CHROM. 12,088

Note

Gas–liquid chromatographic separation of N-trifluoroacetyl n-butyl amino acid derivatives on Silar™ stationary phases

STEVEN NAGY and NANCY T. HALL

U.S. Citrus and Subtropical Products Laboratory (U.S. Department of Agriculture, Science and Education Administration, Agricultural Research), P.O. Box 1909, Winter Haven, Fla. 33880 (U.S.A.)

(Received May 4th, 1979)

Extensive pioneering studies on the use of gas–liquid chromatography (GLC) for analyzing of amino acids have been conducted by Gehrke and co-workers[1–7]. One major research area which continues to receive extensive attention is the selection of stationary phase(s) for the resolution of N-trifluoroacetyl (N-TFA) n-butyl amino acid esters. Early investigations[3,6] showed that polyester stationary phases, such as ethylene glycol adipate (EGA), diethylene glycol succinate (DEGS), neopentyl glycol succinate, and Carbowax 20M, possessed excellent resolving abilities for the N-TFA n-butyl esters. A disadvantage of these phases was high stationary phase bleed which required extensive column conditioning.

We became interested in the GLC technique for amino acids during our studies on the amino acid contents of leaves and leaf protein concentrates[8–10]. Initially, we used the two-column system (0.65% EGA and 2% OV-17–1% OV-210) proposed by Kaiser *et al.*[11], but encountered a number of difficulties with the EGA column. The requirement for extensive conditioning of the EGA phase, and its poor thermal stability at temperatures above 200° were major disadvantages. Also peaks for trifluoroacetic acid (reagent) and trifluoroacetamide (ammonia derivative) interfered with the accurate quantitation of some amino acid derivatives[12].

Because of the disadvantages in the use of EGA and other polyester phases, we decided to investigate non-polyester polar stationary phases. The purpose of this investigation was to study the resolving capabilities of Silar™ (phenyl and cyanoalkyl polysiloxane) stationary phases for separating N-TFA n-butyl esters.

EXPERIMENTAL*

Apparatus

A Hewlett-Packard 5750 gas chromatograph equipped with flame ionization detectors and coupled with an Autolab System IV B chromatography Data Analyzer (Spectra-Physics) was used for the amino acid analyses. Equipment for the preparation of N-TFA n-butyl ester derivatives has been described[13].

Reagents, chromatographic phases and sample preparation

Amino acids were purchased from Mann Research Labs. (New York, N.Y., U.S.A.). The internal standard, *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (tranexamic acid), was obtained from Aldrich (Milwaukee, Wisc., U.S A.), whereas trifluoroacetic acid in methylene chloride (25%) was purchased from Regis (Morton Grove, Ill., U.S.A.). GLC stationary phases, Silar 5CP, 7CP, 9CP, 10C, and solid support (100–120 mesh Gas-Chrom Q) were purchased from Applied Science Labs. (State College, Pa., U.S.A.). The 3 *N* HCl–*n*-butanol for esterifying of the amino acids was prepared with HCl gas bubbled through cooled *n*-butanol in a nitrogen-purged flask[13]. Amino acid derivatives for chromatography were prepared by the method of Roach and Gehrke[14] but at a different esterification temperature[13].

Chromatographic columns and conditions

Each Silar stationary phase was coated onto 100–120 mesh Gas-Chrom Q at a concentration of 0.75% by a described procedure[13]. The dried, coated support was gently packed, under positive nitrogen pressure, into a coiled 183 cm × 0.4 cm I.D. glass column. For conditioning, the column temperature was raised at 2°/min until it reached the desired upper limit of about 240°; it was then maintained at that limit for 15 min.

Chromatography

The smallest possible height equivalent to a theoretical plate (HETP) was determined for each Silar phase. GLC parameters for separation of N-TFA *n*-butyl esters were similar: detector, 245°; injection port, 200°; and helium flow, 60 ml/min. A standard amino acid mixture was injected on-column at 110°. The temperature was held at 110° for 5 min, then programmed at 8°/min to 230°.

