and peptides and composition of protein hydrolysates[3]. The highly colored derivatives are easily detected on the chromatograms and can be eluted and measured colorimetrically[4,5].

However, little has been reported on its application to studies of complex mixtures of free amino acids in plant preparations.

We have found that N-2,4-dinitrophenyl-S-alkyl-(L)-cysteine sulfoxides crystallize readily and can be separated from corresponding dinitrophenyl-S-alkyl-(L)-cysteines and some other neutral amino acids. These findings have enabled us to separate the material on silicic acid columns and to use paper chromatograms to monitor the silicic acid chromatography.

Solvent systems reported in the literature[3–8] were, for the most part, unsatisfactory, because the spots migrated too quickly for effective separation or streaked too badly. Efforts to find a single solvent system that would resolve the mixtures satisfactorily were finally abandoned in favor of several solvent mixtures, which gave excellent results for the purposes to which they were applied.

*Experimental*

Preliminary paper chromatograms indicated that the dinitrophenyl-amino acid mixtures could be separated into a fast fraction containing the S-alkyl-(L)-cysteine derivatives (Table I) and a slow fraction containing the corresponding cysteine sulfoxides (Table II). The fast fraction could be resolved in a 16- to 24-h chromatogram. On the other hand, the slow fraction required a development time of 60 to 72 h with the fastest solvent for full resolution. Because of the long development time, the solvents were allowed to drip off the ends of the chromatograms, and the relative $R_F$ values of the dinitrophenyl derivatives were calculated relative to that of N-2,4-dinitrophenyl-serine as an internal standard (Tables I and II).

Four solvent systems were used:

*Ethyl acetate–phosphate buffer.* Ethyl acetate saturated with pH 6.18 *M*/4 Sørensen buffer[11] was used in the preparative silicic acid column[1,9,10] as well as with

TABLE I

RELATIVE $R_F$'S OF DNP-S-ALKYL-(L)-CYSTEINES AND SOME INTERFERING NEUTRAL AMINO ACIDS (FAST FRACTION)[a]

| *Compound* | *EtOAc–phosphate*[b] | *EtOAc–phthalate*[b] | *Heptane–propanol–phosphate*[c] | *Collidine–isoamyl alcohol–ammonia*[d] |
|---|---|---|---|---|
| S-Methyl-(L)-cysteine | 4.8 | 4.7 | 4.3 | 2.0 |
| S-Allyl-(L)-cysteine | 5.8 | 6.8 | 7.4 | 2.6 |
| S-*n*-Propyl-(L)-cysteine | 5.7 | 7.9 | 9.3 | 2.7 |
| Proline | 5.4 | 8.0 | 6.3 | 1.6 |
| Pipecolic acid | 8.3 | 12.7 | 9.8 | 3.1 |
| Methionine | 4.5 | 6.7 | 6.7 | 2.4 |

[a] Calculated relative to $R_F$ DNP-serine at 23°.
[b] S & S No. 589 Blue Ribbon paper; 8 h.
[c] Whatman No. 1 paper; 24 h.
[d] S & S Blue Ribbon paper; 24 h.

paper chromatograms. In the isolation of pure S-methyl- and S-*n*-propyl-cysteine sulfoxides, this solvent system consistently gave the best results. The fast fractions were eluted off rapidly and effectively, thus eliminating them as possible contaminants. Excessive amounts of asparagine and glutamine in the onion extracts interfered with

the separation of S-*n*-propyl-(L)-cysteine sulfoxide on the silicic acid column. However, the problem was not encountered on paper.

*n-Heptane–n-propanol–phosphate.* Heptane and propanol were mixed with the pH 6.18 *M*/4 Sørensen buffer (60:40:5) to give a single-phase system. Chromatograms developed with this mixture showed a slower migration rate with less distortion of spots. Whenever good resolution of fast-fraction components was desired, the mixture was very satisfactory if used with Whatman No. 1 paper.

*Ethyl acetate–phthalate buffer.* Ethyl acetate saturated with a pH 4.6 *M*/5 phthalate buffer[12] was compared with the phosphate buffered ethyl acetate. Results indicated a similarity in solvent behavior except that with the phthalate buffer the fast fraction moved faster and the slow fraction more slowly. There was less tendency toward streaking. Although the overall degree of resolution was not significantly improved, use of both ethyl acetate mixtures was rewarding in that one could readily resolve components that the other could not.

