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GAS CHROMATOGRAPHIC ANALYSIS OF DIFFERENT HOMOLOGOUS SERIES OF ACIDS ESTERIFIED IN AQUEOUS SOLUTIONS WITH BUTYL AND PROPYL ALCOHOLS

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SUMMARY

The esterification of homologous series of fatty acids, aliphatic and aromatic di- and polycarboxylic acids, with aliphatic alcohols in the presence of water and a mineral acid as catalyst, is described. The efficiency of derivatization as well as the detector responses, referred to the organic carbon content of the derivatized products, obtained with two alcohols are compared to each other to establish the optimum conditions for various series of acids. The molar ratios of water/alcohol which yielded quantitative esterifications are given, as is the maximum water content of the esterifying mixture permitted in order for esterification to proceed to completion. The optimum conditions for a reasonable ester yield in the presence of anhydrous sodium sulphate are also presented.

INTRODUCTION

According to the literature[1], esterification should be carried out under strictly anhydrous conditions. Since the analysis of many natural matrices requires the measurement of acids in aqueous solutions, there is a need for derivatization methods for organic acids in the presence of water. Earlier papers reported methods of esterification in aqueous solutions with water-miscible alcohols such as methanol[2–6] and 1-propanol[7]. Although the results were not quantitative, but facilitated the rapid analysis of biological matrices, they were accepted [4–6].

Recently, a systematic study was undertaken concerning the *n*-butyl[8–15] and 1-propyl[16–20] esterification of various series of acids in the presence of water.

This paper compares the optimum conditions for direct butyl and propyl esterification of C_1–C_{22} fatty acids[9–10,16], of C_2–C_{16} aliphatic dicarboxylic acids[11,17], of aliphatic hydroxy acids[12,18] and of aromatic di- and polycarboxylic acids[13,19], respectively. The optimum elution order of chosen members of homologous series was determined, using the same chromatographic conditions for the corresponding butyl and propyl derivatives. The simultaneous chromatography of members of different homologous series was investigated. Model esterifications were performed: (i) under anhydrous conditions, (ii) in the presence of various amounts of water con-

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taining sulphuric acid and (iii) in the presence of the maximum water content compatible with complete esterification in the presence of anhydrous sodium sulphate.

MATERIALS AND METHODS

All reagents and carboxylic acids were of analytical grade. 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} dicarboylic acids, the trimellitic and pyromellitic aromatic carboxylic acids from Fluka (Buchs, Switzerland) and the support material and liquid phase used in gas chromatography (GC) from Applied Science Labs. (State College, PA, U.S.A.).

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 Model Digint-34 μ computing integrator (Chinoin, Budapest, Hungary). Stainless-steel columns (2 m × 3 mm I.D.) were used. The coating material (15% Dexsil 300) was supported on Chromosorb W AW DMCS (80–100 mesh). The flow-rate of nitrogen was 10 ml/min. Other conditions are detailed in the figure captions.

Model esterifications were carried out with 1–2 ml of the given stock solution adjusted to pH 10-11 (containing 1–2 mg/ml of each acid) in a special glass vessel, containing a stirrer, described earlier[8,15].

The molar ratios of water, alcohol, sodium sulphate, concentrated sulphuric acid and anhydrous sodium sulphate in the esterification mixture are given as, *e.g.*, $[H_2O]/[1\text{-PrOH}]$, $[H_2O]/[n\text{-BuOH}]$, $[Na_2SO_4]/[H_2O]$ and $[H_2SO_4]/[Na_2SO_4]$.

Procedure 1

Esterification under strictly anhydrous conditions, which served as the basis for comparison, was performed according to Gehrke *et al.*[21].

