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Synthesis of Aromatic *S*-Substituted Derivatives of *N*-Acetyl-L-cysteine

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Abstract

A new method for the preparation of aromatic, *S*-substituted derivatives of *N*-acetyl-L-cysteine is described. The method involves nucleophilic substitution of aromatic iodides by *N*-acetyl-L-cysteine in the presence of copper(I) iodide, and provides the first example of nucleophilic substitution of unactivated aromatic halides by *N*-acetyl-L-cysteine. The method has been used to prepare *N*-acetyl-*S*-(2-thienyl)-L-cysteine and *N*-acetyl-*S*-(3-thienyl)-L-cysteine from 2-iodothiophen and 3-iodothiophen respectively. In addition, the method has been successfully applied to iodobenzene and 1-iodonaphthalene. A product yield of about 30% was obtained in each case.

Introduction

In the course of a study of the metabolism of 2-bromo- and 3-bromo-thiophen, authentic samples of the two *S*-thienyl derivatives of *N*-acetyl-L-cysteine were required. These compounds were to be used as analytical standards, and as starting materials for the preparation of *S*-(bromothienyl) derivatives of *N*-acetyl-L-cysteine.

Three different methods have been used for the preparation of aromatic, *S*-substituted derivatives of cysteine. These are: the substitution of 3-halo-2-aminopropanoic acids by aromatic thiols,[1] the addition of aromatic thiols to acetamidoacrylic acid,[2,3] and the reaction of diazotized aromatic amines with the copper(I) salt of L-cysteine.[4] In a study of the metabolism of thiophen, Bray, Carpanini and Waters[1] used the first of these methods to prepare *N*-acetyl-*S*-(2-thienyl)-DL-cysteine, and the second method to prepare *N*-acetyl-*S*-(3-thienyl)-DL-cysteine. In each case the yield obtained was very small (< 1%) and the product was not fully characterized. For this reason, and from the account given of the preparations, it did not seem likely that these methods would provide access to useful quantities of the compounds required. Furthermore, the remaining method of preparation was not promising because the aminothiophens required as starting materials are not readily available, and, in any case, it is reported that they do not form diazonium salts.[5]

[1] Bray, H. G., Carpanini, F. M. B., and Waters, B. D., *Xenobiotica*, 1971, **1**, 157.
[2] Behringer, H., and Fackler, E., *Justus Liebigs Ann. Chem.*, 1941, **138**, 369.
[3] Ondrus, T. A., Christie, B. J., and Guy, R. W., *Aust. J. Chem.*, 1979, **32**, 2313.
[4] West, H. D., and Mathura, G. R., *J. Biol. Chem.*, 1954, **208**, 315.
[5] Hartough, H. D., (Ed.), 'Thiophene and Derivatives' in 'The Chemistry of Heterocyclic Compounds' p. 234 (Interscience: New York 1952).

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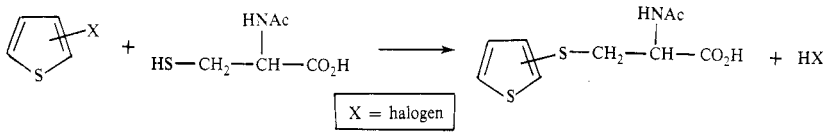
The compound *N*-acetyl-L-cysteine and a number of halothiophens are commercially available, and so a search of the literature was made for reaction conditions under which the following transformation might be achieved.



