Vol. 11, No. 4, 2020
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE www.msletters.org | Mass Spectrometry Letters

Chiral Derivatization of Hydroxycarboxylic Acids Using 2,4,6-Trichlorobenzoyl Chloride as a Highly Efficient Regioselective Esterification Reagent for Gas Chromatography-Mass Spectrometry

Jeong Hyeok Park and Sang Yun Han*

Department of Chemistry, Gachon University, Seongnam-si, Gyeonggi-do 13120, Republic of Korea

Received December 14, 2020; Revised December 23, 2020; Accepted December 23, 2020
First published on the web December 31, 2020; DOI: 10.5478/MSL.2020.11.4.103

Abstract : We report the application of 2,4,6-trichlorobenzoyl chloride (often referred to as Yamaguchi esterification reagent) for the selective derivatization of the carboxylic group for GC-MS with the sample preparation method optimized for GC-MS analysis. The reagent was shown to be capable of selectively turning the carboxylic group into a reaction center, *i.e.,* anhydride, of which the further reaction was directed to a near complete formation of required esters by unique steric and electronic effects of the reagent. Using the developed method, the chiral separation of hydroxycarboxylic acids by GC-MS using non-chiral columns was successfully demonstrated.

Keywords : 2,4,6-trichlorobenzoyl chloride, chiral separation, GC-MS, hydroxycarboxylic acids, *O*-acetylated *L*-menthyl esters

Introduction

Gas chromatography-mass spectrometry (GC-MS) is a powerful technique for characterizing and quantifying volatile non-polar molecules. To expand its range of applications, which is limited by the molecular properties of analytes, various derivatization methods to modify analytes have been utilized. The examples include silylation, *i.e.*, the formation of trimethylsilyl derivatives on reactive functional groups. In most cases, the derivatizations aim to replace active hydrogen atoms in analytes by inert molecular groups. This results in an increase in vapor pressure and a decrease in the polarity of derivatized analytes, which leads to higher sensitivity and better resolving power for GC-MS. In this regard, silylation using *N*, *O*-bistrifluoroacetamide (BSTFA), *tert*-butyldimethylsilyl chloride (TBDMS-Cl), *N*-methyl-*N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), and the like has widely been adopted in the research fields.[1-3]

*Reprint requests to Sang Yun Han
E-mail: sanghan@gachon.ac.kr

Esterification provides another chance for GC-MS analysis. One example offers an opportunity of chiral separation (enantioseparation), which is especially important for production and analysis in industrial areas such as pharmaceutics, pesticides, food additives, and fragrances.[4-6] Although the enantiomers possess identical physical and chemical properties, due to the chirality, they often exhibit significant differences in physiological activity, which activates different metabolic pathways. A notorious example is a drug, thalidomide, of which the *D*-isomer exhibits medicinal effects, whereas the *L*-isomer causes a fatal adverse effect of deformity in pregnant women.[7]

In the chiral separation of racemic mixtures, a well-known strategy is introducing an additional optical center such as *L*-menthol by the esterification of the carboxylic group in the analytes via anhydride formation. For the reaction, acyl chlorides such as acetyl chloride and benzoyl chlorides have been commonly employed as acylating reagents to produce the reaction intermediates of anhydrides for esterification reactions. The esterification transforms the enantiomers into diastereomers, which have different physical and chemical properties that are amenable to separation by common chromatographic techniques using non-chiral columns.[8,9]

However, from a chemistry point-of-view, the use of acetyl chloride and benzoyl chlorides has certain limitations due to the regioselectivity issue in esterification. As shown in Figure 1, the anhydride intermediates that are produced by the reaction of the acyl chlorides with the carboxylic group have two available sites for nucleophilic

