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Characterisation and Simple Synthesis of S-[3-Hydroxy-1-propylpropyl]-L-cysteine

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Abstract: A convenient new synthetic approach has been established to prepare S-[3-hydroxy-1-propylpropyl]-L-cysteine **4** by Michael-addition of enantiomerically pure L-cysteine to (2*E*)-2-hexenoic acid ethyl ester followed by selective reduction of the ester function with sodium trimethoxyborohydride. All compounds were obtained as a diastereomeric mixture in good yields and their structures were determined by NMR and MS analyses.

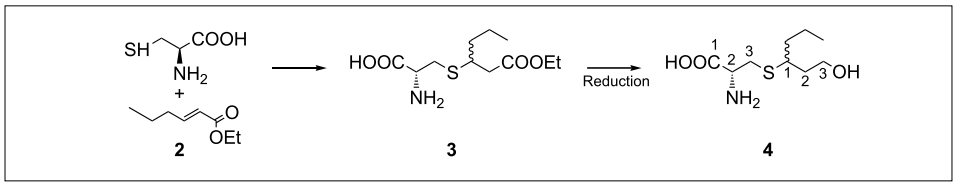
Keywords: Analysis · S-3-(Cysteinyl)-mercaptohexanol · S-3-Cysteinylhexan-1-ol · S-3-(Hexan-1-ol)-L-cysteine · Flavor precursor · 3-Mercaptohexanol · Thiol

Introduction

One of the most efficient mechanisms of biological detoxification is the conjugation with the sulfhydryl group of glutathion.[1] The glutathion conjugate itself generally undergoes a hydrolysis of the γ-glutamic and the glycyl residue, which produce cystein conjugates, most of them being interesting precursors of different aromas of food, like 4-mercapto-4-methylpentane-2-one, *p*-menthene thiol, mercaptohexanol or others.[2]

S-[3-hydroxy-1-propylpropyl]-L-cysteine is a precursor of 3-mercaptohexanol, which is a potent aroma compound in various vegetables like rhubarb, grapefruit, passion fruit and also in wines.[3,4]

In order to investigate the biosynthesis of 3-mercaptohexanol during the wine fermentation, we needed an efficient synthesis of this conjugate, which could enable radioactive labelling of this compound. The cysteine conjugate labelled with radioactive sulfur is intended to be introduced into fermenting grape juice in order to follow the eventual incorporation of the sulfur atom into new compounds.



Scheme. Reaction pathway.

The synthesis of conjugates of cysteine with aromatic or branched hydrocarbons is straightforward, but the synthetic method is known to be difficult to apply to linear hydrocarbons.[5] Another synthetic method has been proposed by Tominaga and coworkers[6,7] and recently by Starkenmann,[8] but the yields were rather low and the products difficult to obtain as pure compounds.

Results

The synthetic method proposed by Tominaga and Starkenmann involves a Michael addition of cysteine to *E*-2-hexenal. The aldehyde is very reactive and the formation of a double addition product or a Schiff base with the amine group of cysteine is likely to occur[5,8] as a side reaction of the Michael addition. We chose another synthetic pathway starting with the corresponding α,β-unsaturated ethylester **2**, (Scheme) as the Michael acceptor and reducing the addition product S-[2-ethoxycarbonyl-1-propylethyl]-L-cysteine **3** to the corresponding alcohol S-[3-hydroxy-1-propylpropyl]-L-cysteine **4**. For the addition step, we found that the pH control is the most important factor. The reaction occurs, critically depending on the exact reaction conditions, with good yield and the product is easy to separate from the reactants by salting out with THF/water/NaH_2PO_4.

The reduction of the ester in the presence of a carboxylic acid group is more difficult to control, mostly due to the poor solubility of the compound in organic solvents. $NaBH_4$ is not strong enough to effect this reduction and $LiAlH_4$ is too strong and

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reduces both the ester and the carboxylic group indiscriminately. We tested trimethoxyborohydride as reducing agent.[8] In this case, the choice of the solvent is crucial. Good yields were only obtained with 1,2-dimethoxyethane.

