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## EXTRACTIVE ALKYLATION OF FATTY ACIDS WITH PHENACYL BROMIDE, STUDIED BY LIQUID CHROMATOGRAPHY

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### SUMMARY

Fatty acids with two to eighteen carbon atoms have been chromatographed as phenacyl esters. The derivatives were prepared by extractive alkylation. The acids were extracted as ion pairs with quaternary ammonium ions from an aqueous phase (phosphate buffer, pH 7) into dichloromethane, where the alkylation took place. The phenacyl esters formed showed good UV-absorption properties, and the detection limits were at the nanogram level. Chromatographic conditions suitable for acids with different chain lengths have been obtained by changing the composition of the mobile phase.

Extraction constants as well as rate constants for the alkylation in single- and two-phase systems have been determined using different kinetic models. With the aid of these constants optimal conditions for the extraction as well as the alkylation reaction were calculated.

Nonanedioic acid has been dialkylated with phenacyl bromide and a reaction model is proposed, where the rate constants involved have been determined.

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### INTRODUCTION

Extractive alkylation is well known for preparative<sup>1,2</sup> as well as analytical purposes, *e.g.* ref. 3. Carboxylic acids have been alkylated with different alkyl halides such as methyl and butyl iodide<sup>4-7</sup>, as well as pentafluorobenzyl bromide<sup>8-10</sup>. Phenacyl bromide has been used for the alkylation of 4-methoxybenzoic acid and 4-methoxyphenylacetic acid<sup>11</sup>.

Fatty acids have been alkylated with methyl iodide<sup>12</sup> and pentafluorobenzyl bromide<sup>13,14</sup> for gas and liquid chromatographic determinations. Durst and co-workers<sup>15,16</sup> have shown the catalytic effect of a crown ether compound to form *p*-bromophenacyl ester derivatives of the fatty acids and different dicarboxylic acids. This technique was also used by Barcelona *et al.*<sup>17</sup> and by Patience and Thomas<sup>18</sup>. Extractive alkylation has been used<sup>19,20</sup> where the derivatives were determined in a gas chromatographic system.

The aim of this work was to find optimal conditions for extractive alkylation of different fatty acids with phenacyl bromide. A straight-phase liquid chromatographic (LC) system was developed where the good UV properties of the esters

formed were utilized. To minimize side-reactions, the high reactivity of phenacyl bromide must be studied, *cf.* refs. 20–23. Earlier<sup>11</sup> a two-phase system was used, and it was shown that phenacyl bromide was reacting by second-order kinetics. The same system was used in this study for the alkylation of fatty acids.

## EXPERIMENTAL AND METHODS

### *Chemicals and reagents*

The fatty acids studied (Table I) were of analytical grade (>99%), and were obtained from Fluka, except C<sub>2</sub> (May & Baker) and C<sub>8</sub> (Merck).

Tetrahexylammonium, tributylmethylammonium and tetrapropylammonium hydrogen sulphate were obtained from Hässle (Mölnädal, Sweden). Tetrabutylammonium hydrogen sulphate was obtained from Fluka as well as phenacyl bromide.

Dichloromethane was washed with water before use, except when used as mobile phase.

Phenacyl hexanoate standard was prepared by extractive alkylation as described earlier<sup>6</sup>. The identification was made by NMR and the LC system described in this paper.

All other chemicals were of analytical grade.

### *Apparatus*

The chromatographic system comprised a LDC Minipump, a W + W recorder, a LDC UV detector Model 1203 measuring at 254 nm and a Rheodyne injector Model 7125 with a 20- $\mu$ l loop. The column and the mobile phase reservoir were thermostatted at 25.0°C in a water-bath. The spectrophotometer and pH meter were the same as in the previous study<sup>6</sup>.

