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DERIVATIZATION OF CARBOXYLIC ACIDS BY REACTION WITH 4'-BROMOPHENACYL TRIFLUOROMETHANESULFONATE PRIOR TO DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The use of 4'-bromo-2-hydroxyacetophenone trifluoromethanesulfonate ester (4'-bromophenacyl triflate) in the preparation of carboxylic acid 4'-bromophenacyl ester derivatives for spectrophotometric detection in high-performance liquid chromatography is described. The reagent is prepared in 66% yield by the reaction of 4'-bromo-2-diazoacetophenone with trifluoromethanesulfonic acid in anhydrous sulfur dioxide and is stable for 3–6 months. Reactions of 10^{-6} *M* carboxylate N,N-diisopropylethylammonium salts with this reagent in acetonitrile at room temperature proceed to completion in 1–5 min. Optimal rates of reaction are obtained with a 10-fold equivalent excess of alkylating agent and 5 equivalents of N,N-diisopropylethylamine present. The process has been applied successfully to mono-, di- and tricarboxylic and sterically hindered carboxylic acids.

INTRODUCTION

Carboxylic acids are important nutritional substrates and metabolites. The carboxyl functional group is a significant structural feature of other compounds of biological interest, notably prostaglandins and related eicosanoids, steroid carboxylates and some pharmaceuticals. This functional group is only weakly chromophoric (λ_{max} = 204 nm, log ε = 1.78[1]); thus, carboxylates with no other adequately chromophoric structural feature must be derivatized for enhancement of the sensitivity of spectrophotometric absorbance detection in their determination at low concentrations by high-performance liquid chromatography (HPLC).

Chromophoric derivatives of carboxylic acids are accessible by several reaction mechanisms. Acylations of aromatic amines to produce N-phenylcarboxamides[2],

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N-4-methylphenylamides[3] and N-4-methoxyphenylamides[4] have been described. Addition of acids to O-substituted isoureas and subsequent rearrangement to carboxylate esters has been employed in the preparation of benzyl[5] and 4-nitrobenzyl esters[6]. However, carboxyl-O-alkylation has been applied most frequently in the analytical-scale preparation of chromophoric acid derivatives. Benzyl esters were prepared on a semi-preparative scale by reaction of alkanoic acids with 1-benzyl-3-(4-tolyl)triazine[7]. 4-Nitrobenzyl esters were also obtained by the reaction of carboxylates with 4′-nitrobenzyl bromide[8]. The derivatives most frequently prepared by this mechanism include a number of analogous aryl hydroxymethyl ketone carboxylate esters. Reports of the preparation and chromatography of 2-naphthacyl[9], 4′-nitrophenacyl[10], 4′-bromophenacyl[8,11,12] and 4′-phenylphenacyl[12] esters appeared in rapid succession as chromatographic techniques were applied to carboxylate determination problems. The particular suitability of these derivatives follows from the greater success of carboxyl-O-alkylation compared with other derivatization reaction processes at low sample concentrations, their chromatographic tractability and their intense absorbance maxima (λ_{max} = 240–260 nm, log ε = 3.8–4.5[8,9,11,13–15]) in solvents useful as reversed-phase chromatographic eluents.

Two important general methods have emerged for the preparation of 4′-bromophenacyl and analogous esters on the analytical scale. In 1975, Durst and co-workers[8,11] exploited current discoveries of the greatly enhanced nucleophilic reactivity of potassium carboxylates in aprotic solvents under conditions of solid–liquid phase-transfer catalysis by macrocyclic polyethers[16–18]. Accordingly, they prepared 4′-bromophenacyl esters in quantitative yield by reaction of 10^{-6}–10^{-2} *M* carboxylate potassium salts with a 2-fold equivalent excess of 2,4′-dibromoacetophenone and 5–10 mol.-% of 18-crown-6 phase-transfer catalyst in acetonitrile at 80°C for 15–30 min. At the same time it was reported that 2-naphthacyl esters[9] and phenacyl esters[13] could be prepared in good yields from similar amounts of carboxylate trialkylammonium salts and similar equivalent proportions of 2-bromoketone alkylating agents in aprotic solvents at or slightly above room temperature in several hours. Subsequently, variations of the former procedure have been used for determinations of carboxylic acids in wastewater and rainwater[14], penicillins[15] and steroid-21-oic acids[19]. The latter procedure has been applied to determinations of carboxylates in rain and river water[20], prostaglandins[10,21], long-chain alkanoates in bacterial culture media[22,23] and prostaglandin precursors in cell culture media[24]. Considerably longer reaction times and higher temperatures are required in both systems for complete derivatization of carboxylic acids at sample concentrations lower than 10^{-4} *M*. These findings are consistent with the expected kinetic behavior of bimolecular reactions under the stated conditions of reagent proportions.

