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Note

Derivatisation of amines with O-acetylsalicyloyl chloride to enhance electrochemical detection in high-performance liquid chromatography*

ROGER M. SMITH*, ASRI ABDUL GHANI, DECLAN G. HAVERTY, GILLIAN S. BAMENT, A. Y. CHAMSI and ARNOLD G. FOGG

Department of Chemistry, Loughborough University of Technology, Loughborough, Leics. LE11 3TU (U.K.)

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Many compounds, such as simple aliphatic amines or alcohols, lack a chromophore and cannot be readily directly analysed by high-performance liquid chromatography (HPLC) using spectrophotometric detection. This problem can often be overcome by derivatisation to introduce a chromophore or fluorophore. However, derivatisation to introduce an electrochemically active group into the analyte has rarely been utilised even though electrochemical detection can be both highly sensitive and selective. Suitable electrochemically active groups include nitrophenyls, which can be detected by reductive electrochemical methods, or phenolic and arylamino groups, which can be detected oxidatively. Oxidative detection is usually preferred because it avoids the need to exhaustively remove oxygen from the sample solution and mobile phase.

Compared with the wide versatility of derivatisation reactions which can be used to introduce a chromophore, only a relatively small number of reactions have been reported for the formation of electrochemically active derivatives. The early methods, which were primarily for the formation of reducible derivatives were surveyed by Kissinger et al.[1]. They also tested many of the nitroaromatic derivatives, such as the 3,5-dinitrobenzoates of aliphatic alcohols, which are also frequently formed in order to enhance spectrophotometric detection[2].

Most interest in recent years has been found in compounds which can be detected electrochemically in the oxidative mode. For example aliphatic isocyanates have been derivatised with 1-(2-methoxyphenyl)-piperazine to provide a sensitive method for environmental monitoring[3], and hydrazines can be detected as the corresponding salicylaldehyde derivatives[4]. These and other methods have been summarised in a recent review by Leroy and Nicolas[5]. However, the introduction of a phenolic group into many compounds can be difficult. This is because the most common route for the derivatisation of hydroxyl or amino groups is the use of reagents containing reactive carbonyl or sulphonyl chlorides, which would also react with any phenolic groups.

The present study describes the application of a protected reagent, O-acetylsal-

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icyloyl chloride, as a pre-column derivatisation reagent for aliphatic amines. Initially this compound will react with an amino group to form an O-acetylsalicylamide, which will be hydrolysed spontaneously under the alkaline conditions of the synthesis to give a 2-hydroxybenzamide. This free phenolic group can then be detected electrochemically following separation by HPLC. While this study was in progress, a related reaction was described by Chou *et al.*[6]. They derivatised amines in a solid phase reaction using a polymeric anhydride substituted with O-acetylsalicyl groups. This reagent gave the corresponding O-acetylsalicylamides, which were usually detected spectrophotometrically. However, it was also found that the derivatives responded in an electrochemical detector despite the absence of a free phenolic group.

EXPERIMENTAL

Reagents

O-Acetylsalicyloyl chloride was purchased from Fluka (Buchs, Switzerland) and was used as received. Sample amines, N-methylaniline, benzylamine and piperidine, were laboratory reagent-grade from a range of suppliers. Buffer reagents were AR-grade and methanol was HPLC-grade from FSA Laboratory Supplies (Loughborough, U.K.). Water was distilled.

Equipment

Separations were carried out using a rapid stroke reciprocating pump ACS Model LC 500 (Applied Chromatography Systems; Macclesfield, U.K.), a Cecil 2012 spectrophotometric detector (Cecil Instruments; Cambridge, U.K.) set at 254 nm and Kipp Analytica 9205 electrochemical detector (Emmen, The Netherlands) with a glassy carbon thin film detector cell at a potential of + 0.90 V with respect to an Ag/AgCl reference electrode. The samples (10 μl) were injected using a Model 7125 Rheodyne valve onto an ODS-Hypersil 5-μm column (100 × 5 mm I.D.). The eluent was methanol–0.025 *M* phosphate buffer, pH 8.0 (50:50) containing 0.001 *M* sodium chloride at a flow-rate of 1 ml min^{-1}.