This approach is unconventional because it is usually held that unactivated aromatic halides do not undergo nucleophilic substitution reactions.[6] Nonetheless the recent literature does contain considerable precedent for such a reaction. For example, Shaw, Kunerth and Swanson[7] have found that sodium methoxide reacts with unactivated aryl halides, in hexamethylphosphoramide as solvent, to form methyl aryl ethers in good yield. Cogolli *et al.*[8] have found that, in the same solvent, unactivated aryl halides undergo nucleophilic substitution reactions with thiolate ions to form aryl thioethers. Bacon and Karim[9] have found that a wide range of aromatic halides, including some which are unactivated, undergo nucleophilic substitution by phthalimide ion when they are treated with potassium phthalimide and copper(I) iodide in dimethylformamide or diethylacetamide at the reflux temperature. This last reaction is particularly significant for two reasons: it is successful when applied to 2-bromothiophen, and it proceeds through the initial formation of a complex of copper(I) and the phthalimide ion. Analogous complexes of cysteine are well known,[10a] and so it seemed quite possible that the compounds which were sought could be obtained by using these reaction conditions but with *N*-acetyl-L-cysteine to serve as the nucleophilic agent instead of potassium phthalimide. This has turned out to be the case, and both *S*-thienyl derivatives of *N*-acetyl-L-cysteine have been prepared in about 30% yield.

Results and Discussion

Four aromatic, *S*-substituted derivatives of *N*-acetyl-L-cysteine have been prepared by a method modelled on the synthetic procedure developed by Bacon and Karim which is cited above. The particular reaction conditions used by Bacon and Karim were modified following the performance of a series of analytical scale experiments in which methyl *N*-acetyl-L-cysteinate was used as the nucleophile and 2-bromothiophen or 2-iodothiophen as the substrate.

In experiments where the reaction was carried out at reflux temperature (153°), a pronounced darkening of the reaction mixture occurred. When temperatures in the range 70–120° were used, there was less darkening of the reaction mixture and substrate was still consumed. Eventually a reaction temperature of 100° was chosen as

[6] Roberts, J. D., and Caserio, M. C., 'Basic Principles of Organic Chemistry' p. 844 (W. A. Benjamin: New York 1965).
[7] Shaw, J. E., Kunerth, D. C., and Swanson, S. B., *J. Org. Chem.*, 1976, **41**, 732.
[8] Cogolli, P., Maiolo, L. T., Testaferri, L., Tingoli, M., and Marcello, T., *J. Org. Chem.*, 1979, **44**(15), 2642.
[9] Bacon, R. G. R., and Karim, A., *J. Chem. Soc., Perkin Trans. 1*, 1973, **1**, 272.
[10] Greenstein, J. P., and Winitz, M., 'Chemistry of the Amino Acids' (*a*) p. 645; (*b*) p. 639 (John Wiley: New York 1961).

being convenient and effective. Use of methyl *N*-acetyl-L-cysteinate as the nucleophile rather than *N*-acetyl-L-cysteine itself allowed the formation of product to be monitored by gas–liquid chromatography. The consumption of substrate was also monitored by this technique.

In the experiments carried out at 100°, it was observed that 30–50% of the aromatic halide initially present in the reaction mixture was consumed within 20 h. Furthermore, a product having a retention time similar to that of methyl *N*-acetyl-*S*-[*p*-(trifluoromethyl)phenyl]-DL-cysteinate, previously prepared in our laboratory,[3] appeared in the reaction mixture. This product, which was assumed to be methyl *N*-acetyl-*S*-(2-thienyl)-L-cysteinate, was detectable within 3 h of commencement of the reaction, and its concentration continued to increase for a further 65 h.

On the basis of these results, an attempt was made to carry out a preparative scale reaction with equimolar quantities of 2-bromothiophen, *N*-acetyl-L-cysteine and copper(I) iodide. The reagents were mixed all at once and heated at 100° for 3 days. No consumption of 2-bromothiophen was observed indicating that *N*-acetyl-L-cysteine, in contrast to its methyl ester, is not a suitable nucleophile for displacement of the bromine atom of 2-bromothiophen.

