Die Synthese von (IV) gelang wie folgt: 2-Amino-5-chlorbenzophenon (I),[1] aus welchem (V) aufgebaut wird,[2] liess sich mittels einer modifizierten Chinolinsynthese nach Skraup—von Campbell et al.[3] zur Herstellung von 8-Carboxychinolin benützt— zu 45 % in 5-Chlor-8-benzoylchinolin (II) überführen. (Andere 2-Aminobenzophenone scheinen bisher nicht nach Skraup umgesetzt worden zu sein, dagegen einige 4-Aminobenzophenone [4]). Die Hydrierung von (II) mit Raney-Nickel in EtOH bei Normaldruck verlief rasch und führte in 80 % Ausbeute zu 6-Chlor-8-benzoyl-1,2,- 3,4-tetrahydrochinolin (III). Trotz wiederholter Versuche unter verschiedenen Bedingungen liess sich (III) mit Bromacetylbromid nicht in das entsprechende N-Bromacetyl-Derivat überführen. Dies, und die ungewöhnlich langwellige Lage der CO-Bande im IR-Spektrum deuten auf das Vorliegen der tautomeren Form (IIIa) hin. Dagegen führte der Umsatz von (III) mit kochendem Essigsäureanhydrid/Pyridin glatt zu 6-Chlor-8-benzoyl-1-acetyl-1,2,3,4-tetrahydrochinolin vom Smp. 118—119°C. (IR-Spektrum: C=O 1650 cm^{-1} und 1665 cm^{-1} für CH$_3$CON<).

Zur Synthese von (IV) wurde (III) mit 4,6 Mol Glycinaethylester-hydrochlorid und katalytischen Mengen Piperidin während 50 Std. in Pyridin gekocht. Den schwarzen Verdampfungsrückstand schüttelte man mit verd. Salzsäure und Aether. Aus dem Letzteren gewann man so 77 % an nicht umgesetztem (III) zurück. Durch Alkalisieren der sauren Phase erhielt man das rohe (IV). Die Ausbeute an reinem Stoff betrug 18 % d. Th.

1. Sternbach, L. H., Reeder, E., Keller, O. und Metlesics, W. J. Org. Chem. 26 (1961) 4488.
2. Sternbach, L. H., Fryer, R. J., Metlesics, W., Reeder, E., Sach, G., Saucy, G. und Stempel, A. J. Org. Chem. 27 (1962) 3788.
3. Campbell, K. N., Kerwin, J. F., LaForge, R. A. und Campbell, B. K. J. Am. Chem. Soc. 68 (1948) 1844.
4. Diesbach, H., Pugin, A., Morard, F., Nowaczinski, W. und Dessibourg, J. Helv. Chim. Acta 35 (1952) 2322.

Separation of *Allium* Sulfur Amino Acids and Peptides by Thin-Layer Electrophoresis and Thin-Layer Chromatography

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In the course of an investigation of the sulfur metabolism in *Allium* species it was found necessary to develop an improved method for the analysis of sulfur amino acids and peptides. Using large amounts of plant material and time-consuming preparative methods, it has been possible to isolate a great number of onion sulfur compounds in this laboratory, and also to determine their structure.[1] After knowledge about the composition of onion in this way has been obtained, the rapid determination of these compounds on a micro scale has become important. The previously used two-dimensional paper chromatography [2-4] is rather time-consuming and does not allow the separation of all such compounds without decomposition, and paper chromatographic separation of some isomers has been difficult or even impossible. Consequently, there was a need for a more sensitive, rapid and non-artefact-producing method for the analysis of these compounds from plant extracts.

In two-dimensional separations, high-voltage electrophoresis in the first direction eliminates the need for removal of carbohydrates and salts from the extracts, thus making any initial ion exchange procedure unnecessary. Thin-layer technique improves the sensitivity about 50 times. Nybom separated plant amino acids by a two-dimensional thin-layer technique involving electrophoresis in the first direction.[5] Bieleski and Turner worked out an improved procedure including facilities for quantitative determination (ninhydrin reaction), autoradiography and heterogeneous liquid scintillation counting.[6] The present method for analysis of *Allium* sulfur compounds is essentially an adaptation of the procedure of Bieleski and Turner. However, several modifications have been made. Extraction and separation is rapid (two working days), no alkaline conditions are involved, and the use of tem-

peratures higher than room temperature may if necessary be avoided.