We have developed a new reagent for the preparation of 4′-bromophenacyl ester derivatives of carboxylic acids. 4′-Bromo-2-hydroxyacetophenone trifluoromethanesulfonate ester (4′-bromophenacyl triflate) may be prepared in 55% overall yield from commercially available starting materials. This reagent reacts completely with low concentrations of mono-, di-, and tricarboxylic acid diisopropylethylammonium salts in acetonitrile at room temperature in 1–5 min. Despite its great reactivity, the compound can be handled easily and stored at room temperature for periods of 3–6 months. A general derivatization procedure has been developed and applied to a number of representative sterically hindered and functionalized carboxylic acids.

EXPERIMENTAL

Equipment

The liquid chromatograph consisted of a Model 6000A pump, a U6K syringe-loading sample injection valve, an RCM-100 radial compression module and a Model 440 fixed-wavelength spectrophotometric detector purchased from Waters Assoc. (Milford, MA, U.S.A.). The chromatographic separation was accomplished on a 10 × 0.5 cm plastic cartridge containing Radial-PAK C_{18} of 10 μm nominal particle diameter (Waters Assoc.). The chromatographic column was protected by a 5 × 0.4 cm I.D. pre-column packed with Co:Pell ODS (Whatman, Clifton, NJ, U.S.A.). The detector was operated at 254 nm and its output signal was recorded by a Linear Instruments (Irvine, CA, U.S.A.) Model 291 chart recorder. A Hewlett-Packard (Avondale, PA, U.S.A.) Model 3354C laboratory automation system was used for chromatographic peak area integration, peak-height measurement and calculations derived from those measurements. A Waters Assoc. WISP-710A automatic sampler was used in experiments related to the stability of reagents and identification of side reaction processes. ^{1}H nuclear magnetic resonance spectra of new compounds were recorded with a Bruker (Billerica, MA, U.S.A.) WH-180/270 pulsed Fourier-transform NMR spectrometer. Electron impact mass spectra were obtained with a Hewlett-Packard Model 5985B gas chromatograph–(quadrupole) mass spectrometer through the heated direct inlet probe. UV absorbance spectra were acquired with an American Instruments (Silver Spring, MD, U.S.A.) DW-2A double-beam spectrophotometer. Infrared spectra were recorded with a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 1420 instrument. Liquid scintillation counting was performed with a Packard (Downers Grove, IL, U.S.A.) PRIAS scintillation spectrometer. Melting points were observed with a Fisher-Johns micro hot-stage apparatus and are uncorrected.

Materials

Acetonitrile (OmniSolv, non-UV grade) was purchased from MCB (Cincinnati, OH, U.S.A.) and filtered through nylon membranes of 0.45 μm nominal pore size before use in the preparation of chromatographic mobile phases. Acetonitrile intended for use in derivatization reaction solutions was distilled from calcium hydride. Water was prepared for use as a chromatographic mobile phase constituent by passage through a Milli-Q water purification unit (Millipore, Bedford, MA, U.S.A.). All reagents used for the preparation of 4′-bromophenacyl triflate and the carboxylic acids and 2,4′-dibromoacetophenone used for the preparation of authentic 4′-bromophenacyl esters were obtained from Aldrich (Milwaukee, WI, U.S.A.). Sulfur dioxide was purchased from the Linde Division of Union Carbide (Somerset, NJ, U.S.A.). Triethylamine, N,N-diisopropylethylamine and tri-*n*-butylamine were purchased from Aldrich and were distilled from potassium hydroxide prior to use in derivatization studies. [1-^{14}C]Hexadecanoic acid, [1,4-^{14}C]succinic acid and [1,5-^{14}C]citric acid were obtained from Amersham Corp. (Arlington Heights, IL, U.S.A).

