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# The Synthesis of Naturally Occurring Sulfinic Acids

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# THE SYNTHESIS OF NATURALLY OCCURRING SULFINIC ACIDS

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This review is a complete and detailed account of synthetic pathways to the known naturally occurring and biologically active sulfinic acids (and their unnatural enantiomers) with additional complete references to their radiolabeled analogs.

*Key words:* Natural sulfinic acids, radiolabeled natural sulfinic acids.



## CONTENTS

## 1. INTRODUCTION

There are eight known naturally occurring sulfinic acids, most of them linked to cysteine metabolism. They have been the subject of numerous biochemical studies. Only two, cysteinesulfinic acid and hypotaurine, are commercially available. In this short review we wish to present the known synthetic procedures for naturally occurring sulfinic acids, with additional references to isotopically labeled preparations, in a coherent and comprehensive manner. Our literature search has also revealed that two naturally occurring sulfinic acids, i.e. sulfinoacetaldehyde and sulfinopyruvic acid, have not been synthesized yet.

The first sulfinic acid found in nature was cysteinesulfinic acid.[1] Even though sulfinic acids are found in all living systems they are not equally distributed in nature. Cysteinesulfinic acid is found in bacteria, plants, and mammals, but hypotaurine and homohypotaurine are not found in plants, and hypotaurocyanamine is only found in invertebrates. Because of the uneven distribution of the natural sulfinic acids their metabolic pathways are not uniform and not all have been fully elucidated.[2-6]

Most of the naturally occurring sulfinic acids are present in relatively high concentrations in the central nervous system (CNS) and a great deal of work has been carried out to prove a physiological role of the sulfinic acids in the CNS as neurotransmitter substances. The naturally occurring sulfinic acids are generally neuroactive towards different types of receptors.[6a-9] Hypotaurine has a well established inhibitory neurotransmitter action. Hypotaurine and homohypotaurine also promote potassium release in brain slices, but so far, as also in the case of the receptor interactions, no significant physiological role nor potential pharmacological use appears obvious.[9]

The common names of the naturally occurring sulfinic acids (including their unnatural stereoisomers) are listed below, together with their CAS Registry Numbers:

Cysteinesulfinic acid [2381-08-0], **2**
D-(−)-Cysteinesulfinic acid [35554-99-5], D-(−)-**2**
L-(+)-Cysteinesulfinic acid [115-65-1], L-(+)-**2**
D-(−)-Homocysteinesulfinic acid [33514-39-5], D-(−)-**8**
L-(+)-Homocysteinesulfinic acid [2686-70-6], L-(+)-**8**
DL-Homocysteinesulfinic acid [14857-75-7], DL-**8**
Homohypotaurine [25346-09-2], **15**
Hypotaurine [300-84-5], **20**
Hypotaurocyanamine [1119-54-6], **31**
Sulfinoacetaldehyde [76425-74-6]
Sulfinoacetic acid, no RN, **36**
Sulfinopyruvic acid [88947-38-0]

The nomenclature of these compounds used by chemists and biochemists throughout the years has varied considerably and includes frequent use of incorrect or controversial names. Considerable confusion arose by the faulty structure assign-

TABLE 1 The naming of some naturally occurring sulfinic acids

| Common name (abbreviation) | CA name | IUPAC name | Other names |
|---|---|---|---|
| cysteinesulfinic acid (CSA) | 3-sulfinoalanine | 2-amino-3-sulfino-propanoic acid | 2-amino-2-carboxyethanesulfinic acid |
| homocysteinesulfinic acid (HCSA) | 2-amino-4-sulfino-butanoic acid | 2-amino-4-sulfino-butanoic acid | — |
| hypotaurine (HT) | 2-aminoethanesulfinic acid | 2-aminoethanesulfinic acid | — |
| homohypotaurine (HHT) | 3-aminopropanesulfinic acid | 3-aminopropanesulfinic acid | — |
| sulfinoacetic acid (none) | 2-sulfinoacetic acid | 2-sulfinoethanoic acid | — |
| hypotaurocyanamine (HTC) | 2-guanidino-ethanesulfinic acid | 2-guanidino-ethanesulfinic acid | — |

ment R-SO-SO-R for the stable oxidation products of symmetrical disulfides which were later shown to possess the structure $R\text{-}SO_2\text{-}S\text{-}R$.[10-16] Thus, the correct name of the oxidation product of cystine is cystine *S,S*-dioxide, not cystine dioxide or cystine *S,S'*-dioxide.



