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MODIFICATIONS IN THE CHEMICAL DERIVATIZATION OF CARBOXYLIC ACIDS FOR THEIR GAS CHROMATOGRAPHIC ANALYSIS

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SUMMARY

Gas chromatographic analysis of (i) C_1–C_{20} fatty acids, (ii) C_2–C_{16} aliphatic dicarboxylic acids, and (iii) in a single run, C_{16} and C_{18} fatty acids, C_2–C_7 aliphatic dicarboxylic acids, and aromatic di- and polycarboxylic acids, was made possible by derivatization with sulphuric acid–butanol. In addition, it has been shown that aqueous solutions of fatty acids, aliphatic dicarboxylic acids, aromatic polycarboxylic acids and aliphatic hydroxy acids can be esterified by sulphuric acid–butanol, with different yields, depending on the amount of water present. The efficiency of the esterification process in the presence of water has been extended by use of anhydrous sodium sulphate.

INTRODUCTION

According to the classical literature[1], esterification must be carried out under strictly water-free conditions. Owing to the fact that many practical tasks require the analysis of aqueous solutions, there is a need for direct derivatization of carboxylic acids (thus avoiding tedious and time-consuming separation procedures).

Earlier studies have been reported on esterification in aqueous solutions using propanol[2] and methanol[3–7]. Although the results were not quantitative, they did facilitate the rapid determination of the lactic and succinic acid content of cerebrospinal fluid[6]. Since alcohols are immiscible with water, there were no literature data available concerning esterification in the presence of water. With experience involving the formation of homogeneous ternary systems[8] (the necessary knowledge to develop the optimal reaction condition here) butyl esterification in aqueous solutions became possible.

This paper describes the most favourable conditions for direct butyl esterification of C_1–C_{20} fatty acids[9,10], of C_{12}–C_{16} aliphatic dicarboxylic acids[11], of aromatic di- and polycarboxylic acids[12] and those of hydroxy acids[13], respectively. The efficiency of esterification was monitored by gas chromatography (GC) in the following way.

The optimum order of elution of selected members of different homologous series (Figs. 1–5) was determined chromatographically. The possibility of simulta-

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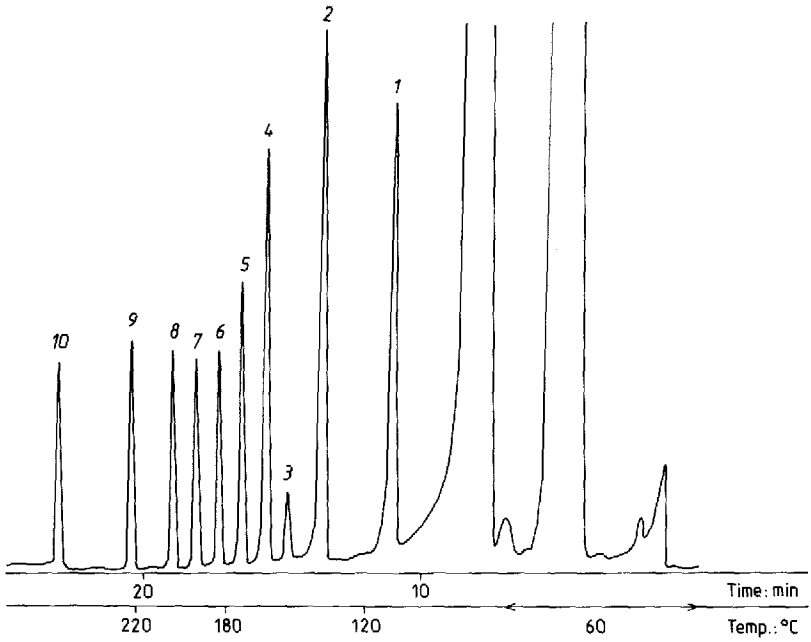


Fig. 1. Chromatogram of the mixture of C_1–C_8 fatty acids *n*-butyl esters. Peaks: 1 = formic; 2 = acetic; 4 = propionic; 5 = isobutyric; 6 = *n*-butyric; 7 = isovaleric; 8 = *n*-valeric; 9 = caproic; 10 = caprylic acid. Peak 3 corresponds to di-*n*-butyl ether. Conditions: column temperature maintained at 60°C for 7 min, then raised at 12°C/min to 220°C, then held 3 min. The temperature of the injector and detector was 280°C.

neous elution of the members of various groups (Fig. 6) was also taken into consideration. With this information model esterifications were then performed (i) in water-free conditions, which serve as the basis for comparison with aqueous esterification[14] (Procedure 1), and (ii) partly in the presence of water. In the latter case, our aim was to evaluate (i) the maximum water content of the esterifying mixture, when esterification in homogeneous solutions containing sulphuric acid proceed to completion (Procedure 2) and (ii) the maximum water content for complete esterification in the presence of anhydrous sodium sulphate (Procedure 3).

