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Relative efficiency of free fatty acid butyl esterification Choice of catalyst and derivatisation procedure

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ABSTRACT

The conversion efficiency of alkanoic, alkenoic, branched, alicyclic, aromatic, keto-substituted, and dioic carboxylic acids to their corresponding butyl esters was compared under different reaction conditions (time, temperature, catalyst). We show that boron trifluoride is generally a more efficient catalyst than sulphuric acid. However, optimum derivatisation conditions vary strongly for different acids and no single derivatisation protocol can be employed without certain losses. Therefore, care must be taken when the simultaneous quantitative analysis of different types of carboxylic acids in one sample is envisaged. Addition of water-scavenging reagents to the reaction mixture caused the formation of artefacts and selectively decreased reaction yields.

1. Introduction

The analysis of long-chain fatty acids (>C_{12}) by gas chromatography coupled to mass spectrometry (GC–MS) is a routine procedure in many branches of biological and Earth sciences (e.g. [1–3]). Separating carboxylated compounds by GC is complicated by their relatively high polarity and therefore derivatisation prior to analysis is commonly needed. A popular method to derivatise carboxylic acids is esterification to fatty acid methyl esters (FAMEs), a technique that has been thoroughly reviewed over the years (e.g. [4–7]). This procedure is however not very suitable for analysing carboxylic acids of low-molecular weight (<C_{12}) since their increased volatility after derivatisation can lead to unquantifiable losses related to evaporation. GC analysis of free fatty acids yields substandard results [8] and is restricted to monocarboxylic acids of low-molecular weight. Interaction with the capillary GC column phase leads to peak tailing and less than optimal separation [9]. Due to sample losses in the GC system, the analysis of free fatty acids cannot be performed reliably when dealing with minor amounts of sample [8]. Although polar stationary phases such as those based on polyethylene glycol (PEG) or acid phases can be used in capillary GC columns to enhance separation, the maximum temperatures at which they can be operated precludes the analysis of compounds with higher boiling points. Derivatisation methods forming propyl or butyl esters have been known for a long time [10,11] but gained popularity recently. Due to their decreased volatility, butyl esters allow the simultaneous analysis of both low- and high-molecular weight fatty acids (e.g. [12,13]).

Derivatisation protocols reported in the literature differ widely in terms of catalyst reagent, derivatisation time, and temperature. Still, very little comparative data are available. The most commonly used catalyst is boron trifluoride (BF_3), which has become popular as a derivatisation reagent since the early 1960s [14,15]. However, it decomposes rapidly when not adequately stored [4], poses a health concern [16], and issues exist about its quality in derivatisation reactions [17,18]. In particular, it can unpredictably form artefacts [16,18], especially when oxidised upon atmospheric exposure. These facts are even annotated in the product specification of commercially available BF_3–butanol mixtures [19]. In theory, any Brønsted or Lewis acid can be used as a catalyst during the alkylation of an organic acid in the presence of an alcohol, and sulphuric acid (H_2SO_4) can be an attractive alternative to BF_3 [5].

In the present study, we examined the relative efficiency of different butylation procedures. The amount of alcohol, catalyst (BF_3 or H_2SO_4), reaction time, and reaction temperature were investi-

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Table 1
Compounds used in this study to monitor the butylation efficiency of free carboxylic acids

	Carboxylic acid	Concentration (mg/mL)	Amount used (mg/50 µL)
1	Acetic	2.320	0.116
2	Butanoic	1.500	0.075
3	Hexanoic	1.165	0.058
4	2-Ethylhexanoic	1.400	0.070
5	Cyclopentylacetic	1.685	0.084
6	Benzoic	0.660	0.033
7	2-Methyl-1-cyclohexane	1.480	0.074
8	Malonic (1,3-propanedioic)	2.910	0.146
9	Pyruvic (α-keto propionic)	3.125	0.156
10	2,3-Dimethylsuccinic	0.700	0.035
11	Tetradecanoic	1.145	0.057
12	Octadecanoic	0.500	0.025
13	Octadecenoic	1.650	0.083
14	Docosenoic	0.810	0.041
	Sum	21.050	1.053