### *Liquid chromatographic method*

The separation column (150  $\times$  4 mm I.D.) was packed with Spherosil XOA-

TABLE I  
STUDIED SUBSTANCES AND  $pK'_{HA}$  VALUES

<i>Acid</i>	<i>Abbreviation</i>	$pK'_{HA}$ *		
Acetic acid	C <sub>2</sub>	4.76		
Butyric acid	C <sub>4</sub>	4.80		
Hexanoic acid	C <sub>6</sub>	4.8		
Octanoic acid	C <sub>8</sub>	4.89**		
Decanoic acid	C <sub>10</sub>			
Dodecanoic acid	C <sub>12</sub>			
Myristic acid	C <sub>14</sub>			
Palmitic acid	C <sub>16</sub>			
Stearic acid	C <sub>18</sub>			
			$pK'_{H_2A}$	$pK'_{HA}$
Nonanedioic acid		4.56		5.53

\* From ref. 25.

\*\* From ref. 26.

600 (mean particle diameter 5  $\mu\text{m}$  and surface area 600  $\text{m}^2/\text{g}$ ). The mobile phase consisted of *n*-hexane, dichloromethane, tri-*n*-butyl phosphate (TBP) and acetic acid in various volume ratios.

Peak heights were measured manually and the concentrations were calculated as follows. The peak heights were measured after complete reaction and set to 100%. The concentrations at different times were then related to this peak height taken as representing infinite time. In the case of hexanoic acid the prepared phenacyl ester was used as standard.

#### *Determination of partition ratio*

An aqueous phase, containing known amounts of the analyte and the quaternary ammonium ion, was equilibrated with dichloromethane using equal phase volumes. The pH was 7 (phosphate buffer) or 13 (sodium hydroxide), the ionic strength 0.1 throughout, and the temperature 25.0°C. After centrifugation and phase separation the concentration of the quaternary ammonium ion was determined by the picrate method<sup>24</sup> in one or both phases, after repeated extractions.

#### *Single-phase alkylation*

The acids were extracted at pH 7 to dichloromethane as ion pairs with different quaternary ammonium ions. After centrifugation and phase separation, phenacyl bromide was added to the organic phase. The reaction was performed in a water-bath at 25.0°C. At certain well-defined times, 1.00-ml samples were withdrawn. The reaction was stopped by adding 9.00 ml of *n*-hexane, dichloromethane, TBP and acetic acid, in different proportions in order to obtain samples as similar as possible to the mobile phase. The analysis were performed on the LC system described above, and repeated injections were used to control whether the reaction had stopped.

#### *Two-phase alkylation*

The two-phase alkylations were performed in centrifuge tubes: 4.00 ml of aqueous phase (pH 7) containing the quaternary ammonium ion was equilibrated with 2.00 ml dichloromethane in which the acid was dissolved. The reaction was started by adding 2.00 ml of organic phase containing phenacyl bromide. One tube was used for each reaction time and the reaction was stopped, at certain well-defined times, by adding 2 *M* sulphuric acid to the tube. After centrifugation and phase separation, 1.00-ml samples of the organic phase were mixed with *n*-hexane, TBP and acetic acid for analysis in the straight-phase LC system.

### RESULTS AND DISCUSSION

#### *Determination of extraction constants*

The acids  $C_2$ ,  $C_4$ ,  $C_6$  and  $C_8$  were extracted at pH 7 or 13 into dichloromethane, with TBA as counter-ion. The concentration range was varied between 0.01 and 0.1 *M* and between 0.1 and 20 *mM* for the acid and the counter-ion, respectively.

The conditional extraction constant increased with decreasing concentration of the ion pair in the organic phase. This was interpreted as being due to dissociation of the ion pair in the organic phase<sup>24</sup>, when the following expression will be valid.

$$K_{\text{ex(QA)}}^* = K_{\text{ex(QA)}} + [K_{\text{diss(QA)}}K_{\text{ex(QA)}}(C_{\text{Q,aq}}C_{\text{A,aq}})]^{1/2} \quad (1)$$

Straight-line relationships were found for  $C_2$ – $C_8$ , and extraction as well as dissociation constants for the acids were determined by slope analysis based on the equation.