Methods. The tissue (about 100 mg) is extracted in the cold with methanol/chloroform/ water by a procedure similar to that of Bieleski and Turner.[6] An aliquot of the extract, equivalent to 5—10 mg fresh tissue, is placed as a narrow band (Fig. 1) on a cellulose-silica gel thin-layer plate (15 g cellulose powder MN 300 + 2.5 g silica gel H Merck + 100 ml water). Electrophoresis is performed at pH 2.0 in a formic acid-acetic acid buffer, the permissible voltage gradient being limited mainly by the efficiency of the cooling system. A Varsol cooling bath with an attached cooling coil provides efficient heat removal, and this coolant is compatible with formic and acetic acid buffers. 15—20 min runs are usually performed at 2100 V and 60 mA. Alternatively the plate may be cooled on a perspex box filled with crushed ice, according to Katz and Lewis.[7] This system, which is compatible with any kind of buffer, is sufficient for a 25 min run at 1500 V and 25—30 mA.

After electrophoresis, the plate is dried in a stream of air and the bands are concentrated to spots by a water run (Fig. 1). The chromatographic development in the second direction is in most cases performed efficiently by using two successive solvents. The first solvent is methyl ethyl ketone/pyridine/water/acetic acid
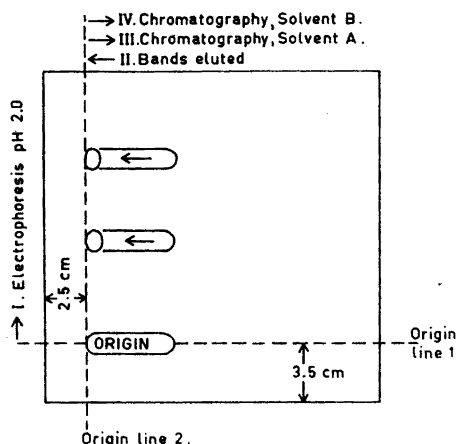

Fig. 1. Diagram of the procedure used in two-dimensional separation of amino acids by thin-layer electrophoresis and chromatography, according to Bieleski and Turner.[6]

710 + 5 + 15 + 2 (v/v), and the second propanol/ water/propyl acetate/acetic acid/pyridine 120 + 60 + 20 + 4 + 1 (v/v).[6]

In radiotracer work, plates are run in duplicate. Autoradiograms are produced on X-ray films with exposure time of about 3 days which is enough to reveal activities of 0.1 nC soft β-emitter. One plate is sprayed with ninhydrin for identification of the radioactive spots, and the second plate is used for quantitative determinations. The radioactive spots are marked through the film with a ballpoint pen, the plate is covered with cellulose acetate solution and allowed to dry. The spots are cut out and counted directly by liquid scintillation.

Thin-layer methods have also been used in the synthesis of labelled amino acid derivatives and in micropreparative isolation of labelled metabolites from tissues. In these cases, the reaction mixture or extract is pipetted as a long streak on one or several plates. After electrophoresis, the bands are eluted and further purified by chromatography.

Results. The separation of the sulfur amino acids and peptides of onion by this method is superior to that obtained by paper chromatography. For most purposes, the running conditions used by Bieleski and Turner[6] are quite sufficient for separating the compounds of interest. Fig. 2 shows the relative positions of some of the compounds. (+) and (−) Isomers of the short-chained alkyl cysteine sulfoxides are well separated. (+)-*S*-Allylcysteine sulfoxide and *trans*-(+)-*S*-(propen-1-yl) cysteine sulfoxide are slightly separated and easily identified by colour differences (brownish and blue-lilac, respectively). The method made it possible to demonstrate the presence of *trans*-(+)-*S*-(propen-1-yl) cysteine sulfoxide in garlic (*Allium sativum*) and chives (*Allium schoenoprasum*). The four isomers of synthetic (±)-*cis*/*trans*-*S*-(propen-1-yl) cysteine sulfoxide are readily separated. (+)-*S*-Methylcysteine sulfoxide and γ-glutamyl-*trans*-(+)-*S*-(propen-1-yl)-cysteine sulfoxide do not separate well at pH 2.0, but at pH 1.5 no problem is encountered. Cysteine and reduced glutathione are stable during the electrophoresis, but are degraded in a somewhat irregular way during the subsequent operations. None of the other known compounds studied is significantly degraded during the separation. Acid compounds are easily differentiated by electrophoresis at pH 6.5. Using larger thin-layer plates (20 × 30 cm) and electrophoresis at pH 1.5, it is possible to