Compounds **3** and **4** were obtained as diastereomeric mixtures at C(1). The diastereomeric ratio was found to be close to 1:1 as judged by ^{13}C-NMR spectral data. Attempts to separate the compounds by column chromatography on silica gel using a variety of eluents were not successful.

Characterization of the Compounds

The structural assignment of all compounds was carried out by thorough investigation of ^{1}H, COSY, ^{13}C-NMR, and mass spectra.

Michael Addition Leading to Product 3

In the proton NMR spectrum of the hexanoate adducts **3**, the proton of the carbon attached to the sulfur atom (H-C(1)) gives a broad complex multiplet due to quadrupolar coupling. The chemical shift of ~2.95 ppm strongly supports the structure of the final compounds **3**, in which the cysteine is bound to the hexanoate moiety through its sulfur atom. This linkage is confirmed by the proton decoupled ^{13}C NMR spectrum, which exhibits eleven different resonances in accordance with the expected product. On close inspection of the carbon spectrum, it turns out that some peaks are doubled, indicating the presence of a diastereomeric mixture of **3**. This is not surprising since the addition of L-cysteine to the double bond of the hexenoate can occur on both sides. The chemical shift differences between the corresponding carbon resonances of the two diastereomeric forms are directly proportional to the distance to the stereocenters next to the sulfur. The very low diastereomeric differentiation of the cysteine addition is peculiar and, according to the ^{13}C NMR data, the ratio is about 45:55 in accordance with the observations of Wakabayashi.[5]

The ^{1}H-NMR spectra of the two diastereomers of **3** are virtually identical and consist of three subspectra, one of the cysteine moiety and two of the hexanoate. The first subspectrum exhibits a characteristic ABX-spin system typically attributed to the amino acid fragment of the product (cysteine: -S-CH_2-CHR_2); where the single proton signal at 3.83 ppm corresponds to the α-hydrogen of the aminocarboxylic function. The other two groups of signals correspond to a linear chain $CH_2CHCH_2CH_2CH_3$ and the typical triplet and quartet of the ethylester function of **3**.

