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METHOD FOR THE ESTERIFICATION OF CARBOXYLIC ACIDS IN GAS CHROMATOGRAPHIC ANALYSIS

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SUMMARY

A method for the esterification of carboxylic acids with the aid of N,N'-dicyclohexylcarbodiimide is described.

The time needed for complete esterification with methanol of some aliphatic, aromatic and heterocyclic carboxylic acids was determined. A series of ten determinations of the content carried out on samples of pure pyrazinoic acid was carried out in order to evaluate the reproducibility of the method. Examples of quantitative esterification are also described for other alcohols that can be used instead of methanol in difficult gas chromatographic separations, for example, of isomers.

INTRODUCTION

The gas chromatography (GC) of free carboxylic acids is not generally applicable because of their high polarity and low volatility (for many acids with high molecular weight) and because they are subject to side-reactions such as decarboxylation and transformation into anhydrides, which produces strongly asymmetric peaks, ghosting and non-reproducible results[1].

These difficulties were partly overcome in some instances by using phosphoric acid[1,2] to deactivate the support or special liquid phases, such as Carbowax 20M terminated with terephthalic or isophthalic acid, and Tween 80–phosphoric acid, or by packing the columns with divinylbenzene–styrene[3,4] copolymers. However, the best results in GC are obtained by converting the free acids into their corresponding methyl esters. Many methods have been described for this esterification, such as the use of catalysts of the BF_3 type, acids (HCl, $HClO_4$, H_2SO_4, etc.) in methanol[1,5], or diazomethane[1,5].

BF_3 facilitates the addition of methanol to double bonds[1] and must be removed at the end of the reaction by evaporating the solvent, with the risk of losses through volatilization. The dehydrating acids do not guarantee a constant yield of esterification products. Reaction with diazomethane is usually quantitative[5], but this reagent must be used with particular care in order to prevent the formation of polymethylenes[6] or addition to double bonds[7].

Silyl esters[5,8,9] have been proposed but the reaction needs an absolutely anhydrous medium owing to ready hydrolysis, which makes their use in GC very difficult.

During a study on the metabolism and pharmacokinetics of the antitubercular compounds pyrazinamide and morphazinamide, we developed a method for the esterification of pyrazinoic acid with N,N'-dicyclohexylcarbodiimide (DIC) as dehydrating agent. This method was extended to other aliphatic, aromatic and heterocyclic carboxylic acids in order to make it generally applicable in routine GC analysis. DIC[10–13] has been used for about 20 years as a reagent for the synthesis of peptides, nucleotides, etc.

With acids, esterification occurs with elimination of one mole of water according to the following reaction[12]:

$$RN{=}C{=}NR \xrightarrow{H^+} RN{=}C{=}\overset{+}{N}HR \xrightarrow{R_1COO^-} RN{=}C(O{-}C{=}O{-}R_1){-}NHR \xrightarrow{R_2OH} R_1COOR_2 + RNH{-}CO{-}NHR$$

The reaction is enhanced by the addition of small amounts of a tertiary amine (tributylamine or, preferably, pyridine) and by increasing the temperature, and is also dependent on the type of acid, alcohol or phenol used[13,14]. By using this reaction, a large number of esters of various acids and alcohols can be obtained in virtually quantitative yields.

Esters other than methyl esters are often useful when the latter do not permit adequate GC separations of very similar compounds, such as optical or positional isomers.

EXPERIMENTAL

Gas chromatography

A Carlo Erba Fractovap GV 200 gas chromatograph equipped with a flame ionization detector was used. The peak areas were calculated by manual measurement or with a Carlo Erba Model 75 integrator attached to a Leeds and Northrup Speedomax W recorder.

Glass U-shaped columns of I.D. 3 mm were used; their lengths were chosen depending on the retention times of the esters under examination, and were 0.75 or 2 m. The columns were packed with 5% Versamid 900 + 3% Carbowax 20M (column A), 5% neopentyl glycol succinate (column B) or 10% SE-30 silicone polymer (column C), all on silanized Chromosorb W, 80–100 mesh, or with 5% Bentone 34 + 5% trimer acid on acid-washed Chromosorb G, 80–100 mesh (column D).

Temperatures between 140° and 180° were used, depending on the retention times of the esters, and nitrogen was used as the carrier gas at a constant flow-rate of 50 ml/min.

Reagents

All of the reagents were of analytical grade, obtained from E. Merck (Darmstadt), BDH (Poole, Dorset) or Schuchardt (Munich); pyrazinoic acid was ob-

TABLE I

PERCENTAGE ESTERIFICATION OF CARBOXYLIC ACIDS AS A FUNCTION OF TIME

Acids as methyl esters	*Temp. of esterification*	*Time (min)*						*Column*			*Internal standard**
		5	*15*	*30*	*60*	*120*	*260*	*Type*	*Length (m)*	*Temp. (°C)*	
Palmitic	Amb.	8	30	50	75	98–100	–	B	0.75	180	1
Stearic	Amb.	–	–	–	–	98–100	–	B	0.75	180	6
Oleic	Amb.	–	–	–	–	98–100	–	B	0.75	180	6
Adipic	Amb.	23	70	86	95	98–100	–	B	2	150	2
Benzoic	Amb.	20	30	40	65	83	89	B	2	150	2
Benzoic	40°	50	85	97	98–100	–	–	B	2	150	2
o-Chlorobenzoic	Amb.	95	96	98–100	–	–	–	D	2	140	2
Nicotinic	Amb.	90	97	98–100	–	–	–	A	2	150	3
Pyrazinoic	Amb.	85	98–100	–	–	–	–	A	2	150	2
m- and *p*-chlorobenzoic	Amb.	–	–	98–100	–	–	–	D	2	140	4
2,4-Dichlorobenzoic	Amb.	–	–	98–100	–	–	–	D	2	140	4
o-, *m*-, *p*-bromobenzoic	Amb.	–	–	98–100	–	–	–	D	2	160	5
o-, *m*-, *p*-iodobenzoic	Amb.	–	–	98–100	–	–	–	D	2	160	5

