QUANTITATIVE ANALYSIS OF AMINO ACIDS BY GAS CHROMATOGRAPHY:ACYLATION OF ARGININE[1]

David L. Stalling[2,3] and Charles W. Gehrke

Department of Agricultural Chemistry
University of Missouri
Columbia

Received January 3, 1966

Gehrke, et al., 1965, developed a method for the analysis of amino acids by gas chromatography which gave quantitative conversion of eighteen of the natural protein amino acids to their n-butyl N-trifluoroacetyl esters. These esters were shown to be satisfactory derivatives by Lamkin and Gehrke, 1965. With this method, however, no peak was observed for arginine. This was due to the formation of an arginine trifluoroacetate salt which does not give a good chromatographic peak. Other investigators have reported chromatographic peaks for arginine but acylation of arginine to give a volatile derivative has in general proved to be difficult. A quantitative procedure for the acylation of arginine without decomposition to ornithine was reported by Stalling and Gehrke, 1965.

As reported by Zomzely, et al., 1962, the addition of dimethyl formamide to the acylation mixture gave two peaks for arginine. We were unable to find a concentration of

---

[1]Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 3029. Approved by the Director. Supported in part by grants from the National Science Foundation (G-18722 and GB-1426).

[2]Experimental data taken from doctoral dissertation.

[3]NASA predoctoral research fellow.

dimethyl formamide which prevented the partial conversion of arginine to ornithine. However, acylation of arginine n-butyl ester·HCl at room temperature in the presence of anhydrous Na_2CO_3 gave a single chromatographic peak, but this method required 18 hrs. for maximum conversion to the desired derivative (Stalling, et al., 1964). The observations by other investigators (Zomzely et al., 1962; Cruickshank and Sheehan, 1964; and Hagen and Black, 1965) that arginine could be converted into a suitable peak on acylation were not confirmed. In these reports a common experimental factor was apparent. The gas chromatographs they used were equipped with flash heaters heated to 225-300° C. The gas chromatograph employed in our investigations was not operated with a heated injection port but we employed direct "on column" injection and temperature programming (F and M Model 402 Biomedical Analyzer).

When the gas chromatograph was equipped with a metal flash heater and solutions of the n-butyl arginine ester·HCl dissolved in trifluoroacetic anhydride (TFAA) + CH_2Cl_2 were injected two peaks were produced. This solution was acylated for 2 hrs. at room temperature (r.t.) prior to injection. As shown later the desired volatile derivative was synthesized in the hot metal flash heater. This is apparently what happened in the experiments reported by the above named investigators. These two peaks were not observed when the same solution was injected directly on the chromatographic column.

Room temperature acylation of arginine with TFAA forms a non-volatile trifluoroacetate salt of the guanido group. This was confirmed by IR analysis. This salt was observed to dissociate on chromatography to a compound not suitable

for measurement (an extremely broad peak at a retention temperature of 235°C). The synthesis and subsequent breakdown of the arginine derivative in the hot flash heater is shown in Figure I. Methyl stearate (20 mg) and 60 mg of arginine·HCl were converted to their respective n-butyl esters, then dissolved in 4.0 ml of CH_2Cl_2 + 1.0 ml of TFAA, and acylated for 2 hrs. at r.t. The relative peak areas as a function of the metal flash heater temperature are given in Figure I and the chromatographic conditions in Table I. Two major peaks were formed when this stock solution was injected into the flash heater at different temperatures, a peak for arginine

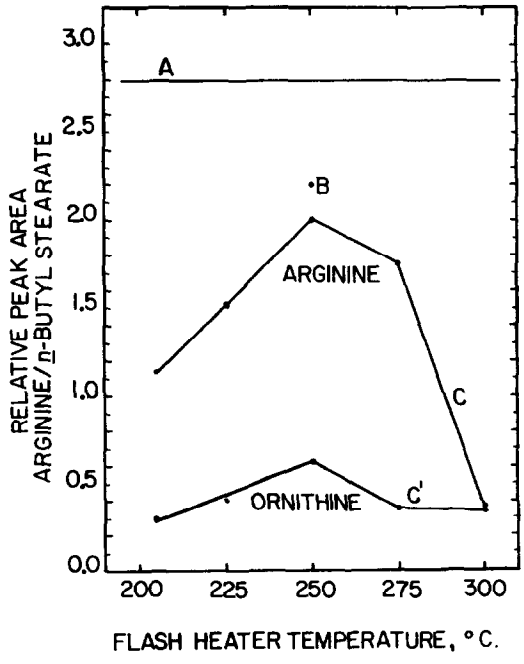


Figure I. Acylation of Arginine -- Effects of on Column and Flash Heater Injection.

