



Derivatization-Gas Chromatographic Determination of Hydroxylic and Ketonic Acids with Pentafluorobenzyl ρ -Toluenesulfonate as the Reagent

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Derivatization-Gas Chromatographic Determination of Hydroxylic and Ketonic Acids with Pentafluorobenzyl *p*-Toluenesulfonate as the Reagent

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ABSTRACT

A new gas chromatographic method for the determination of hydroxylic and ketonic acids is presented. In the method, hydroxylic and ketonic acids are derivatized to their pentafluorobenzyl esters with pentafluorobenzyl *p*-toluenesulfonate, which was recently developed by the author and his co-workers as the derivatizing reagent for the gas chromatographic determination of inorganic anions, etc., and the resulting pentafluorobenzyl esters are subsequently determined by flame ionization gas chromatography. Lactic and levulinic acids were selected as the model hydroxylic and ketonic acids, and the calibration curves for the acids were constructed, which show straight lines passing through the origin in the concentration ranges of 2-20 mM for lactic acid and 1-10 mM for levulinic acid. The effects of reaction temperature, reaction time and the concentrations of pentafluorobenzyl *p*-toluenesulfonate and phase transfer catalyst on the derivatization of the two acids were studied.

Key Words: Gas Chromatography, Derivatization with Pentafluorobenzyl *p*-Toluenesulfonate, Determination of Hydroxylic and Ketonic Acids

Introduction

To date, gas chromatography has been widely used for separation and analyses of volatile compounds. By using derivatization technique, furthermore, gas chromatography can be used for the determination of compounds which are not accessible to direct gas chromatographic determination, for example, non-volatile and thermally unstable compounds. Derivatization has been employed not only to increase the volatility of the analyte but also to introduce a detector-oriented tag into the molecule^{1,2}. Electron capture detection has been studied in conjunction with derivatization to introduce a detector-oriented tag, due to its high sensitivity, and several derivatizing reagents have been developed for electron capture gas chromatography³. Recently, we have developed a new derivatizing reagent, pentafluorobenzyl *p*-toluenesulfonate, which can be used for the determination of carboxylic acids, phenols⁴, mercaptans^{5,6}, and inorganic anions^{4,7} by electron capture gas chromatography. The reagent has been synthesized to enhance the responses of the electron capture detector as well as the volatility of the above chemicals.

As previously reported⁴, carboxylic acids can be derivatized to their pentafluorobenzyl esters with pentafluorobenzyl *p*-toluenesulfonate, and the resulting pentafluorobenzyl esters can be subsequently determined by gas chromatography. From the results, it was expected that the reagent also derivatize hydroxylic and ketonic acids to their pentafluorobenzyl esters. In the present work, the author has investigated the applicability of the reagent to the determination of hydroxylic and ketonic acids. In the presence of phase transfer catalyst, hydroxylic and ketonic acids are made to react with pentafluorobenzyl *p*-toluenesulfonate, and the resulting derivatives are subsequently determined by flame ionization gas chromatography. As the phase transfer catalyst, 18-crown-6 is used. The final object of this work, of course, is to develop a new gas chromatographic method for the determination of trace amounts of hydroxylic and ketonic acids by using the electron capture detector.

Experimental

Apparatus.

A Shimadzu GC-3BF gas chromatograph equipped with a dual flame ionization detectors system (Shimadzu, Kyoto, Japan) was used together with a glass coiled tube (2.1 m X 3 mm I.D.) packed with 5% PEG-HT on 60-80 mesh Uniport HP, which

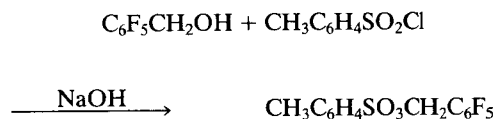
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was obtained from Gasukuro Kogyo (Tokyo, Japan). Nitrogen was used as the carrier gas at a constant flow rate of 30 mL/min. The column and injection port temperatures were maintained isothermally at 200 and 275°C in the determination of lactic and levulinic acids, respectively. In the determination of the mixture of several hydroxylic and ketonic acids, the temperatures were kept at 180 and 225°C, respectively. A Shimadzu Chromatopac C-R3A data processor was used as the recorder and integrator. The pentafluorobenzyl derivatives of the acids were identified with a Shimadzu GCMS-QP1000 gas chromatograph-quadrupole mass spectrometer with an ionization-source temperature of 200°C and an electron energy of 70 eV.