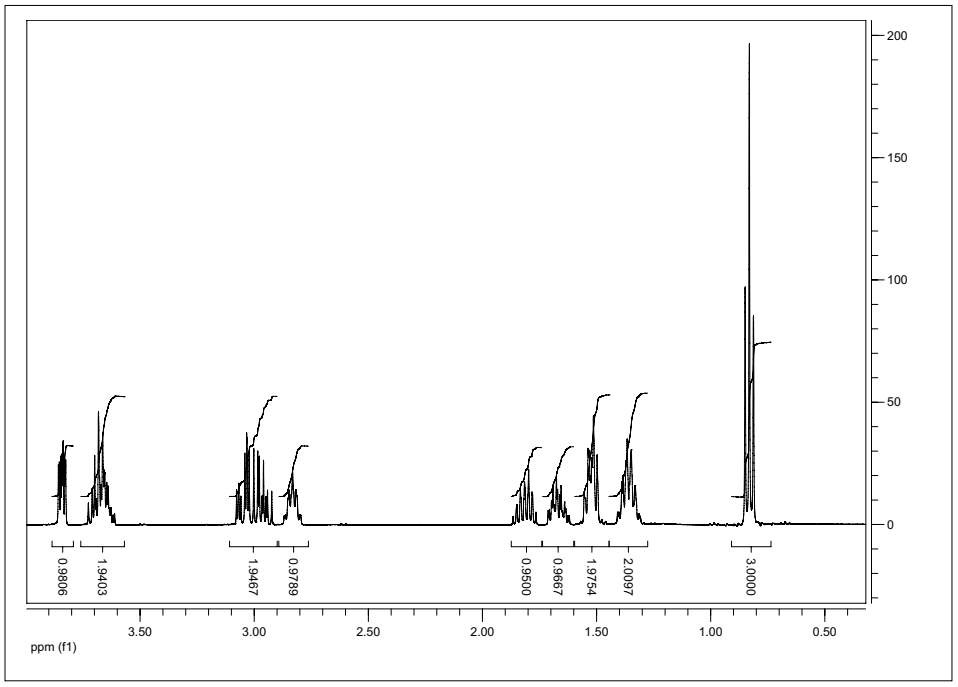

Fig. ^{1}H-NMR spectrum of S-[3-Hydroxy-1-propylpropyl]-L-cysteine

The question of at which site of the cysteine the addition took place remains to be solved. Theoretically, three different nucleophiles are present: the carboxylate group, the amino-nitrogen and the sulfur atom. Amongst these three possibilities, the sulfur atom exhibits the best nucleophilicity. However the carboxylate might be a potent competitor in its deprotonated form. The nitrogen atom seems not to be able to act as a nucleophile, especially not in its protonated form (-NH_3^+). The chemical shift of the protons adjacent to the heteroatom (~3 ppm) is not in accordance with those found in isopropyl esters,[10] eliminating the carboxylate option. For the two possibilities still open, it is easy to distinguish which of them has reacted by measuring the free thiol residue according to Ellmann.[11] This measurement shows the disappearance of the sulfhydryl group as the reaction progresses confirming thus the formation of the proposed product.

Reduction of the Ester 3 to S-[3-Hydroxy-1-propylpropyl]cysteine 4

The proton-decoupled ^{13}C-spectrum of the alcohol consists of nine peaks, some of them appearing as a doublet, in accordance with the diastereomeric character of the mixture. The selectivity of the reduction is clearly demonstrated by the disappearance of three signals of the starting material: one carboxyl resonance at ~170 ppm, one CH_2 resonance at ~20 ppm and one methyl resonance at ~14 ppm and by the appearance of a new peak, which can be attributed to an alcohol and thus revealing the success of the reduction.

The ^{1}H-NMR spectrum (Fig.) consists of two subspectra corresponding to the reduced cysteine product. The first subspectrum exhibits a characteristic ABX-spin system typically belonging to the amino acid part of the product (cysteine: -S-CH_2-CHR_2). The other signal group corresponds to a n-hexyl chain with a relatively broad quintet at 2.81 ppm suggesting the presence of the quadrupolar sulfur atom.

The spectroscopic data are close to those published and corrected by Starkenmann[8] and we may therefore conclude that we indeed have prepared S-[3-hydroxy-1-propylpropyl]-L-cysteine **4** in good yield. All reactions are performed under mild conditions, the work up of all the reaction products is simple.

The results of a study on radioactive metabolites after the use of the cysteine conjugate labelled with radioactive sulfur in the fermentation of grape juice and the syntheses of other hexenal derivatives of gluthathione will be reported later.

Experimental

General: NMR Spectra: Brucker XP-400 spectrometer at 400 (^{1}H) or 100 MHz (^{13}C), HPLC quadrupolar MS (HP-Serie 4, Palo Alto, USA) using a direct inlet for solid sample analyses.

2-Amino-3-[(2-ethoxycarbonyl-1-propylethyl)sulfanyl]propionic Acid

260 mg of L-cysteine-HCl (1.27 mM, Fluka, Buchs, Switzerland, Nr 30210) were dissolved in 5 ml of water in a 25 ml round

bottom flask, N_2 was bubbled through the solution, 1.8 ml of a 1M solution of NaOH was added to adjust the pH to 7.5. An excess of ethyl (2*E*)-2-hexenoate **2** (0.96 g, 6.75 mM) was added and the solution was stirred for 2 h at 50 °C.

The reaction can be monitored either by the disappearance of cysteine according to Ellmann[10] or by TLC on silica gel (isopropanol:ethylacetate:water 2:2:1) and detection with a solution of 0.5% ninhydrine in acetone.

