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# RAPID ESTERIFICATION FOR GAS CHROMATOGRAPHY

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## SUMMARY

A new and convenient esterification procedure for both synthetic and analytical purposes has been developed. The basic reaction is the $S_N2$ attack of a soluble organic anion on an alkyl iodide in a highly polar solvent system. The reaction is both fast and extremely mild. Because of its generality, this procedure can be substituted for more complex and lengthier reactions, such as transesterifications, which are often employed. These factors make this technique extremely useful for both analytical and synthetic purposes, especially with sensitive compounds.

## INTRODUCTION

Esterification is an extremely valuable procedure for the alkylation of acidic compounds to increase volatility for gas chromatographic (GC) analysis, to protect the acidic group during other reactions, and as a specific synthetic step in itself. However, the toxic and dangerous reagents often required and the difficulties involved in forming esters of the higher alcohols have led many workers to prefer other derivations (*e.g.*, silylation) for many purposes.

In the work described below, a new method of derivation has been developed, which overcomes many of the problems common to traditional esterification reactions. The reaction developed here is virtually quantitative, utilizes extremely mild conditions and non-acidic reagents, is complete, for analytical purposes, in less than 10 min, and can be used to prepare any of a variety of esters from any acidic compound.

## MATERIALS AND METHODS

GC analysis was performed on a Hewlett-Packard Model 402 gas chromatograph (Hewlett-Packard, Avondale, Pa., U.S.A.) employing a 6 ft. × 2 mm I.D. glass column packed with 2% OV-17 on 80–100 mesh Chromosorb W HP.

Measurements of NADH in the enzymatic assay for cholic acid were performed on a Gilford Model 300 spectrophotometer.

N,N-Dimethylacetamide (spectroquality), methanol (spectroquality), tetramethylammonium hydroxide (24% in methanol), N,N-dimethylformamide, aceto-

nitrile, and iodomethane were obtained from Matheson, Coleman and Bell (East Rutherford, N.J., U.S.A.). Phenyltrimethylammonium hydroxide (0.1 *M* in methanol), stearic acid, diphenylhydantoin, cholic acid, 1-iodohexadecane, iodoethane, 1-iodobutane, 2-iodobutane, and cholesterol were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Barbiturates were obtained from the UCSF Hospital Pharmacy, benzoic acid from J. T. Baker (Phillipsburgh, N.J., U.S.A.), deoxycholic acid from Matheson, Coleman and Bell, and lithocholic acid from Sigma (St. Louis, Mo., U.S.A.).

## EXPERIMENTAL AND RESULTS

*1. Derivation for GC with phenyltrimethylammonium hydroxide*

A total of 14.2 mg of stearic acid (0.050 mmole) was dissolved in 4 ml of N,N-dimethylacetamide and 1.0 ml of 0.1 *M* phenyltrimethylammonium hydroxide in methanol (0.10 mmole). To this solution was added 0.10 ml of 1-iodobutane (0.88 mmole) and the system was mixed thoroughly. This quantity of 1-iodobutane is a great excess and was chosen solely for ease of measurement.

GC analysis after 10 min for unreacted stearic acid, side products, and butyl stearate showed that 98.1% of the stearic acid had been converted to the butyl ester (see Table I for results with other iodides).

TABLE I

DERIVATION OF STEARIC ACID WITH PHENYLTRIMETHYLAMMONIUM HYDROXIDE

| *Iodide* | *Yield (%)* | *Reaction time (min)* |
|---|---|---|
| Methyl | 100 | 10 |
| Ethyl | 100 | 10 |
| 1-Butyl | 98.1 | 10 |
| 2-Butyl | 70.1 | 30 |

*2. Derivation for GC with tetramethylammonium hydroxide*

A total of 25 mg of stearic acid (0.088 mmole) was dissolved in 4 ml of N,N-dimethylacetamide and 0.95 ml of methanol. To this was added 0.050 ml of tetramethylammonium hydroxide solution (0.104 mmole). Then 0.10 ml of 1-iodobutane (0.88 mmole) was added and the mixture was thoroughly agitated. Tetramethylammonium iodide precipitated as the reaction proceeded.

Analysis of the supernatant, as above, demonstrated 99.4% conversion of the stearic acid to the butyl ester (see Table II for results with other iodides).

*3. Synthesis of ethyl stearate*

A total of 2.014 g of stearic acid (7.08 mmoles) was dissolved in 15 ml of N,N-dimethylacetamide, 5 ml of tetramethylammonium hydroxide solution (9.56 mmoles), and 2 ml of methanol. The system was agitated to ensure complete solution.

To this solution was then added 2 ml of iodoethane (25 mmoles), and the sys-

TABLE II

DERIVATION OF STEARIC ACID WITH TETRAMETHYLAMMONIUM HYDROXIDE

| *Iodide* | *Yield (%)* | *Reaction time (min)* |
|---|---|---|
| Methyl | 99.8 | 3 |
| Ethyl | 98.8 | 10 |
| 1-Butyl | 99.4 | 10 |
| 2-Butyl | 98.8 | 13 |

tem was agitated again to ensure complete mixing. After 2 h, the product was isolated by addition of 20 ml of toluene and filtration under vacuum through a fritted glass funnel. The tetramethylammonium iodide precipitate and the reaction vessel were washed with another 40 ml of toluene.

The combined organic solution was extracted three times with 40 ml of water, twice with 40 ml of half-saturated sodium bicarbonate, and again with 40 ml of water. After drying with sodium sulfate and filtration, the organic phase was concentrated to give 2.075 g (94% yield) of ethyl stearate.