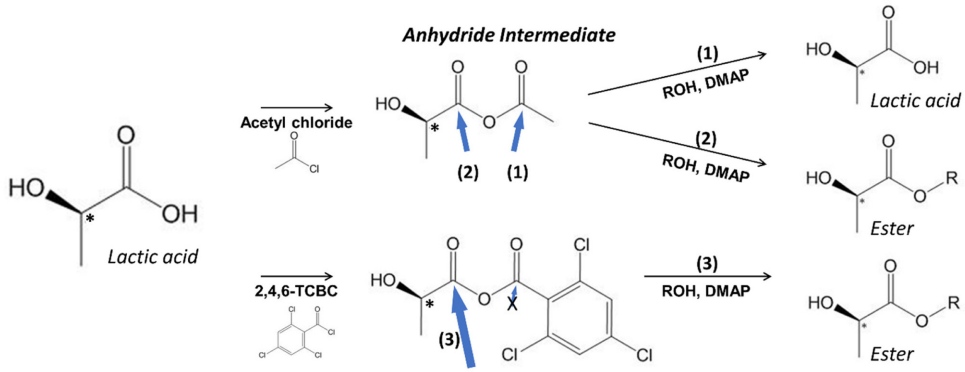

Figure 1. Esterification of lactic acid via anhydride formation using acetyl chloride and 2,4,6-TCBC.

attacks (1 and 2), of which one pathway (1) turns the reaction back to the starting materials, which simply consumes the acyl chloride reagents. In contrast, the attack by (2) leads to the esterification intended for chiral derivatization.

Due to the regioselectivity issue, the efficacy of chiral derivatization using the conventional acylating reagents may be limited. In this regard, this study examined the use of 2,4,6-trichlorobenzoyl chloride (2,4,6-TCBC), often referred to as Yamaguchi esterification reagent as a highly efficient regioselective reagent for esterification reactions (Figure 1),[10,11] which is driven by the steric effect and the electronic induction effect supported by a catalysis, 4-dimethylaminopyridine (DMAP).[12]

The synthetic method for esterification using 2,4,6-TCBC has been known to be successful only in non-polar organic solvents such as toluene.[11] However, such solvents are not suitable for polar analytes like hydroxycarboxylic acids due to their limited solubilities. Because of this, we examined various solvent systems as well as various experimental parameters, including reaction time and temperature. As a result, we obtained the following derivatization method using ethyl acetate as solvent, which is suitable for GC-MS analysis.

Herein, we report the optimized sample preparation method for the chiral derivatization of hydroxycarboxylic acids using 2,4,6-TCBC. This method was highly selective to the carboxylic group in the analytes and also highly efficient for esterification (> 95%), which is in this regard superior to the methods that used the conventional acyl chlorides such as acetyl chloride. The detailed reaction scheme is given in the supplementary information (Figure S1).

Experimental

Reagents

Dodecanoic acid, *L*-lactic acid, *D*-mandelic acid, *L*-mandelic acid, diisopropylethylamine (DIEA), acetyl chloride, MTBSTFA, and ethyl acetate were commercially obtained from Sigma Aldrich, Co. 1-Dodecanol, *D*-lactic acid, *D*-, *L*-3-hydroxybutyric acid, DMAP, 2,4,6-TCBC, and *L*-menthol were purchased from Tokyo Chemical Industry. Sodium hydrogen carbonate was purchased from Daejung Chemicals & Metals, Co.2

Chiral derivatization of hydroxycarboxylic acids using 2,4,6-TCBC

The procedure of chiral derivatization using the 2,4,6-TCBC optimized in this study consisted of three steps: (1) 2,4,6-TCBC (1.5 eq.) and DIEA (2.5 eq.) were added to the hydroxycarboxylic acid solution in ethyl acetate, which was then incubated at 40°C for 10 min. (2) Acetyl chloride (1.3 eq) was added, and the solution was incubated at 40°C for 10 min. This step masks the hydroxy group to avoid any possible nucleophilic attack to the anhydride intermediates formed with 2,4,6-TCBC. (3) *L*-menthol (2 eq.) was added with DMAP (1.5 eq.), and the solution was incubated at 40°C for 5 min. Finally, the solutions were washed with an equal volume of the saturated aqueous solution of sodium hydrogen carbonate. Then, one microliter of the supernatant was taken and diluted, which was subject to GC-MS analysis.

Derivatization of hydroxycarboxylic acids using MTBSTFA

The silylation of hydroxycarboxylic acids using MTBSTFA was also performed for comparison, which utilized the well-known protocols for TBDMS derivatization.[3] The solutions of hydroxycarboxylic acids were prepared in ethyl acetate, which included DIEA (2.5 eq.), DMAP (1.5 eq.), and excess MTBSTFA. The solutions were incubated at 40°C for 1 hr in order for derivatization to occur. To wash the solution, an equal volume of the saturated aqueous solution of sodium hydrogen carbonate was added, and the mixture was vortexed for 3 min. one microliter of the supernatant was taken and diluted, which was then analyzed by GC-MS.

