THE GAS CHROMATOGRAPHIC DETERMINATION OF ASCORBIC ACID IN THE FORM OF ITS TRIMETHYLSILYL ETHER DERIVATIVE

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(Received May 4th, 1966)

Various poorly vaporizable substances can be made available for gas chromatographic analysis by transformation into appropriate derivatives. Trimethylsilyl ether derivatives in particular have recently acquired prominence.

Even sugars, which are normally destroyed at higher temperatures[1,2], can be analyzed by gas chromatography in the form of their trimethylsilyl ethers. We felt it desirable to apply this useful approach to the study of ascorbic acid in gas chromatographic analysis.

EXPERIMENTAL

Apparatus and experimental conditions

The analyses were carried out using a Perkin-Elmer gas chromatograph Model 801 fitted with an all-glass system. Occasionally a Pye gas chromatograph was used. The experimental conditions are given in Table I.

Derivatives

In order to prepare the trimethylsilyl ether of ascorbic acid we tried two different silylating agents: (a) hexamethylenedisilazane, utilizing the method of SWEELEY

TABLE I

APPARATUS AND EXPERIMENTAL CONDITIONS

Apparatus	Pye	Perkin-Elmer No. 801
Detector	β-ionisation	Flame ionisation
Sensitivity	× 10	X50
Tension	1250 V	
Temperature:		
(a) Column	135°	170°
(b) Injection port	240°	240°
Column/column dimension	Glass, 1.2 m, 4 mm Ø	Glass, 2 m, 2.3 mm Ø
Carrier gas	Argon	Helium
Gas flow	60 ml/min	40 ml/min
Stationary phase	10 % Silicone XE60	3 % Silicone SE30
Carrier material	Anachrom ABS 100–120 mesh	Anachrom ABS 100–120 mesh
Paper advance	6 in./h	40 in./h
Quantity injected	0.8 μl	1 μl
Diluting fluid	Pyridine	Pyridine