Procedure 2

Esterification in aqueous solution in the absence of anhydrous sodium sulphate at $[H_2O]/[\text{alcohol}] = 0.02\text{–}14$ 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*-butanol or 1-propanol, 0.2–1 ml of sulphuric acid and different amounts of water (0.1–2 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 was placed in a water-bath. Esterification proceeded at 100°C for various periods depending on the homologous series of the acids under investigation. The solution was cooled to room temperature and transferred to a separatory funnel together with 300 ml water and 15 ml of dichloromethane. Extraction was completed with two additional 15-ml portions of dichloromethane. (For quantitative extraction of aliphatic hydroxy acids, 25 ml of water and 5 ml of dichloromethane were used, and the extraction was completed with two additional 5-ml portions of dichloromethane.) The pooled dichloromethane 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 from the residue by addition of dichloromethane and 5- to 10-μl aliquots of the stock solution were injected into the gas chromatograph.

Procedure 3

In the course of esterification at $[H_2O]/[alcohol] = 2.2–10.4$, carried out in the presence of anhydrous sodium sulphate, 1–2 ml aqueous solution of the acids were mixed with 1–2 ml *n*-butanol or 1-propanol and 4–12 g anhydrous sodium sulphate. Esterification was performed in the presence of 0.5–2 ml conc. sulphuric acid. Subsequently, procedure 2 was followed.

RESULTS AND DISCUSSION

The elution orders of different homologous series of acids offer the following possibilities (Figs. 1–5). The C_1–C_{22} fatty acids can be separated as 1-propyl and *n*-butyl esters in a single step (Fig. 1, chromatograms A and B). The GC of propyl and butyl esters of aromatic di- and polycarboxylic acids is, according to our knowledge, reported for the first time (Fig. 4, chromatograms A and B). A utilizable procedure for the GC analysis of aromatic acids as their propyl or butyl esters is important for the simultaneous analysis of the members of various homologous series, since GC analysis of C_1–C_5 carboxylic acids is possible by using their corresponding propyl[16,22] or butyl esters[9,10,14].

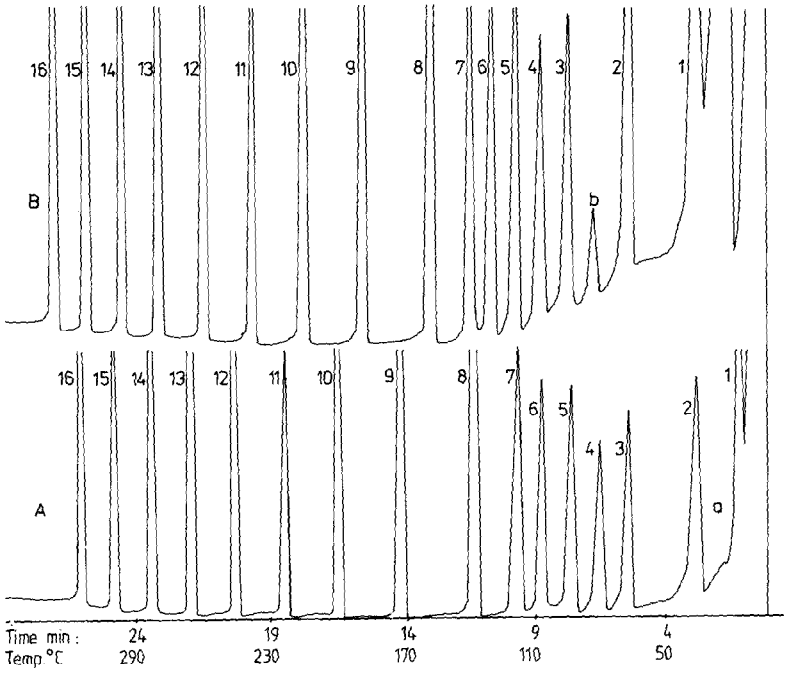

Fig. 1. Chromatograms of C_1–C_{22} fatty acids as their 1-propyl (A) and *n*-butyl (B) esters. Peaks: 1 = formic; 2 = acetic; 3 = propionic; 4 = isobutyric; 5 = *n*-butyric; 6 = isovaleric; 7 = *n*-valeric; 8 = caproic; 9 = caprylic; 10 = capric; 11 = lauric; 12 = myristic; 13 = palmitic; 14 = stearic; 15 = arachidic; 16 = behenic acid. Peaks a and b correspond to propyl and butyl ethers, respectively. Conditions: column temperature maintained at 50°C for 4 min, then raised at 12°C/min to 340°C, finally held constant for 5 min. The temperatures of the injector and detector were invariably 290 and 310°C, respectively.