After the disappearance of the starting material, 10 ml of a buffer solution of NaH_2PO_4 20% were poured into the reaction flask, the pH of the reaction mixture was adjusted to about 5-6. The solution was transferred to a separating flask and the unreacted ethylhexenoate was extracted with two 5 ml portions of dichloromethane. 2-Amino-3-[(2-ethoxycarbonyl-1-propylethyl)sulfanyl]propionic acid **3** could be extracted by salting out in THF.

The THF fractions were evaporated under vacuum and dried overnight at 50 °C under high vacuum. The yield of a colourless powder was 325 mg (75% of the max. theoretical yield). For larger quantities the extraction of the buffered aqueous phase with THF in a Kutscher-Steudel extractor gave better yields.

^{1}H-NMR (D_2O): δ 0.81 (t, 3H, *J* = 7.3Hz, CH_3), 1.11 (t, 3H, *J* = 7.2 Hz, ester-CH_3), 1.27–1.40, (m, 2H, CH_2), 1.48–1.57, (m, 2H, CH_2), 2.55 (dxdxd, 1H, *J* = 7.8, 11.4 and 15.7 Hz, **A**BX), 2.66 (dxd, 1H, *J* = 5.6 and 15.7 Hz, A**B**-X), 2.91–3.00 (m, 1H, CH–S), 3.03–3.14 (m, 2H, CH_2), 3.83 (dxdxd, 1H, *J* = 4.2, 7.5 and 11.7 Hz CH-N), 4.11 (q, 2H, *J* = 7.2 Hz, C*H*$_2$-CH_3); ^{13}C-NMR (D_2O): δ 14.2 (q), 14.6 (q), 20.7 (t), 32.1 (t), 37.7 (t), 41.2 (d), 43.2 (t), 55.3 (d), 63.3 (t), 173.6 (s), 175.6 (s), APCI$^+$ $[M+H]^+$ 264.

Reduction of the Ester 3 to 2-Amino-3-[(3-hydroxy-1-propylpropyl)sulfanyl] propionic Acid

760 mg of **3** (2.89 mM) were dissolved in 10 ml 1,2-dimethoxyethane (Fluka, Buchs Switzerland, Nr 38570), the mixture was heated to reflux in a round-bottom flask of 100 ml. A suspension of 2.6 g sodium trimethoxyborohydrate, $NaB(OCH_3)_3H$ (20mM, Sigma-Aldrich, Buchs, Switzerland, Nr 217-956-10g), in 20 ml of 1,2-dimethoxyethane was slowly added to this reaction mixture and the reflux maintained for 2 h.

The reaction mixture was then cooled down to room temperature. 1 ml of water was added in order to precipitate the reducing agent. The white precipitate was decanted and the solution was neutralized to pH 6–7 with HCl 1M. The solution was evaporated almost to dryness and the product was flash-chromatographed over silica gel with isopropanol:ethylacetate:water 2:2:1, yielding after drying over night under vacuum 294 mg (46% of the max. theoretical yield) of a colourless powder.

^{1}H NMR (D_2O): δ 0.80, (t, 3H, *J* = 7.2 Hz, CH_3), 1.26–1.38 (m, 2H, CH_2), 1.44–1.53, (m, 2H, CH_2), 1.58–1.69 and 1.72–1.84, (m, 2H, CH_2), 2.76–2.85 (m, 1H, CH-S), 2.93 (dxdxd, 1H, *J* = 7, 9, 14.5 Hz, ABX), 3.08 (dxt, 2H, J = 3.6 and 14.5 Hz, ABX), 3.57–3.70 (m, 2H, CH_2-OH), 3.80 (dxdxd, 1H, J = 1.7, 4.2 and 7.5 Hz, CH-N); ^{13}C NMR (MeOH): δ 13.5 (q), 19.6 (t), 30.1 (t), 36.5 (t), 36.8 (t), 43.2 (d), 53.5 (d), 59.5 (t), 173.7 (s), APCI$^+$, $[M+H]^+$ 222 and APCI$^-$ $[M-H]^-$ 220.

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