MATERIALS AND METHODS

The mole ratios of water to alcohol, sodium sulphate to water, and sulphuric acid to anhydrous sodium sulphate, indicating the concentration of water, the anhydrous sodium sulphate and the sulphuric acid in the esterification mixture, respectively, are given as $[H_2O]/[n\text{-BuOH}]$, $[Na_2SO_4]/[H_2O]$ and $[H_2SO_4]/[Na_2SO_4]$. In each case the data refer to concentrated sulphuric acid.

Materials and reagents

All reagents and carboxylic acids were of analytical purity. The reagents and most of the carboxylic acids were obtained from Reanal (Budapest, Hungary). The C_7–C_{12}, C_{13}, C_{14} and C_{16} dicarboxylic acids, the hemimellitic, trimellitic, trimesic

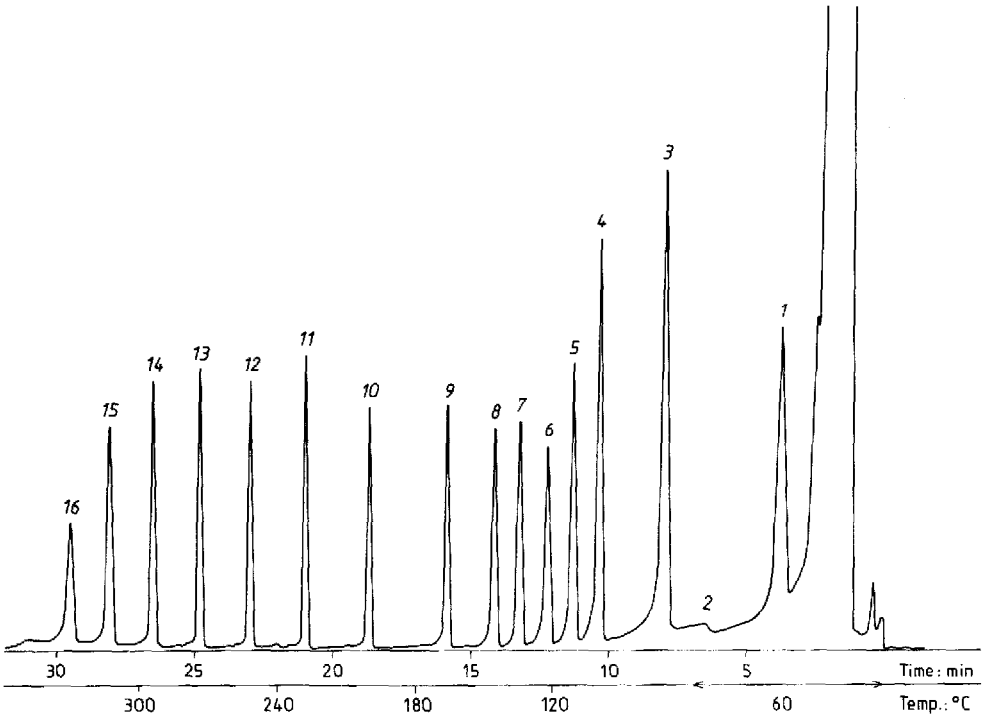


Fig. 2. Chromatogram of the mixture of C_1–C_{20} fatty acid isobutyl esters. Peaks (isobutyl esters of): 1 = formic; 3 = acetic; 4 = propionic; 5 = isobutyric; 6 = *n*-butyric; 7 = isovaleric; 8 = *n*-valeric; 9 = caproic; 10 = caprylic; 11 = capric; 12 = lauric; 13 = myristic; 14 = palmitic; 15 = stearic; 16 = arachidic acid. Peak 2 corresponds to diisobutyl ether. Conditions: column temperature maintained at 60°C for 7 min, then programmed at 12°C/min to 340°C, then held 5 min. The temperatures of the injector and detector were 290°C and 310°C, respectively.

and pyromellitic aromatic carboxylic acids were purchaised from Fluka (Buchs, Switzerland). The support material and liquid phase used in GC were obtained from Applied Science Labs. (State College, PA, U.S.A.).