In their work, Bacon and Karim had found that aromatic iodides underwent reaction with phthalimide ion as readily as aromatic bromides did, and so a sample of 2-iodothiophen was subjected to the reaction conditions. This compound underwent reaction with *N*-acetyl-L-cysteine to form the corresponding *S*-thienyl derivative. The experiment was repeated with dropwise addition of a solution of *N*-acetyl-L-cysteine and copper(I) iodide in dimethylformamide to a solution of 2-iodothiophen in dimethylformamide maintained at 100°. This change in procedure gave an improved yield of product, and it was used in all subsequent preparations.

Table 1. Nucleophilic substitution of aromatic iodides

Aromatic iodide	Iodide consumed (%)	Product yield (%)[A]
2-Iodothiophen	38	30 [78]
3-Iodothiophen	50	29 [58]
Iodobenzene	29	28 [96]
1-Iodonaphthalene	46	31 [78]

[A] Yield in brackets is expressed as a percentage of the iodide consumed.

The reaction conditions have also been applied successfully to 3-iodothiophen, iodobenzene and 1-iodonaphthalene. For each of the four iodides studied, Table 1 lists the consumption of iodide which occurred, the yield of product isolated and, in brackets, this yield expressed as a percentage of the iodide consumed. The bromides corresponding to the compounds listed in Table 1 were all unreactive toward *N*-acetyl-L-cysteine, but each of them did react with the methyl ester.

The melting point values obtained for *N*-acetyl-*S*-phenyl-L-cysteine and *N*-acetyl-*S*-(1-naphthyl)-L-cysteine are very similar to those reported in the literature. The two thienyl derivatives have only been prepared previously as components of racemic mixtures, and so no comparison can be made between their melting points and the literature values. However, a correct microanalysis has been obtained for each of these two compounds, and their spectral properties are consistent with the structures assigned to them.

The data given in brackets in Table 1 show that the aromatic iodide consumed in these reactions is converted quite efficiently into product. This suggests that the low absolute yields of product obtained are due to destruction of *N*-acetyl-L-cysteine under the reaction conditions. In attempts to increase the amount of aromatic iodide which is converted into product, some reactions were carried out by using the aromatic iodide, *N*-acetyl-L-cysteine and copper(I) iodide in the molar ratio 1 : 2 : 2 rather than in equimolar quantities. This produced only a slight increase in the proportion of aromatic iodide which was converted into product, and so the efficient conversion of unchanged aromatic iodide into product requires more than the simple provision of additional *N*-acetyl-L-cysteine and copper(I) iodide with which it might react.

It is well known that cysteine reacts with oxygen in the presence of some metal ions and that this reaction takes place even when there is only a trace amount of oxygen present in the reaction system.[10b] It is therefore possible that the rigorous exclusion of oxygen from the atmosphere of the reaction system is required to improve the yield of product. Investigations of this particular matter and of the effects which additional substituents of the aromatic nucleus exert upon the course of the reaction are proceeding in these laboratories.

Experimental

General

Melting points were determined on a Gallenkamp melting point apparatus and are corrected. Infrared spectra were recorded on a Perkin–Elmer 337 grating infrared spectrophotometer. ^{1}H nuclear magnetic resonance spectra were determined either as a solution in (D_4)methanol or a solution in deuterium oxide made basic by the addition of sodium hydroxide. The instrument used was a Perkin–Elmer R12B spectrometer. For the spectrum determined in (D_4)methanol, $SiMe_4$ was used as an internal standard. For the spectrum determined in deuterium oxide, the instrument was adjusted so that the protons of water had δ 4·8. Mass spectra were measured with an AEI MS30 mass spectrometer. Microanalyses were performed by the Australian Microanalytical Service.

All aromatic halides and *N*-acetyl-L-cysteine were commercial samples used without further purification. 3-Iodothiophen was obtained from Pfaltz & Bauer Inc., whereas *N*-acetyl-L-cysteine and all the other aromatic halides were obtained from Aldrich Chemical Co. The copper(I) iodide used was of technical grade and obtained from Ajax Chemicals. Analytical grade dimethylformamide was refluxed with calcium hydride for 4 h, distilled, and stored over molecular sieve. *N*-Acetyl-L-cysteine and copper(I) iodide were stored in a vacuum desiccator with concentrated sulfuric acid as drying agent.