Preparation of standard derivatives

Samples of the derivatives were prepared by condensation of the free amine with O-acetylsalicyloyl chloride in 0.1 *M* aqueous sodium hydroxide (Schotten–Baumann conditions). The product of the reaction was extracted and recrystallised. The structures of the products were confirmed by NMR and IR spectroscopy.

Derivatisation of dilute aqueous solutions of amines

O-Acetylsalicyloyl chloride (20 mg) was mixed with a dilute solution of the amine in 0.1 *M* sodium hydroxide solution (1.0 ml). After shaking and standing for 1 h, samples (10μl) were analysed directly by HPLC.

RESULTS AND DISCUSSION

Amines and amino acids are usually derivatised for HPLC analysis by using either a pre- or post-column reaction. For small numbers of samples the former method is easier and a wide range of reagents to form derivatives with enhanced

spectrophotometric or fluorescence properties have been described. A common derivatisation route is the conversion of the amine into an amide with an acyl or sulphonyl chloride under alkaline conditions. As the products no longer possess basic amino groups they usually suffer less interactions with the stationary phase and the derivatives have better chromatographic properties.

However, the use of acylation to introduce an electrochemically active group, such as an aromatic amine or phenolic group, directly into an aliphatic amine would be difficult. The problem is that the reagent may also react with the electrochemically active group. The present study sets out to demonstrate that if the phenolic group is protected as an ester, the acylation reaction of the amine can proceed. The protecting group could then be easily removed using alkali to reveal the phenolic group. These conditions should have little effect on the amido group.

The present study therefore examined the reaction of the commercially available reagent O-acetylsalicyloyl chloride with amines under alkaline Schotten–Baumann conditions. In trial experiments, even under mild conditions, the reaction was found to give the corresponding 2-hydroxybenzamides directly. The intermediate O-acetylsalicylamide appeared to be spontaneously hydrolysed during the synthesis (Fig. 1).

Pure samples of the derivatives of a series of simple amines, piperidine, benzylamine and N-methylaniline, were therefore prepared. These derivatives were examined by HPLC on an ODS-Hypersil column with a methanol–0.025 *M* aqueous phosphate buffer, pH 8.0 (50:50) eluent and were found to be well retained (capacity factors of piperidine, N-methylaniline and benzylamine were 2.7, 3.5 and 11.5, respectively) and to give good peak shapes. The peaks could be readily detected using spectrophotometric detection at 254 nm. This eluent was selected because at lower pH the phenolic groups gave only a poor electrochemical response.

In order to study the electrochemical properties of these phenolic derivatives and determine the optimum potential for detection, a series of replicate injections of a $1 \cdot 10^{-3}$ *M* solution of the derivative of piperidine was carried out using an electrochemical detector with a thin-film glassy carbon electrode. The peak heights were measured at different applied potentials (Fig. 2). There was no evidence of electrode deterioration or contamination and good reproducibility was obtained, although in previous studies there had been problems with electrode contamination using other phenolic compounds[7]. The results suggested that a working potential of + 0.90 V would be suitable and this was used in all subsequent work. Using these conditions, linear calibration curves were obtained for dilute solutions of each of the derivatives (*e.g.* 2-hydroxybenzamide of piperidine gave a correlation coefficient of 0.9950 from $1 \cdot 10^{-7}$ to $1 \cdot 10^{-6}$ *M*). The derivatives could be detected down to concentrations of

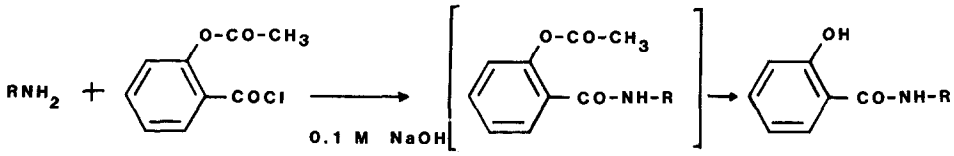

Fig. 1. Reaction of O-acetylsalicyloyl chloride with an amine in alkaline solution.