## 2. SYNTHESES OF CYSTEINESULFINIC ACID

$$HO_2C\text{-}CH(^+NH_3)\text{-}CH_2\text{-}SH \xrightarrow{CoCl_3,\ KOH}$$

**1**

$$K_3[Co(O_2C\text{-}CH(NH_2)\text{-}CH_2\text{-}S)_3]\cdot H_2O \xrightarrow{H_2O_2}$$

$$K_3[Co(O_2C\text{-}CH(NH_2)\text{-}CH_2\text{-}SO_2)_3]\cdot 3H_2O \xrightarrow{H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2}$$

$$[Co(H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2)_3]\cdot[Co(O_2C\text{-}CH(NH_2)\text{-}CH_2\text{-}SO_2)_3]\cdot 7H_2O$$

$$\xrightarrow{BaCl_2} Ba(O_2C\text{-}CH(NH_2)\text{-}CH_2\text{-}SO_2)\cdot H_2O \xrightarrow{H_2SO_4}$$

$$HO_2C\text{-}CH(^+NH_3)\text{-}CH_2\text{-}SO_2^-\cdot H_2O$$

**2**

### 2.1. *L-(+)-Cysteinesulfinic Acid L-(+)-***2**[17]

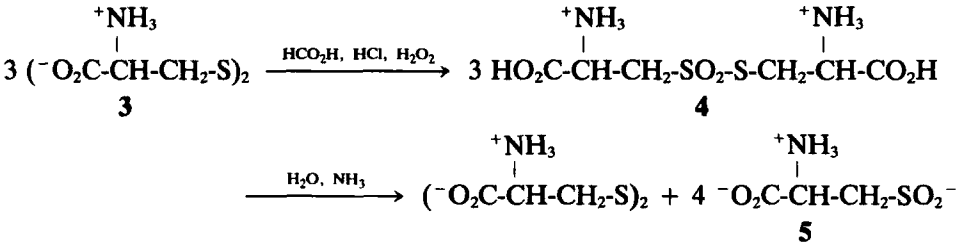
L-(+)-Cysteine hydrochloride **1** (25.0 g, 0.180 mol) is dissolved in 52 mL (52 mmol) 4 N cobalt(III) chloride, 100 mL (0.660 mol) 13.2 N potassium hydroxide added, the mixture cooled, air bubbled through for ca. 5 h, and then 200 mL ethanol added. The mixture is kept at r.t. for a few hours and then filtered, dried by suction, and redissolved in 150 mL water. The solution is filtered and 150 mL ethanol slowly stirred in. After a few hours standing a green mass of crystals separates out; the crystals are collected, washed with 50% ethanol and

dried at 1 mm Hg over sulfuric acid. Yield of potassium cobalt(III) tris(L-cysteinate), $K_3[Co(SCH_2CHNH_2CO_2)_3]\cdot 3H_2O$, 64%.

Potassium cobalt(III) tris(L-cysteinate) (10.0 g, 17 mmol) is dissolved in 100 mL water and while the solution is kept well cooled under running water 13 mL (0.115 mol) 30% hydrogen peroxide, diluted with 13.2 mL water, is added dropwise. The color of the solution changes to yellow. After 30 min the solution is heated to 50–60 °C and 35 mL (0.21 mol) 6 N hydrochloric acid is added slowly with vigorous stirring until precipitation takes place. The crystals are filtered, suspended in 25 mL water, and just enough 13.2 N potassium hydroxide (5–7 mL) is added to cause complete dissolution. The solution is filtered, 100 mL ethanol slowly stirred in, and the solution kept on ice for a few hours. The yellow crystals are collected and washed with ethanol. Yield of potassium cobalt(III) tris(L-cysteinesulfinate) trihydrate, $K_3[Co(SO_2CH_2CHNH_2COO)_3]\cdot 3H_2O$, 43%.

Potassium cobalt(III) tris(L-cysteinesulfinate) trihydrate (12.5 g, 18 mmol) is dissolved in 60 mL water and 8 mL (12.8 mmol) ethylenediamine and warmed at 45 °C for 10 min. The mixture is cooled rapidly, the crystals filtered off and 40 mL (40 mmol) 2 N barium chloride is added to the filtrate. A gummy precipitate appears and 50 mL ethanol is added slowly with stirring. The mixture is filtered and 150 mL ethanol added to the filtrate. This causes a separation of the barium sulfinate in an almost pure state. It can be dissolved in water and reprecipitated with ethanol for further purification. After drying the yield of barium L-cysteinesulfinate hydrate, $Ba(SO_2CH_2CHNH_2CO_2)\cdot H_2O$, is 36%.

