*Journal of Chromatography*, 132 (1977) 148–151


CHROM. 9651

## Note

# Gas chromatography of the pyrazoline derivative of trimethyl aconitate

SERGIO DILLI

*Department of Analytical Chemistry, School of Chemistry, University of N.S.W., Kensington, N.S.W., 2033 (Australia)*

and

JOSEF SZYSZKA

*I.C.I. Australia Limited, Villawood, 2163 (Australia)*

(Received August 27th, 1976)

The gas chromatographic (GC) analysis of 1,2,3-propenetricarboxylic acid (*cis*-aconitic acid), a member of the Krebs-cycle acids is normally carried out by esterification usually with methanol[1–6]. Diazomethane has been found to be among the best esterification reagents for carboxylic acids because of quantitative conversion obtainable at room temperature[3].

A secondary reaction of diazomethane with unsaturated acids involves its cycloaddition to a carbon–carbon double bond to form a pyrazoline compound. Whereas this reaction is well known for *trans*-butenedioic acid (fumaric acid) which appears to be stable[3,7] during GC, little information is available for the pyrazoline compound derived from the aconitate ester. However, McKeown and Read[3] found that *cis*-aconitic acid was isomerised completely to the *trans* ester with diazomethane. On prolonged exposure of *cis*- and *trans*-aconitic acid with diazomethane, no well-defined peak was obtained on a diethylene glycol succinate (DEGS) column. Other workers[1,2,4] reported no apparent difficulty using excess diazomethane in the analysis of the aconitic acid trimethyl ester except for variations in the proportions of the *cis* and *trans* forms due to isomerization.

This paper presents the results of an examination of the reaction of diazomethane with aconitic acid and its trimethyl ester. It is shown that a pyrazoline derivative is formed in the presence of excess diazomethane and this compound decomposes on chromatographic columns at moderately low temperatures. Depending upon the stationary phase, the various reaction products complicate the analysis of the Krebs-cycle acids.

## EXPERIMENTAL

Microanalyses were carried out by Unisearch Ltd. Microanalytical Services. Infrared spectra were obtained on a Jasco IRA-1 instrument using paraffin mulls. A Shimadzu Model GC-4B gas chromatograph fitted with a flame ionization detector and borosilicate glass columns were used. The columns were packed with coated Chromosorb W (80–100 mesh, AW, DMCS treated) as detailed in Table I. Both column

TABLE I

GAS CHROMATOGRAPHIC RETENTION DATA FOR TRIMETHYL *trans*-ACONITATE AND RELATED COMPOUNDS ON VARIOUS COLUMNS

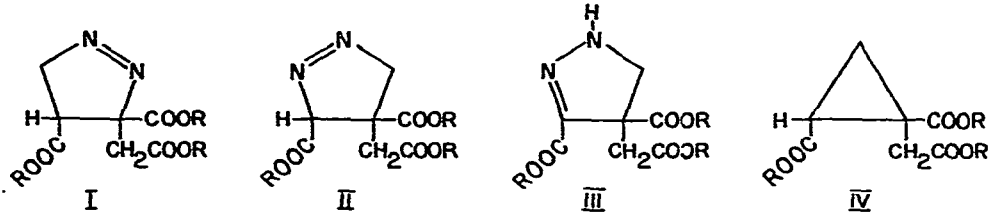
Column 1: 1.5 m, 10% Apiezon L + 5% OV-17 mixed support 2:1 by weight; column 2: 1 m, 5% Carbowax 20M; column 3: 1 m, 5% DEGS; column 4: 17 cm 4% SE-30; column 5: 0.5 m, 5% QF-1. Flow-rate 25 ml/min, unless stated otherwise.

| *Column* | *Column temp. (°C)* | *Injection port temp. (°C)* | *Retention time (min)* | | |
|---|---|---|---|---|---|
| | | | *Trimethyl trans-aconitate* | *Pyrazoline derivative (I)* | *Cyclopropane derivative (IV)* |
| 1a | 160 | 200 | 9.5 | 10.4*** | 10.4 |
| 1b | 160 | 160 | 9.5 | 10.6*** | 10.4 |
| 1c* | 130 | 130 | 19.6 | 22.6*** | 21.6 |
| 2 | 130 | 180 | 12.2 | 12.0*** | 12.0 |
| 3 | 130 | 180 | 20.0 | 20.5 | 20.0 |
| 4 | 90 | 130 | 2.4 | 7.4 | 3.0 |
| 5 | 130 | 130 | 3.2** | 12.8 | 3.2 |

* Flow-rate, 50 ml/min.
** Retention time for trimethyl *cis*-aconitate is 4.0 min.
*** Slow decomposition evident in the tailing of the chromatographic peak.

and injection port temperatures were varied (see Table I) and the detector temperature, alone, held constant at 220°. All products examined by GC were introduced by direct on-column injection.