Authentic 4′-bromophenacyl esters of hexadecanoic, octadecanoic, succinic and citric acids were prepared for use as experimental external standards in derivatization reaction optimization studies[25,26]. The products were identified by comparison of their melting points with published values[25,27] and they were shown to be of >99% chromatographic purity at 254 nm.

Preparation of 4′-bromophenacyl triflate

The reagent was synthesized according to the method developed by Vedejs *et al.*[28] for the preparation of 2-hydroxymethyl ketone trifluoromethanesulfonate esters. Thus, 50 ml of anhydrous sulfur dioxide were condensed in a 100-ml round-bottomed reaction flask containing a magnetic stirring bar and fitted with a Claisen head, gas-inlet tube and calcium sulfate-filled drying tube. The solvent was cooled to the temperature of a dry-ice–acetone bath and 2.25 g (10^{-2} mol) of 4′-bromo-2-diazoacetophenone[29,30] were added. The solution was stirred for 5 min and 1.5 g (0.9 ml, 10^{-2} mol) of anhydrous trifluoromethanesulfonic acid (Aldrich) measured from a freshly opened bottle was added in one portion. The flask was closed and its contents were stirred for 15 min, then the cooling bath was removed. After 30 min the reaction flask was warmed over an ice–water bath to evaporate the solvent.

The brown product residue was dissolved in 100 ml of boiling methylene chloride, treated twice with 5 g of decolorizing carbon and filtered. The filtrate solvent was evaporated; recrystallization of the residue from pentane–methylene chloride (80:20, v/v) afforded 2.3 g ($6.6 \cdot 10^{-3}$ mol, 66%) of 4′-bromophenacyl triflate as colorless, transparent plates, m.p. 137–138°C. Product identity was confirmed by ^{1}H NMR spectrometry, mass spectrometry (MS), IR spectrophotometry, UV spectrophotometry and elemental analysis. NMR ($CDCl_3$ solution, $CHCl_3$ as secondary internal reference taken as 7.24 ppm relative to Me_4Si): 5.60 ppm (s), 7.71 ppm (q, J = 8.1 Hz). MS (EI, 70 eV, ion source temperature 200°C) [m/z (relative intensity)]: 69 (100), 185 (44), 183 (43), 155 (15), 157 (16), 225 (10), 227 (9). IR [ν (cm^{-1})]: 2955 (w), 1715 (s), 1590 (s), 1420 (s), 1145 (s), 1050 (m), 960 (s). UV (acetonitrile): λ_{max} = 259.5 nm, log ε = 4.37. Elemental analysis (Galbraith Labs., Knoxville, TN, U.S.A.): calculated for $C_9H_6BrF_3O_4S$: C 31.13, H 1.73, Br 23.03, F 16.43%; found: C 31.03, H 1.70, Br 23.09, F 16.29%.

Optimization of derivatization reaction conditions

Stock solutions of tertiary amines and alkylating agents in acetonitrile were prepared daily and serially diluted to provide working solutions of appropriate concentrations. In typical optimization trials, 75 μl of acetonitrile containing $2.5 \cdot 10^{-8}$ mol of carboxylic acid substrate was combined in a 100 × 13 mm disposable glass test-tube with 200 μl of acetonitrile containing $2.5 \cdot 10^{-8}$ mol of a homologous carboxylate 4′-bromophenacyl ester external standard. To this were added 25 μl of tertiary amine working solution, thereby fixing the equivalent ratio of base to carboxylic acid. The tube was vortexed and 250 μl of the freshly prepared 4′-bromophenacyl triflate working solution were added, thus fixing the initial concentration ratio of alkylating agent to carboxylic acid and starting the derivatization reaction. The tube was capped; at fixed times thereafter aliquots of the solution were chromatographed and the extent of the reaction was monitored for at least 1 h by observation of the peak-height ratios of derivative product to external standard 4′-bromophenacyl ester. These experiments typically were conducted on groups of five or six samples.