Thin-layer chromatography was carried out on plastic sheets precoated with a 0·2-mm layer of Keiselgel 60 and produced by Merck. The developing solvent was ethyl acetate/chloroform/formic acid (6 : 4 : 1 v/v), and spots were detected by spraying the dry plates with a 1 : 1 mixture of potassium dichromate (0·1 mol l^{-1}) and acetic acid (1 mol l^{-1}) followed by silver nitrate (0·1 mol l^{-1}).

Column chromatography was carried out by using column loadings of 1% on silica gel G. The column was developed with the same solvent as was used for thin-layer chromatography, with a flow rate in the range 10–15 ml h^{-1}. Collection of fractions began about 2 h after development had started, and the emergence of product from the column was monitored by spotting a portion of each fraction onto glass plates coated with fluorescent silica (Kieselguhr F-254). Spots from fractions which contained appreciable concentrations of product appeared dark when viewed under ultraviolet light of wavelength 254 nm. All fractions containing product were then examined by thin-layer chromatography, and those which contained no sign of compounds other than the desired product were combined and evaporated.

Gas–liquid chromatography was carried out on a Shimadzu GC-6AM gas–liquid chromatograph fitted with a flame ionization detector and one or other of the following columns: column *1*, 5% QF1

on Gas Chrom Q (2 m by 2 mm); column *2*, 5% SE30 on Gas Chrom Q (2 m by 2 mm). The carrier gas (nitrogen) flow rate was 40 ml min^{-1}. The percentage of aromatic iodide consumed in each preparation was estimated by using naphthalene as an internal standard. Before commencement of each reaction, a 50-μl aliquot of the aromatic iodide solution was taken and diluted with 50 μl of dimethylformamide. To this mixture was added a 100-μl aliquot of a solution of naphthalene in dimethylformamide (8 mg l^{-1}). After the reaction had been carried out, a 50-μl aliquot of the reaction mixture was added to 50 μl of naphthalene solution. The two mixtures prepared in this way were examined by using column *1* and an oven temperature of 90°. Peak areas for the aromatic iodide and for naphthalene were determined by multiplying peak height by width at half height. The ratio of these areas was taken as a measure of the concentration of aromatic iodide in each mixture and used to estimate the percentage which had been consumed by the reaction.

In analytical scale experiments where methyl *N*-acetyl-L-cysteinate was used as nucleophile, the corresponding products were detected by using column *2* and an oven temperature of 180°.

Synthetic Procedure

This is described in detail for *N*-acetyl-*S*-(2-thienyl)-L-cysteine. For the other compounds prepared, the only variation required was in the choice of solvent for recrystallization of *N*-acetyl-*S*-(3-thienyl)-L-cysteine.