Solutions of diazomethane in ether were prepared by the alkaline decomposition of "Diazald" (Aldrich, Milwaukee, Wisc., U.S.A.). The diazomethane was removed from the reaction tube, and trapped in a second tube containing ether, by a stream of nitrogen passing through the reaction mixture.




*Synthesis of the pyrazoline compound (3-carbomethoxymethyl-[3,4-dicarbomethoxy]-1-pyrazoline, I)*

Trimethyl *trans*-aconitate (0.61 g), previously prepared by esterification of *trans*-aconitic acid (Calbiochem, Los Angeles, Calif., U.S.A.) with methanol containing sulphuric acid as catalyst, was reacted with portions (6 × 60 ml) of a diazomethane solution (generated from 5 g Diazald). The conversion of the aconitate to the pyrazoline (I) was followed by CC on column 1a (see Table I) on which the pyrazoline was decomposed to the cyclopropane derivative (IV). Evaporation of the solvent and vacuum drying (30°, 4 h) yielded a pale yellow liquid (0.70 g, 98%). (Found: C, 46.5; H, 5.5; N, 10.7; calculated for $C_{10}H_{14}O_6N_2$: C, 46.5; H, 5.5; N, 10.8%.)

Examination of the pyrazoline under conditions which do not cause it to de-

compose (on column 5) revealed the presence of a second minor compound (retention time 18.0 min; peak area ratio of 6.7:1 for the two components). This compound has not been identified but it is probably the pyrazoline II or its tautomer (III).

Reaction of *trans*-aconitic acid (38 mg) in methanol (5 ml) with excess diazomethane solution over several days showed that the same compounds were present when the product was examined on column 5. Similar treatment of *cis*-aconitic acid (40 mg) with diazomethane (at 20°) gave three chromatographic peaks on column 5 (retention times 12.8, 16.0 and 18.0 min). Of these, the first two peaks were equal in area, while the third peak appeared as a shoulder on the second peak. The first peak is identified as I, produced evidently by isomerization of *cis*- to *trans*-aconitate during esterification and subsequent cycloaddition of diazomethane. The second peak is assumed to be due to the *cis*-isomer of pyrazoline I, while the third peak corresponds to the same compound present in the pyrazoline I, and referred to above. Diazomethylation of *cis*-aconitic acid at a lower temperature (—20°) did not increase the proportion of the *cis* analogue of I. Treatment of *cis*-aconitic acid with diazomethane (carefully avoiding excess) showed that the *cis*-aconitate trimethyl ester initially formed was completely isomerized to the *trans* ester on standing overnight. However, as no pyrazoline was observed, this suggests that the cycloaddition reaction is much slower than the esterification reaction.

*Synthesis of the cyclopropane derivative (1-carbomethoxymethyl-1,2-dicarbomethoxy-cyclopropane, IV)*

The pyrazoline (I, 0.1 g) was heated in air at 160° for 30 min. The infrared spectrum of the residue showed loss of the –N=N– at 1560 $cm^{-1}$. (Found: C, 51.8; H, 6.0; N, 0.5; calculated for $C_{10}H_{14}O_6$: C, 52.2; H, 6.1; N, 0.0%). The amount of nitrogen indicated that about 5% of the pyrazoline remained, however further heating at 140° for 50 min did not reduce the nitrogen content. GC also showed, under high sensitivity, that some pyrazoline compound remained.

In addition, the cyclopropane derivative IV appeared as two peaks on column 1a (retention times 10.4 and 12.0 min with peak area ratio 7:1), and on column 2 the first of these components was separated further into two peaks (with retention times 12.0 and 14.0 min, and 24.0 min, respectively; peak area ratios 5:3:1). The additional compound has not been identified but the peak with retention time of 14.0 min is believed to be due to the *cis* form of IV.