The acid dissociation constants for some of the acids are listed in Table I and the determined extraction constants are given in Table II. A good correlation with an earlier determined<sup>27</sup> constant for  $C_2$  was obtained. The extraction constant increases by *ca.* 0.6 log units for each additional carbon atom of the acid, which is in good agreement with earlier reported values of alkylammonium ions<sup>28,29</sup>. Dissociation of the ion pair in dichloromethane is an important side-reaction at lower concentrations ( $< 10^{-4}$  M) of the ion pair in the organic phase. Estimated values of the extraction constants for the  $C_{10}$ – $C_{18}$  acids, as well as found or estimated distribution constants, are also listed in Table II.

#### Preparation of phenacyl hexanoate

Phenacyl hexanoate was prepared by extractive alkylation, followed by distillation in vacuum. The product was identified by NMR. The absorption spectrum in dichloromethane was recorded, and an absorbance maximum at 243 nm with a molar absorptivity of  $1.21 \cdot 10^4$  l mol<sup>-1</sup> cm<sup>-1</sup> was obtained. The alkylation was first performed at a pH between 10 and 13. Unidentified peaks in the chromatograms were probably due to degradation of the reagent, *e.g.* ref. 20.

In an earlier work<sup>11</sup>, the alkylation between 4-methoxyphenylacetic acid and phenacyl bromide at pH 7 was investigated. A second-order kinetic model verified that no side-reactions occurred and, as a consequence, the alkylations in this work were carried out at pH 7 throughout.

#### Choice of chromatographic conditions

A normal-phase LC system was chosen for the determination of the phenacyl ester. This was most convenient as the derivatization took place in dichloromethane and thus the reaction mixture could be analysed without any further treatment. The polarity of the organic phase was regulated by *n*-hexane and TBP in order to obtain optimal separation conditions and suitable retention times.

TABLE II  
COMPILATION OF EQUILIBRIUM CONSTANTS

Acid	$Q^+$	Log $K_{ex(QA)}$ (batch)	log $K_{diss}$	log $K_{D(HA)}$
$C_2$	TBA	-1.80	-4.14	-1.54**
$C_4$	TBA	-0.770	-4.29	-0.27**
$C_6$	TBA	0.441	-4.02	0.90**
$C_8$	TBA	1.57	-4.24	2.1*
$C_{10}$	TPrA	0.40*	—	3.3*
$C_{12}$	TPrA	1.6*	—	4.5*
$C_{14}$	TrBMA	3.4*	—	5.7*
$C_{16}$	TrBMA	4.6*	—	6.68***
$C_{18}$	TrBMA	5.8*	—	8.1*

\* Estimated values, according to ref. 30.

\*\* From ref. 31, organic phase chloroform.

\*\*\* From ref. 32.

TABLE III

COMPILATION OF MOBILE PHASES AND CAPACITY RATIOS FOR PHENACYL ESTERS OF FATTY ACIDS FORMED AFTER ALKYLATION

LC system as described under Experimental.

<i>Phenacyl ester</i>	<i>Mobile phase n-hexane-dichloromethane-TBP</i>	<i>Capacity ratio</i>	
C <sub>2</sub>	50:50:0/60:40:1	9.8/2.6	
C <sub>4</sub>	50:50:0	4.1	
C <sub>6</sub>	40:60:0/50:50:0/60:40:0	2.3/3.1/3.4	
C <sub>8</sub>	50:50:0	2.3	
C <sub>10</sub>	50:50:0	2.0	
C <sub>12</sub>	50:50:0	1.7	
C <sub>14</sub>	60:40:0/80:20:0	2.2/3.2	
C <sub>16</sub>	80:20:0/83:17:0*	3.0/5.0	
C <sub>18</sub>	83:17:0*	4.2	
Nonanedioic	80:20:1*	7.2**	—
Nonanedioic	90:10:7.5:1*,***	3.7**	4.6§

\* Dichloromethane saturated with water.

\*\* Dialkylated end product (C).

\*\*\* 1 refers to acetic acid.

§ Monoalkylated intermediate (B).