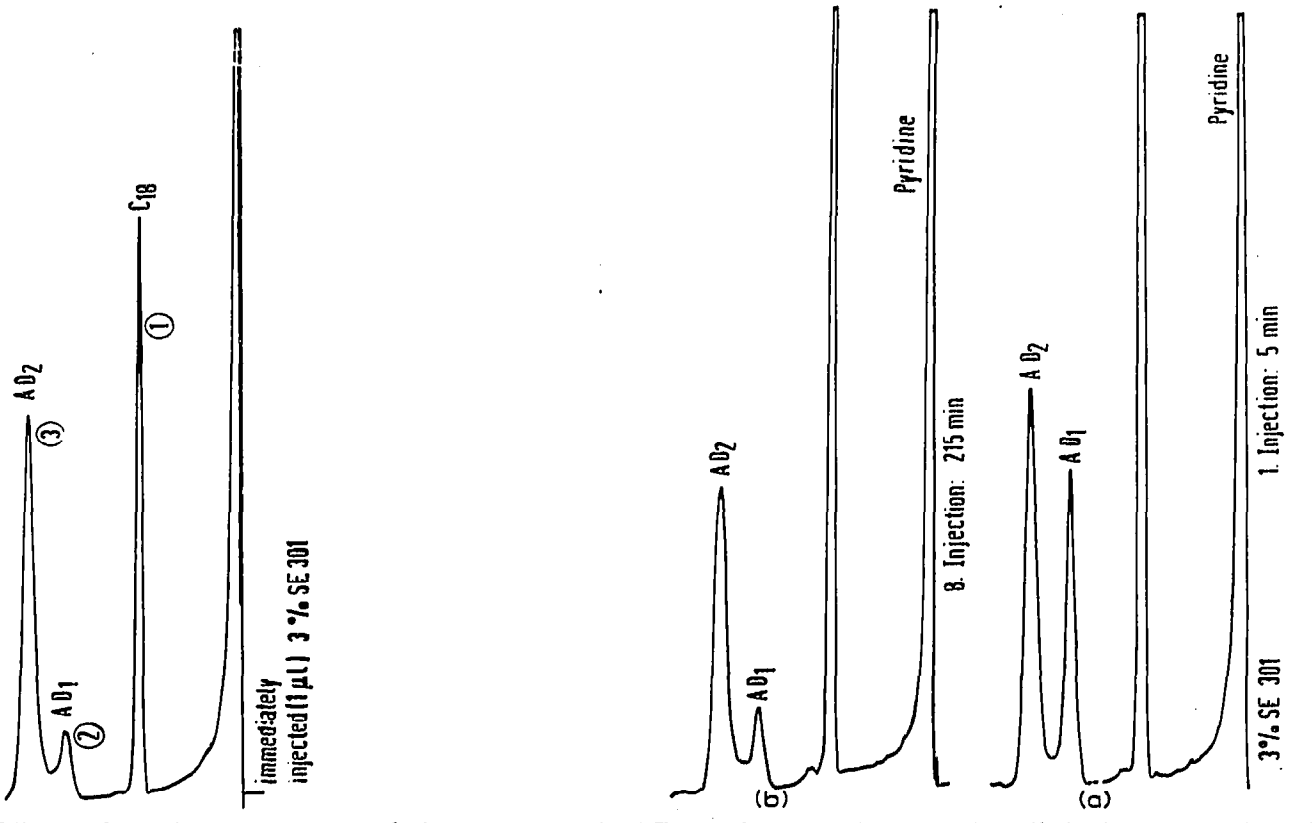


Fig. 1. Gas chromatogram of the compounds AD_1 and AD_2, obtained by silylating ascorbic acid. Silylating agent: hexamethylenedisilazane. See also Table II.

Fig. 2. Gas chromatograms of the compounds AD_1 and AD_2, obtained by silylating ascorbic acid with a small excess of N-trimethylsilylacetamide. Samples drawn after 5 min (a) and 215 min (b), respectively.

et al.[1] and (b) N-trimethylsilylacetamide, a substance which has been successfully employed by BIRKHOFER[3].

(*a*) *Hexamethylenedisilazane as silylating agent.* Hexamethylenedisilazane yielded unsatisfactory results when employed as a silylating agent. Gas chromatographic analysis of the reaction solution showed that two reaction products are formed which we have designated ascorbic acid derivative 1 (AD_1) and ascorbic acid derivative 2 (AD_2) — *cf.* Fig. 1 and Table II.

The concentration of these reaction products, however, drops after a short

TABLE II

RETENTION TIME AND RELATIVE RETENTION TIME OF THE PRODUCTS AD_1 AND AD_2 WHICH ARE FORMED BY REACTION BETWEEN ASCORBIC ACID AND HEXAMETHYLENEDISILAZANE

	Retention time (min)	*Retention time relative to octadecane*
Peak 1: octadecane (C_{18}) (int. standard)	5.74	1.00
Peak 2: ascorbic acid derivative 1 (AD_1)	9.53	1.66
Peak 3: ascorbic acid derivative 2 (AD_2)	11.88	2.06

time (*cf.* Table III). The peak area of the reaction products AD_1 and AD_2, respectively, are plotted in relation to the peak area of the internal standard. We did not attempt to determine whether decomposition of AD_1 or AD_2 occurs or whether these products enter into further chemical reactions.

TABLE III

DECREASE IN CONCENTRATION OF AD_1 AND AD_2 IN THE REACTION MIXTURE AS A FUNCTION OF TIME

Hexamethylenedisilazane was used as silylating agent.

Reaction time (min)	$\frac{F_{AD_1}}{F_{C_{18}}}$	$\frac{F_{AD_2}}{F_{C_{18}}}$
30	0.30	1.62
60	0.27	0.74
90	0.26	0.73

(*b*) *Silylation with N-trimethylsilylacetamide.* When a small excess of the silylating agent is used the reaction products AD_1 and AD_2 can again be demonstrated on the gas chromatogram, *cf.* Fig. 2. Analysis of the reaction solution was repeated at various time intervals. The data obtained are shown in Table IV. The peak area of reaction product AD_2 is plotted in relation to the peak area of the reaction product AD_1. It is apparent that the reaction product AD_1 can be considered the precursor of the ascorbic acid derivative AD_2.

TABLE IV

RELATIONSHIP BETWEEN PEAK AREAS OF AD_1 AND AD_2 AFTER DIFFERENT REACTION TIMES

N-Trimethylsilylacetamide was used as silylating agent.