Care must be taken to assure that all traces of N,N-dimethylacetamide are removed before the isolation is considered complete. If the extraction procedure per se occasionally does not remove all of the N,N-dimethylacetamide, redissolving the product in petroleum ether, extracting with water, and concentrating will do so.

## DISCUSSION

The work leading to the development of this technique began as an evaluation of a little used procedure for the preparation of methyl esters[1,2]. The literature cited described a reaction utilizing N,N-dimethylacetamide as solvent with methyl iodide and sodium bicarbonate as reagents. The reaction, as described, can require several days to reach completion.

The original reaction has been significantly modified here to make it both rapid and quantitative. The conditions which proved most effective for preparing the derivatives for GC analysis are described in parts 1 and 2 of Experimental and results, while conditions found applicable to synthesis are described in part 3.

$$\mathrm{RCOOH} \xrightarrow{\mathrm{R'_4N^+\ OH^-}} \underset{\mathrm{I}}{\mathrm{RCOO^-\ R'_4N^+}} \xrightarrow[\mathrm{S_N2}]{\mathrm{R''I}} \underset{\mathrm{II}}{\mathrm{RCOOR''}} \qquad \text{(A)}$$

In essence, the reaction employs an organic base such as tetramethylammonium hydroxide and a highly polar solvent system. The reaction mechanism is outlined above. The base–solvent system appears to be relatively stable, and can therefore often be prepared in advance[3].

When the acidic compound is dissolved in the base–solvent system, a soluble salt (I) is immediately formed. In the solvent systems used here (4:1 or 3:1 N,N-dimethylacetamide–methanol), which have very high dielectric constants, the anion of the soluble salt and any primary alkyl iodide will undergo a rapid $S_N2$ reaction to form the corresponding ester (II).

A number of parameters were studied in the development of this procedure. Both sodium and potassium bicarbonates were evaluated as bases, but neither was acceptable. When they were used, the reaction rate was quite variable.

Phenyltrimethylammonium hydroxide was deemed the best organic base for GC derivation, both because its salts have a high solubility in the organic solvent system, and because there is no precipitation of the corresponding iodide in the concentration ranges used.

However, phenyltrimethylammonium hydroxide has two very definite drawbacks. First, it is only available commercially in the relatively dilute 0.1 *M* solution in methanol, and, secondly, it has only limited stability over a period of time[3].

$$R'_4N^+OH^- + R''I \rightarrow R''OH + R'_4N^+I^- \qquad (B)$$

On the other hand, salts of tetramethylammonium hydroxide are less soluble, complicating the isolation in synthetic reactions. It was found, however, that after allowing some additional time for the secondary reaction (B) to reach completion, all the precipitated tetramethylammonium iodide could be removed by filtration and the ester product could be isolated in good yield by classical extraction techniques.

The actual composition of the solvent system is not critical and is, in fact, a compromise. A solvent containing 80% N,N-dimethylacetamide and 20% methanol was selected for most of the above work. Three factors were involved in this selection. First, the adverse effects of methanol on GC columns (including stripping of the liquid phase) is reduced by limiting the amount of methanol in the solvent. Secondly, the addition of at least 10–20% methanol is necessary to increase the solubility of the intermediate salts I. Thirdly, the soluble salts are formed from quaternary bases, which are usually supplied in methanolic solutions. Preliminary evaluation indicates that N,N-dimethylformamide can be substituted for N,N-dimethylacetamide without adversely affecting the reaction, but acetonitrile (also possessing a high dielectric constant) is not acceptable.

There are two definite limitations of the system. First, the solvent should be anhydrous. Although the reaction of phenobarbital and 1-iodobutane, for instance, was observed by GC to go to completion in a system containing 5% water, the reaction time was extended from less than 10 min to over 1 h.

Secondly, it is essential that the order of addition described earlier be adhered to. The soluble bases utilized in this reaction will react directly and rapidly with the alkyl iodides; thus, the acid–base reaction must take place before the alkyl iodide is added.

In order to verify the generality of this reaction, a number of alkyl iodides were reacted with stearic acid salts of tetramethylammonium hydroxide and phenyltrimethylammonium hydroxide. The results are recorded in Tables I and II. A yield of greater than 98% was observed with each of the primary iodides, regardless of the base employed. However, 2-iodobutane gave anomalous results, which may be due to a competing dehydrohalogenation.

The derivation of bile acids with alkyl iodides was examined by assaying for unreacted starting material with a modification of the enzyme method of Iwata and Yamasaki[4]. Less than 2% of the bile acids remained unreacted under the conditions described.

## APPLICATIONS

The first and probably most important application of this procedure is the formation of esters using virtually any primary iodide to supply the alkyl group. For example, 1-iodohexadecane was used in the derivation of benzoic acid, thus forming an ester easily analyzed by GC. This reaction exemplifies how this procedure can be used in the analysis of volatile acids by the formation of esters not easily obtained by conventional methods.

Another related possibility is the preparation of esters for mass spectral analysis. Substitution of trideuterioiodomethane for iodomethane in the reaction, and comparison of the analysis of this derivative and that of iodomethane can greatly facilitate the interpretation of the spectra obtained.

Preliminary evaluations indicate that the reaction described here is applicable not only to carboxylic acids, but to the derivation of diphenylhydantoin and the barbiturates as well. Studies in this area will be described at a later date.

An examination of the use of this reaction in the analysis of bile acids from physiological systems has been made. A common difficulty in GC analysis of bile acid methyl esters from a physiological system is the interference caused by massive amounts of cholesterol. However, the bile acid butyl esters, which can be conveniently prepared by this technique, are eluted later than the methyl esters and are far more easily separated from cholesterol.

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