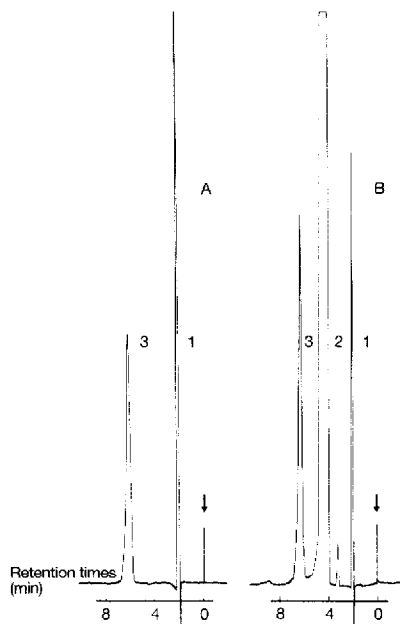


Fig. 1. Alkylation of hexanoic acid with phenacyl bromide. Chromatographic system as described under Experimental and in Table III; 20  $\mu$ l injected. (A) Standard sample,  $3 \cdot 10^{-5}$  M; (B) alkylated sample,  $2 \cdot 10^{-4}$  M. Peaks: 1 = solvent front; 2 = phenacyl bromide; 3 = phenacyl hexanoate.

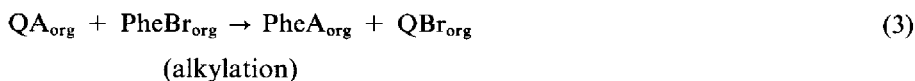
When the same mobile phases were used as in the previous studies<sup>6,11</sup> no separation was obtained between the phenacyl ester and the excess of phenacyl bromide. Thus, different mobile phases had to be used (Table III), consisting in most cases of *n*-hexane and dichloromethane. Table III shows that an increase in the number of carbon atoms gave a decrease in the capacity ratio. Dichloromethane was saturated with water for C<sub>16</sub> and C<sub>18</sub>, which gave an increase of the capacity ratio, but simultaneously the peak symmetry was improved. Chromatograms from a standard solution, as well as for an alkylated sample, are shown in Fig. 1.

Phenacyl hexanoate standards showed good linearity between 10<sup>-3</sup> and 10<sup>-7</sup> M, the lowest levels corresponding to 2 pmole acid injected. The relative standard deviation (*n* = 6) was 0.59% when six different samples of hexanoic acid (3 · 10<sup>-4</sup> M) were alkylated in the two-phase system and chromatographed.

The polarity of the mobile phase was increased when the mono- and dialkylated esters of nonanedioic acid were determined, *cf.* Table III.

#### Determination of rate constants

The extractive alkylation process can be described by two steps:



Reaction 2 illustrates the ion pair extraction of the acid into dichloromethane, which is governed by the extraction constant  $K_{ex(QA)}$ . Reaction 3 shows the alkylation reaction in dichloromethane forming the corresponding phenacyl ester, a process governed by the second-order rate constant  $k_A$ .

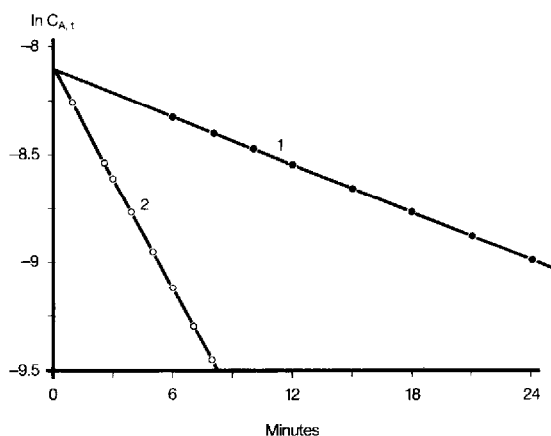


Fig. 2. Alkylation of 3.1 · 10<sup>-4</sup> M hexanoic acid at pH 7 with phenacyl bromide, performed in single-phase (line 2) and in a two-phase (line 1) system. Line 1, C<sub>Q</sub> = 0.10 M TBA, C<sub>PheBr</sub> = 10 mM. Line 2, C<sub>Q</sub> = 0.01 M THA, C<sub>PheBr</sub> = 10 mM.