Comparative alkylation rate experiments with 4′-bromophenacyl triflate and bromide were conducted under concentration conditions found to be optimal for the reaction of carboxylates with the new reagent. In 100 × 13 mm glass test-tubes were combined $1.5 \cdot 10^{-7}$ mol of acid substrate and $1.5 \cdot 10^{-7}$ mol of an analogous

carboxylate 4′-bromophenacyl ester in a total volume of 460 μl of acetonitrile. The acid was neutralized by addition of 5 equiv. of N,N-diisopropylethylamine in 250 μl of acetonitrile. The tube was vortexed and 10 equiv. of alkylating agent in 360 μl of acetonitrile were added to start the derivatization reaction. Sample aliquots of 15 μl were withdrawn for injection into the chromatograph after 30 sec, 5 min and at intervals of 5 min thereafter for 1 h. In experiments with 4′-bromophenacyl bromide, injections were made at hourly intervals for another 8 h after the first hour of the reaction period, and subsequently at intervals of 24 h for several days. The effect of water on the success of the derivatization procedure was evaluated by systematic replacement of some fraction of the acetonitrile solvent with an equal amount of water.

Verification of completion of reaction

The absolute extent of the reaction of 4′-bromophenacyl triflate with representative carboxylic acids under the suggested derivatization conditions was evaluated by demonstration of the conversion of chromatographically pure samples of ^{14}C-labeled carboxylic acids (10^5 dpm) into a single radioactive derivative peak that co-chromatographed with the chromophoric derivative peak of the same unlabeled carboxylic acid. Eluent fractions of 0.5 ml were collected in 5.5-ml scintillation vials; scintillation cocktail was added and the radioactivity present was determined by liquid scintillation counting. Recovery of sample radioactivity through the chromatograph was established by comparison of the radioactivity found in the derivative peak with that detected in an aliquot of reaction solution equal in volume to that chromatographed.

General procedure for derivatization of isolated carboxylic acids

Sample residues amounting to 10^{-9}–10^{-6} mol of isolated carboxylic acids (including chemically analogous internal standards) in disposable 100 × 13 mm glass test-tubes are neutralized with at least 5 equiv. of N,N-diisopropylethylamine in 100 μl of acetonitrile. To this are added 100 μl of a freshly prepared solution of 4′-bromophenacyl triflate in acetonitrile containing a minimum of 10 equiv. of the reagent. The tubes are capped, vortexed for 10 sec and allowed to stand for at least 2 min. If necessary, excess of derivatization reagent may be removed from the reaction solution by subsequent addition of an excess of carboxylic acid (N,N-diisopropylethylammonium salt in acetonitrile) either more or less polar than the most or least polar acid among the analytical substrates. Sample aliquots may be injected directly into the liquid chromatograph.

RESULTS AND DISCUSSION

Our interest in the biosynthesis and metabolism of some sterically hindered ω-trialkylammonio acids necessitated the development of a method for their determination by HPLC. The compounds reacted only slowly with 2,4′-dibromoacetophenone under conditions similar to those recommended for analytical-scale derivatizations[11,13]. Longer reaction times at elevated temperatures resulted in decomposition of the ammonio acids and the derivative products. O-Alkylation of carboxylates with 2,4′-dibromoacetophenone is generally a thermodynamically favored pro-

cess, as evidenced by reports of nearly quantitative yields of 4′-bromophenacyl esters derived from equimolar amounts of acid and alkylating agent at concentrations of 10^{-1} *M* or greater[25–27]. We reasoned that a reaction involving a better nucleofuge should proceed more rapidly and provide better derivative yields at mild reaction temperatures, thus circumventing the destructive side reaction processes that occur under forcing conditions.

Correspondence of measured S_N2 solvolysis rates of primary alkyl substrates with leaving group electronegativity has been established[31]. The solvolytic substrates employed in these experiments have included a number of readily accessible sulfonate esters varying greatly in the electronegativity of their substituents at sulfur. These display a range of leaving group aptitudes, the perfluoroalkanesulfonates being the most reactive[32]. In these reaction systems, estimates of the ratio of pseudo-first-order solvolytic rate constants of alkyl trifluoromethanesulfonates and the corresponding bromides range from 10^4 to 10^8, depending on the solvent ionizing strength and other structural features of the substrate[33,34]. This suggested that some sulfonate ester formally derived from 4′-bromophenacyl alcohol might show greater alkylative reactivity than the corresponding bromide toward ω-trialkylammonio acids.