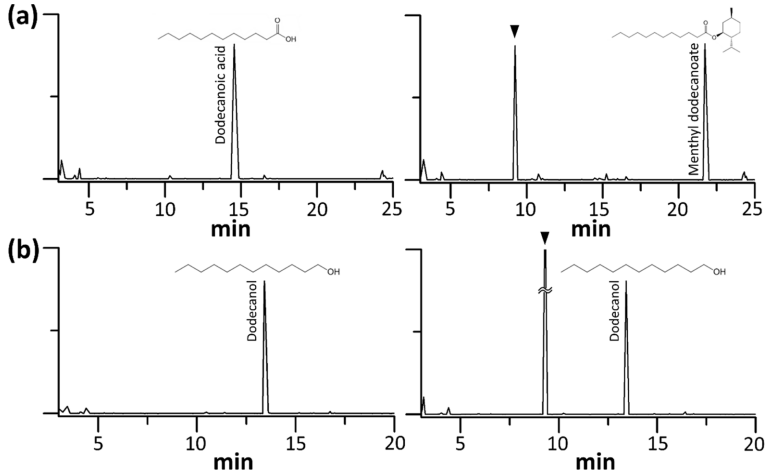

Figure 2. GC-MS chromatograms of (a) dodecanoic acid and its reaction products, (b) dodecanol and its reaction products using 2,4,6-TCBC. ▼ indicates the excess *L*-menthol.

GC-MS analysis

The experiments used a GC-MS instrument (QP2020, Shimadzu Co.) that operated in the electron impact ionization mode at 70 eV, which was equipped with an autosampler (AOC-20s), autoinjector (AOC-20i), and a gas chromatography (GC-2010) interfaced to a quadrupole mass spectrometer. A non-chiral column (Rtx-5MS capillary column, 30.0 m × 0.25 mm × 0.25 μm, 5% diphenyl, 95% dimethyl polysiloxane bonded phase) was used. He (99.999%) was used as carrier gas at a flow rate of 1.0 mL/min, and the analysis was performed in the splitless mode. The temperatures of injector, ion-source, and interface were 250, 270, and 270°C, respectively. The column oven was initially set at 70°C and increased up to 300°C at a rate of 10°C/min.

Results and Discussion

Selective esterification at carboxylic group

Acyl chloride is a reactive reagent that can react with functional groups such as hydroxy and carboxylic groups. Its reaction with a hydroxy group forms a direct linkage between the hydroxy group and acyl chloride, an esterification. In contrast, the reaction with a carboxylic group creates a new reaction center, *i.e.,* anhydride functionality, which can be exploited for further reactions such as esterification with selected alcohols, *e.g., L*-menthol, to turn enantiomers into diastereomers, as in this study. Likewise, the selective reactivity of acyl chloride reagents to the carboxyl group is critical in order to control the derivatization reactions of hydroxycarboxylic acids, in which both functional groups coexist in the analytes.

Figure 2 demonstrates the selectivity of the 2,4,6-TCBC reagent toward the carboxylic group. In the test runs, the optimized procedure was examined with respect to a carboxylic acid (1-dodecanoic acid) and an alcohol (1-dodecanol). The derivatization was performed through the same procedure, which was optimized for esterification using the 2,4,6-TCBC described in the Experimental Methods section. As evident in Figure 2, after the process was completed, the 1-dodecanoic acid was completely transformed to *O*-acetylated *L*-menthyl esters (menthyl dodecanoate) (Figure 2(a)), whereas 1-dodecanol was still there without undergoing esterification (Figure 2(b)). This indicates that the optimized method of this study using 2,4,6-TCBC has a distinct selectivity for the carboxylic group against the hydroxy group, which is a strong advantage for the chiral derivatization of hydroxycarboxylic acids.

