Tetrahedron Letters No.27, pp. 3119-3122, 1968. Pergamon Press. Printed in Great Britain.

# GAS CHROMATOGRAPHY OF AMINO ACIDS AS N-THIOCARBONYL ESTER DERIVATIVES

B. Halpern, Virginia A. Close, Annemarie Wegmann, and J. W. Westley

Department of Genetics, Stanford Medical Center

Palo Alto, California

(Received in USA 11 March 1968; received in UK for publication 4 April 1968)

Protein hydrolysates are now routinely analysed by ion exchange chromatography (1) but a complete determination takes at least 3 hours. In addition, the automated amino acid analyzers now used are expensive and have limited application to other problems.

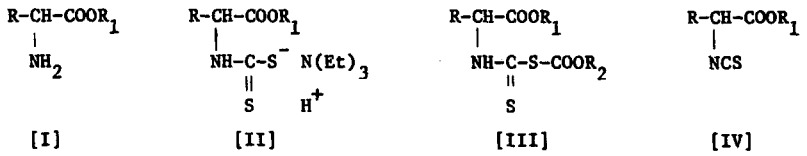
Gas chromatography (g.l.c.) offers potential advantages in both speed and sensitivity at a greatly reduced instrumentation cost. However, despite many attempts (2), no entirely satisfactory g.l.c. procedure has been developed.

The best published method is by Gehrke (3), who converts the amino acids into N-trifluoracetyl butyl esters. However, several problems remain: (a) the rapid hydrolysis of the hydroxy amino acid derivatives which prevents removal of inorganics by water washing; (b) the formation of both mono and di-substituted trifluoracetyl compounds from histidine and (c) the poor separation of the compounds on a single g.l.c. column.

The difficult g.l.c. of the TFA butyl esters is, we believe, due to the large increase in molecular weight (154) introduced during the derivatisation. Thus, the chromatography of neutral amino acids, which should be based on molecular weight differentials of 14% per $-CH_2-$ now depends on the differential volatility of compounds whose m.w. differs by only 6 per cent, e.g., 240 (glycine) and 254 (alanine).

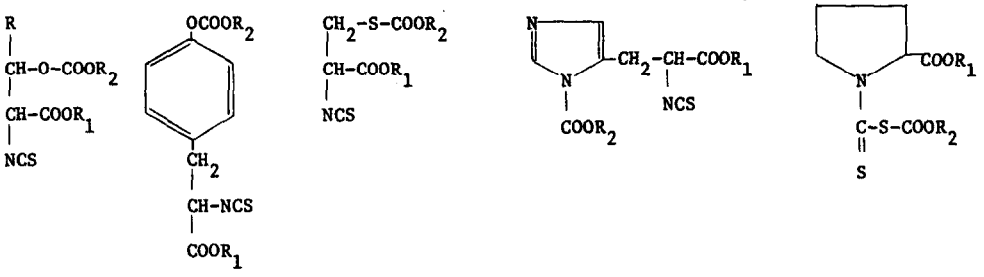
We now describe a g.l.c. analysis of the amino acids based on their conversion to the more volatile, stable N-thiocarbonyl alkyl esters, by a modified Kaluza reaction (4). This derivative adds only 56 -(methyl esters) or 84 (propyl esters) to the molecular weight and g.l.c. of the amino acids, with the exception of arginine, is greatly simplified.

The reaction sequence involves esterification of the amino acids [I] followed by a treatment with carbon disulfide and triethylamine to yield a dithiocarbamate intermediate [II]. Reaction of [II] with a chloroformate ester results in the formation of a carboalkoxy dithiocarbamate [III] which decomposes into the N-thiocarbonyl derivative [IV].



[I] [II] [III] [IV]

During treatment with the chloroformate ester, the hydroxy-amino acids and tyrosine react further to give carbonate esters [V] and [VI], cysteine and cystine yield the same thiocarbonate [VII] and the imidazole nitrogen of histidine is protected [VIII]. The indole function of tryptophan is unchanged, and proline remains as the stable carboalkoxy dithiocarbamate [IX].