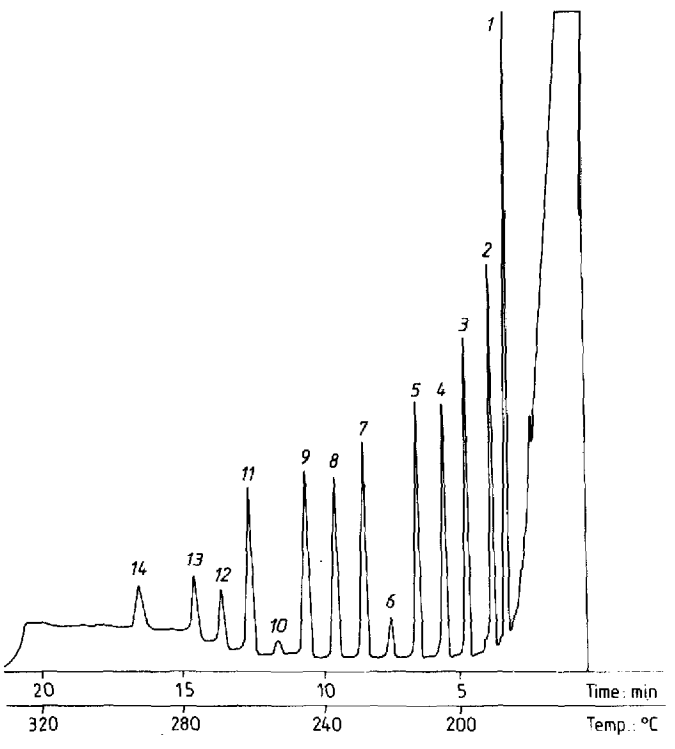
Apparatus

The gas chromatograph was a Model G.C.H.F. 18.3 instrument (Chromatron, Berlin, G.D.R.) equipped with a flame-ionization detector. Chromatographic peak area determinations were made with a Chinoin Model Digint-34μ computing integrator. Stainless-steel columns of 15% Dexsil 300 (2 m × 3 mm I.D.) were used. The packing material was supported on 80–100 mesh Chromosorb W AW DMCS.

Esterification procedures

Model esterifications were carried out with 1–2 ml of the given stock solution neutralized to pH 10–11 (containing 1–2 mg/ml acid of each) in a special glass vessel containing a stirrer (Fig. 7).

Procedure 1. Esterification under strictly water-free conditions, which served as the basis for comparison, was performed according to Gehrke *et al.*[14].



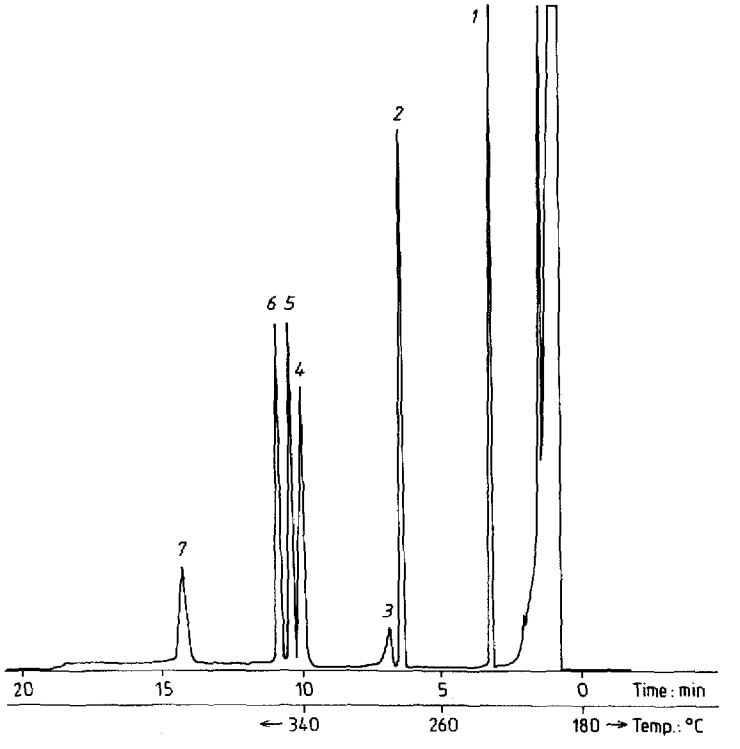


Fig. 3. Chromatogram of the mixture of C_2–C_{16} dicarboxylic acid *n*-butyl esters. Peaks (*n*-butyl esters of): 1 = oxalic; 2 = malonic; 3 = succinic; 4 = glutaric; 5 = adipic; 6 = pimelic; 7 = suberic; 8 = azelaic; 9 = sebacic (decanedioic); 10 = undecanedioic; 11 = dodecanedioic; 12 = brassylic (tridecanedioic); 13 = tetradecanedioic; 14 = hexadecanedioic acids. Conditions: column temperature was programmed at 8°C/min from 160°C to 320°C. The temperatures of the injector and detector were 320°C and 340°C, respectively.