Reagents.

The derivatizing reagent, pentafluorobenzyl *p*-toluenesulfonate, was prepared from pentafluorobenzyl alcohol and *p*-toluenesulfonyl chloride by modification of the literature method⁸⁾ and identified by mass spectrometry and infrared spectrometry, as



reported previously^{4,6)}. Analytical reagent grade lactic acid and *p*-bromonitrobenzene, and commercial grade 2-hydroxyisobutyric acid and 1,2,3,4-tetrachlorobenzene were obtained from Wako Pure Chemical Industries (Osaka, Japan). Analytical grade levulinic acid, commercial grade 3-hydroxy-*n*-butyric acid and *ca.* 50% aqueous solution of 3-hydroxypropionic acid were from Tokyo Kasei Kogyo (Tokyo, Japan). Analytical reagent grade 18-crown-6 was obtained from Aldrich (Milwaukee, Wisconsin, USA), respectively. Acetonitrile and deionized water were distilled before use for analysis.

Procedure.

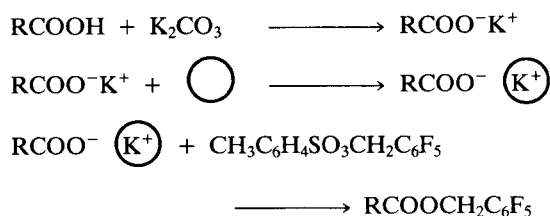
The recommended procedure for the determination of hydroxylic and ketonic acids was as follows. A brown-colored test tube with a screw cap (*ca.* 10 mL) was used as the reaction vessel in order to protect the content from the light. To a reference standard solution of hydroxylic and/or ketonic acids in acetonitrile added were three acetonitrile solutions of pentafluorobenzyl *p*-toluenesulfonate (0.10 M), 18-crown-6 (5.0 mM) and an internal standard (each 0.50 mL).

As the internal standard, *p*-bromonitrobenzene (2.5×10^{-2} M) or 1,2,3,4-tetrachlorobenzene (2.5×10^{-2} M) was used in the determination of lactic or levulinic acid, respectively. Then, anhydrous potassium carbonate was added, and the mixture was stirred magnetically for 30 min in an oil-bath controlled at 40°C. After the reaction period, an aliquot (0.6 μL) was injected into the gas chromatograph.

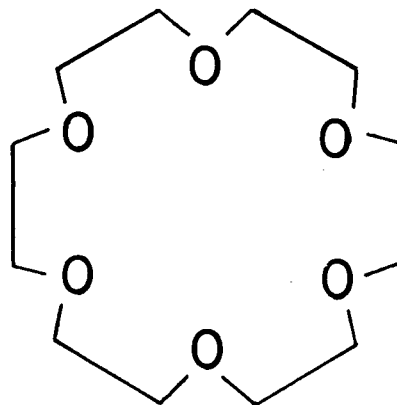
Results and Discussion

Pentafluorobenzylation.

The reaction mechanism of the derivatization used in this work is as follows. Hydroxylic and/or ketonic acids are changed to their potassium salts by adding potassium carbonate. As potassium cation of the salts is included in 18-crown-6, the counter anions are lead to "naked anions". By the naked anion effect, the reactivity of the anions increases, and the anions easily react with pentafluorobenzyl *p*-toluenesulfonate to produce pentafluorobenzyl esters of hydroxylic and ketonic acids. The reactions are formulated as follows:



Alkyl group (R) contains hydroxyl or carboxyl group, and \bigcirc is abbreviation of 18-crown-6;



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Optimum derivatization conditions.

In order to perform the pentafluorobenzylation of hydroxylic and ketonic acids under optimum reaction conditions, we have first examined the effects of reaction temperature, of reaction time and of the concentration of each of pentafluorobenzyl *p*-toluenesulfonate and 18-crown-6 on the derivatization of 2.0×10^{-2} M lactic acid or 1.0×10^{-2} M levulinic acid solution, which has been selected as the model hydroxylic or ketonic acid, respectively. The results are given in Figures 1-4.

