CHROM. 6070

METHYLATION OF DIBASIC ACIDS WITH DIAZOMETHANE IN GAS CHROMATOGRAPHY

OTAKAR MLEJNEK
Cables and Insulating Materials Research Institute, Bratislava (Czechoslovakia)
(Received March 3rd, 1972)

SUMMARY

The conditions required for the qualitative and quantitative methylation of saturated and unsaturated aliphatic and aromatic polybasic acids with diazomethane were studied. The presence of side reactions of maleic, fumaric and itaconic acids at room temperature was confirmed. The extent of these reactions depends on the temperature of the medium; at −60°, methylation predominated over the side reactions. It was found to be advantageous to replace diethyl ether by dioxan as the solvent for both sample and reagent for the quantitative methylation of aliphatic saturated and aromatic acids.

INTRODUCTION

The gas chromatography of free polybasic organic acids, with the exception of some lower dibasic acids, is possible only after their transformation into more volatile, less polar derivatives. For this purpose, esterification with diazomethane (DAM) has been used, in addition to other methods, for many years, and is characterized by its simplicity and speed. Although this method is completely successful for the diazomethylation of monobasic acids, the derivatization of some unsaturated dibasic acids is accompanied by undesirable side reactions. Our intention was to investigate the behaviour of selected dibasic acids that are used in the synthesis of polyesters and in the curing of epoxides.

The diazomethylation of free carboxylic groups is currently carried out by the introduction of gaseous DAM[1,2] or by the addition of DAM solution in ether to an ethereal solution of the sample[3,4]. The former is the more effective method[1]. The reaction proceeds at room temperature, or at 0°, and requires only a short period of time; the rate of methylation can be increased by adding various catalysts, such as methanol or water[1] or alkaline hydroxides or by irradiation with UV light[3]. The main advantage of this method is, however, that the products do not need to be isolated.

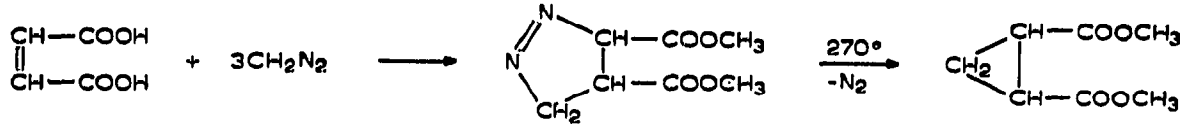
The problem of diazomethylation of most organic acids consists in the preparation of the reagent itself. The commercial solutions of DAM in ether are not sufficiently stable and therefore the laboratory preparation of fresh reagent by the decomposition of certain compounds is recommended.

Gaseous DAM can be generated from nitrosomethylurea (NMU)[2,5,6] or from N-methyl-N-nitroso-*p*-toluenesulphonamide (NTSA)[4,7,8] by decomposition with

alkaline hydroxides. NMU can be prepared by the saturation of methylurea with the reaction products of nitrous acid and arsenic[5], or by the reaction of urea, methylamine and sodium nitrite[6]. NTSA is prepared from methylamine and sodium nitrite by reaction with *p*-toluenesulphonyl chloride[7]; a commercial product is also available.

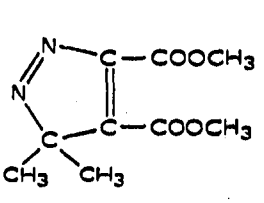
The side reactions that accompany diazomethylation are undesirable, *e.g.*, the production of polymers and the addition of DAM to double bonds. Polymer formation has been described by some workers[2,9,10]. MORRISON *et al.*[10] analyzed the polymer formed during the diazomethylation of higher fatty acids and found the presence of 54.4 % C, 6.4 % H, 38.0 % O and less than 1 % N. It is the predominating opinion that the polymer formed is polymethylene. In order to prevent the formation of polymer, BARTSCH *et al.*[9] recommended the use of a reaction temperature of 0°; the losses of DAM are simultaneously decreased. SCHLENK AND GELLERMAN[1] suggested that the amount of polymer formed is a function of the reaction time; they therefore recommend the use of a catalytic acceleration of the methylation reaction by the addition of methanol.