Comparison between the reactions using acetyl chloride and 2,4,6-TCBC

In chiral derivatization methods using acylating reagents, acetyl chloride has been widely employed to produce anhydride functionality for further esterification. However, as discussed in the Introduction (Figure 1), the anhydride intermediates possess two reaction pathways, of which one causes alcoholysis of the anhydride back to the starting materials, which only consume acetyl chloride reagents. Thus, it is hard to expect for the chiral derivatization to be completely achieved using acetyl chloride. However, the merits of using 2,4,6-TCBC include the anhydrides mostly undergoing alcoholysis toward ester formation, which is directed by the steric hindrance and electronic effect induced by the catalysis (DMAP) because of the aromatic compounds.[10] The effects under experimental conditions are clear in Figure 3. When acetyl chloride was used, about one third of the dodecanoic acid was transformed into the esters, and a large amount of acid remained unreacted in the GC-MS chromatogram (Figure 3(b)). However, it is evident in Figure 3(c) that the developed preparation

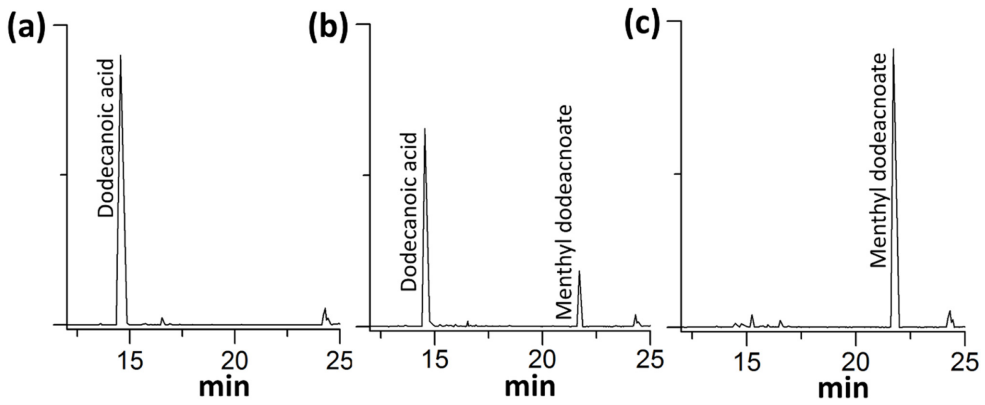

Figure 3. GC-MS chromatograms of (a) dodecanoic acid, (b) the reaction products of esterification of dodecanoic acid using acetyl chloride, (c) the reaction products of esterification of dodecanoic acid using 2,4,6-TCBC.

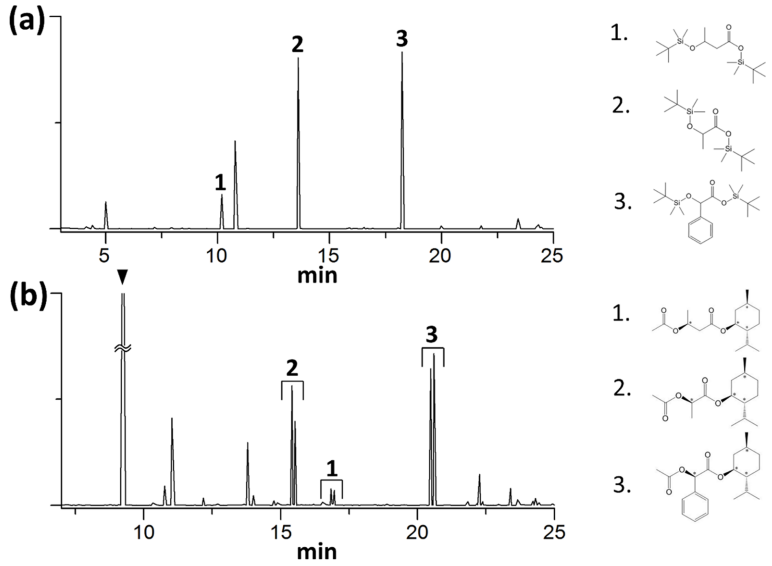

Figure 4. GC-MS chromatograms of (a) silylated compounds of racemic mixtures and (b) chiral derivatized compounds of *D*-, *L*-3-hydroxybutyric acids (1), *D*-, *L*-lactic acids (2), *D*-, *L*- mandelic acids (3). ▼ indicates the excess *L*-menthol.

method that employed 2,4,6-TCBC converted essentially all acids into esters. This indicates that the regioselectivity of 2,4,6-TCBC is indeed beneficial for the complete derivatization, for which the optimized method of this study works properly. This aspect of the present method further suggests its applicability for quantitative analysis as well.