TABLE II

RELATIVE $R_F$'S OF DNP-S-ALKYL-(L)-CYSTEINE SULFOXIDES AND SOME INTERFERING NEUTRAL AMINO ACIDS (SLOW FRACTION)[a]

| *Compound* | *EtOAc–phosphate*[b] | *EtOAc–phthalate*[c] | *Heptane–propanol–phosphate*[d] | *Collidine–isoamyl alcohol–ammonia*[e] |
|---|---|---|---|---|
| S-Methyl-cysteine sulfoxide | 0.10 | 0.08 | 0.64 | 0.58 |
| S-Allyl-cysteine sulfoxide | — | 0.31 | — | 1.12 |
| S-*n*-Propyl-cysteine sulfoxide | 0.49 | 0.30 | 2.4 | 1.51 |
| Asparagine | 0.28 | 0.28 | 0.43 | 0.43 |
| Glutamine | 0.28 | 0.74 | 0.63 | 0.49 |

[a] $R_F$'s calculated relative to $R_F$ DNP-serine at 23°.
[b] S & S No. 589 Blue Ribbon paper; 16 h.
[c] S & S No. 589 Blue Ribbon paper; 24 h.
[d] Whatman No. 1 paper; 24 h.
[e] S & S No. 589 Blue Ribbon paper; 48 h.

*Collidine–isoamyl alcohol–ammonia.* This solvent mixture is adapted from one described by BISERTE AND OSTEUX[3]. The substitution of collidine for pyridine (without changing the 6:14:20 ratio), gave improved resolution. The migration rates were very slow, especially on Schleicher & Schüll, No. 589 Blue Ribbon paper. Chromatograms at 23° produced significant resolution of the fast fractions in 24 h, but 48 h were required for maximum resolution. For the slow fractions, even longer periods, up to 72 h, were required. However, these chromatograms were the most definitive and gave a resolution of the mixtures not obtainable with the other solvents.

For resolving the dinitrophenyl-S-alkyl-cysteine derivatives, the ethyl acetate–phthalate buffer solvent was particularly good for components of the fast fraction (Table I). The results, when viewed in conjunction with paper chromatograms of the slow fraction (Table II) developed with either the ethyl acetate–phosphate buffer or collidine solvent systems, presented a more complete picture of the behavior of these compounds. The collidine solvent system could probably be used to demonstrate the entire series of derivatives described, but the operation was much too slow. In

addition, there was only a partial resolution of dinitrophenyl S-allyl- and S-propyl-cysteines.

Actually, the mixture, *n*-heptane–*n*-propanol–phosphate buffer, approached closest to being the ideal single solvent. This was particularly true when the solvent was used to develop chromatograms on Whatman No. 1 paper, as the results show in Tables I and II. In spite of its apparent usefulness on paper, the *n*-heptane mixture was not satisfactory for silicic acid column work. When applied to the silicic acid column, development and elution of the various fractions were prohibitively slow. Furthermore, the dinitrophenyl-cysteine derivatives were only sparingly soluble in this medium. Since the primary objective was to prepare pure samples in sufficient amounts for further studies, the limitation on capacity negated use of the solvent.

The effect of paper was examined. Whatman No. 4 and No. 3MM[6] proved too fast, giving chromatograms with poor separation and bad streaking. Whatman No. 1 streaked badly with the phosphate-buffered ethyl acetate and somewhat with ethyl acetate–phthalate, but was very good when used with the heptane–propanol solvent. On the other hand, Schleicher & Schüll No. 589 Blue Ribbon paper (an acid washed paper) was particularly good with the buffered ethyl acetate solvents. Streaking was eliminated and the speed of the solvents tempered with resultant good resolution. This paper was used with the collidine mixture and, though very slow, gave the excellent results mentioned earlier. When a buffered solvent was used, the paper was dipped in a solution of the buffer and air dried before being spotted with the samples. Untreated papers were invariably unsatisfactory.

It is interesting to note that, in contrast to the behavior of methionine, the 2,4-dinitrophenyl-cysteines did not appear to undergo oxidation to corresponding sulfoxides during the time required to chromatograph them on the silicic acid columns.

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