The evaluation of the reaction order and the rate constants were performed according to the principles given earlier in this series for the extractive alkylation of carboxylic acids<sup>6</sup>.

*Single-phase systems.* The alkylation reaction (reaction 3) was studied kinetically using pseudo-first-order conditions, (excess of reagent). The following equation is then valid:

$$\ln C_{A,t} = \ln C_{A,0} - k'_A t \quad (4)$$

The reactions were followed to completion, *i.e.* for more than seven half lives.

The ester formed was assumed to be the only reaction product; thus

$$C_{A,t} = [\text{PheA}]_{\infty} - [\text{PheA}]_t \quad (5)$$

According to eqns. 4 and 5 a plot of  $\ln ([\text{PheA}]_{\infty} - [\text{PheA}]_t)$  versus  $t$  should give a straight line with a slope equal to  $-k'_A$ . This is demonstrated in Fig. 2, line 2. The second-order rate constants were calculated from eqn. 6:

$$k'_A = k_A C_{\text{PheBr}} \quad (6)$$

and the results for the alkylation of C<sub>2</sub>–C<sub>18</sub> are listed in Table IV.

The high reactivity of phenacyl bromide is demonstrated by the values of the second-order rate constants, which are of the same order of magnitude as for 4-methoxybenzoic acid and 4-methoxyphenylacetic acid<sup>11</sup> and far higher than the rate constants determined when methyl iodide was used as alkylating agent (*cf.* ref. 6). The variation of the rate constants between the different acids is quite small, however, as noted earlier<sup>6</sup>. This contrasts to the case for substituted phenols<sup>3,3</sup>, where large differences in magnitude of the rate constant were observed.

*Two-phase systems.* The alkylation can also be performed in two-phase systems, illustrated by eqns. 1 and 2. The extraction of the acid is influenced by different side-reactions, such as partition of the acid in uncharged form, protolysis and ion-pair dissociation. This is demonstrated in Fig. 3, where the different equilibria are illustrated.

TABLE IV

## SECOND-ORDER RATE CONSTANTS FOR SINGLE-PHASE ALKYLATION

Alkylating agent: 0.010 M phenacyl bromide.

Acid	Q <sup>+</sup>	C <sub>Q</sub> · 10 <sup>2</sup> (M)	C <sub>A</sub> · 10 <sup>4</sup> (M)	k <sub>A</sub> (min <sup>-1</sup> · M <sup>-1</sup> )
C <sub>2</sub>	THA	1	15	12.3
C <sub>4</sub>	THA	1	1	15.5
C <sub>6</sub>	THA	1	3	17.6
C <sub>8</sub>	TBA	5	2	16.1
C <sub>10</sub>	TBA	5	4	15.8
C <sub>12</sub>	TBA	5	4	13.2
C <sub>14</sub>	TBA	5	4	17.8
C <sub>16</sub>	TBA	5	4	13.1
C <sub>18</sub>	TBA	5	4	14.1

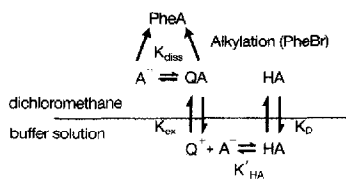


Fig. 3. Simplified compilation of important equilibria in a two-phase system.

The acids are alkylated only when the carboxylic group is deprotonized in dichloromethane. High values of the distribution constant and a low pH of the buffer solution will thus lower the observed reaction rate for the alkylation.

The kinetic model used for two-phase alkylation is given by eqn. 7, (*cf.* ref. 34):

$$\ln ([\text{PheA}]_{\infty} - [\text{PheA}]_t) = \ln C_{A,0} - k'_{A,obs}t \quad (7)$$

where  $k'_{A,obs}$  is the observed pseudo-first-order rate constant when the alkylating agent and the quaternary ammonium ion are present in excess. Plots of  $\ln ([\text{PheA}]_{\infty} - [\text{PheA}]_t)$  versus  $t$  gave straight lines with a slope equal to  $-k'_{A,obs}$ .