N-*Acetyl*-S-(*2-thienyl*)-L-*cysteine*

A solution of 2-iodothiophen (1·05 g, 0·005 mol) in dimethylformamide (12·5 ml) was magnetically stirred and maintained at 100° by immersion in a boiling-water bath. The reaction vessel containing the solution of 2-iodothiophen was fitted with a water-cooled condenser, dropping funnel, and drying tube ($MgSO_4$). A slow stream of helium was passed through the apparatus, and a solution of *N*-acetyl-L-cysteine (0·81 g, 0·005 mol) and copper(I) iodide (0·95 g, 0·005 mol) in dimethylformamide (12·5 ml) was added. This addition took place over a 2-h period after which time stirring was stopped and the stream of helium replaced by sealing the apparatus with a nitrogen-filled balloon. Heating at 100° was continued for a further 68 h. The reaction mixture was cooled and then centrifuged at 3000 r.p.m. for 10 min. The supernatant liquid was poured slowly into ice-cold water (75 ml) which had been adjusted to pH 1–2 with hydrochloric acid. A dense precipitate formed which was separated from the light-orange supernatant liquid by centrifugation at 3000 r.p.m. for 20 min. The supernatant liquid was extracted with chloroform (4 × 25 ml); the combined chloroform extracts were dried ($MgSO_4$), and the chloroform was removed by concentration on a rotary evaporator. Residual dimethylformamide was removed by freeze drying to give 0·43 g of crude product. This crude product was subjected to column chromatography as described above to obtain *N*-acetyl-*S*-(2-thienyl)-L-cysteine as a light-yellow solid (0·35 g, 28%). After recrystallization from ethyl acetate, the *compound* had m.p. 160–160·5° (Found: C, 44·0; H, 4·4; N, 5·7; S, 26·2. $C_9H_{11}NO_3S_2$ requires C, 44·1; H, 4·5; N, 5·7; S, 26·1%). I.r. (Nujol) 3350s (NH), 1710s (C=O, acid), 1620s cm^{-1} (C=O, amide). N.m.r. δ (D_2O/NaOH) 1·85, s, $COCH_3$; 3·2–2·9, m, CH_2; 4·3–4·1, m, CH; 7·5–6·9, m, 3H, 2-thienyl. *m/e* 245 (6%), 186 (36), 130 (58), 71 (52), 45 (49), 43 (100).

N-*Acetyl*-S-(*3-thienyl*)-L-*cysteine*

The quantities of reagents used were as follows: 3-iodothiophen, 1·05 g (0·005 mol); *N*-acetyl-L-cysteine, 0·81 g (0·005 mol); copper (I) iodide, 0·95 g (0·005 mol). After freeze drying, 0·47 g of crude product was obtained which, on column chromatography, gave *N*-acetyl-*S*-(3-thienyl)-L-cysteine as a light-yellow solid (0·36 g, 29%). After recrystallization from ethyl acetate/chloroform, the *compound* had m.p. 123–125° (Found: C, 44·1; H, 4·5; N, 5·7; S, 25·8. $C_9H_{11}NO_3S_2$ requires C, 44·1; H, 4·5; N, 5·7; S, 26·1%). I.r. (Nujol) 3250s (NH), 1710s (C=O, acid), 1620s cm^{-1} (C=O, amide). N.m.r. δ (CD_3OD) 1·95, s, $COCH_3$; 3·4–3·2, m, CH_2; 4·7–4·3, m, CH; 7·6–7·0, 3H, 3-thienyl. *m/e* 245 (11%), 186 (75), 141 (20), 129 (43), 116 (32), 85 (48), 83 (72), 71 (32), 45 (41), 43 (100).

N-*Acetyl*-S-*phenyl*-L-*cysteine*

The quantities of reagents used were as follows: iodobenzene, 1·07 g (0·005 mol); *N*-acetyl-L-cysteine, 0·81 g (0·005 mol); copper(I) iodide, 0·95 g (0·005 mol). After freeze drying, 0·49 g

of crude product was obtained which, on column chromatography, gave *N*-acetyl-*S*-phenyl-L-cysteine as a colourless solid (0·35 g, 28%). After recrystallization from ethyl acetate, the compound had m.p. 140·5–141·5° (lit.[11] 142°).

N-*Acetyl*-S-(*1-naphthyl*)-L-*cysteine*

The quantities of reagents used were as follows: 1-iodonaphthalene, 1·26 g (0·005 mol); *N*-acetyl-L-cysteine, 0·81 g (0·005 mol); copper(I) iodide, 0·95 g (0·005 mol). After freeze drying, 0·62 g of crude product was obtained which, on column chromatography, gave *N*-acetyl-*S*-(1-naphthyl)-L-cysteine as a colourless solid (0·45 g, 31%). After recrystallization from ethyl acetate, the compound had m.p. 170° (lit.[4] 170–172°).

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