The reaction of DAM with olefins and unsaturated acids has been described in various publications. WHITMORE[11] described the origin of pyrazoline derivatives from maleic and fumaric acids. KARRER[12] suggested that the pyrazoline derivatives lose nitrogen by heating to form cyclopropane-1,2-dicarboxylic acids:

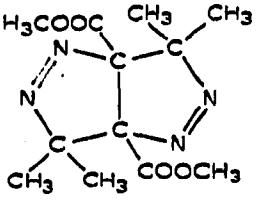


HARMON AND DOELLE[13] assumed that 4,5-dicarboxymethylpyrazoline is formed from fumaric acid in order to explain the very long elution time of dimethyl fumarate as found by LUKE *et al.*[14]. The reaction of DAM with maleic acid can also be used for the microsynthesis of 4,5-dicarboxymethylpyrazoline[2].

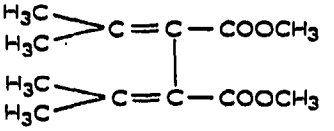
FRANC-NEUMAN[15] studied the addition of diazopropane to triple bonds during the reaction with the dimethyl ester of acetylenedicarboxylic acid. The formation of 3,4-bis(methoxycarbonyl)-5,5-dimethylpyrazoline (I) was suggested, which leads photochemically to 1,5-bis(methoxycarbonyl)-4,4,8,8-tetramethyl-2,3,6,7-tetraazabicyclo[3,3,0]octa-2,6-diene (II) and by heating at 270° to products represented by formula (III).



I



II



III

The addition of DAM to double bonds is a characteristic reaction of dibasic acids; it was not observed with monobasic acids[1,3,16]. Thus, SCHLENK AND GELLERMAN[1] found no activity after the reaction of ethyl esters of C_{16}–C_{18} fatty acids with ^{14}C-labelled DAM and after their chromatographic separation. The IR spectrum of soya-bean oil reacted with DAM did not show any changes that would indicate the

presence of pyrazoline derivatives or cyclopropane structures, and ORD AND BAMFORD[3] did not observe the formation of cyclopropane rings or *cis–trans* isomerization when phosphatidic fatty acids reacted with DAM, after their TLC separation and UV detection. Equally, the results obtained by VORBECK *et al.*[16] in the methylation of oleic and linoleic acids by comparing theoretical and practical yields were negative. All the investigated methods of derivatization (DAM, esterification with methanol in the presence of HCl, BF_3 or ion exchanger) led to the same theoretically predicted results.

From the given survey, it follows that diazomethylation represents a convenient and effective method for the derivatization of monobasic and saturated dibasic acids. The methylation of unsaturated dibasic acids is, however, problematic and requires additional investigation.

EXPERIMENTAL

Gas chromatography

The gas chromatograph used was a Carlo Erba Fractovap P-AID/f instrument provided with a flame ionization detector and a linear temperature programmer. A stainless-steel column, 80 cm long and 5 mm I.D., was used, containing 0.1 % mixed phase (60 % polyethylene glycol succinate and 40 % Carbowax 20M) on 0.20–0.25 mm glass beads treated with HF (ref. 17). The column temperature was programmed from 70 to 120° at 4.5°/min, and the inlet pressure of the carrier gas (nitrogen) was 0.2 kp/cm^2.

Reagents

All chemicals, obtained from Lachema or Chemapol, were of reagent grade and were used without further purification, except for dioxan. Dioxan was refluxed with aqueous 1 *N* HCl, shaken with dilute KOH, refluxed with metallic sodium and distilled through a 1-m column.

Preparation of DAM

The preparation of DAM was carried out by a modified method of SCHLENK AND GELLERMAN[1], comprising the decomposition of NTSA with KOH. For this purpose we used two solutions, one containing 0.7 g of NTSA in 5 ml of diethyl ether or dioxan and the other 10 g of KOH in 10 ml of distilled water and 20 ml of methanol.

Stock solutions

The stock solutions of individual acids were prepared by dissolving 100 mg of sample in 2 ml of methanol containing 100 μl of diphenylmethane as the internal standard. Then 10 μl of these solutions, after dilution with 50 μl of ether or dioxan, were saturated with DAM. The reactions were carried out at room temperature, at 0° (cooling with ice-water) and at −60° (cooling with solid CO_2). The excess of DAM was expelled with a stream of nitrogen at the reaction temperature until the solutions were colourless.

The standard methyl esters of the selected acids were prepared by heating 100 mg of the free acid with 300 μl of methanol containing 2 % H_2SO_4 for 2 h at 60° in a sealed ampoule. After cooling and diluting with water, the esters were separated by extraction with *n*-hexane.