Sealed tube acylation: 150°C., 15 min.,

A--On Column Injection B--Flash Heater Injection

Room temperature acylation: C and C'-- Flash Heater Injection.

TABLE I.

STABILITY OF n-BUTYL-N-TRI-TRIFLUOROACETYL DERIVATIVE OF ARGININE AS A FUNCTION OF TIME

Sample[a] No.	Acylation Time, min.	Acylation Temp., °C.	Relative Area[b] Standing Time, hrs.[c] 0	24	72
A-1	5	150	2.72	2.72	2.72
2	15		2.74	2.78	2.79
3	30		2.76	2.76	2.79
		Mean =	2.74	2.75	2.76
B-1	5	170	2.76	2.73	2.78
2	15		2.72	2.76	2.78
3	30		2.75	2.74	2.77
		Mean =	2.74	2.74	2.78

[a]Each 10 ml. of stock solution contained 10 mg. of phenylalanine and 60 mg. of arginine converted to the n-butyl ester. (8 ml. of CH_2Cl_2 + 2 ml. of TFAA).

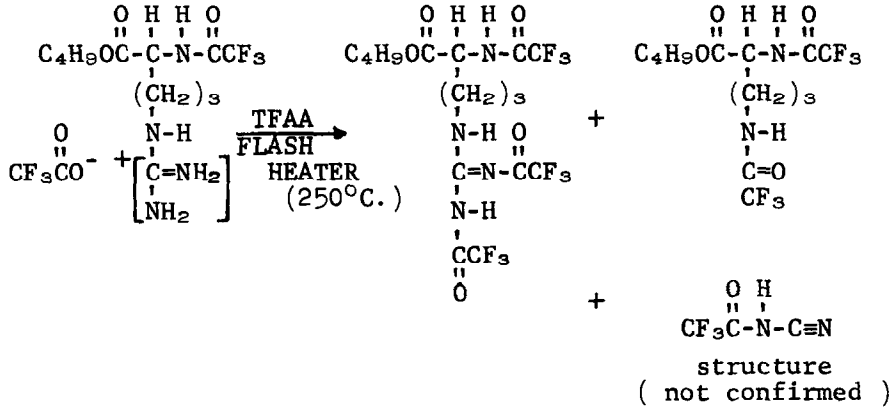
One-half ml. of TFAA was added to 0.5 ml. of stock solution and acylated in a sealed tube. (0.5 mg. of phe and 3 mg. of arg). 3 µl. injected directly on 1 m. x 3 mm. i.d. glass column of 0.75/0.25 w./w.% of DEGS/EGSS-X on 60-70 mesh Chromosorb G at 125°C. and programmed at 7.9°C./min. to 235°C.

[b]Relative area of arginine to phenylalanine, each value is a single result.

[c]Acylated solutions stored in sealed vials at room temperature.

and one for ornithine. As the temperature increased, the desired derivative partially decomposed to ornithine. The formation of an ornithine derivative was non-linear and at temperatures above 250°C again decreased. Elemental analysis of the desired derivative of arginine showed it to be the n-butyl N-tri-trifluoroacetyl ester. The metal flash heater reactions of arginine are postulated in Figure II.