Fig. 4. Chromatogram of the mixture of aromatic carboxylic acids. Peaks (*n*-butyl esters of): 1 = benzoic; 2 = *o*-phthalic; 3 = iso- and/or terephthalic (impurity of *o*-phthalic acid); 4 = hemimellitic; 5 = trimellitic; 6 = trimesic; 7 = pyromellitic acid. Conditions: column temperature was programmed from 180°C to 340°C at 16°C/min. The temperatures of the injector and detector were 340°C and 360°C, respectively.

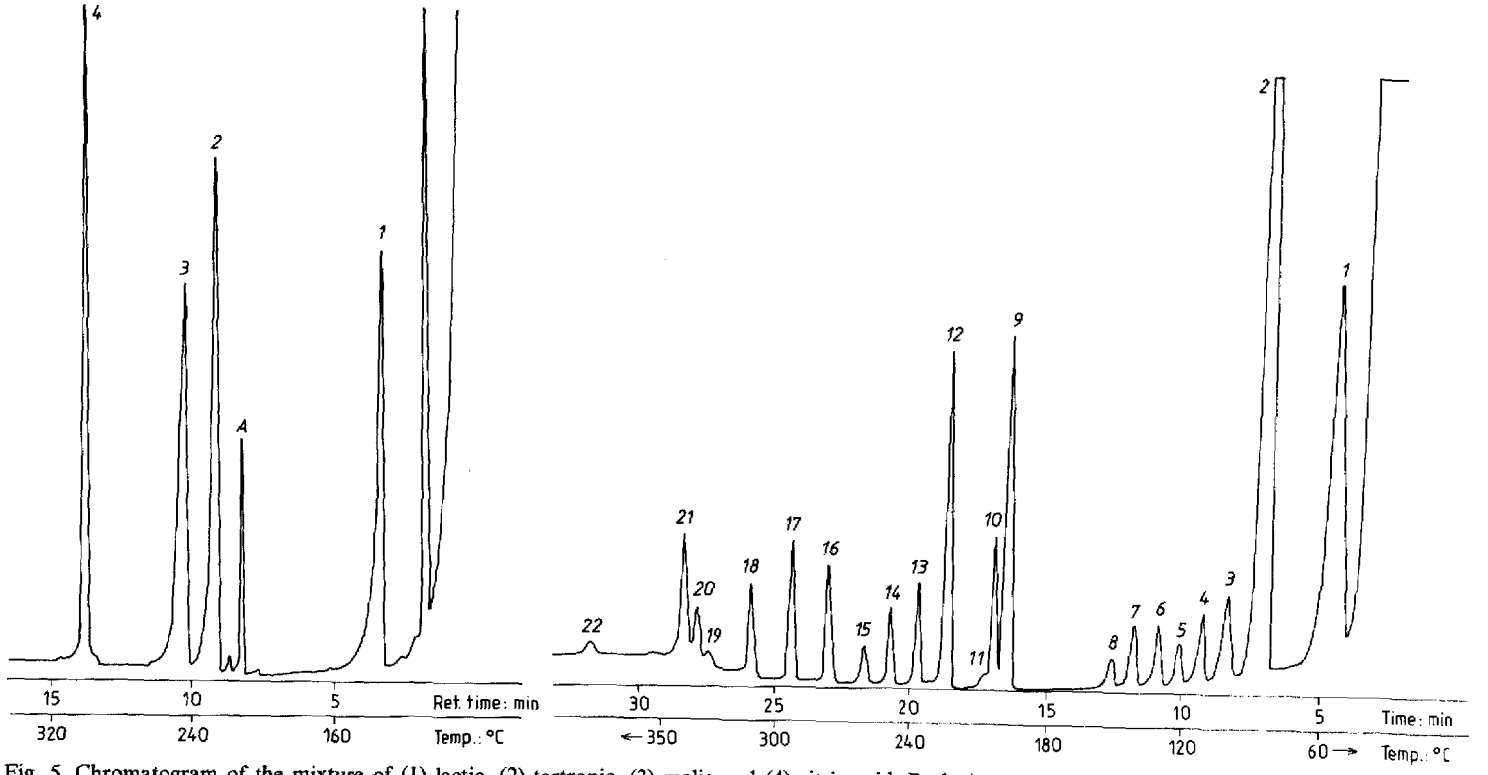
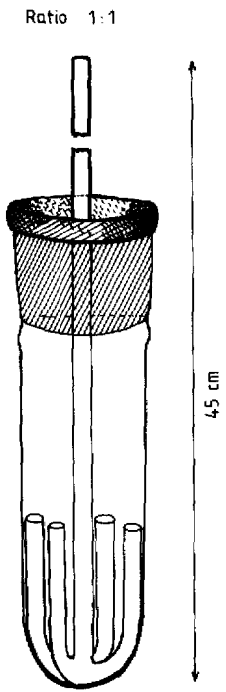
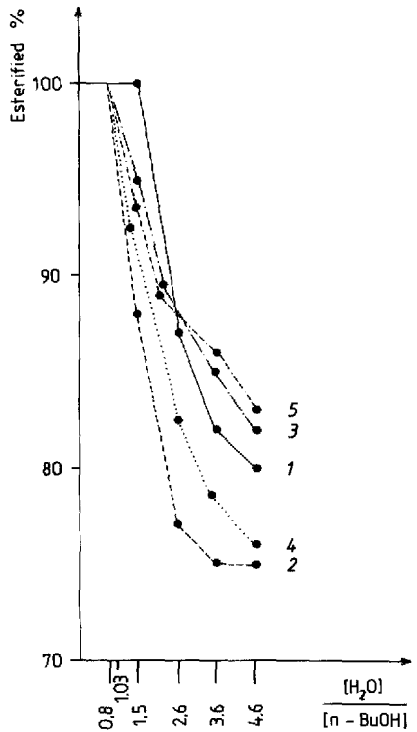