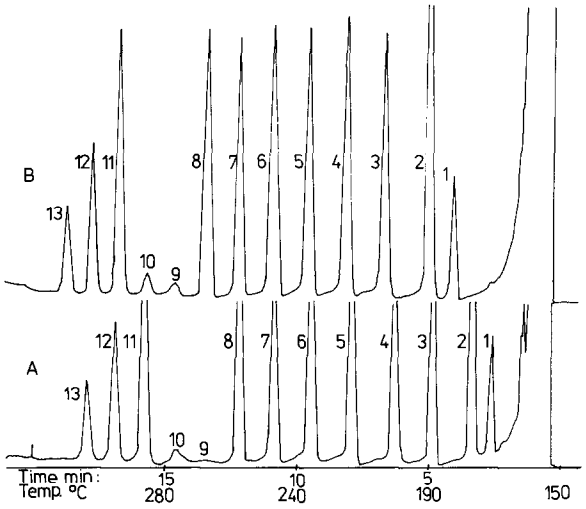

Fig. 2. Chromatograms of C_2–C_{16} dicarboxylic acid esters as in Fig. 1. Peaks: 1 = oxalic; 2 = malonic; 3 = succinic; 4 = glutaric; 5 = adipic; 6 = pimelic; 7 = suberic; 8 = azelaic; 9 = sebacic; 10 = undecanedioic; 11 = dodecanedioic; 12 = tetradecanedioic; 13 = hexadecanedioic acid (peaks 9 and 10 were due to impurities). Conditions: column temperature raised at 8°C/min from 150 to 320°C. The temperature of the injector and detector were invariably 320 and 340°C respectively.

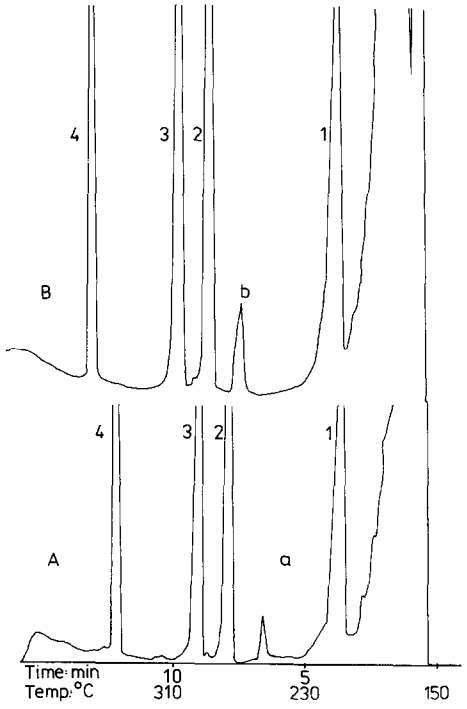

Fig. 3. Chromatograms of lactic (1), tartronic (2), malic (3) and citric acid (4). Peaks: a and b are due to dipropyl and dibutyl sulphates. Conditions: column temperature raised from 80°C at 16°C/min to 320°C, then held constant for 4 min. The temperatures of the injector and the detector were invariably 290 and 320°C. respectively.