The hydrate of L-(+)-**2** can be obtained by dissolution of the barium salt in water and addition of the calculated amount of sulfuric acid, centrifuging off of the barium sulfate, evaporation of the solution to a small volume, and precipitation of L-(+)-**2**·$H_2O$ with ethanol. It separates as colorless rectangular plates, $[\alpha]_D^{25}$ + 24.0° (c = 1.0; 1 N HCl).[18]



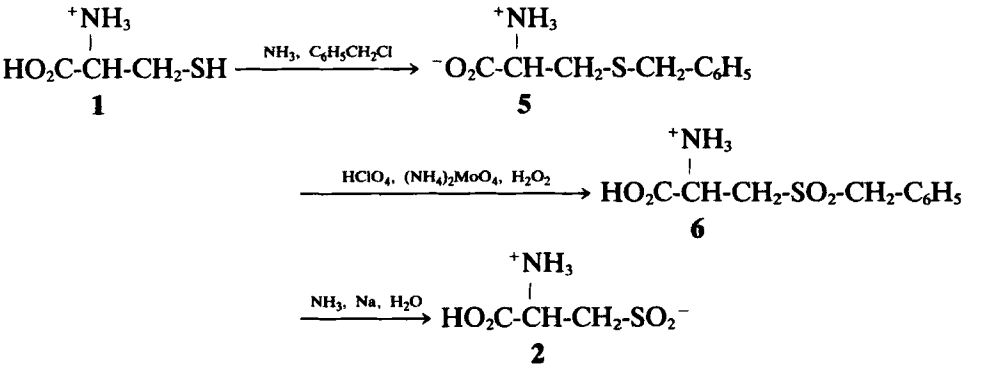

2.2. *L-(+)-Cysteinesulfinic Acid L-(+)-***2**[19]

L-(+)-Cystine **3** (4.8 g, 20 mmol) is stirred with 100 mL 90% formic acid and 4 mL 22% hydrochloric acid and the mixture cooled in an ice bath. Stirring is continued until the temperature reaches 20 °C and then 5 mL (48 mmol) 33% hydrogen peroxide added. After 2.5 h the suspension is evaporated to dryness under reduced pressure. The residue is dissolved in 50 mL distilled water and the pH adjusted to 3–3.5 by addition of concentrated aqueous ammonia with

stirring and cooling. A lower pH immediately causes precipitation. The solution is kept overnight in a refrigerator. The precipitate is collected and washed 3 times with 5 mL distilled water. After drying in a desiccator over phosphorus pentoxide the yield of L-cystine *S,S*-dioxide **4** is 85%, m.p. 179–182 °C.[20]

L-Cystine *S,S*-dioxide **4** (4.0 g, 15 mmol) is suspended in 10 mL distilled water, 10 mL conc. ammonia is added, and the mixture left for 1 h at r.t. The suspension is evaporated at 20 °C on a rotatory evaporator, the residue dissolved in 10 mL distilled water, and evaporated to dryness again. This operation is continued until the pH of the suspension is 6–7. The residue is then suspended in 10 mL distilled water, the precipitate collected, and washed 2 times with 2 mL distilled water. The filtrate is loaded on a 20 × 1.4 cm ion exchange column charged with Dowex 50 X 12, the sulfinic acid eluted with distilled water, and found in the first 80 mL of the eluate. A possible discoloration of the solution can be removed with charcoal. Yield 93%. Pure L-(+)-**2** can be obtained by precipitation from 50% aqueous ethanol.

Chromatography on Whatman paper No. 1 with the solvent mixture *t*-butanol/formic acid/water (0.75:0.15:10) gives a positive ninhydrin response at $R_f$ 0.2–0.3, $[\alpha]_D^{21}$ + 28 ± 1° (c = 0.8; 1 N HCl).




2.3. *L-(+)-Cysteinesulfinic Acid L-(+)-***1**[21]

L-(+)-Cysteine hydrochloride **1** (48.0 g, 0.339 mol) is added to 600 mL liquid ammonia and stirred mechanically. Benzyl chloride (44.3 g, 0.350 mol) is added in 5 mL portions during 15 min. When the mixture is homogeneous the stirring is stopped and the ammonia allowed to evaporate, the last traces being removed *in vacuo*. The residue is suspended in 2 L water and the pH adjusted to 4 with glacial acetic acid. Then the suspension is boiled, filtered, and left overnight at 4 °C. The crystals are filtered off, washed with 200 mL water, 150 mL ethanol, 50 mL diethyl ether, and dried *in vacuo* over phosphorus pentoxide at 62 °C yielding 91%. The L-(+)-*S*-benzylcysteine **5** is recrystallized from hot water, m.p. 214–218 °C, $R_f$ 0.80 (Whatman paper No. 4 with *n*-butanol/acetic acid/water), $[\alpha]_D^{26}$ + 24.5° (c = 1.0; 0.1 N NaOH).