4′-Bromophenacyl triflate was prepared in two synthetic operations from commercially available precursors. The structure of this new compound was confirmed spectroscopically. The yield of the second synthetic step is consistently about 66% when fresh reagents are used. The reagent has been stored at room temperature under vacuum dessiccation for periods of 3–6 months, during which time the crystals may develop a discernable yellow color and minor chromatographic impurities visible at 254 nm. These may be removed by treatment with decolorizing carbon in methylene chloride and recrystallization from pentane–methylene chloride as described. The reagent rapidly alkylates dry N,N-dimethylformamide and dimethyl sulfoxide, giving products chromatographically distinct from the expected 4′-bromo-2-hydroxyacetophenone hydrolysis product. Its reaction with acetonitrile is far slower than with the two aforenamed solvents; solutions of the reagent in acetonitrile are stable for periods of at least 16 h, but preparation just before use is recommended.

Our preliminary experiments indicated that the crown ether catalyzed alkylation of potassium carboxylates by 2,4′-dibromoacetophenone in acetonitrile at room temperature was measurably faster than the reaction of trialkylammonium carboxylates with the bromoketone under the same temperature and solvent conditions. However, we were unable to observe any rate difference between the two reaction systems when 4′-bromophenacyl triflate was employed as the alkylating agent. We therefore chose to neutralize samples conveniently with solutions of tertiary alkylamines[9,13] rather than with solid alkali metal hydroxides or carbonates[8,11].

We decided on external standardization of the peak-height measurements to facilitate recognition of the zero-slope region of reaction progress curves obtained in reaction optimization experiments. Peak-height ratios obtained from nine replicate injections of a solution of $1 \cdot 10^{-4}$ *M* 4′-bromophenacyl hexadecanoate and $1.3 \cdot 10^{-5}$ *M* 4′-bromophenacyl octadecanoate varied with a relative standard deviation of 0.7%. Apparent differences in peak-height ratio at successive time points smaller than this value were then held to be insignificant. In separate experiments these esters were shown to be entirely stable for 24 h under the conditions of reaction by injection of a reaction mixture containing initially all system components except underivatized

carboxylic acid. Hence the reaction progress could be assessed by noting the increase with time in the chromatographic peak-height ratios of the acid derivative product to authentic 4'-bromophenacyl ester external standard. Partial reaction could be estimated by comparison of peak-height ratios obtained in trials with low reagent to substrate concentration ratios with the larger ratios obtained under higher reagent to substrate conditions. The absolute extent of the derivatization reaction under the reaction conditions that produced the largest chromatographic acid derivative to external standard peak-height ratios was confirmed by separate experiments with analogous ^{14}C-labeled carboxylates. Recovery of injected radioactivity through the chromatograph was quantitative.

Fig. 1 shows the dependence of derivative yield on the equivalent ratio of alkylating agent to carboxylic acid. In the presence of a 10-fold or greater excess of alkylating agent and a 5-fold equivalent excess of N,N-diisopropylethylamine, the reaction proceeds to completion at rates nearly unaffected by further increases in the reagent to substrate ratio. In Fig. 2, the effect on the derivative yield of systematic manipulation of the equivalent ratio of N,N-diisopropylethylamine to carboxylic acid in the presence of a 10-fold equivalent excess of 4'-bromophenacyl triflate is shown. Increases in the concentration of tertiary alkylamine in the reaction solution did not noticeably reduce the time required for completion of the derivatization reaction. Notably, increases in the concentrations of both reagents to equivalent excesses of 100-fold do not affect the outcome of the process adversely. Thus, the reaction may be applied to experimental specimens varying considerably in their total carboxylate concentrations without adjustment of the amounts of reagents employed. Comparative reaction progress curves for the reaction of a solution of $1.7 \cdot 10^{-4}$ *M* aconitic acid, $1.7 \cdot 10^{-4}$ *M* citric acid tris(4'-bromophenacyl) ester, $2.7 \cdot 10^{-3}$ *M* N,N-diisopropylethylamine and $6.0 \cdot 10^{-3}$ *M* alkylating agent at room temperature are shown