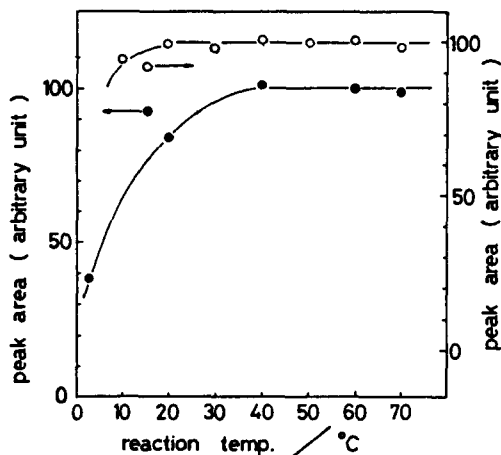


Figure 1 Effect of reaction temperature on the derivatization of lactic (○) and levulinic (●) acids.

Figure 1 shows the effect of reaction temperature on the peak area of the derivative of lactic and levulinic acids. The derivatization yields for the acids increase with an increase in reaction temperature and become constant at reaction temperatures higher than 20°C in the case of lactic acid and 40°C in that of levulinic acid. Considering the simultaneous determination of hydroxylic and ketonic acids, the reaction temperature was fixed at 40°C. Figure 2 shows the effect of reaction time; this study was performed by derivatizing the acids in the presence and absence of 18-crown-6. The derivatization without 18-crown-6 requires longer reaction time than that with 18-crown-6. By using 18-crown-6, the derivatization of lactic acid proceeds within only a few minutes, while that of levulinic acid needs 30 min. Therefore, reaction time was fixed at 30 min, and 18-crown-6 was used in the subsequent study. The effect of pentafluorobenzyl *p*-toluenesulfonate concentration on the yields was examined. As shown in Figure 3, the yields reach constant values when pentafluorobenzyl

p-toluenesulfonate concentration is higher than 0.040 M for both acids. Therefore, further work was performed using 0.10 M pentafluorobenzyl *p*-toluenesulfonate solution. Finally, I have tested the effect of 18-crown-6 concentration (Figure 4). From the result, 5.0 mM 18-crown-6 solution was used for the derivatization in the subsequent study.

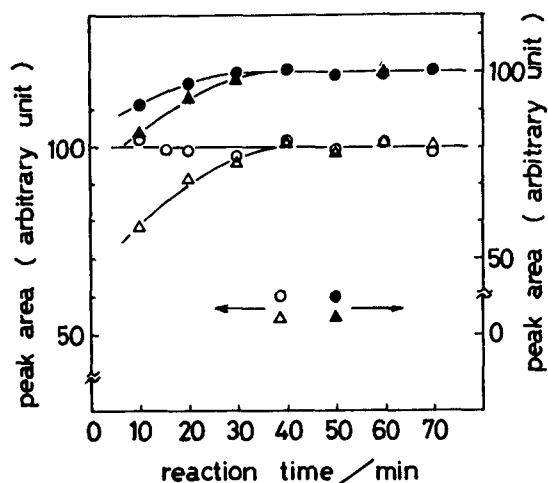


Figure 2 Effect of reaction time on the derivatization of lactic acid with (○) and without 18-crown-6 (△) and levulinic acid with (●) and without 18-crown-6 (▲).

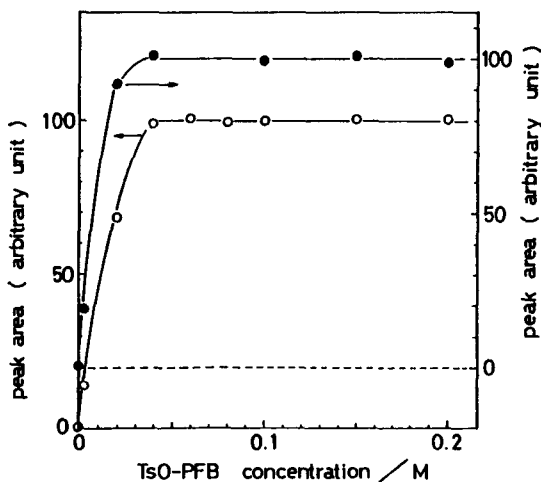


Figure 3 Effect of the concentration of pentafluorobenzyl *p*-toluenesulfonate on the derivatization of lactic (○) and levulinic (●) acids.