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                        ●Gly

                             ●Ala

                    ●Ser            ●Val
                                     ●Ileu
  ↑              ●Asn  ●Thr            ●Leu
  0.2    OCys2   ●Gln ●Glu
  pH                                ●Phe
  ELECTROPHORESIS  ●Asp          OMeCys
                                OCIPC  Otrans-Pren Cys
                           OCEC  O2-CPC     OPrCys
                      OCMC
                   ●(+)-MeCySO OCPG
                      OO● O(+)-PrCySO
                              trans-(+)Pren CySO
                        OCyc  (+)All CySO
                              cis-(+)-Pren CySO



        ⊂⊃ Origin      ⟨  Area with            ⟩
                          organic acids and sugars

  CHROMATOGRAPHY
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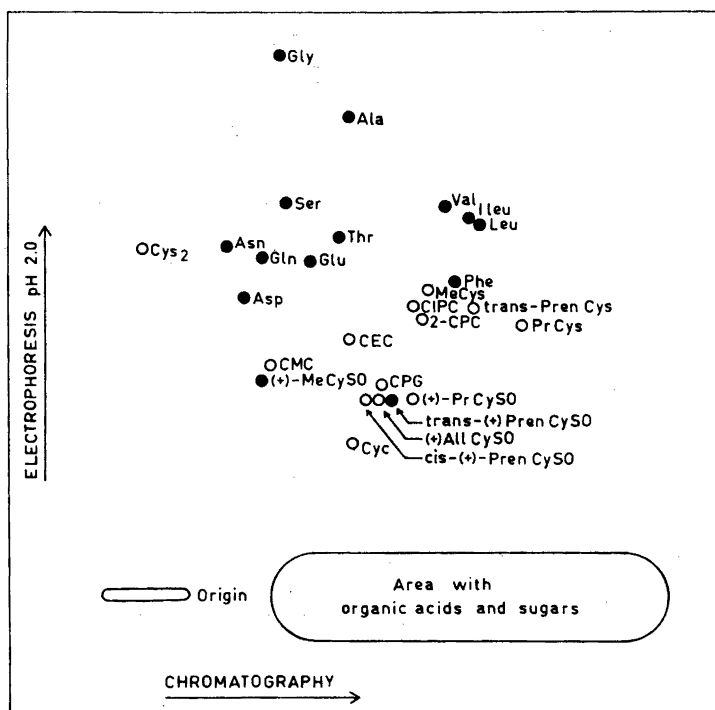
Fig. 2. Approximate positions of onion free amino acids (black spots) and some sulfur compounds of interest (circles) after thin-layer electrophoresis (pH 2.0, 60 mA, 2100 V, 20 min) and chromatography. Arginine, histidine, and lysine have moved off the plate. CMC=S-(carboxymethyl) cysteine, CEC=S-(2-carboxyethyl)-cysteine, 2-CPC=S-(2-carboxypropyl)cysteine, CIPC=S-(carboxyisopropyl)cysteine, CPG=S-(2-carboxypropyl)-glutathione, MeCys=S-methylcysteine, PrCys=S-propylcysteine, PrenCys=S-(propen-1-yl)cysteine, MeCySO=S-methylcysteine sulfoxide, PrCySO=S-propylcysteine sulfoxide, PrenCySO=S-(propen-1-yl) cysteine sulfoxide, AllCySO=S-allylcysteine sulfoxide, Cyc=cycloalliin.

map the distribution of label among amino acids, peptides, organic acids, and inorganic ions on a single plate.

A detailed report on this method will be published elsewhere.

1. Virtanen, A.I. *Phytochemistry* **4** (1965) 207.
2. Ettala, T. and Virtanen, A.I. *Acta Chem. Scand.* **16** (1962) 2061.
3. Suzuki, T., Sugii, M. and Kakimoto, T. *Chem. Pharm. Bull. (Tokyo)* **10** (1962) 328.
4. Granroth, B. and Virtanen, A.I. *Acta Chem. Scand.* **21** (1967) 1654.
5. Nybom, N. *Physiol. Plantarum* **17** (1964) 434.
6. Bieleski, R. L. and Turner, N.A. *Anal. Biochem.* **17** (1966) 278.
7. Katz, S. and Lewis, A. *Anal. Biochem.* **17** (1966) 300.