Fig. 5. Chromatogram of the mixture of (1) lactic, (2) tartronic, (3) malic and (4) citric acid; Peak A corresponds to dibutyl sulphate. Conditions: column temperature was programmed from 80°C at 16°C/min to 320°C, then held for 1 min. The temperatures of the injector and the detector were 290°C and 320°C, respectively.

Fig. 6. Chromatogram of the mixture of fatty, aliphatic and aromatic di- and polycarboxylic acids. Peaks (*n*-butyl esters of): 1 = formic; 2 = acetic; 4 = propionic; 5 = isobutyric; 6 = *n*-butyric; 7 = isovaleric; 8 = *n*-valeric; 9 = oxalic; 10 = benzoic; 12 = succinic; 13 = glutaric; 14 = adipic; 15 = pimelic; 16 = *o*-phthalic; 17 = palmitic; 18 = stearic; 19 = hemimellitic; 20 = trimellitic; 21 = trimesic; 22 = pyromellitic acid. Peaks 3 and 11 correspond to diisobutyl ether and dibutyl sulphate, respectively. Conditions: column temperature held at 60°C for 5 min, then programmed at 12°C/min to 350°C. The temperatures of the injector and detector were 350°C and 370°C, respectively.





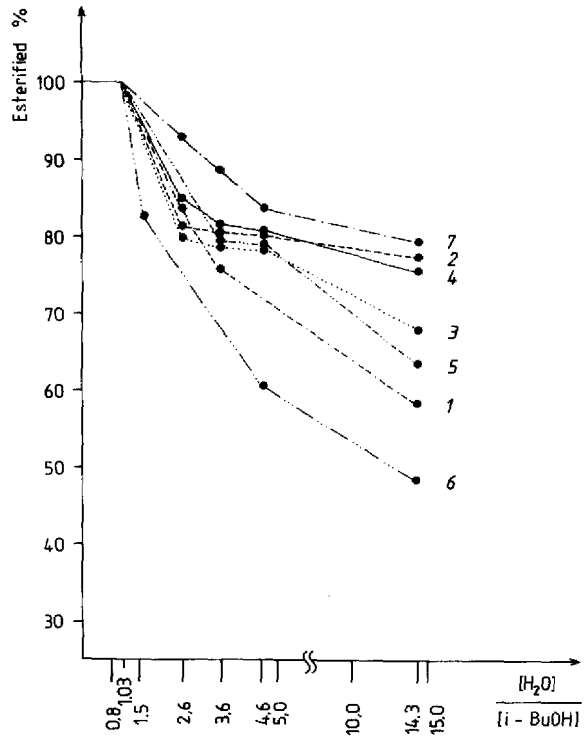


Fig. 7. Vessel and stirrer used for esterification.

Figs. 8. Esterifications of C_1–C_8 fatty acids in solutions containing different amounts of water. Time of esterification, 60 min. Plots: 1 = C_1; 2 = C_2; 3 = C_3; 4 = iso-C_4; 5 = n-C_4–C_8 fatty acids.

Fig. 9. Esterification of C_1–C_{20} fatty acids (60 min). Plots: 1 = C_1; 2 = C_2; 3 = C_3; 4 = n-C_4 + n-C_5; 5 = iso-C_4; 6 = iso-C_5; 7 = C_6–C_{20} fatty acids.