RESULTS AND DISCUSSION

Qualitative evaluation

We prepared and analyzed the products of diazomethylation of the following polybasic acids; succinic, maleic, fumaric, itaconic, adipic, sebacic, *o*-, iso- and terephthalic, tetrahydrophthalic and trimellitic. The products of all these acids, except for maleic, fumaric and itaconic, appeared on the chromatograms as single well defined peaks, and elution times agreed well with the elution times of corresponding standards. Moreover, elution time is independent of the diazomethylation conditions.

Maleic, fumaric and itaconic acids, however, gave a number of peaks according to the diazomethylation temperature used. The results for relative elution times and relative peak areas as a function of the reaction temperature are summarized in Table I. The chromatographic spectra are shown in Figs. 1–3.

On the basis of these results, one can assume that in the course of the diazomethylation of unsaturated aliphatic dibasic acids with a double bond conjugated with at least one carboxylic group, side products arise as shown by the number of peaks on the chromatogram.

The absolute magnitudes of the individual peaks depend on the temperature and the arrangement of the injection port. Inserting a glass-wool plug into this port favours the appearance of peaks of side products. The maximum peak height was obtained at injection port temperatures above 200°.

The relative peak ratio of individual acids varies with the diazomethylation temperature. On lowering the temperature, the magnitude of the peaks of products of side reactions decreases and that of the methyl ester peaks increases. At —60°, the peaks of the methyl esters predominate, which indicates that the side reactions are supressed to a minimum without adversely affecting the esterification reactions.

Although maleic and fumaric acids differ in the elution times of their methyl

TABLE I

RELATIVE PEAK AREAS OF DIAZOMETHYLATION PRODUCTS OF MALEIC, FUMARIC AND ITACONIC ACIDS

Acid	*Peak*		*Relative peak area at reaction temp.*		
	No.	t_R	*22°*	*0°*	*—60°*
Maleic	1	0.30	32.2	14.7	2.2
	2	0.37	29.1	11.2	2.0
	3[a]	0.44	13.9	61.1	94.4
	4	0.68	24.8	13.0	1.4
Fumaric	1[a]	0.30	29.7	30.2	91.9
	2	0.37	27.5	28.5	2.8
	3	0.44	15.0	14.7	2.2
	4	0.68	27.7	25.7	3.1
Itaconic	1[a]	0.46	3.9	11.7	80.7
	2	0.58	66.1	60.3	13.2
	3	0.63	22.2	21.1	4.3
	4	0.71	7.8	6.9	1.6

[a] Corresponding methyl esters.

esters, their side products give identical chromatographic spectra, indicating that *cis-trans* isomerization occurs.

As the small amounts of methyl esters that are found during diazomethylation at room temperature could be due to their volatilization during the bubbling of nitrogen enriched with DAM through the ethereal solution of the sample, we compared the yields of the methyl esters of maleic and succinic acid. We found that the methyl ester formed by maleic acid is only a small proportion of that given by succinic acid. That means that volatilization is not the main reason for the low yield of methyl esters of unsaturated acids.