The reactions were mostly followed during seven half lives; as an example, the alkylation of hexanoic acid ( $3 \cdot 10^{-4} M$ ) is shown in Fig. 2, line 1.

Graphical computation illustrates that the alkylation rate is slower for the two-phase system. This indicates that the ion pair is not quantitatively extracted into the organic phase. However, both lines have the same intercept, which supports the kinetic models.

The results from the two-phase alkylations of acids  $C_2$ – $C_{18}$  are listed in Table V. The acids have been alkylated with different counter-ions, thus the degree of the ion-pair extraction is different for each acid. The most hydrophilic acid, acetic acid,

TABLE V

SECOND-ORDER RATE CONSTANTS FOR TWO-PHASE ALKYLATION

Alkylating agent: 0.01–0.2  $M$  phenacyl bromide.  $C_A = (2.6 \cdot 10^{-4}$ – $30 \cdot 10^{-4} M$  at pH 7;  $C_Q = 0.1 M$ .

Acid	$Q^+$	$k_{A,obs}$ ( $\text{min}^{-1} \cdot M^{-1}$ )
$C_2$	TBA	0.018
$C_4$	TBA	0.27
$C_6$	TBA	3.7
$C_8$	TBA*	8.5
$C_{10}$	TPrA	0.96
$C_{12}$	TPrA	1.1
$C_{14}$	TrBMA	2.0
$C_{16}$	TrBMA	2.6
$C_{18}$	TrBMA	2.7
$C_{18}$	TrBMA	4.0**

\*  $C_Q = 0.05 M$ .

\*\*  $C_A = 1.3 \cdot 10^{-4} M$ .



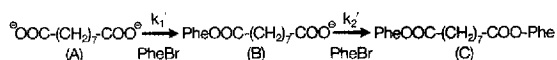


Fig. 4. Proposed reaction model for the dialkylation of nonanedioic acid with phenacyl bromide in dichloromethane.

gave a very low extraction degree, which resulted in a small value of  $k_{A,\text{obs}}$ ; if an increase in reaction rate is desired, a larger counter-ion, such as tetrahexylammonium, must be used.

From Tables I and II it can be estimated that  $A^-$  is the dominating species for the smallest acids and  $[\text{HA}]_{\text{org}}$  will dominate for the largest fatty acids at the start (*cf.* Fig. 3). However, this will not influence the end result of the alkylation, as long as a small part of the acid is extracted as ion pair and the reaction thereby continues.

The dissociation of the ion pair in the organic phase will influence the alkylation. The extraction of the acid as ion pair  $\text{QA}_{\text{org}}$  and dissociation to  $A_{\text{org}}^-$  will increase the observed alkylation rate<sup>34</sup>. This is supported by the results obtained when the initial concentration of the acid was decreased ( $\text{C}_{18}$ , Table V).

The results in Table V show that it is possible to form phenacyl esters of fatty acids in two-phase systems with buffer solutions (pH 7). The reaction time can be controlled by the kind and concentration of the counter-ion and by the concentration of phenacyl bromide.

#### Determination of rate constants, dialkylation

Nonanedioic acid, has been alkylated extractively, using tetrahexylammonium as counter-ion. The acid has two reactive sites, forming a dialkylated ester with phenacyl bromide. In earlier work<sup>7</sup> phenol-carboxylic acids were dialkylated with alkyl halides. A reaction model was proposed that involved two pathways giving the same end product.

Fig. 4 shows the assumed reaction model for the alkylation of nonanedioic acid. The acid is extracted to dichloromethane as  $\text{Q}_2\text{A}$  ( $pK_{\text{HA}}$  in Table I) and reacts to a monoalkylated intermediate, B. The reaction continues, forming a dialkylated end product, C. The steps are governed by the pseudo-first-order rate constants  $k'_1$  and  $k'_2$ , respectively. The model is verified in Fig. 5, showing chromatograms at two different times of reaction, and obviously the intermediate B is completely transformed into the end product C. Interfering peaks appear in the chromatograms if the extraction is performed at a pH greater than 7.5.