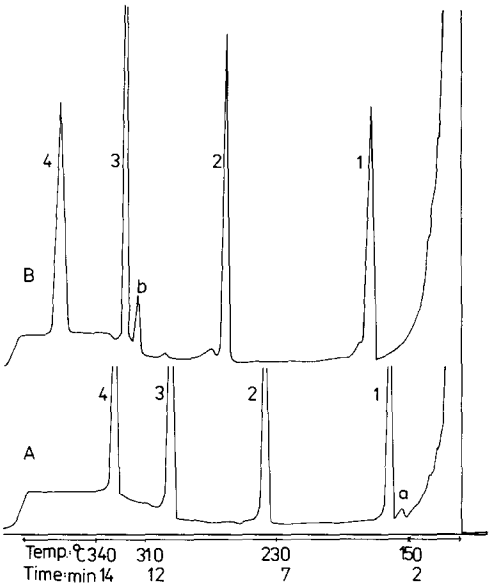

Fig. 4. Chromatograms of aromatic carboxylic acids. Peaks: 1 = benzoic; 2 = *o*-phthalic; 3 = trimellitic; 4 = pyromellitic acid. Peaks a and b correspond to monopropyl and dibutyl sulphates, respectively. Conditions: column temperature raised from 150 to 340°C at 16°C/min. The temperature of the injector and detector were invariably 340 and 360°C, respectively.

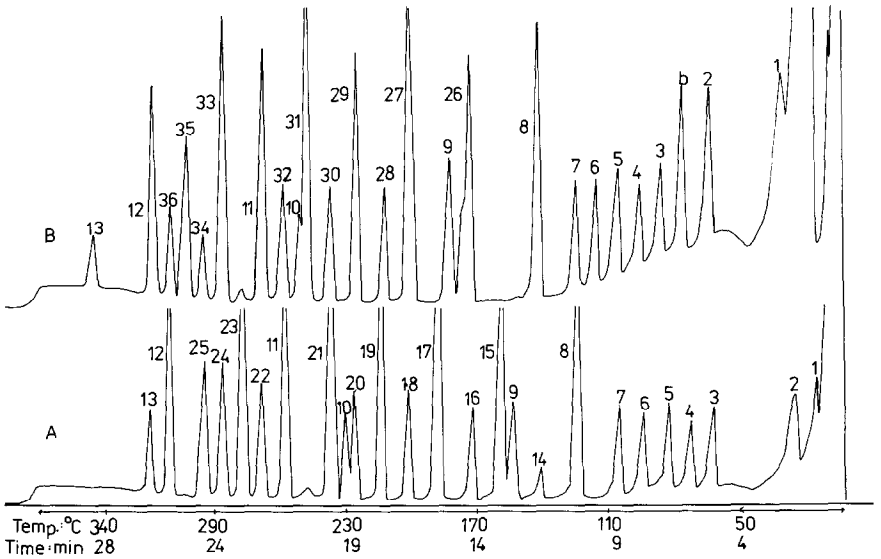

Fig. 5. Chromatograms of fatty, aliphatic and aromatic di- and polycarboxylic acids. Peaks: 1 = formic; 2 = acetic; 3 = propionic; 4 = isobutyric; 5 = *n*-butyric; 6 = isovaleric; 7 = *n*-valeric; 8 = caproic; 9 = malonic; 10 = *o*-phthalic; 11 = palmitic; 12 = behenic; 13 = pyromellitic; 14 = oxalic; 15 = caprylic + benzoic; 16 = succinic; 17 = capric + glutaric; 18 adipic; 19 = lauric + pimelic; 20 = suberic; 21 = myristic + azelaic; 22 = dodecanedioic; 23 = stearic + tetradecanedioic; 24 = hexadecanedioic + trimellitic; 25 = arachidic; 26 = caprylic + oxalic + benzoic; 27 = capric + succinic; 28 = glutaric; 29 = lauric + adipic; 30 = pimelic; 31 = myristic + suberic; 32 = azelaic; 33 = stearic + dodecanedioic; 34 = tetradecanedioic; 35 = arachidic + hexadecanedioic; 36 = pyromellitic. Peak b corresponds to dibutyl ether. Conditions: column temperature held at 50°C for 4 min, then raised at 12°C/min to 340°C. The temperatures of the injector and detector were invariably 320 and 350°C, respectively.

TABLE I

YIELDS IN PROCEDURES 1 AND 2 (SEE TEXT) FOR DIFFERENT HOMOLOGOUS SERIES OF ACIDS IN AQUEOUS SOLUTIONS AS THEIR 1-PROPYL (A) AND *n*-BUTYL ESTERS (B) AS A FUNCTION OF THE COMPOSITION OF THE ESTERIFICATION MIXTURE

Esterification yields are expressed as percentages of those obtained by procedure 1.