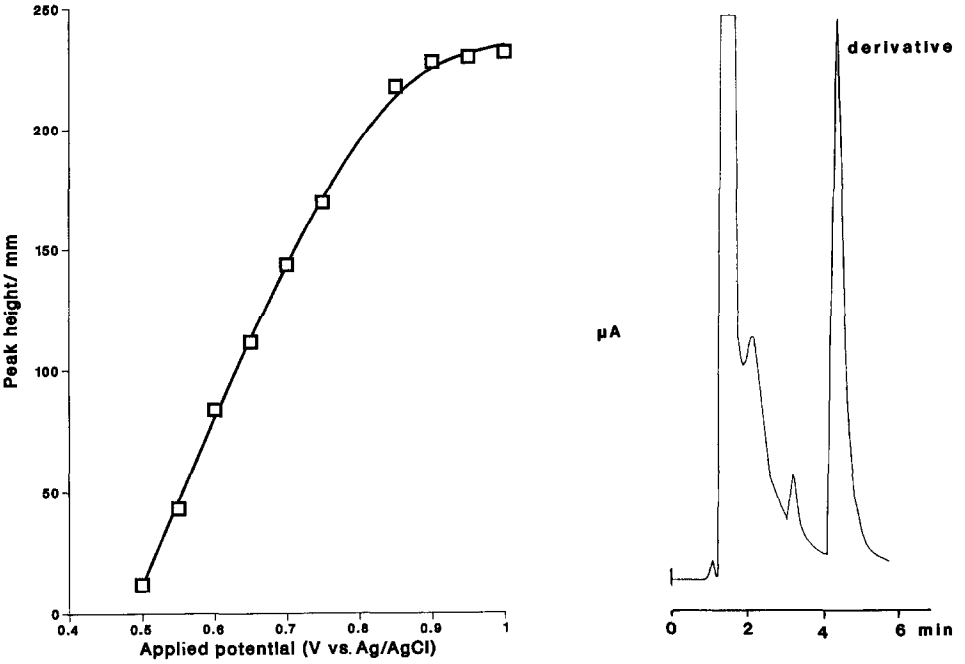

Fig. 2. Effect of measurement potential of the electrochemical detector on peak height on injecting replicate samples of 10 μl of a $1 \cdot 10^{-3}$ *M* solution of the 2-hydroxybenzamide of piperidine.

Fig. 3. Separation of the reaction mixture from the addition of 20 mg of O-acetylsalicyloyl chloride to an aqueous alkaline solution of $1 \cdot 10^{-4}$ *M* piperidine (1.0 ml). Column ODS-Hypersil 100 mm × 5 mm I.D.. Mobile phase methanol–phosphate buffer, pH 8.0 (50:50). Electrochemical detection at +0.90 V, sensitivity range 1 μA.

$1 \cdot 10^{-7}$ *M* in the analyte solution using a 10-μl injection. At these concentrations no reponse was observed using an ultraviolet spectroscopic detector at 254 nm.

The interest in this reaction lay in its ability to be applied to the determination of low concentrations of aliphatic amines in aqueous solution. A series of experiments was therefore carried out to determine the amount of reagent required in order to ensure a high yield of the derivative. A series of dilute solutions of piperidine ($1 \cdot 10^{-4}$ *M*) in 0.1 *M* sodium hydroxide were prepared and different amounts of O-acetylsalicyloyl chloride were added and left for 1 h at room temperature. The derivative was not extracted but an aliquot of the reaction solution was injected directly onto the HPLC column. In the separation the excess of the reagent and its hydrolysis products were well separated from the derivatives of the amines (Fig. 3). It may be possible to use shorter reaction times but this was not investigated. In order to determine the efficiency of the reaction the peak heights of the derivatives were compared with standard solutions of the pure derivative.