#### Calculation of kinetics

The kinetics were derived as for consecutive reactions (*cf.* ref. 35). The rate of consumption of nonanedioic acid and the rates of formation of B and C are given by eqns. 8–10:

$$-dC_A/dt = k'_1 C_A \quad (8)$$

$$dC_B/dt = k'_1 C_A - k'_2 C_B \quad (9)$$

$$dC_C/dt = k'_2 C_B \quad (10)$$

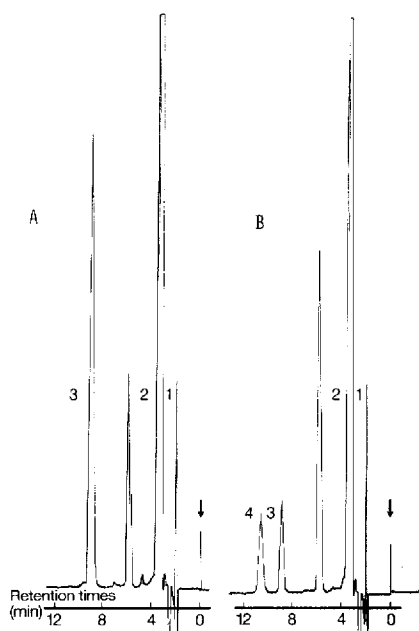


Fig. 5. Alkylation of nonanedioic acid  $2.6 \cdot 10^{-4} M$  with phenacyl bromide after 60 min (A) and 4 min (B) reaction time. Chromatographic system as described under Experimental and in Table III;  $20 \mu l$  injected. Peaks: 1 = solvent front; 2 = phenacyl bromide; 3 = dialkylated acid (C); 4 = monoalkylated acid (B).

when these equations are integrated and proper substitutions made, we obtain

$$C_{A,t} = C_{A,0} e^{-k'_1 t} \quad (11)$$

$$C_{B,t} = \frac{C_{A,0} k'_1}{k'_2 - k'_1} (e^{-k'_1 t} - e^{-k'_2 t}) \quad (12)$$

$$C_{C,t} = C_{A,0} \left[ 1 + \frac{1}{k'_1 - k'_2} \left( k'_2 e^{-k'_1 t} - k'_1 e^{-k'_2 t} \right) \right] \quad (13)$$

The increase of the concentration of the end product C is demonstrated by eqn. 13. This equation was used to calculate the rate constants  $k'_1$  and  $k'_2$ .  $C_{C,t}$  was plotted *versus* time, and the constants were calculated by non-linear curve-fitting. The second-order rate constants were calculated from obtained data, *cf.* eqn. 6, and are listed in Table VI. From the three single-phase runs the average rate constants  $k_1 = 45.7 \text{ min}^{-1} M^{-1}$  and  $k_2 = 13.2 \text{ min}^{-1} M^{-1}$  were obtained. These values were inserted in eqns. 11–13. Fig. 6 shows the concentration profiles for A, B and C, as well as the results from one single-phase experiment ( $C_{\text{PheBr}} = 0.019 M$ ). Found concentration of B and C are in good agreement with calculated rate constants.

TABLE VI

## ALKYLATION OF NONANEDIOIC ACID WITH PHENACYL BROMIDE

 $C_A = 2.6 \cdot 10^{-4} M$ ;  $C_Q = 0.025 M$  THA.

Alkylation	$C_{PhBr} \cdot 10^2 (M)$	$k_1 (min^{-1} \cdot M^{-1})$	$S_{rel}(\%)$	$k_2 (min^{-1} \cdot M^{-1})$	$S_{rel}(\%)$
Single-phase	0.8	42.1	4	14.4	2
	1	56.2	14	10.2	5
	2	38.7	4	14.9	2
Two-phase (pH 7)	2	39.1	10	16.9	5

The alkylation was also performed in a two-phase system. The results (Fig. 6) show good agreement with the calculated line. This verifies the assumed kinetic model, as well as a quantitative extraction of the acid. The magnitude of  $k_2$  is of the same order as for the fatty acids in this paper and as for the earlier determined rate constants for carboxylic acids<sup>11</sup>. The high value of  $k_1$  illustrates the reactivity of both carboxylic groups in nonanedioic acid.