Molar ratios of the components				*Esterified carboxylic acids (%)*					*Composition of the esterification mixture**			
Test	$\frac{[H_2O]}{[alcohol]}$	$\frac{[H_2SO_4]}{[Na_2SO_4]}$	$\frac{[Na_2SO_4]}{[H_2O]}$	C_1C_5 *fatty acids*	C_6–C_{22} *fatty acids*	C_2–C_{16} *dicarboxylic acids*	*Aliphatic hydroxy acids*	*Aromatic polycarboxylic acids*	*Water (ml)*	*Alcohol (ml)*	H_2SO_4 *(ml)*	Na_2SO_4 *(g)*
A/1	0.03	1.0	7.9	100	100	100	100	100	0.008	1	0.2	0.5
B/1	0.04		7.9	100	100	100	100	100	0.008	1	0.2	0.5
A/2	0.03	1.0		100	100	100	100	100	0.008	1	0.2	0
B/2	0.04			100	100	100	100	100	0.008	1	0.2	0
A/3	0.45			100	100	100		84–90	0.108	1	0.2	0
B/3	0.51			100	100	100	80–90	72–100	0.108	1	0.2	0
A/4	0.86			100	100				0.208	1	0.2	0
B/4	1.06			100	100				0.208	1	0.2	0
A/5	1.06			100	100				0.308	1	0.2	0
B/5	1.28			100	100				0.308	1	0.2	0
A/6	4.26				100				1.02	1	0.5	0
B/6	5.16				100				1.02	1	0.5	0

A/7	8.51				100				2.04	1	1	0
B/7	10.31				100				2.04	1	1	0
A/8	4.20	0.2	0.7	100	100				1.02	1	0.5	6
B/8	5.16	0.2	0.7	100	100				1.02	1	0.5	6
A/9	8.51	0.2	0.7	100	100				2.04	1	1.0	12
B/9	10.37	0.2	0.7	100	100				2.04	1	1.0	12
A/10	2.20	0.6–1.0	0.5–0.7			100			1.06	2	1.5	4–6
B/10	2.53	0.43				100			1.02	2	0.5–1.0	3–6
A/11	2.15	0.6	0.5				74–90		1.04	2	1	4
B/11	2.53	0.6	0.5				80–90		1.04	2	1	4
A/12	2.23	0.4–1.3	0.5–1.5					84–90	1.08	2	2	4–12
B/12	2.71	0.5–1.0	0.5–1.0					80–100	1.07	2	1.8	4–8

* Also taking into consideration the water content of concentrated sulphuric acid. The sodium sulphate employed was anhydrous.

Esterification in solutions with 1-propanol (n-butanol)–sulphuric acid in the absence of anhydrous sodium sulphate

At the molar ratios of $[H_2O]/[alcohol] = 0.03$ and 0.04, *i.e.*, in the presence of water originating only from the sulphuric acid, quantitative esterification was ob-

TABLE II

DETECTOR RESPONSES OF VARIOUS HOMOLOGOUS SERIES OF ACIDS MEASURED AS 1-PROPYL (A) AND *n*-BUTYL (B) ESTERS

A_1 and B_1 are the detector responses toward the 1-propyl and *n*-butyl esters, respectively, taking into consideration the amount of the underivatized carboxylic acid employed, expressed in terms of their carbon contents. A_2 and B_2 are the corresponding values calculated from the effective carbon number of the derivatized product, *i.e.*, regarding the carboxylic carbon as $C = 0.0$ the primary, secondary and tertiary alcoholic carbons as $C = -0.5$, -0.75 and -0.25, respectively, according to Scanlon and Willis[22].