Reaction time (min)	$\frac{F_{AD_2}}{F_{AD_1}}$
5	1.49
35	1.93
65	3.41
95	4.61
125	5.30

If the silylation is carried out with a 15-fold excess of N-trimethylsilylacetamide, only the reaction product AD_2 is found in the reaction solution (see Fig. 3a and 3b).

The progress of the reaction was also studied in terms of time. The results obtained are presented in Table V and Fig. 4. It is evident from these that the silylation reaction, at room temperature, is not complete until after 210 min.

We ascertained that a further increase in the concentration of N-trimethylsilylacetamide does not improve the yield. This suggests that the reaction proceeds

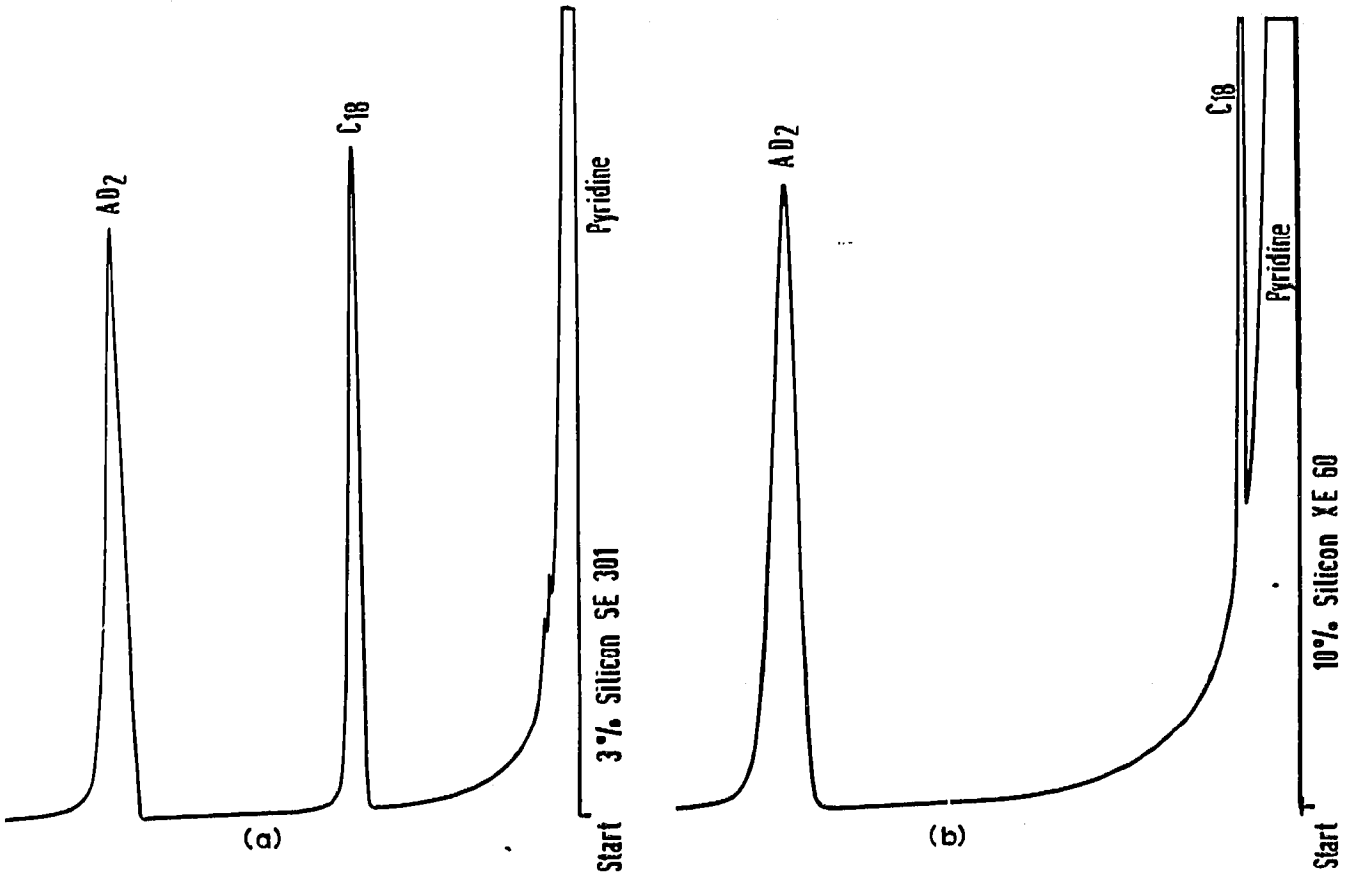