## CONCLUSION

Fatty acids can be transformed into UV-absorbing species by extractive alkylation with phenacyl bromide. Side-reactions can be avoided by performing the reactions in a neutral environment. The products obtained from homologues can be effectively separated in normal-phase LC systems. The retention can flexibly be adjusted by applying mixtures of organic solvents of different characteristics, such as the non-polar *n*-hexane, the dipolar dichloromethane and the hydrogen-acceptor tributylphosphate.

Detection limits were in the picomole range. Determination of the values of the rate constants showed the high reactivity of phenacyl bromide, and thus it seems to be possible to choose reaction conditions so that precolumn, on-line, or post-column derivatization procedures may be possible.

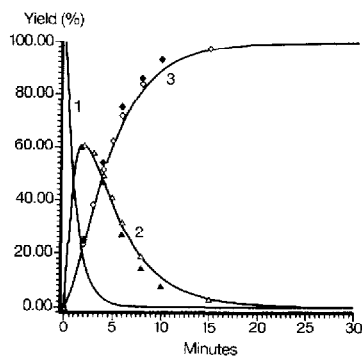


Fig. 6. Single- and two-phase alkylation of nonanedioic acid with  $0.019 M$  phenacyl bromide. THA as counter-ion and an aqueous phase of pH 7. The calculated values of A, B and C are indicated as lines 1, 2 and 3, respectively. Found concentrations:  $\triangle$  = B, single-phase;  $\diamond$  = C, single-phase;  $\blacktriangle$  = B, two-phase;  $\blacklozenge$  = C, two-phase.

## SYMBOLS

HA	carboxylic acid
Q <sup>+</sup>	quaternary ammonium ion
THA	tetrahexylammonium ion
TBA	tetrabutylammonium ion
TrBMA	tributylmethylammonium ion
TPrA	tetrapropylammonium ion
PheBr	phenacyl bromide
PheA	phenacyl ester of A
[A <sup>-</sup> ]	molar concentration of A <sup>-</sup> in the aqueous phase
[QA] <sub>org</sub>	molar concentration of QA in the organic phase
[PheA] <sub>∞</sub>	molar concentration of PheA at infinite phase
C <sub>A,aq</sub>	total concentration of A in the aqueous phase
C <sub>A</sub>	total molar concentration of the acid
C <sub>A,0</sub>	total concentration of A at time zero
C <sub>A,t</sub>	total concentration of A after <i>t</i> min of reaction
k <sub>A</sub>	second-order rate constant M <sup>-1</sup> · min <sup>-1</sup>
k' <sub>A</sub>	pseudo first-order rate constant min <sup>-1</sup>
k <sub>A,obs</sub>	observed second-order rate constant (M <sup>-1</sup> · min <sup>-1</sup> )
K <sub>D(HA)</sub>	[HA] <sub>org</sub> /[HA] = distribution constant of HA
K <sub>ex(QA)</sub>	[QA] <sub>org</sub> /[Q <sup>+</sup> ][A <sup>-</sup> ] = extraction constant of QA
K <sub>ex(QA)</sub> *	C <sub>[QA]org</sub> /C <sub>Q,aq</sub> C <sub>A,aq</sub> = conditional extraction constant of the ion pair QA
K <sub>diss(QA)</sub>	[Q <sup>+</sup> ] <sub>org</sub> [A <sup>-</sup> ] <sub>org</sub> /[QA] <sub>org</sub> = dissociation constant of the ion pair QA
K' <sub>HA</sub>	a <sub>H+</sub> [A <sup>-</sup> ]/[HA] = apparent acid dissociation constant of HA
a <sub>H+</sub>	hydrogen ion activity

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