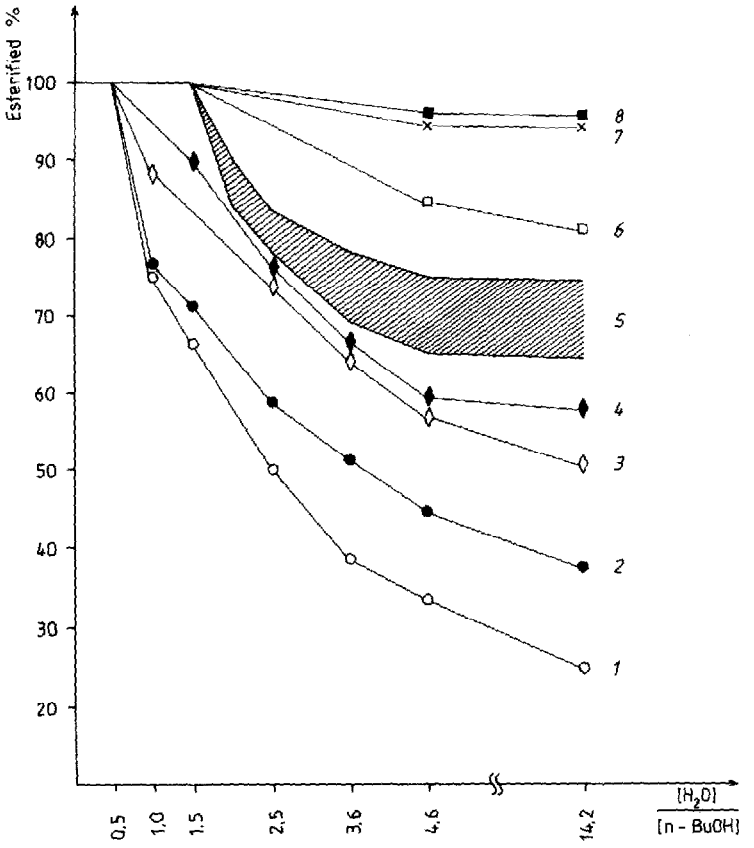


Fig. 10. Esterification of C_2–C_{16} aliphatic dicarboxylic acids (60 min). Plots: 1 = C_2; 2 = C_3; 3 = C_4; 4 = C_5; 5 = C_6–C_{12} (C_6, C_7, C_8, C_9, C_{10} and C_{12}); 6 = C_{13}; 7 = C_{14}; 8 = C_{16} dicarboxylic acids.

Procedure 2. Esterification in aqueous solution in the absence of anhydrous sodium sulphate at $[H_2O]/[alcohol]$ = 0.01–15 was carried out as follows: 1–2 ml of the aqueous solutions of the given acid salts were evaporated to dryness under vacuum. To the residue, 1–2 ml of *n*- or 2-butanol, 0.1–0.4 ml of sulphuric acid and different amounts of water (0.1–3 ml) were added. The ground joint of the vessel, wetted with one drop of sulphuric acid, was fitted to the reflux condenser, and the apparatus placed into the water-bath. Esterification was continued at 100°C for various times depending on the homologous series of the acids under investigation (Figs. 8–12). The solution was cooled to room temperature and transferred into a separatory funnel together with 300 ml of water and 15 ml of chloroform. Extraction was completed with two 15-ml portions of chloroform. The combined chloroform extracts were evaporated under vacuum in a water-bath kept at room temperature to a volume of 2–2.5 ml. A stock solution of 3 ml was then prepared and 5–10 μl aliquots of the stock solution were injected into the gas chromatograph.

Procedure 3. In the course of esterification at $[H_2O]/[alcohol]$ = 2.5–10.3, carried out in the presence of anhydrous sodium sulphate, 1–2 ml aqueous solution of the acids were mixed with 1–2 ml butanol and 0.5–8 g anhydrous sodium sulphate.