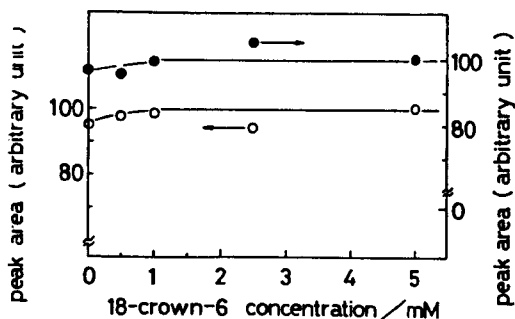


Figure 4 Effect of the concentration of 18-crown-6 on the derivatization of lactic (O) and levulinic (●) acid.

Gas chromatograms and calibration curves.

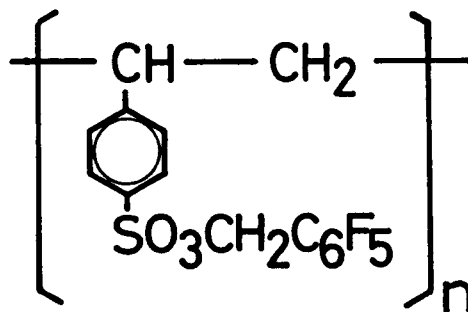
From these results, the optimum derivatization conditions described in the Experimental section were chosen. Typical gas chromatograms of the conversion products in the reacted solutions are shown in Figure 5, where 20 mM lactic acid, 10 mM levulinic acid or the mixture of lactic, 3-hydroxypropionic, 3-hydroxy-*n*-butyric, 2-hydroxyisobutyric and levulinic acids is made to react with pentafluorobenzyl *p*-toluenesulfonate in the presence of 18-crown-6 under the optimum derivatization conditions. The gas chromatogram obtained from the mixture (Figure 5C) has been measured by keeping the column temperature isothermally at 180°C. Better separation may be expected by using temperature programming technique. The peaks of the derivatives of the five acids (i.e., peaks 5-9 in Figure 5C) have been identified by gas chromatography-mass spectrometry. The mass spectra obtained exhibited that the derivatives are pentafluorobenzyl esters of 2-hydroxyisobutyric, lactic, 3-hydroxy-*n*-butyric, levulinic and 3-hydroxypropionic acids (i.e., $R\text{COOCH}_2\text{C}_6\text{F}_5$; $R = (\text{CH}_3)_2\text{C}(\text{OH}), \text{CH}_3\text{CH}(\text{OH}), \text{CH}_3\text{CH}(\text{OH})\text{CH}_2, \text{CH}_3\text{C}(\text{=O})\text{CH}_2\text{CH}_2$ and $\text{CH}_2(\text{OH})\text{CH}_2$). In the present work, positive ion electron impact ionization mass spectrometer was used, but pentafluorobenzylation is a very useful for the structural analysis in negative ion chemical ionization mass spectrometry, because pentafluorobenzyl derivatives give the strong negative ion peaks derived not from the pentafluorobenzyl moiety but from the analytes⁹⁻¹¹. This reagent, therefore, may be used for gas chromatography-negative ion chemical ionization mass spectrometry.

because pentafluorobenzyl derivatives give the strong negative ion peaks derived not from the pentafluorobenzyl moiety but from the analytes 9-11). This reagent, therefore, may be used for gas chromatography-negative ion chemical ionization mass spectrometry.

Calibration curves for lactic and levulinic acids were constructed, plotting the concentration of the acid (X) against the peak area of the derivative (Y). As shown in Figure 6, good linear relationships were obtained ($Y = 5130X + 0.26$, correlation coefficient, $r = 0.997$ for lactic acid; $Y = 10231X + 2.6$, $r = 0.998$ for levulinic acid).

Conclusion

It is found that several hydroxylic and ketonic acids can be derivatized to their pentafluorobenzyl esters by reaction with pentafluorobenzyl *p*-toluenesulfonate in the presence of 18-crown-6 and subsequently determined by flame ionization gas chromatography. This technique will be applicable to other hydroxylic and ketonic acid. Furthermore, the present technique based on pentafluorobenzylation is an electron capture detector-oriented designed to enhance the sensitivity. The development of this method is under investigation for the determination of trace amounts of hydroxylic and ketonic acids by electron capture gas chromatography. One of the major problem for the development is that relatively high concentration reagent solution cannot be used for the derivatization, because the injection of large amounts of pentafluorobenzyl *p*-toluenesulfonate produces some troubles; the contamination and the decrease in the sensitivity of the electron capture detector. Therefore, the author and his coworkers are studying the development of a new polymer reagent, the construction of which is as follows¹²:



As this polymer reagent is non-volatile, the injection of it do not produce the troubles of the detector mentioned above at all.