RESULTS AND DISCUSSION

Silar stationary phases were thermally stable at the elution temperatures of the least volatile amino acid derivatives. After a short column conditioning period Silar phases showed excellent resolving capabilities and minimal bleed at high temperatures (< 230°). The short conditioning time is in contrast to the many days required to condition the polyester phases (DEGS, EGA, SP-1000) that we tested in preliminary studies.

The optimum helium flow-rate for all Silar columns was 60 ml/min. Using the N-TFA *n*-butyl ester of proline, we found the optimum HETP values for the four Silar phases to be: 0.9 (5CP and 7CP), 1.1 (9CP) and 1.0 mm (10C).

The Silar phases are manufactured at various polarities indicated by their McReynolds number: 319 (5CP), 440 (7CP), 489 (9CP) and 523 (10C)[15,16]. We were interested in determining whether these phases of different polarities would increase the resolution of specific types of amino acids. Table I lists the relative retention times (*RRT*, relative to the retention time of the internal standard) of 17 α-amino acids and 1 γ-amino acid on the four Silar phases. N-TFA *n*-butyl ester derivatives of arginine, histidine, and cystine could not be quantitatively eluted from the Silar phases; therefore, their *RRT* values are not tabulated. Gehrke *et al.*[12] also could not quantitatively elute these three derivatives from various polyester phases. For the separation and

TABLE I

RELATIVE RETENTION TIMES OF AMINO ACID N-TFA *n*-BUTYL ESTERS ON 0.75%
SILAR COLUMNS

Amino acid	Relative retention time			
	5CP	7CP	9CP	10C
Alanine	0.192	0.195*	0.186*	0.168
Valine	0.219	0.195*	0.186*	0.161
Threonine	0.278	0.264*	0.265	0.231
Isoleucine	0.305	0.264*	0.247	0.208
Glycine	0.343	0.355	0.369	0.351
Leucine	0.364	0.329	0.319	0.276
Serine	0.421	0.431	0.448	0.433
Proline	0.547	0.520	0.517	0.489
Cysteine	0.562	0.560	0.572	0.561
Hydroxyproline	0.662	0.621	0.631	0.621
γ-Aminobutyric acid	0.633	0.636	0.648	0.640
Aspartic acid	0.734*	0.711	0.710	0.695
Methionine	0.734*	0.733	0.741	0.737
Phenylalanine	0.765	0.752	0.755	0.749
Glutamic acid	0.873*	0.853	0.849	0.841
Tyrosine	0.873*	0.873	0.878	0.878
Internal standard**	1.000	1.000	1.000	1.000
Tryptophan	1.191*	1.116	1.147	1.121
Lysine	1.191*	1.165	1.180	1.202

* Unresolved.
** Internal standard: tranexamic acid.
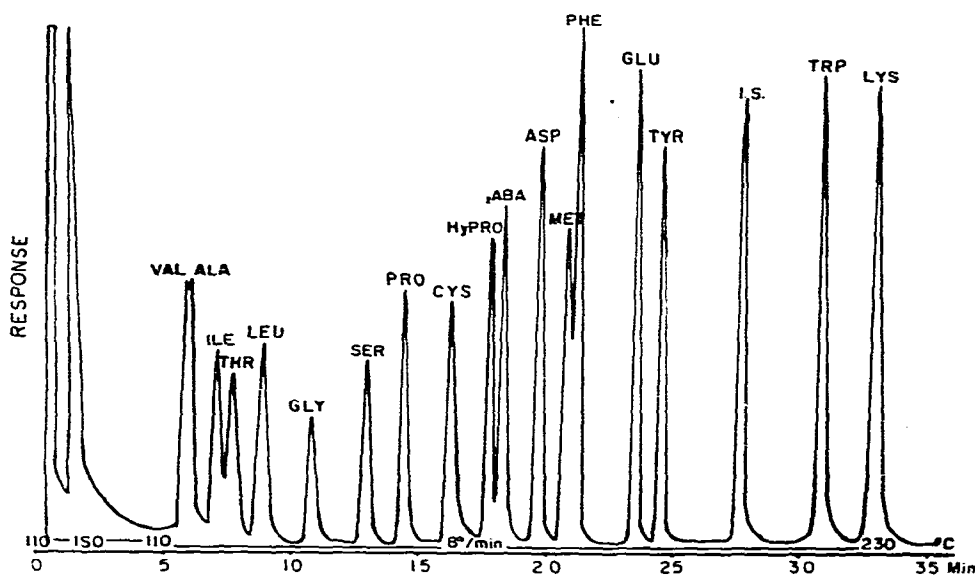
Fig. 1. Separation of 1 γ- and 17 α-amino acids on 0.75% Silar 10C.