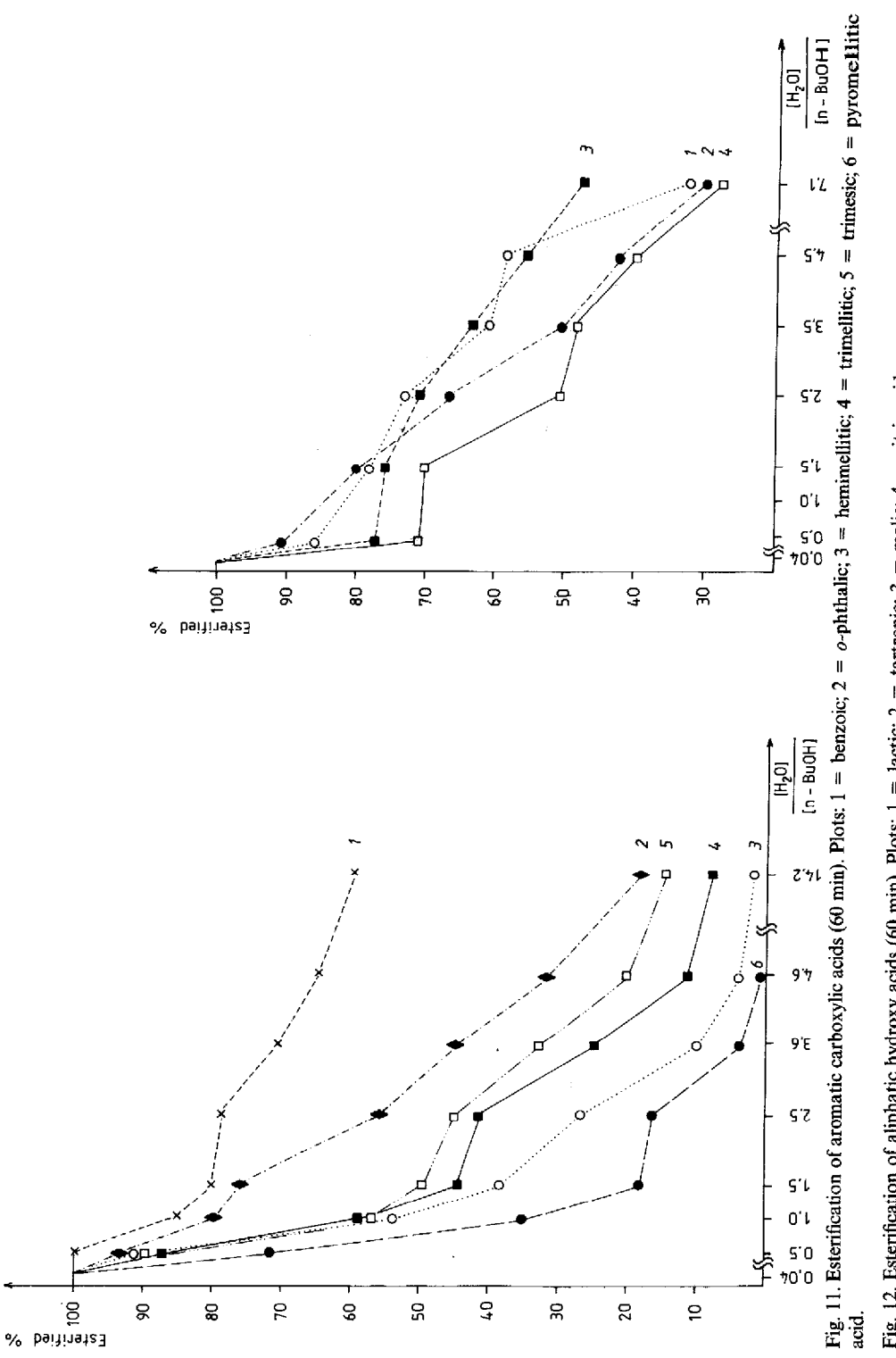


Fig. 11. Esterification of aromatic carboxylic acids (60 min). Plots: 1 = benzoic; 2 = *o*-phthalic; 3 = hemimellitic; 4 = trimellitic; 5 = trimesic; 6 = pyromellitic acid.

Fig. 12. Esterification of aliphatic hydroxy acids (60 min). Plots: 1 = lactic; 2 = tartronic; 3 = malic; 4 = citric acid.

Esterification was performed in the presence of 0.1–1 ml, conc. sulphuric acid. Subsequently Procedure 1 was followed.

RESULTS AND DISCUSSION

From the GC of different homologous series of acids, the following observations can be made.

(i) The C_1–C_{20} fatty acids as normal and isobutyl esters can be separated in a single run (Figs. 1 and 2).

(ii) With reference to butyl esterification of aromatic di- and polycarboxylic acids, no relevant information can be found in the literature (Fig. 4).

(iii) The availability of a convenient method for the GC analysis of aromatic carboxylic acids as their butyl esters is significant, since several practical applications require the simultaneous analysis of members of different homologous series, and the optimum conditions for determining every C_1–C_5 carboxylic acid is provided by using their respective normal or isobutyl esters[1–3]. The simultaneous quantitation of C_1–C_5 fatty acids and C_2–C_7 aliphatic dicarboxylic acids, together with the aromatic di- and polycarboxylic acids (esterifiable with sulphuric acid–butanol[15]) can also be used in the analysis of natural matrices, particularly those of geological origin[16].

Derivatization in aqueous solutions with butanol–sulphuric acid without the use of anhydrous sodium sulphate

In the presence of water originating from the sulphuric acid only, quantitative esterifications were measured in all cases at $[H_2O]/[BuOH] = 0.01$–0.04, depending on the composition of the esterification mixture applied and the homologous series of acids studied (Figs. 8–12 and Table I). Studies performed using solutions with increasing water content (in accordance with the dissociation constants[17]) and increasing chain lengths of the acids, yielded 100% esterification as follows:

$[H_2O]/[iso\text{-}BuOH] \leqslant 1.03$ (C_1–C_{20} fatty acids)

$[H_2O]/[n\text{-}BuOH] \leqslant 0.8$ (C_1–C_8 fatty acids)

$[H_2O]/[n\text{-}BuOH] \leqslant 0.27$ (C_2–C_{16} aliphatic dicarboxylic acids)

Derivatization in aqueous solutions with butanol–sulphuric acid in the presence of anhydrous sodium sulphate

The efficiency of esterification of carboxylic acids in 1–2 ml of water-containing solutions was increased by the use of anhydrous sodium sulphate.