[V] [VI] [VII] [VIII] [IX]

The derivatives have been fully characterized by mass spectrometry using a EAI Quad 300 mass spectrometer coupled to a Varian Aerograph 600C gas chromatograph. The correct interpretation of the mass spectral fragmentation was established by comparing several derivatives of each amino acid (e.g. methyl and propyl esters; methyl and ethyl carbonates). (Tables I and II)

In a typical analysis, the dry protein hydrolysate (0.2-0.5 mg) is twice esterified with propanolic HCl (1 ml, 25-30% w/w) at 80°C. After removal of the solvent in vacuo, methylene chloride (100 µl), carbon disulfide (10 µl) and triethylamine (8 µl) were added at -5°C. After 1 hour at 20°C the reaction is cooled to -5°C and methyl chloroformate (2 µl) is added. After a further hour at 20°C, the solution is washed into citric acid (1 ml, 20 w/w) with more methylene chloride (5 ml). After a further water wash (1 ml), the solution is dried, concentrated (0.2 ml) and part of the sample (3 µl) injected into the gas chromatograph.

Separation of the derivatives can be obtained on heavily loaded, silicone type columns. For quantitative analysis of mixtures, a temperature-programmed 6' x 1/8" glass column (5% QF-1 DMCS treated Gaschrom P) separates all the amino acid propyl ester derivatives (with the exception of the leucine-isoleucine pair) in 60 minutes (Table III). These can be separated on other columns such as SE52 or XE60 on DMCS treated Chromosorb W. A quantitative study with several of the analytically pure derivatives showed that $10^{-10}$ M of an amino acid

TABLE I

Mass Spectral Fragmentation of the Neutral and Acidic Amino Acid Derivatives (IV)

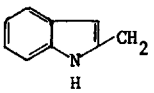
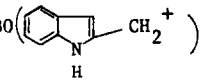
| R | $R_1$ | Major Fragments (m/e) $M^+$ | $M^+-COOR_1$ | |
|---|---|---|---|---|
| H | $CH_3$ | 131 | 72 | |
| H | $C_3H_7$ | 159 | 72 | |
| $CH_3$ | $CH_3$ | 145 | 86 | |
| $CH_3$ | $C_3H_7$ | 173 | 86 | |
| $CH(CH_3)_2$ | $CH_3$ | 173 | 114 | |
| $CH(CH_3)_2$ | $C_3H_7$ | 201 | 114 | |
| $CH_2CH(CH_3)_2$ | $CH_3$ | 187 | 128 | |
| $CH(CH_3)CH_2CH_3$ | $CH_3$ | 187 | 128 | |
| $CH(CH_3)CH_2CH_3$ | $C_3H_7$ | 215 | 128 | |
| $CH_2C_6H_5$ | $CH_3$ | 221 | 162 | 91($PhCH_2^+$) |
| $CH_2CH_2$-S-$CH_3$ | $CH_3$ | 205 | - | 158($M^+-SCH_3$) |
| $CH_2CH_2$-S-$CH_3$ | $C_3H_7$ | 233 | - | 186($M^+-SCH_3$) |
| $CH_2$-$COOR_1$ | $CH_3$ | 203 | 144 | 171($M^+-CH_3OH$) |
| $(CH_2)_2$-$COOR_1$ | $CH_3$ | 217 | - | 185($M^+-CH_3OH$) |
|  | $CH_3$ | 260 | | 130  |