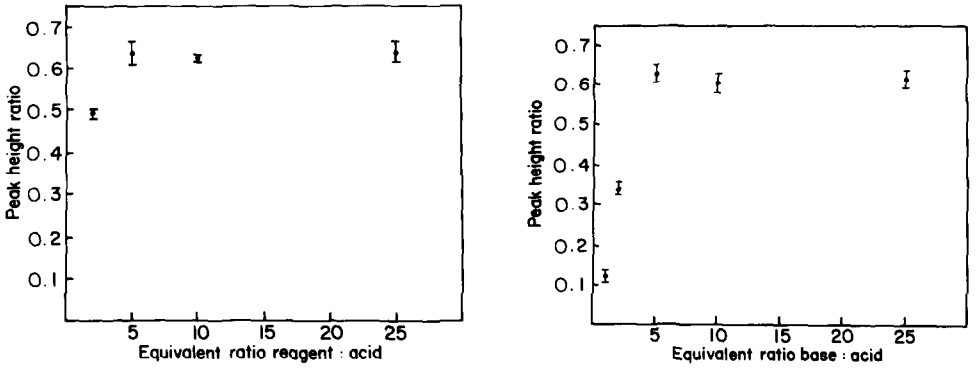


Fig. 1. Variation of derivative yield with the concentration ratio of alkylating agent to carboxylic acid. Bis-4'-bromophenacyl succinate was used as a procedural external standard for the derivatization of glutaric acid ($5 \cdot 10^{-5}$ *M*) at room temperature in the presence of a 5-fold equivalent excess of N,N-diisopropylethylamine. Plotted data points represent the means ± standard deviations (s.d.) for groups of five samples.

Fig. 2. Variation of derivative yield with the concentration ratio of N,N-diisopropylethylamine to carboxylic acid. Bis-4'-bromophenacyl succinate was used as a procedural external standard for the derivatization of glutaric acid ($5 \cdot 10^{-5}$ *M*) at room temperature with 10 equiv. of 4'-bromophenacyl triflate.

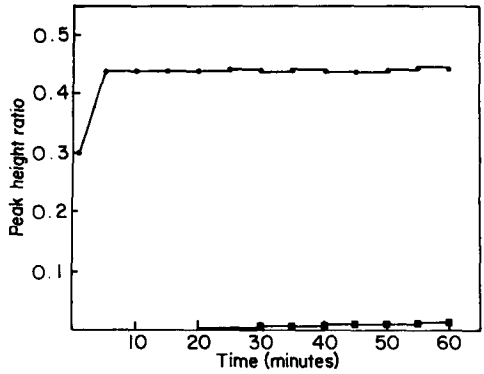


Fig. 3. Comparison of reaction progress for the alkylation of aconitic acid by 4′-bromophenacyl triflate (●) and 2,4′-dibromoacetophenone (■) at room temperature under the recommended reaction conditions. Citric acid tris-4′-bromophenacyl ester was used as an external standard. Data points represent arithmetic means (n = 6). Reaction completion under these conditions was confirmed by experiments with [1,5-^{14}C]citric acid. The reaction of aconitic acid with 2,4′-dibromoacetophenone under these conditions was 50% complete after 5 days.

in Fig. 3. In comparison, the reaction with 2,4′-dibromoacetophenone was only 50% complete after 5 days.

It has been reported that the rates of alkylation of prostaglandin trialkylammonium salts by 4′-nitrophenacyl bromide depend on the identity of the tertiary amine used for sample neutralization[10]. In preliminary experiments with 2,4′-dibromoacetophenone, we also found that the initial carboxylate alkylation rates increased with greater steric crowding at nitrogen in the trialkylammonium counter ion. Although no difference in the rate of carboxylate alkylation by 4′-bromophenacyl triflate with variation in counter ion identity could be detected, chromatographic interference could result from a competing reaction between excess alkylating agent and base present in the reaction medium. The time course of this process was examined by combining equimolar amounts of 4′-bromophenacyl triflate and individual tertiary amines in acetonitrile and periodically chromatographing the reaction mixture until the reagent peak could no longer be detected. Two or more hours were required for completion of this side reaction process. The sterically hindered N,N-diisopropylethylamine was alkylated least rapidly of all the amines tested, and therefore was used for sample neutralization in subsequent experiments. Carboxyl-O-alkylation by 4′-bromophenacyl triflate is a far more rapid reaction than the competing N-alkylation of the tertiary amine base. Quantitative yields of carboxylic acid 4′-bromophenacyl esters were obtained in a few minutes regardless of the base identity, and even when the ratio of amine base to alkylating agent in the reaction solution exceeded 10.