Carboxylic acid	*Detector responses*					
	A_1	B_1	F_1 (B_1/A_1)	A_1	B_1	F_1 (B_2/A_2)
Formic	269	487	1.81	90	122	1.36
Acetic	347	547	1.58	173	219	1.27
Propionic	290	498	1.72	174	249	1.43
Isobutyric	246	403	1.64	164	230	1.40
n-Butyric	305	440	1.44	203	251	1.23
Isovaleric	281	387	1.38	201	242	1.20
n-Valeric	300	387	1.29	214	242	1.13
Caproic	329	389	1.18	247	259	1.05
Caprylic	281	313	1.11	225	228	1.01
Capric	326	351	1.08	271	270	1.00
Lauric	310	325	1.05	266	260	0.98
Myristic	294	311	1.06	257	256	0.99
Palmitic	323	340	1.05	287	287	1.00
Stearic	247	261	1.06	223	224	1.01
Arachidic	283	301	1.06	257	262	1.02
Behenic	293	306	1.05	269	270	1.00
Oxalic	231	382	1.65	77	95	1.24
Succinic	359	481	1.34	180	194	1.07
Glutaric	316	437	1.38	175	198	1.13
Adipic	274	399	1.46	164	200	1.22
Pimelic	263	387	1.47	168	208	1.24
Suberic	247	358	1.45	165	205	1.24
Azelaic	233	328	1.40	161	197	1.22
Dodecanedioic	248	326	1.32	186	218	1.17
Tetradecanedioic	138	213	1.55	107	149	1.39
Hexadecanedioic	144	211	1.46	115	154	1.33
Lactic	208	323	1.55	192	228	1.19
Tartronic	260	560	2.16	148	232	1.56
Malic	229	412	1.80	146	200	1.36
Citric	306	470	1.53	171	205	1.20
Benzoic	310	329	1.06	241	230	0.95
o-Phthalic	309	379	1.23	206	217	1.05
Trimellitic	377	470	1.25	226	235	1.04
Pyromellitic	356	423	1.19	198	192	0.97

tained in all cases with both alcohols (Table I, A,B/1,2). Derivatization was carried out in solutions with increasing water content in the case of C_6–C_{22} fatty acids; 100% yield can be obtained up to $[H_2O]/[alcohol] = 8.50$ for 1-propyl and 10.31 for *n*-butyl derivatives (Table I, A,B/6,7).

Regarding the esterification yield, at the same $[H_2O]/[alcohol]$ molar ratios, the decrease with increasing amounts of water was greater with 1-propanol than with *n*-butanol.

Derivatization in aqueous solutions with 1-propanol (n-butanol)–sulphuric acid in the presence of anhydrous sodium sulphate

The esterification yield of carboxylic acids in water-containing solutions was increased by the use of anhydrous sodium sulphate. The optimum conditions regarding the ratios of $[Na_2SO_4]/[H_2O]$ and $[H_2SO_4]/[Na_2SO_4]$, as well as the maximum esterification yields, are summarized in Table I. The increase in the efficiency of derivatization using anhydrous sodium sulphate is in close accord with the acid strength, chain length and substituents of the carboxylic acid to be esterified and with the chain length of the esterifying alcohol (Table I, A,B/8–12).

Calculations of detector responses as a function of the esterifying alcohol

The response factors of propyl (A values) and butyl (B values) esters calculated for different series of acids are shown in Table II.

Regarding the effective integrator units for the same amounts of the corresponding propyl and butyl esters, measured under strictly identical circumstances, the butyl esters gave on the average 1.5 times greater sensitivity compared with the corresponding propyl esters.

As to the response factors, based on calculation of the "effective carbon number" (ECN) detailed in Table II of the acids, (which are of only theoretical importance), they are closer to one another than the respective A_1 and B_1 values; however, the sensitivity factors are in most cases equal to 1. Based on the results detailed above, it can be stated that for GC of all the carboxylic acid series tested, the *n*-butyl esters are preferred to the 1-propyl esters.

As to the many-sided usefulness of our method, two tasks have been accomplished, *i.e.*, the GC analysis of citrus juices[23] and the determination of the carboxylic acid composition of valeriana extracts[24].

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