Fig. 3. Gas chromatograms of the compound AD_2 which is formed by the reaction of ascorbic acid with a 15-fold excess of N-trimethylsilylacetamide. (a) Gas chromatogram obtained with 3 % SE 30 on Gaschrom Q. (b) Gas chromatogram of the same sample analysed with the polar phase XE 60 on Gaschrom Q.

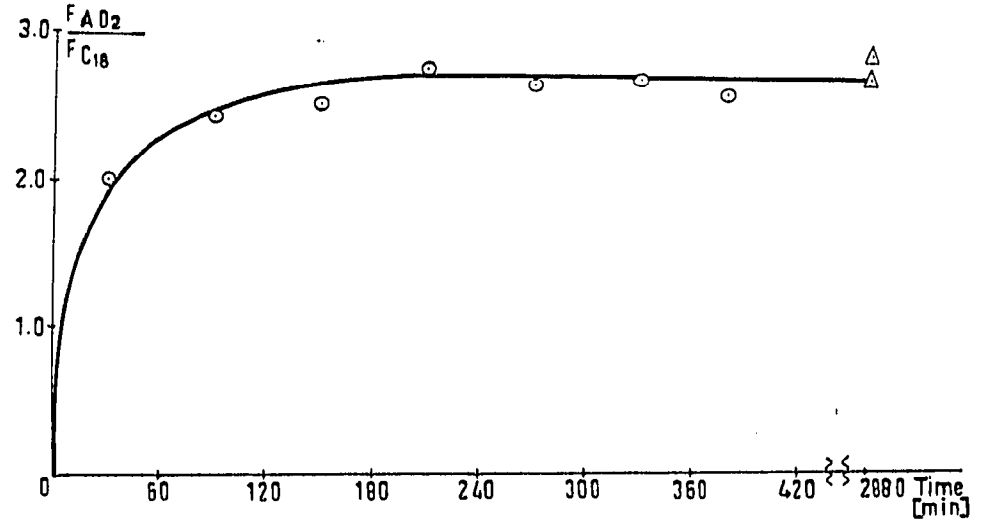


Fig. 4. Formation of AD_2 as a function of time. Ordinate: relationship between peak area AD_2 and peak area internal standard; abscissa: time in minutes.

quantitatively. No decomposition of the ascorbic acid derivative AD_2 could be demonstrated under the experimental conditions set forth below.

TABLE V

RELATIONSHIP BETWEEN PEAK AREA OF AD_2 AND INTERNAL STANDARD AFTER DIFFERENT REACTION TIMES

The silylation was carried out with a 15-fold excess of N-trimethylsilylacetamide.

Reaction time (min)	$\frac{F_{AD_2}}{F_{C_{18}}}$
30	2.01
90	2.45
150	2.54
210	2.79
270	2.70
330	2.75