We investigated the dependence of the outcome of the reaction procedure on the presence of water. It was surprising in the light of the great electrophilic reactivity of 4′-bromophenacyl triflate that the derivatization reaction succeeded reliably until the volume fraction of water in the reaction solution exceeded 5%. This suggested that better grades of commercial acetonitrile from freshly opened bottles might be used in this procedure without additional purification. We encountered no problem in the derivatization when using unpurified acetonitrile as the reaction solvent. How-

ever, the low-molecular-weight organic acid contaminants in commercial acetonitrile[35] can interfere in the determination of similar acids if they are not first removed from the reaction solvent.

The derivatization procedure was applied to a number of acids representing several biologically important classes of carboxylates (Table I). The reaction of monocarboxylic acids under these conditions proceeds to completion in less than 2 min. Reactions of di- and tricarboxylic and sterically hindered acids were slower, as evidenced by the lesser extents of completion after 30 sec (Fig. 3). Still, no difference in peak-height ratios was observed after the third experimental time point in trials with these acid classes. Thus, a 5-min reaction time is recommended for the quantitative derivatization of polyfunctional carboxylic acids at these concentrations.

The electrophilic reactivity of 4′-bromophenacyl triflate caused some concern that competing hydroxyl-O-alkylation might occur in applications of the reagent to

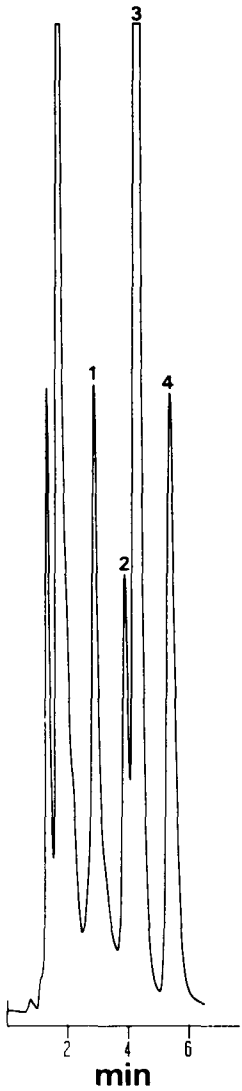
TABLE I

CARBOXYLIC ACIDS ALKYLATED BY 4′-BROMOPHENACYL TRIFLATE

The compounds listed (10^{-6}–10^{-3} *M* in acetonitrile) were derivatized according to the general procedure given in the text.

Compound	*Note**
n-Alkanoic acids (C_1–C_{10})	1
Hexadecanoic acid	1,2
Octadecanoic acid	1
Bromoacetic acid	1
Trichloroacetic acid	1
ω-Dicarboxylic acids (C_2–C_{10})	3, 4
Citric acid	5, 6, 8
Aconitic acid	5
Cyclopropanecarboxylic acid	7
2,2-Dimethylpropanoic acid	7
2,2-Dimethylpropanedioic acid	7
2,2-Dimethylbutanedioic acid	7
2-Propylpentanoic (valproic) acid	7
2,4,6-Trimethylbenzoic acid	7
Hydroxyacetic acid	1, 8
2-Hydroxypropanoic (lactic) acid	1, 8
3-Hydroxybutanoic acid	1, 8
2-Hydroxysuccinic (malic) acid	3, 8
2-Hydroxybenzoic (salicylic) acid	1, 8
Cholic acid	1, 8
Deoxycholic acid	1, 8

* 1 = Reaction of monocarboxylic acids at dilute concentrations was complete after 2 min. 2 = Completion of reaction was verified by experiment with [1-^{14}C]hexadecanoic acid. 3 = Reaction of ω-dicarboxylic acids at dilute concentrations was complete after 5 min. 4 = Completion of reaction was verified by experiment with [1,4-^{14}C]succinic acid. 5 = Reaction of tricarboxylic acids tested was complete after 5 min. 6 = Completion of reaction was verified by experiment with [1,5-^{14}C]citric acid. 7 = Reaction of sterically hindered acids tested was complete after 5 min. 8 = No evidence of a competing hydroxyl-O-alkylation was observed.