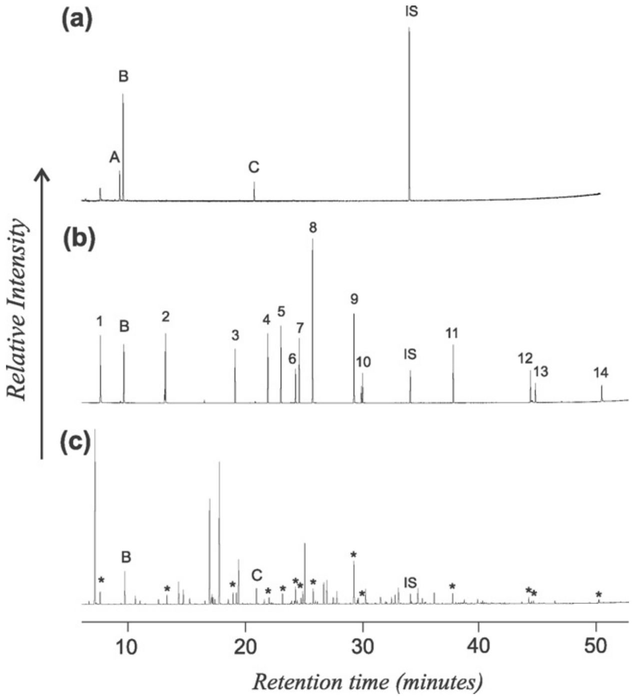

Fig. 1. Total ion chromatograms of (a) a procedural blank exhibiting the catalytic rearrangement products of *n*-butanol, (b) the butylated acid standard mixture, and (c) the butylated acid standard mixture, derivatised with addition of 2,2-dimethoxypropane. For the identification of carboxylic acids see Table 1. Asterisks in (c) correspond to butyl esters in (b). A, 4-heptanone; B, di-*n*-butyl ether; C, 1,1′-dibutoxybutane; IS, internal standard (*n*-octadecane).

gated. In addition, we studied the importance of water-scavenging additives in butylation reactions.

2. Experimental

2.1. Materials

Dichloromethane (DCM, analytical-reagent grade) and *n*-pentane were purchased from Mallinckrodt (Sydney, Australia). Diethyl ether (photospectroscopic grade, inhibitor free), 1-butanol, and *n*-butanol/BF_3 (10%, w/w) were purchased from Sigma–Aldrich (Sydney, Australia), concentrated sulphuric acid from Mallinckrodt, and carboxylic acids (Table 1) from Sigma–Aldrich and Fluka (Sydney, Australia). All solvents were used without further purification, except *n*-pentane, which was distilled before usage. Magnesium sulphate ($MgSO_4$) was pre-extracted with DCM and dried for >24 h at 240 °C. Water was purified using an *Elga* (Clayton, Australia) 'Purelab ultra' apparatus. Glassware was annealed at 600 °C and cleaned with DCM before use.

2.2. Analytical protocol

An aliquot of a mixture of 14 organic acid standards dissolved in DCM (Table 1) was processed under varying conditions of esterification to compare fatty acid butyl ester yields. Reactions varied in terms of temperature (65 or 100 °C) and time (15, 60, or 120 min). Additionally, one reaction was carried out at 100 °C for 15 h. This resulted in seven different reaction schemes, labelled alphabetically from A to G (Tables 2 and 3). Each reaction scheme was conducted with three different amounts of *n*-butanol (100, 200, or 500 µL) and two different catalysts (BF_3 or H_2SO_4), resulting in a total of 42 measurements of butyl ester yield for each carboxylic acid. In addition, reaction schemes C and B were repeated with 100 µL *n*-butanol and 200 µL *n*-butanol, respectively, and with 200 mg of anhydrous $MgSO_4$ added to the reaction vessel.

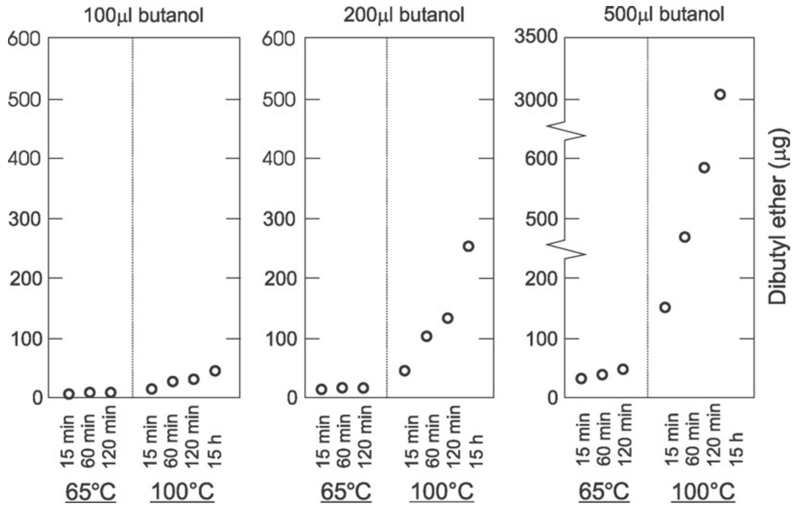

Fig. 2. The amount of di-*n*-butyl ether, a rearrangement product of *n*-butanol, increases parallel to reaction time, temperature, and the amount of *n*-butanol used. Values are averages of reaction schemes using BF_3 and H_2SO_4 as catalysts.