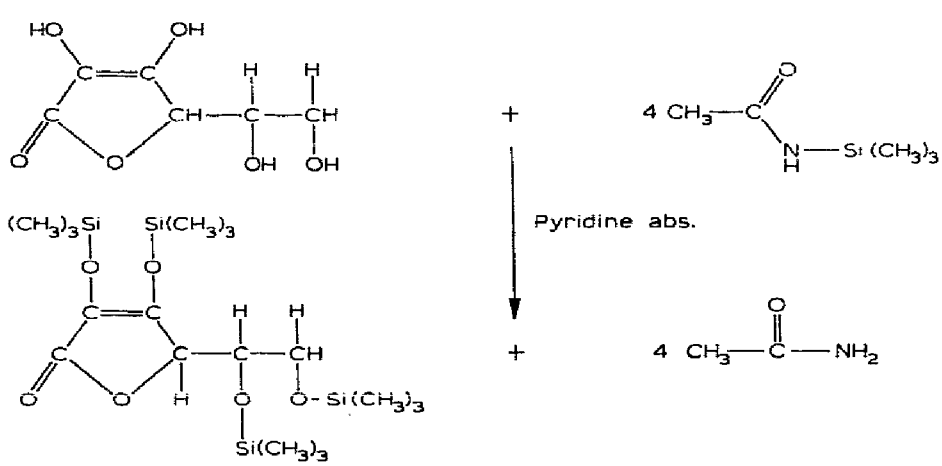
Procedure

Silylation is carried out as follows. 10 ml absolute pyridine are poured into a small serum bottle filled with pure nitrogen. 50 mg ascorbic acid and 50 mg octadecane are then dissolved in this. The octadecane serves as internal standard. 1.5 g N-trimethylsilylacetamide are then added, the mixture is again perfused with nitrogen or argon and the bottle is covered with an ordinary serum stopper. The reaction is allowed to proceed for at least 240 min at room temperature. The sample can then be obtained without exposure to air by drawing an aliquot through the rubber cap by means of a syringe.

RESULTS AND DISCUSSION

Mass spectrometric study of the ascorbic acid derivative AD_2

Having found conditions of silylation which produced only one reaction product in quantitative yield, it remained to determine whether the reaction proceeded according to the following equation:



For this purpose, a sample of the ascorbic acid derivative AD_2 was retrieved from the detector exit of a Pye gas chromatograph (*cf.* Fig. 5) and forthwith studied by mass spectrometry. The spectrum exhibited a molecular peak of 464, exactly the molecular weight of the tetra-(trimethylsilyl ether) of ascorbic acid. This compound is an oily colorless liquid which is, transformed in air into a crystalline product which could not be further studied.

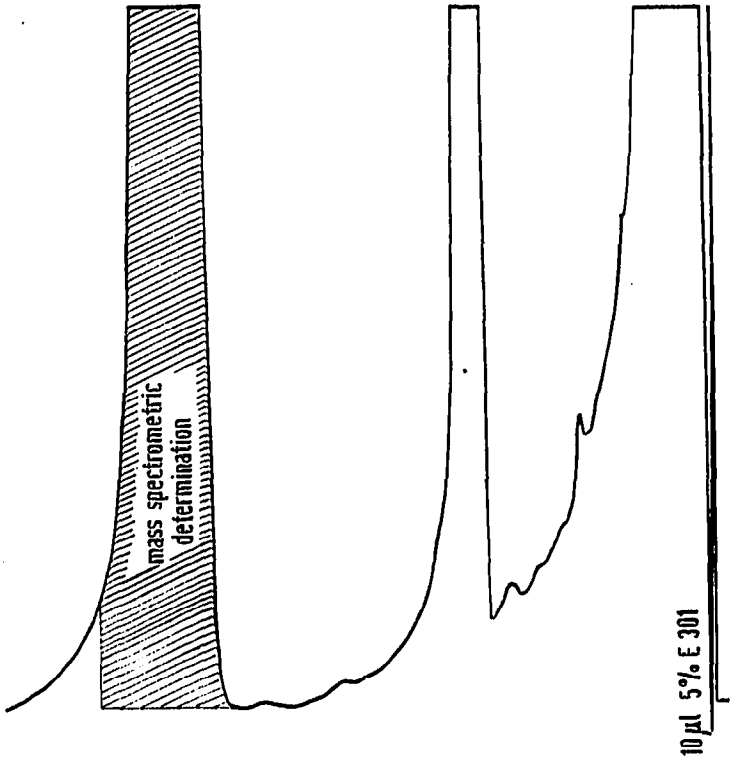


Fig. 5. Chromatogram obtained with a preparative gas chromatograph. The product corresponding to the hatched peak area has been collected for mass spectrometric analysis. Flow: 60 ml/min. Temperature: column 165°, injection port 235°.

Reproducibility of the analyses

Aliquots were repeatedly removed and analyzed from a charged of 75 mg ascorbic acid, 1.5 g N-trimethylsilylacetamide, 50 mg octadecane and 10 ml pyridine following a reaction period of 12 h. An assessment of the analyses is given in Table VI.

The variation of the results is of an order of magnitude which excludes the possibility of decomposition during gas chromatographic analysis.