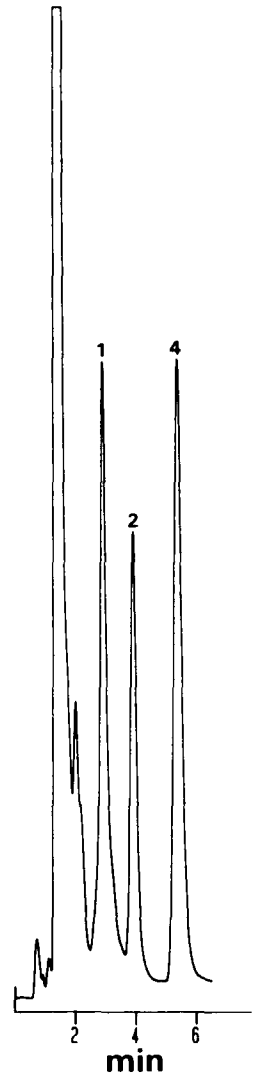


Fig. 4. Chromatogram obtained after derivatization reaction of C_3–C_5 *n*-alkanoic acids. The column used was a 10 × 0.5 cm I.D. radially compressed cartridge of Radial-PAK C_{18} (10 μm nominal particle diameter). The eluent was 60% (v/v) acetonitrile–water and was pumped at 1.5 ml/min. The absorbance detector was operated at 254 nm. Full scale of the ordinate is 0.1 absorbance unit. Peaks: 1 = 4′-bromophenacyl propanoate; 2 = 4′-bromophenacyl butanoate; 3 = 4′-bromophenacyl triflate; 4 = 4′-bromophenacyl pentanoate.

Fig. 5. Chromatogram obtained on treatment of the same reaction solution as in Fig. 4 with a solution of hydroxyacetic acid N,N-diisopropylethylammonium salt in acetonitrile for 2 min at room temperature. Chromatographic conditions as in Fig. 4. Peaks: 1 = 4′-bromophenacyl propanoate; 2 = 4′-bromophenacyl butanoate; 4 = 4′-bromophenacyl pentanoate.

hydroxy acids. No hydroxyl-O-alkylation products were observed in trials with the hydroxy acids listed in Table I. No slow production of palmitic acid 4′-bromophenacyl ester was observed when the derivatization procedure was applied to cholesteryl palmitate. Thus, the suggested reaction conditions may be useful for the selective alkylation of carboxylates in the presence of cholesteryl and glyceryl esters. 4′-Bromophenacyl triflate immediately and quantitatively S-alkylated benzenethiol.

4′-Bromophenacyl triflate is well suited for use in procedures involving isolation of carboxylic acids by solvent extraction or by chromatographic sample preparation with apolar polymeric non-ionic adsorbents. The reagent reacts with any nucleophilic anions present in solution at rates not appreciably different from the rate of reaction with carboxylates. Therefore, sample preparation schemes should be designed for minimization of the amounts of non-carboxylate nucleophiles isolated in sample residues. Variations of a reported procedure for the isolation of volatile short-chain *n*-alkanoates[36,37] might prove useful. In other work on isolated sample residues containing comparatively large amounts of non-nucleophilic inorganic salts, we found that reaction rate and completion are limited by the rate of dissolution of the solid materials (which occlude the carboxylate of interest) in the reaction medium. Continuous vortexing of the test-tubes for 5–10 min may be required for complete derivatization with such samples.

Some workers have described troublesome chromatographic interference by residual 2,4′-dibromoacetophenone[14], its impurities and its side-reaction products[14,36,38] in determinations of carboxylic acids with that derivatization reagent. Fig. 4 is a chromatogram obtained 2 min after reaction of $1 \cdot 10^{-7}$ mol each of propanoic, butanoic and pentanoic acids with $1.5 \cdot 10^{-6}$ mol of N,N-diisopropylethylamine and $3 \cdot 10^{-6}$ mol of 4′-bromophenacyl triflate in 900 μl of acetonitrile according to the recommended procedure. The butanoic acid ester peak is obscured by the enormous chromatographic peak of residual alkylating agent. Fig. 5 is a chromatogram of the same reaction solution 2 min after addition of $6 \cdot 10^{-6}$ mol of hydroxyacetic acid diisopropylethylammonium salt in 300 μl of acetonitrile. The alkylating agent peak has disappeared from the chromatogram, revealing the butanoic acid ester derivative peak; a new chromatographic peak of a functionally more polar sample constituent has appeared in the region of the solvent front. Alternatively, an acid with a hydrocarbon chain length greater than 5 could have been used to remove the interfering reagent peak from this exemplary chromatogram. This experimental tactic may also be employed to quench relatively slow alkylative side reaction processes which otherwise might cause chromatographic interference in high-sensitivity applications.

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