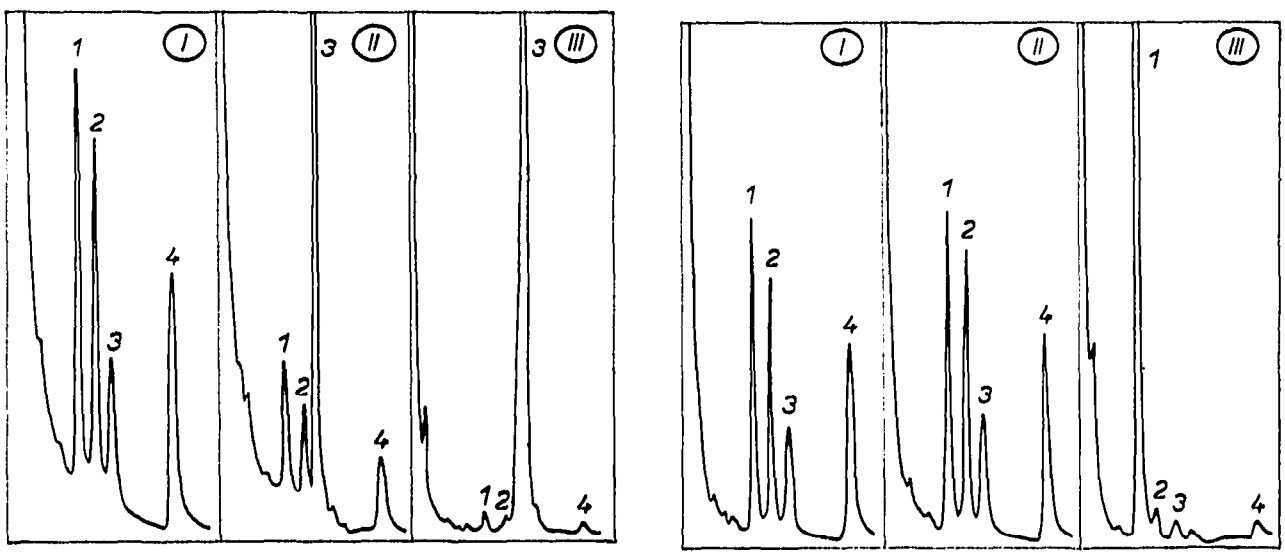


Fig. 1. Chromatogram of derivatization products of maleic acid at various diazomethylation temperatures (I = 22°; II = 0°; III = −60°). Conditions as described in EXPERIMENTAL section. Vapourizer temperature, 200°. Peak No. 3 represents the corresponding methyl ester, the other peaks the products of side reactions.

Fig. 2. Chromatogram of derivatization products of fumaric acid at various diazomethylation temperatures. Conditions as in Fig. 1. Peak No. 1 represents the corresponding methyl ester.

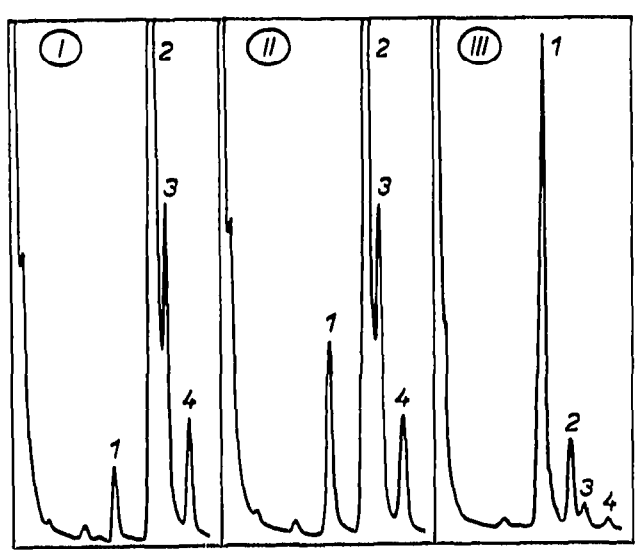
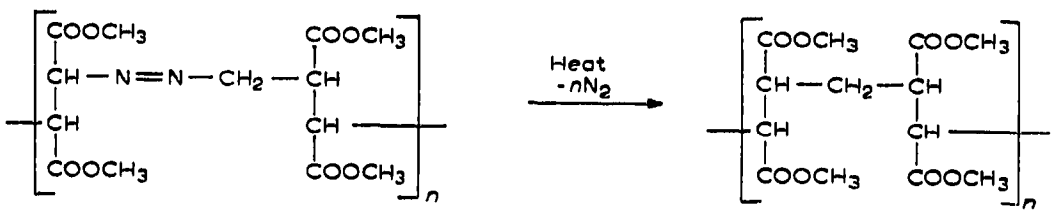


Fig. 3. Chromatogram of derivatization products of itaconic acid at various diazomethylation temperatures. Conditions as in Fig. 1. Peak No. 1 represents the corresponding methyl ester.

On the basis of the information acquired, it appears that during diazomethylation at temperatures higher than —60°, side reactions take place that lead to the formation of thermolabile products, which partly decompose in the flush heater of the gas chromatograph. Maleic and fumaric acids thus produce *cis*- and *trans*-3,4- or 4,5-bis(methoxycarbonyl)pyrazoline, which decompose to the corresponding 1,2-cyclopropane derivatives with the same proportions of *cis* and *trans* isomers. Under the same conditions, itaconic acid gives 3-methylbis(methoxycarbonyl)pyrazoline and after decomposition the dimethyl ester of 3-methylene-1,1-cyclopropanecarboxylic acid.

It is interesting that under no conditions were we able to obtain a peak of a pyrazoline derivative on the chromatogram. Therefore, it can be assumed that another reaction mechanism also occurs, giving less volatile products, such as polymers. With maleic and fumaric acids, the following equation can be taken into consideration:



The side reactions that occur during diazomethylation can be prohibited by cooling the reaction mixture to —60° with solid CO_2 and removing the excess of DAM by bubbling nitrogen through the solution at the same temperature. The rate of the methylation itself is not decreased significantly under these conditions.