Furthermore, we have been investigating the development of new high performance liquid

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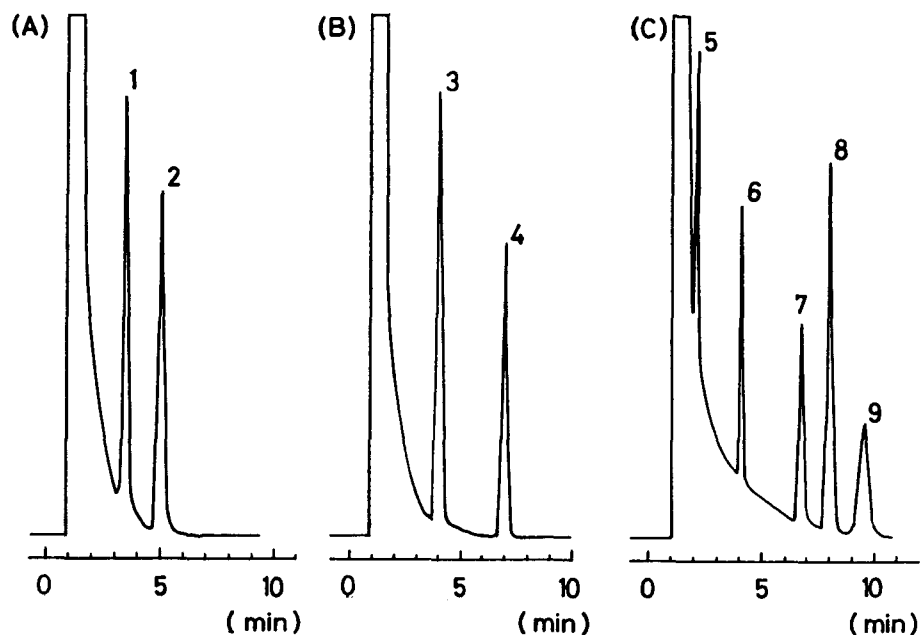


Figure 5 (A) Gas chromatogram obtained in the determination of only lactic acid. (B) Gas chromatogram obtained in the determination of only levulinic acid. (C) Gas chromatogram obtained in the determination of the mixture of hydroxylic and ketonic acids. Peaks: 1 = derivative of lactic acid, 2 = *p*-bromonitrobenzene (internal standard), 3 = 1,2,3,4-tetrachlorobenzene (internal standard), 4,5,6,7,8,9 = derivatives of levulinic, 2-hydroxyisobutyric, lactic, 3-hydroxy-*n*-butyric, levulinic and 3-hydroxypropionic acids, respectively.

chromatographic derivatizing reagents used for labelling for ultraviolet spectrometric and/or fluorescent detection, the structure of which is *p*-toluenesulfonate¹³. In full length papers, we will publish the polymer reagent for gas chromatography and the derivatizing reagents for high performance liquid chromatography in near future^{14,15}.

Acknowledgment

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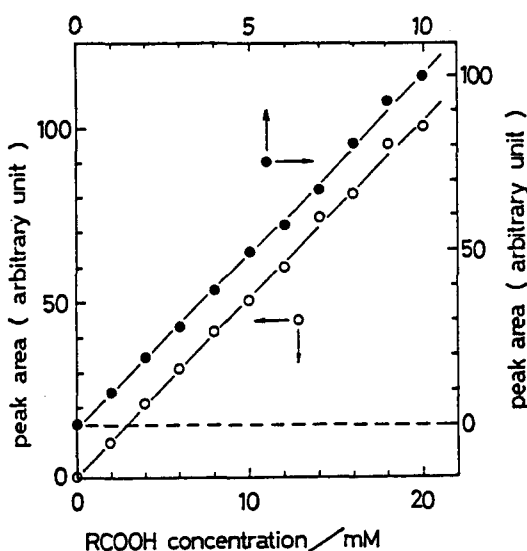


Figure 6 Calibration curves of lactic (○) and levulinic (●) acids.

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