* Internal standards: 1 = methyl stearate; 2 = methyl nicotinate; 3 = methyl pyrazinoate; 4 = methyl *o*-chlorobenzoate; 5 = *o*-bromobenzoic acid as standard for *m*- and *p*-bromobenzoic acid; *p*-bromobenzoic acid as standard for *o*-bromobenzoic acid and analogously for iodobenzoic acids; 6 = methyl palmitate.

tained with a high degree of purity by recrystallizing it from ethanol; and the *cis–trans* isomers of 2-ethyl-3-(3-nitrophenyl)acrylic acid were separated by means of preparative chromatography on a layer of Silica Gel HF, Merck (solvent, ethyl acetate–isopropanol–25% ammonia solution, 55:35:20) and by crystallizing the products from ethanol.

The various methyl esters were prepared by the reaction of the acids at 0° with diazomethane in an ethereal solution and the esters of the other alcohols by esterification of the acids with an excess of the alcohol in the presence of H_2SO_4, and purification of the esters by fractional vacuum distillation.

Procedure

A 10-mequiv. amount of the acid to be determined is dissolved in 25 ml of the alcohol and 4 ml of pyridine is added as catalyst. DIC is added in excess of the theoretical amount (12 mequiv.) or in even larger amounts if the reagents and the acid used are not dry or contain water of crystallization that reacts with DIC. The mixture is then stirred gently. If the esterifying alcohol is solid (for example, L-menthol), the amount of pyridine is increased so that it acts as a solvent, reducing the amount of alcohol to 20 mequiv.

For most of the acids used, esterification with methanol is quantitative at room temperature; but for some acids and alcohol with high molecular weights it is necessary to heat the mixture at 40–80° for 30–120 min. If during the reaction a precipitate of N,N'-dicyclohexylurea is formed, it is allowed to settle, and after addition of the internal standard, 0.5 μl of the supernatant clear solution is injected into the gas chromatograph.

In easy esterifications, the amount of pyridine used can be reduced to one tenth, with the advantage that less tailing of the pyridine peak occurs. Furthermore, if the excess of DIC should interfere with the peak of an ester, it can be decomposed at the end of the esterification by adding acetic acid.

TABLE II

REPRODUCIBILITY DETERMINED FROM TEN REPLICATE ESTERIFICATIONS OF PYRAZINOIC ACID USING METHYL NICOTINATE AS INTERNAL STANDARD

Determination	*Found (%)*	*Deviation from theoretical (%)*
1	99.5	0.5
2	100.5	0.5
3	100.5	0.5
4	99.5	0.5
5	99.5	0.5
6	100.5	0.5
7	99.8	0.2
8	100.0	0.0
9	100.3	0.3
10	100.0	0.0
Average	100.0	

RESULTS AND DISCUSSION

Table I shows the variation with time of the extent of esterification giving methyl esters of various acids. Table I also shows some acids for which it was established only whether complete esterification could be obtained within a reaction time of 30–120 min, which is acceptable for a routine analysis.

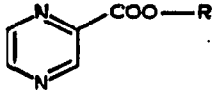
In order to check the reproducibility of the proposed method, ten esterifications of pyrazinoic acid were carried out, using methyl nicotinate as the internal standard, and the areas of the peaks were compared with those obtained by injecting a methanolic solution containing precisely weighed amounts of pure methyl pyrazinoate and methyl nicotinate (Table II). A standard deviation of ± 0.45% was found.

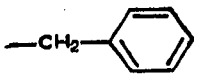
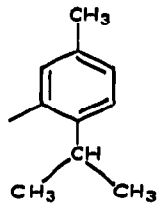
Pyrazinoic acid was also esterified with various alcohols, complete esterification being obtained under the conditions reported in Table III.

The method was also applied with success in the gas chromatographic determination of a mixture of the *cis–trans* isomers of 2-ethyl-3-(3-nitrophenyl)acrylic

TABLE III

ESTERIFICATION OF PYRAZINOIC ACID WITH VARIOUS ALCOHOLS



R	*Temp. of esterification (°C)*	*Esterification time (min)*	*Column Type*	*Length (m)*	*Temp. (°C)*	*Relative retention times*
$-CH_3$	Amb.	15	A	2	150	1.00
$-CH_2-CH_3$	Amb.	< 30	A	2	150	1.20
$-CH_2-CH_2-CH_3$	50	30	A	2	150	1.90
$-CH(CH_3)_2$	50	60	A	2	150	1.12
 (pyridine solvent)	50	60	A C	2 0.75	150 180	> 2 h 1.00
 (pyridine solvent)	50	60	A C	2 0.75	150 180	2 h 1.48

acid, which, after esterification, were separated on a 75-cm column packed with 10% SE-30 silicone fluid on silanized Chromosorb W, 80–100 mesh. The relative retention times were 1.00 for the *cis* form and 1.23 for the *trans* form.

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