Chiral derivatization of hydroxy carboxylic acids

Finally, we applied this method for the chiral separation of racemic mixtures of hydroxycarboxylic acids using non-chiral columns. In this demonstration, the enantioseparation of the racemic mixture of *D*-, *L*-lactic acids, *D*-, *L*-mandelic acids, and *D*-, *L*-3-hydroxybutyric acids were examined. As shown in Figure 4(a), when the sample was treated with MTBSTFA, the silylated compounds of the three racemates were clearly separated but were not resolved for their respective enantiomers in the GC-MS chromatogram. However, when this method that used 2,4,6-TCBC was applied, the enantiomers of the acids were further resolved as doublets in the chromatogram with the measured enantiomeric resolution value (R_s) of 2.56 and 2.38 for *L*-lactic acid and *D*-mandelic acid, respectively (Figure 4(b)). This was achieved by the derivatization of the enantiomers into diastereomers by esterification with optically active *L*-menthol under the optimized procedure in this study. In addition, this method was highly quantitative in terms of linearity (R^2 = 0.9995 and 0.9992 for *L*-lactic acid and *D*-

mandelic acid, respectively), LOD (0.02 and 0.007 μM), and LOQ (0.066 and 0.023 μM), which is beneficial from the high reactivity and regioselectivity provided by the use of 2,4,6-TCBC (Table S1).

Conclusions

This study reports the chiral derivatization method for GC-MS using 2,4,6-TCBC that serves as a highly efficient regioselective esterification reagent. By virtue of its excellent selectivity to the carboxylic group and regioselectivity toward esterification, which featured near complete derivatization, this method may offer a robust way of investigating chiral molecules in a highly quantitative way.

Supporting Information

Supplementary Information is available at https://drive.google.com/file/d/1K3JlC3yyme506rHmD8k8XT8smb4Vi2gP/view?usp=sharing.

Acknowledgements

This work was supported by the National Research Foundation of Korea with grant number NRF-2019R1A2C1089580. This work was supported by Korea Basic Science Institute (NFEC) grant funded by the Ministry of Education (2020R1A6C101A184).

References

1. Stalling, D. L.; Gehrke, C. W.; Zumwalt, R. W. *Biochem. Biophys. Res. Commun.* **1968**, 31, 616, DOI: 10.1016/0006-291x(68)90523-8.
2. Schummer, C.; Delhomme, O.; Appenzeller, B. M. R.; Wennig, R.; Millet, M. *Talanta* **2009**, 77, 1473, DOI: 10.1016/j.talanta.2008.09.043.
3. Jimenez-Martin, E. ; Ruiz, J.; Perez-Palacios, T.; Silva, A.; Antequera, T. *J. Agric. Food Chem.* **2012**, 60, 2456, DOI: 10.1021/jf2052338.
4. Armstrong, D. W.; Chang, C. D.; Li, W. Y. *J. Agric. Food Chem.* **1990**, 38, 1674, DOI: 10.1021/jf00098a010.
5. Toyo'oka, T. *J. Biochem. Biophys. Methods* **2002**, 54, 25. DOI: 10.1016/S0165-022X(02)00127-6.
6. Gorog, S.; Gazdag, M. *J. Chromatogr. B.* **1994**, 659, 51, DOI: 10.1016/0378-4347(94)00124-3.
7. Kim, J. H.; Scialli, A. R. *Toxicol. Sci.* **2011**, 122, 1, DOI: 10.1093/toxsci/kfr088.
8. Ding, X.; Lin, S.; Weng, H.; Liang, J. *J. Sep. Sci.* **2018**, 41, 2576, DOI: 10.1002/jssc.201701555.
9. Ionue, Y.; Shinka, T.; Ohse, M.; Ikawa, H.; Kuhara, T. *J. Chromatogr. B.* **2006**, 838, 37, DOI: 10.1016/j.jchromb.2006.02.019.
10. Dhimitruka, I.; SantaLucia, *Org. Lett.* **2006**, 8, 47, DOI: 10.1021/ol0524048.
11. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamacughi, M. *Bull. Chem. Soc. Japan* **1979**, 52, 1989, DOI: 10.1246/bcsj.52.1989.
12. Sakakura, A.; Kawajiri, K.; Ohkubo, T.; Kosugi, Y.; Ishihara, K. *J. Am. Chem. Soc.* **2007**, 129, 14775, DOI: 10.1021/ja075824w.