The yield of the derivative increased with the amount of reagent added up to about 19 mg/ml of amine solution. Thus to ensure complete reaction a considerable excess of the reagent was required. Apparently at lower levels much of the reagent was being hydrolysed before reaction with the amine was complete. In subsequent studies on more dilute amine solutions a ratio of 20 mg of reagent to 1 ml of dilute amine solution was used as the standard method.

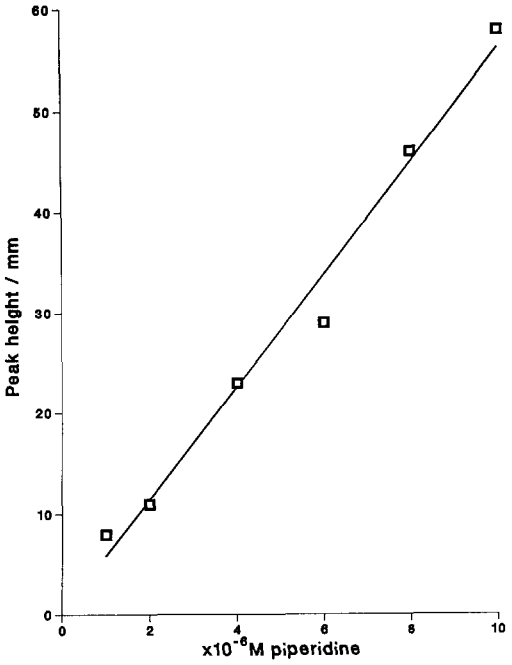

Fig. 4. Calibration graph for peak for 2-hydroxybenzamide of piperidine formed from piperidine in dilute aqueous alkaline solution following addition of O-acetylsalicyloyl chloride.

A series of dilute solutions containing different concentrations of the amines were then analysed. The aliphatic amine, piperidine, gave virtually quantitative yields at the 10^{-4} to 10^{-6} *M* levels, when compared with the separation of a pure derivative. The derivatisation of four samples of a $1 \cdot 10^{-5}$ *M* solution of piperidine showed good reproducibility (relative standard deviation 1.4%). Derivatisation of both piperidine and benzylamine gave a linear response for the amine (*e.g* piperidine from $1 \cdot 10^{-6}$ to $10 \cdot 10^{-6}$ *M* correlation coefficient 0.9915, Fig. 4). The limit of detection (signal-to-noise ratio of 2) of piperidine was about $6 \cdot 10^{-7}$ *M* in the aqueous solution. A lower limit of detection could probably have been reached if the product had been extracted and concentrated before chromatography but this would have been more time consuming.

However, the yield of the derivative of aromatic amine N-methylaniline was much lower, 42% at $1 \cdot 10^{-4}$ *M* and 55% at $1 \cdot 10^{-5}$ *M*. This level of reaction was unaffected by further increasing the amount of reagent added to the solution. It appeared that the reaction was slower because of the lower basicity of the aromatic amino group and as a consequence hydrolysis of the reagent was competing with derivatisation. The separation in this case was also more complicated as unreacted N-methylaniline also gave an electrochemically active peak, which confused the chromatogram. Despite the poor yield the reaction with N-methylaniline still gave a linear response at 10^{-4} *M* levels in the aqueous sample solution. However, direct electrochemical detection would probably be a more suitable method for the detection of the arylamines.

CONCLUSION

Aliphatic amines on reaction with O-acetylsalicyloyl chloride are readily converted into the corresponding 2-hydroxybenzamides, which can be detected at low concentrations in aqueous solution using HPLC with electrochemical detection.

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