With a knowledge of the optimum conditions concerning the competitive ratios of water to anhydrous sodium sulphate and those of sulphuric acid to anhydrous sodium sulphate[9–13], it can be shown that the increase in the efficiency of esterification using anhydrous sodium sulphate is in close agreement with acid strength, with the chain length and with the substituents of the carboxylic acids to be esterified.

Thus esterification yields of 100% have been obtained with C_1–C_{20}, C_1–C_8 fatty acids, and C_2–C_{16} aliphatic dicarboxylic acids, respectively[10,9,11], under the conditions (see also Table I):

$[H_2O]/[BuOH] = 10.27$, 5.3 and 2.53

$[H_2SO_4]/[Na_2SO_4] = 0.09$–$0.43$, $\geqslant 0.3$, and 0.32–1.3

$[Na_2SO_4]/[H_2O] = 0.2$–0.8, $\geqslant 0.2$ and 0.25–0.75

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF THE CHOSEN REPRESENTATIVES OF HOMOLOGUE SERIES ESTERIFIED UNDER DIFFERENT CONDITIONS

All peak areas listed represent the mean of at least three determinations. In parentheses ( ): esterification yields of trimellitic acid (%) compared to 100%.

Carboxylic acid	*Conditions*	*Peak area obtained, equivalent to 1 μg substance*	*Composition of the esterification mixture*						
			Mole ratios of the components			*Amount of components*			
			[H_2O]/[BuOH]	*[Na_2SO_4anh.]/[H_2O]*	*[H_2SO_4]/[Na_2SO_4anh.]*	*Water* (cm^3)*	*Alcohol (cm^3)*	*Sulphuric acid (cm^3)*	*Anhydr. sodium sulphate (g)*
Propionic	a_1**	1650	0.01	0	0	0.004***	1	0.1	0
	a_2	1643	0.01	0	0	0.004***	1	0.1	0
	b_1	1682	0.01	15.8	0.5	0.004***	1	0.1	0.5
	b_2	1658	0.01	15.8	0.5	0.004***	1	0.1	0.5
	c_1	1661	0.8	0	0	0.154	1	0.1	0
	c_2	1648	1.03	0	0	0.204	1	0.1	0
	d_1	1650	5.3	0.5	0.31	1.02	1	0.5	4
	d_2	1661	5.3	0.5	0.31	1.02	1	0.5	4
	e_2	1649	10.3	0.5	0.31	2.04	1	1.0	8
	$\bar{x}$	1655							
	S.D.	11.6							
	S.D. (%)	0.7							

Succinic	a	982	0.02	0	0	0.008***	2	0.2	0
	b	985	0.02	0	0	0.008***	2	0.2	0.5
	c	984	2.53	0.25	0.65	1.02	2	0.5	2
	d	984	2.53	0.50	0.32	1.02	2	0.5	4
	e	981	2.67	0.25	1.30	1.04	2	1.0	2
	f	986	2.67	0.50	0.65	1.04	2	1.0	4
	g	986	2.67	0.75	0.43	1.04	2	1.0	6
	$\bar{x}$	984							
	S.D.	1.9							
	S.D. (%)	0.2							
Trimellitic	a	346	0.04	0	0	0.016***	2	0.4	0
	b	348	0.04	7.9	1.0	0.016***	2	0.4	0
	c	299 (86)	0.54	0	0	0.216	2	1.0	0
	d	298 (86)	0.54	0.23	0.64	0.216	2	1.0	4
	e	299 (86)	0.54	0.47	0.32	0.216	2	1.0	8

* Present in total.

** a_1, a_2–d_1, d_2, e_2 refer to *n*-butyl esters (index$_1$) and isobutyl esters (index$_2$) of propionic acid, respectively.

*** Originating from the sulphuric acid applied as catalyst, only.

Less than 100% but analytically reproducible esterification yields have been measured for aromatic di- and polycarboxylic acids (72–100%)[12] and for aliphatic hydroxy acids (85%)[13], on average.

The reproducibility of the determinations carried out under different esterification conditions with the chosen representatives of various homologous series of acids is presented in Table I.

Investigations are continuing in order to extend the esterification in aqueous solutions to the homologous series of carboxylic acids using *n*- and 2-propanol as esterifying agents, and also to make calculations concerning 'esterification equilibria' in the presence of water.

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