quantitative estimation of histidine, arginine and cystine, we use a mixed phase, 0.5% OV-210–0.5% OV-17–0.4% OV-7 (ref. 13). The mixed phase might also be used to separate valine from alanine when resolution and accurate quantitation of these two amino acids on Silar 9CP or 10C are questionable.

The least polar phase, 5CP, separated 12 amino acids but could not resolve Asp from Met; Glu from Tyr and Trp from Lys. The order of elution of the first six amino acid derivatives (Ala, Val, Thr, Ile, Gly, Leu) on 5CP was different from that on 7CP, 9CP and 10C (Val, Ala, Ile, Thr, Leu, Gly). Whereas 5CP resolved the first six amino acids quite effectively, resolution of these six on the other Silar phases was not as efficient.

Silar 7CP separated 14 amino acids but could not resolve Val from Ala and Ile from Thr. Silar 9CP resolved 16 amino acids but could not separate Val from Ala. Silar 10C separated all 18 amino acids (Fig. 1), although separation between Val and Ala was not always definitive.

The use of two columns for the separation of the 20 natural protein amino acids is recommended. Silar 10C can be used for the separation of all amino acids except histidine, arginine and cystine. For the separation of these last three, a mixed phase 0.5% OV-210–0.5% OV-17–0.4% OV-7 (ref. 13) is preferred.

REFERENCES

1 C. W. Gehrke, W. M. Lamkin, D. L. Stalling and F. Shahrokhi, *Biochem. Biophys. Res. Commun.*, 13 (1965) 328.
2 W. M. Lamkin and C. W. Gehrke, *Anal. Chem.*, 37 (1965) 383.
3 C. W. Gehrke and F. Shahrokhi, *Anal. Biochem.*, 15 (1966) 97.
4 C. W. Gehrke and D. L. Stalling, *Separ. Sci.*, 2 (1967) 101.
5 D. L. Stalling, G. Gille and C. W. Gehrke, *Anal. Biochem.*, 18 (1967) 118.
6 C. W. Gehrke, R. W. Zumwalt and L. L. Wall, *J. Chromatogr.*, 37 (1968) 398.
7 D. Roach and C. W. Gehrke, *J. Chromatogr.*, 43 (1969) 303.
8 N. T. Hall, S. Nagy and R. E. Berry, *Proc. Fla. State Hort. Soc.*, 88 (1975) 486.
9 S. Nagy, L. Telek, N. T. Hall and R. E. Berry, *J. Agr. Food Chem.*, 26 (1978) 1016.
10 L. Telek, G. Telek, N. T. Hall and J. M. Smoot, *J. Agr. Univ. P.R.*, (1979) in press.
11 F. E. Kaiser, C. W. Gehrke, R. W. Zumwalt and K. Kuo, *Amino Acid Analysis: Hydrolysis, Ion-Exchange Clean-Up, Derivatization, and Quantitation by Gas–Liquid Chromatography*, Analytical and Biochemical Laboratories, Columbia, Mo., 1974.
12 C. W. Gehrke, D. Roach, R. W. Zumwalt, D. L. Stalling and L. L. Wall, *Quantitative Gas–Liquid Chromatography of Amino Acids in Proteins and Biological Substances*, Analytical Biochemical Laboratories, Columbia, Mo., 1978.
13 N. T. Hall and S. Nagy, *J. Chromatogr.*, (1979) 171 (1979) 392.
14 D. Roach and C. W. Gehrke, *J. Chromatogr.*, 44 (1969) 269.
15 W. O. McReynolds, *J. Chromatogr. Sci.*, 8 (1970) 685.
16 *Chromatography Products 1978 Catalog*, Applied Science Labs., State College, Pa., 1978.