Linearity of the detector

The quantitative turnover and the linearity of the detector employed were tested with three charges containing differing concentrations of ascorbic acid, but the same quantity of the silylating agent, *viz.*:

(1) 50.40 mg ascorbic acid;
(2) 79.18 mg ascorbic acid;
(3) 99.91 mg ascorbic acid.

These were each converted to the trimethylsilyl ether with 1.5 g N-trimethylsilylacetamide and 10 ml pyridine, which contained 50 mg octadecane as internal

TABLE VI

REPRODUCIBILITY OF ANALYSIS OF THE TETRA-(TRIMETHYLSILYL ETHER) OF ASCORBIC ACID

The statistical evaluation was carried out with the help of the approximation equations of DEAN AND DIXON[4].

Injection No.	$\frac{F_{AD_2}}{F_{C_{18}}}$
1	1.75
2	1.69
3	1.74
4	1.69
5	1.75
6	1.69
7	1.75
Average	1.72
Standard deviation S	±0.03/±1.74%
Variation of individual values at 95% confidence limit	±0.07/±4.07%
Variation of duplicate determination	±0.05/±2.9%

standard. After a reaction time of 12 h we carried out triplicate determinations on each charge. The values obtained are given in Table VII. A standard curve (Fig. 6) may be constructed by plotting the surface quotient against the concentration of ascorbic acid. Its linearity permits the conclusion that the conversion occurs quantitatively.

TABLE VII

VALUES TAKEN FOR LINEAR RESPONSE TEST

Concentration of ascorbic acid	*5.040 (mg/ml)*	*7.918 (mg/ml)*	*9.991 (mg/ml)*
$\frac{F_{AD_2}}{F_{C_{18}}}$	1.19	1.96	2.40
	1.19	1.91	2.59
	1.15	1.92	2.36
Average	1.18	1.93	2.38

ACKNOWLEDGEMENTS

We wish to thank Dr. W. VETTER for measuring and interpreting the mass spectra, and Dr. R. BARNER and Mr. O. BISCHOFF for synthesizing the N-trimethylsilylacetamide.

SUMMARY

A method of determining vitamin C by gas chromatography by means of its silyl ether derivative has been investigated. N-Trimethylsilylacetamide proved a

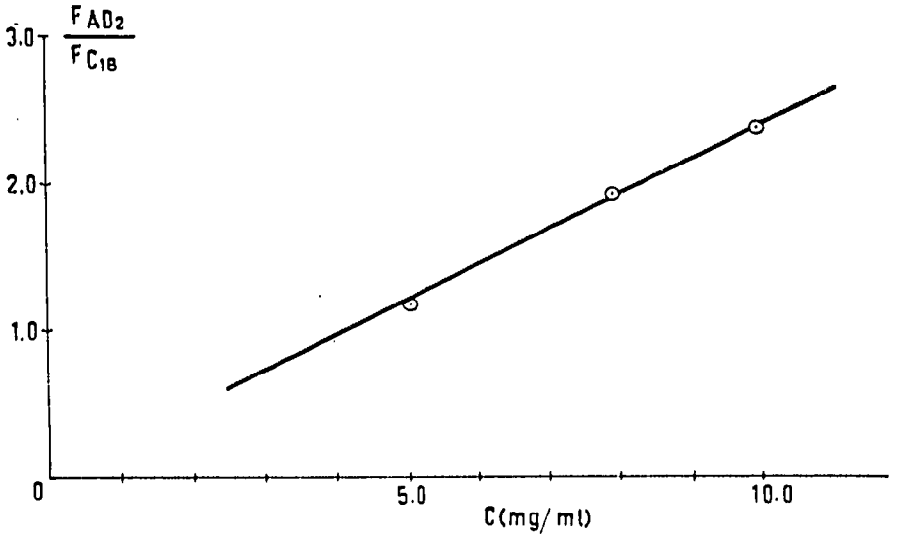


Fig. 6. Standard curve. The surface quotient is plotted against the concentration of ascorbic acid

suitable silylating agent. Analysis by mass spectrometry demonstrated formation of the tetra-(trimethylsilyl ether) of ascorbic acid. Kinetic studies and analysis by gas chromatography indicate that the reaction proceeds almost quantitatively. The results are well reproducible.

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