Quantitative evaluation

During the determination of the yields of the methyl esters of aliphatic saturated and aromatic dibasic acids, we found that for quantitative purposes it is advantageous to replace diethyl ether by dioxan as the solvent for both sample and reagent. This follows also from Table II, which gives the results of bubbling DAM

TABLE II

QUANTITATIVE DIAZOMETHYLATION OF ADIPIC AND ISOPHTHALIC ACIDS

Sample[a]	*Solvent*	*Temperature* (°C)	*Found* (%)
DMA	Diethyl ether + methanol, 9:1	22	95.2 ± 2.4
AA			93.3 ± 2.1
DMA	Diethyl ether + methanol, 9:1	—60	94.3 ± 1.5
AA			92.7 ± 1.7
DMA	Dioxan + methanol, 2:1	22	100.2 ± 2.5
AA			98.7 ± 2.6
DMIP	Dioxan + methanol, 2:1	22	101.9 ± 1.6
IPA			100.7 ± 1.1

[a] DMA = dimethyl adipate; DMIP = dimethyl isophthalate; AA = adipic acid; IPA = isophthalic acid.

through the ethereal solutions of either free adipic and isophthalic acids or their methyl esters. These acids were chosen for their importance in the production of electrical insulating enamels.

It is evident that during the reaction in diethyl ether, losses in the dimethyl esters and non-quantitative methylation occur, whereas the temperature has little effect. On replacing diethyl ether by dioxan, the theoretical value (100 %) lies within the experimental confidence interval of the results, calculated from six parallel determinations. The probable relative error is small enough and does not exceed the limits that are currently used in gas chromatography.

The use of dioxan as a solvent for NTSA is also advantageous in that it has good miscibility with water and methanol (solvent for KOH in the preparation of DAM). The good mixing of both reagents results in a homogeneous solution, providing more intensive generation of DAM. The reaction time is thus shortened and the extent of the side reactions is decreased.

However, because of the comparatively high melting point of dioxan, it is not a suitable solvent for the diazomethylation of unsaturated aliphatic dibasic acids, where the derivatization takes place at —60°. In this case, the use of methanol as the solvent is more preferable.

ACKNOWLEDGEMENT

The author would like to thank Miss H. SEČKÁŘOVÁ for her technical assistance.

REFERENCES

1 H. SCHLENK AND J. L. GELLERMAN, *Anal. Chem.*, 32 (1960) 1412.
2 R. ROPER AND T. S. MA, *Microchem. J.*, 1 (1957) 245.
3 W. O. ORD AND P. C. BAMFORD, *Chem. Ind.*, (1957) 2115.
4 G. ZWEIG, T. E. ARCHER AND D. RAZ, *Agric. Food Chem.*, 10 (1962) 191.
5 H. MEYER, *Analyse und Konstitutions-Ermittlung Organischer Verbindungen*, Springer, Prague, 1916, p. 614.
6 F. ARNDT, *Organic Syntheses*, Vol. 2, John Wiley, New York, 1943, p. 165.
7 J. TH. DE BOER AND H. J. BACKER, *Recl. Trav. Chim. Pays-Bas*, 73 (1954) 229.
8 G. ZWEIG, D. L. GUTNICK, R. GULLI, T. E. ARCHER AND H. T. HARTMANN, *Agric. Food Chem.*, 12 (1964) 59.
9 W. R. BARTSCH, F. D. MILLER AND F. M. TRENT, *Anal. Chem.*, 32 (1960) 1101.
10 W. R. MORRISON, T. D. V. LAWRINE AND J. BLADES, *Chem. Ind. (London)*, (1961) 1534.
11 F. C. WHITMORE, *Organic Chemistry*, Van Nostrand, New York, 1951, p. 769.
12 P. KARRER, *Lehrbuch der Organischen Chemie*, Thieme, Stuttgart, 1950, p. 707.
13 M. A. HARMON AND H. W. DOELLE, *J. Chromatogr.*, 42 (1969) 157.
14 N. H. LUKE, T. E. FREEMAN AND L. B. KIER, *Anal. Chem.*, 35 (1963) 1916.
15 M. FRANC-NEUMAN, *Angew. Chem.*, 79 (1967) 98.
16 M. L. VORBECK, L. R. MATTICK, F. A. LEE AND C. S. PEDERSON, *Anal. Chem.*, 33 (1961) 1512.
17 O. MLEJNEK AND N. KLIMAN, *Chem. Zvesti*, 18 (1964) 99.