Figure II
FLASH HEATER REACTIONS OF ARGININE



Sealed Tube Acylation

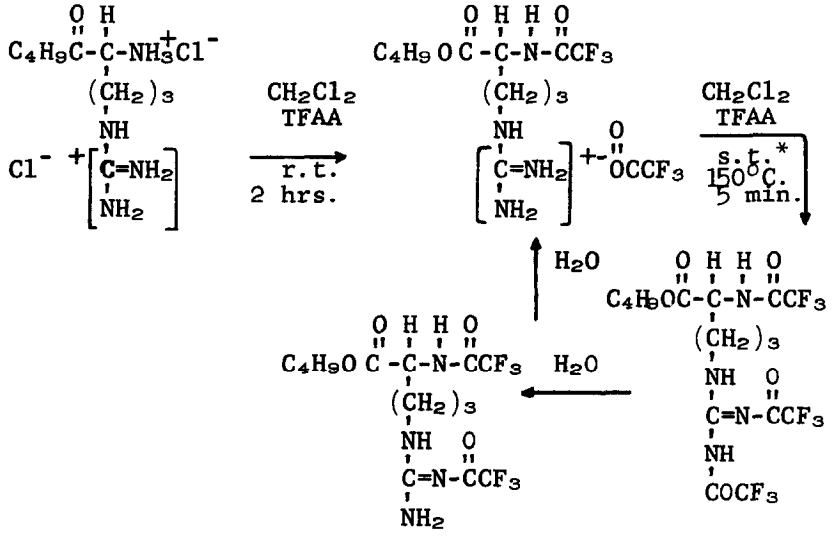
A satisfactory method for the quantitative formation of the desired derivative of arginine (99%) was developed. Less than one per cent decomposition to ornithine occurred. The acylation reaction was conducted at 150°C for 5 min. in a heavy walled, screw top, 12 ml centrifuge tube, sealed with a teflon lined cap. A stock solution identical to that employed in the above flash heater experiments was used in the sealed tube (s.t.) acylation studies. The results are shown in Figure I, line A, using direct "on column" injection. A quantitative, reproducible formation of the desired derivative of arginine was achieved. This sealed tube acylated solution was then injected into the hot flash heater and gave the relative response shown in Figure I, point B. Only 68 per cent as much derivative was formed in the hot flash heater as compared to the amount formed in the sealed tube. A sealed tube acylation experiment of a similar solution of the arginine methyl ester·HCl resulted in 5 times as much derivative

as compared to acylation at r.t. for 2 hrs. Direct on column injection was employed for both samples.

The acylation reactions of the n-butyl ester of arginine are summarized in Figure III. The bis-trifluoroacetyl derivative has not been isolated or identified but probably exists as an intermediate. The n-butyl-N-tri-trifluoroacetyl ester must be maintained under anhydrous conditions or hydrolysis to the salt occurs. This can be accomplished by storage in screw capped vials with teflon cap liners. The stability of the tri-trifluoroacetyl derivative is very good under anhydrous conditions. As presented in Table I the acylation temperature (150 or 170°C) or time does not affect the yield or stability of the derivative. Precision as a function of time or temperature was excellent. Also, sealed tube acylation studies have been conducted individually on the 20 protein amino acids and the results show that no decomposi-

Figure III

ACYLATION REACTIONS OF n-BUTYL ESTER·HCl OF ARGININE



*sealed tube

tion occurred. Identical relative peak areas were obtained for the other eighteen protein amino acids using sealed tube acylation at 150°C for five min. as compared to acylation at room temperature for 2 hrs. Tryptophan is also quantitatively converted to the diacyl derivative. Sealed tube acylation of mixtures of amino acids have been investigated to determine if interactions or adverse side reactions occur, none were evident.

This sealed tube acylation method results in a significant saving of time in the conversion of amino acids to their volatile n-butyl N-trifluoroacetyl derivatives. The acylation reaction time has been decreased from 2 hrs. to 5 mins. Direct "on column" injection is necessary to obviate breakdown of the N-acyl ester derivatives of the amino acids in a hot metal flash heater. A single, reproducible, quantitative derivative has been formed for arginine and tryptophan and the other 18 natural amino acids.

References

Cruickshank, P. A., and Sheehan, J. C., Anal. Chem. 36, 1191 (1964).

Gehrke, C. W., Lamkin, William M., Stalling, David L., and Shahrohki, Frank, Biochem. Biophys. Res. Comm. 19, 328 (1965).

Hagen, P., and Black, W., Fed. Proc. 23, 371 (1964).

Lamkin, William M. and Gehrke, C. W., Anal. Chem. 37, 383 (1965).

Stalling, David L. and Gehrke, C. W., 150th Meeting Am. Chem. Soc., Atlantic City, N. J., Sept. (1965).

Stalling, David L., Gehrke, C. W., and Shahrohki, Frank, 16th Annual Midwest Chemistry Conference, Kansas City, Mo., Nov. (1964).

Zomzely, C., Marco, G., and Emery, E., Anal. Chem. 34, 1414 (1962).