## RESULTS AND DISCUSSION

The trimethyl ester of *trans*-aconitic acid reacted with diazomethane to form mainly a 1-pyrazoline compound (I). A second product was not positively identified but is believed to be either compound II or III.

Identity of I was deduced from its infrared spectrum which displayed the characteristic absorption[8] due to –N=N– at 1560 $cm^{-1}$. In addition, the absence of N–H absorption at 3100–3600 $cm^{-1}$ indicated that tautomerism was absent. Although pyrazolines of type II usually tautomerise[9] to 2-pyrazolines such as III, a similar 1-pyrazoline was formed[10] upon the addition of diphenyldiazomethane to the ester of aconitic acid. In contrast, with dimethyl fumarate cycloaddition of diazomethane gives a 2-pyrazoline which is stable[3,7] during GC.

Differential thermal analysis of I showed that an exothermic reaction involving the loss of nitrogen began at 140°. Pyrolysis of I at 130° for 30 min showed little loss of nitrogen. At 140° for 40 min, almost total elimination of nitrogen occurred, resulting with the formation mainly of the cyclopropane derivative IV in which the infrared absorption for –N=N–, –C=C– (in the region 1600–1700 $cm^{-1}$) were both absent. The observation has been made that the pyrolysis of 1-pyrazolines gives rise to two main types of products, namely, isomeric cyclopropanes and olefins[8,10–11].

The behaviour of I, IV and the aconitate ester on polar and non-polar stationary phases is indicated by the retention times given in Table I. With columns approaching normal length (see Columns 1–3), the pyrazoline was decomposed on the column to form the cyclopropane derivative. On shorter columns (see columns 4 and 5) which allowed the use of lower temperatures no decomposition of I was observed. On polar columns 2, 3 and 5 there was little or no separation between trimethyl aconitate and the cyclopropane derivative so that their differentiation based on retention times alone was difficult. There was only a small effect attributable to the injection port temperature.

Following injection and decomposition, the degree of tailing exhibited by the resultant cyclopropane derivative was dependent on the stationary phase. Thus, at 160° slight tailing was observed on columns 1a and 1b whereas symmetrical peaks were obtained on column 3. At 130°, extensive tailing (for as much as 20 min) on columns 1c and 2 indicated a slower decomposition of the pyrazoline. On columns 4 and 5, the pyrazoline gave symmetrical peaks also. With all columns the cyclopropane derivative yielded symmetrical chromatographic peaks.

Clearly this evidence shows the possibility of mis-judging the pyrazoline I or its decomposition product IV for the methyl ester of aconitic acid, depending upon the selection of the stationary phase. Furthermore, in the analysis of Krebs-cycle acids as the methyl esters, the cyclopropane derivative IV formed by the on-column pyrolysis of the pyrazoline I interfered with the estimation of the citric acid ester on non-polar but not on polar columns. However, a non-polar column was preferable because it caused less interference from long-chain ($C_{12}$–$C_{18}$) fatty acid methyl esters. We conclude that, although convenient to use, diazomethane is unsuitable as the esterification reagent for the total analysis of the Krebs-cycle acids.

## REFERENCES


1 A. Kuksis and P. Vishwakarma, *Can. J. Biochem. Physiol.*, 41 (1963) 2353.
2 H. H. Luke, T. E. Freeman and L. B. Kier, *Anal. Chem.*, 35 (1963) 1916.
3 G. G. McKeown and S. I. Read, *Anal. Chem.*, 37 (1965) 1780.
4 F. L. Estes and R. C. Bachmann, *Anal. Chem.*, 38 (1966) 1178.
5 A. Kuksis and P. Prioreschi, *Anal. Biochem.*, 19 (1967) 468.
6 D. S. Zaura and J. Metcoff, *Anal. Chem.*, 41 (1969) 1781.
7 C. A. Atkins and D. T. Canvin, *Can. J. Biochem.*, 49 (1971) 949.
8 T. V. van Auken and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 84 (1962) 3736.
9 K. V. Auwers and O. Ungemach, *Chem. Ber.*, 66 (1933) 1198.
10 J. van Alphen, *Rec. Trav. Chim. Pays-Bas*, 62 (1943) 334.
11 W. M. Jones and T. Wun-Ten, *J. Org. Chem.*, 27 (1962) 1030.