TABLE II

Mass Spectral Fragmentation of the Polyfunctional Amino Acid Derivatives

| NCS derivative V | $M^+$ | Major Fragments (m/3) $(M-COOR_1)^+$ | $(M-COOR_1-COOR_2)^+$ | $(M-COOR_1-CO_2)^+$ (5) |
|---|---|---|---|---|
| R=H, $R_1$=$CH_3$, $R_2$=$CH_3$ | 219 | 160 | 102 | 116 |
| R=H, $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 233 | 160 | 102 | 130 |
| R=$CH_3$, $R_1$=$CH_3$, $R_2$=$CH_3$ | 233 | 175 | 116 | 130 |
| R=$CH_3$, $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 247 | 175 | 116 | |
| NCS derivative VI | $M^+$ | $(M-COOR_1)^+$ | $(M-CH(NCS)-COOR_1)^+$ | $(M-COOR_1-CO_2)^+$ (5) |
| $R_1$=$CH_3$, $R_2$=$CH_3$ | 295 | 236 | 165 | 121 |
| $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 309 | 236 | 179 | 135 |
| NCS derivative VII | $M^+$ | $(M-COOR_1)^+$ | $(M-COOR_1-COOR_2)^+$ | $(M-COOR_1-CO_2)^+$ (6) |
| $R_1$=$CH_3$, $R_2$=$CH_3$ | 235 | 176 | 118 | 132 |
| $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 249 | 176 | 118 | 146 |
| NCS derivative VIII | $M^+$ | $(M-COOR_1)^+$ | $(M-CH(NCS)-COOR_1)^+$ | |
| $R_1$=$CH_3$, $R_2$=$CH_3$ | 269 | 210 | 139 | |
| $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 283 | 224 | 153 | |
| NCS derivative IX | $M^+$ | $(M-COOR_2)^+$ | $(M-COOR_1-COOR_2)^+$ | $(M-CO_2)^+$ (6) |
| $R_1$=$CH_3$, $R_2$=$CH_3$ | 263 | | 145 | 219 |
| $R_1$=$CH_3$, $R_2$=$C_2H_5$ | 277 | 204 | 145 | 233 |
| NCS derivative IV | $M^+$ | $(M-COOR_1)^+$ | $(M-NCS)^+$ | $(M-COOR_1-NCS)^+$ |
| R=$NCS(CH_2)_4$, $R_1$=$CH_3$ | 244 | 185 | 186 | 127 |

TABLE III

Gas-Liquid Chromatographic Analysis of Amino Acids in a Mixture.[a]

| | Retention Time (min) | Retention Temperature °C | | Retention Time (min) | Retention Temperature °C |
|---|---|---|---|---|---|
| alanine | 8.0 | 126 | glutamic acid | 29.4 | 211.5 |
| glycine | 9.5 | 132 | proline | 31.6 | 220 |
| valine | 12.0 | 142 | threonine | 35.4 | 235 |
| leucine | 14.5 | 152 | cysteine / cystine | 36.3 | 235 |
| isoleucine | 14.5 | 152 | serine | 36.9 | 235 |
| norleucine[b] | 15.9 | 159 | lysine | 40.4 | 235 |
| methionine | 23.5 | 188 | histidine | 43.0 | 235 |
| aspartic acid | 25.4 | 195.5 | tyrosine | 49.6 | 235 |
| phenylalanine | 26.3 | 199.5 | tryptophan | 55.8 | 235 |

(a) G.l.c. analyses were carried out on a Wilkens 600 c Aerograph chromatograph equipped with a flame ionization detector. The 6' x 1/8" glass column was packed with 5% QF-1 on DMCS treated Gaschrom P, and during the analysis the carrier gas flow was 25 ml/min and the temperature was programmed from 94-235°C at a rate of 4°/min.

(b) Internal standard.

can be detected with the flame ionization detector (1-3 cm peak height at $4 \times 10^{-11}$ afs) and $10^{-13}$ M can be seen with an electron capture detector.

Reproducibility of response was found to be within 5% for derivatives carried through the entire chemical and chromatographic procedure and no problems were indicated in the analysis of mixtures containing 0.2 μM of each protein amino acid.

A detailed description of the experimental procedure for quantitative amino acid analysis will be published elsewhere.

Acknowledgment. This investigation was supported by National Aeronautics and Space Administration Grant NsG 81.

REFERENCES

1. S. Moore, D. H. Spackman and W. H. Stein, Anal. Chem., 30, 1185 (1958).
J. V. Benson Jr., and J. A. Patterson, Anal. Chem., 37, 1108 (1965).
2. B. Weinstein, Methods in Biochemical Analysis, 14, 203, J. Wiley, New York (1966).
3. C. W. Gehrke and D. L. Stalling, Separation Science, 2, 101-138 (1967).
4. D. L. Garmaise, R. Schwartz and A. F. McKay, J. Am. Chem. Soc., 80, 3332 (1958).
5. P. Brown and C. Djerassi, J. Am. Chem. Soc., 88, 2469 (1966).
6. J. B. Thomson, P. Brown and C. Djerassi, J. Am. Chem. Soc., 88, 4049 (1966).