Perchloric acid (60 % w/v, 4 mL, 24 mmol) is added dropwise to finely ground ammonium molybdate (0.5 g, 3.38 mmol), suspended in 30 mL water, boiled for 5 min, and filtered. L-(+)-*S*-Benzylcysteine **5** (20 g, 95 mmol) is added to the filtrate on an ice bath and 38 mL (0.335 mol) 30% w/v hydrogen peroxide is added dropwise with stirring. The suspension is left at r.t. for 14 h and the crystals collected, washed with 100 mL water, 50 mL ethanol, and 20 mL diethyl ether, and then dried *in vacuo* over anhydrous calcium chloride, yield 91%, m.p. 171 °C. The dry material is recrystallized from hot water (2.0 g in 250 mL, 81% recovery): L-(+)-*S*-benzylcysteine sulfone **6**, m.p. 175 °C, $R_f$ 0.71 (Whatman paper No. 4 with *n*-butanol/acetic acid/water), $[\alpha]_D^{23}$+ 19.8° (c = 1.0; 5 N HCl).

L-(+)-*S*-Benzylcysteine sulfone **6** (4.55 g, 19 mmol) is dissolved in 400 mL liquid ammonia and small pieces of sodium are added until a permanent blue color appears. Excess sodium is destroyed with glacial acetic acid. The ammonia is allowed to evaporate and the solid residue extracted with 100 mL diethyl ether before dissolution in 100 mL water. The aqueous solution is passed through a water washed 2 × 27 cm column charged with the cation exchanger Dowex AG X 8 (200–400 mesh) in the $H^+$ form which is loaded with the crude sulfinic acid and eluted with water. The product containing fractions are collected and concentrated to 10 mL and upon cooling fine colorless needles precipitate. Ethanol (15 mL) is added and the mixture cooled to 4 °C for 6 h. The mixture is filtered, the crystals of L-(+)-**2** washed with methanol, and dried *in vacuo* over anhydrous calcium chloride. Yield 77%, $R_f$ 0.31 (Whatman paper No. 4 developed with *n*-butanol/acetic acid/water), $[\alpha]_D^{23}$ + 24.0° (c = 1, 1 N HCl), m.p. 152–153 °C, $pK_a$ 1.50, 2.38.[22]



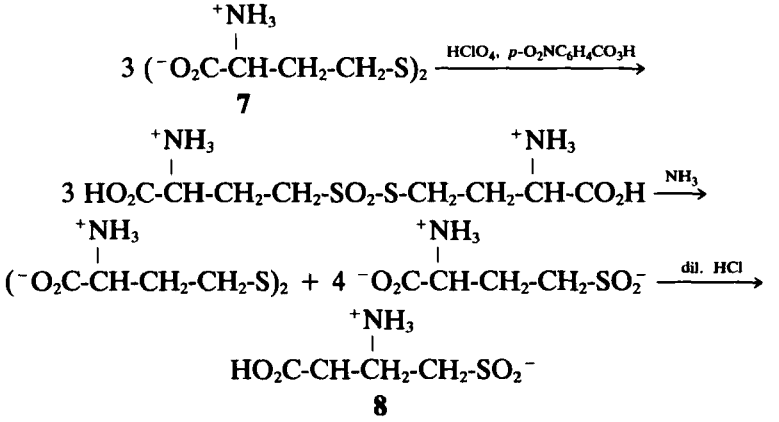
2.4. *D-(−)-Cysteinesulfinic acid D-(−)-***2**[23,24]

D-Cystine (12.01 g, 5 mmol) is suspended in 250 mL acetonitrile, 0.10 mol 70 % perchloric acid in 300 mL acetonitrile added with cooling, and 21.5 mL acetic anhydride added to remove the water contained in the perchloric acid. The mixture is shaken until complete solution and warmed to r.t. Perbenzoic acid[25] (16.6 g, 120 mmol), dissolved in 150 mL chloroform, is added with shaking and slight cooling, and the volume increased to 1 L with acetonitrile. After standing for 1 h the solution is extracted with 250 mL 1 N hydrochloric acid and the organic layer extracted once again with 100 mL 1 N hydrochloric acid. The two aqueous layers are combined and extracted five times with 50 mL chloroform. After filtration the aqueous phase is slowly neutralized with 8 N aqueous ammonia until beginning precipitation, and the neutralization to pH 7 completed with 1 N aqueous ammonia. After 10 min standing the suspension is filtered by suction and the precipitate washed with water, ethanol, and diethyl ether, and finally dried *in vacuo*. Yield of D-cystine *S*,*S*-dioxide **4** 73%.

D-Cystine *S*,*S*-dioxide (2.72 g, 0.102 mol) is dissolved in 20 mL 2 N aqueous ammonia, left overnight, and filtered. The yellow filtrate is treated with charcoal, hydrochloric acid added until pH 2, and the solution evaporated to dryness. The solid is redissolved in 4 mL water and 5 mL ethanol with formation of two layers.

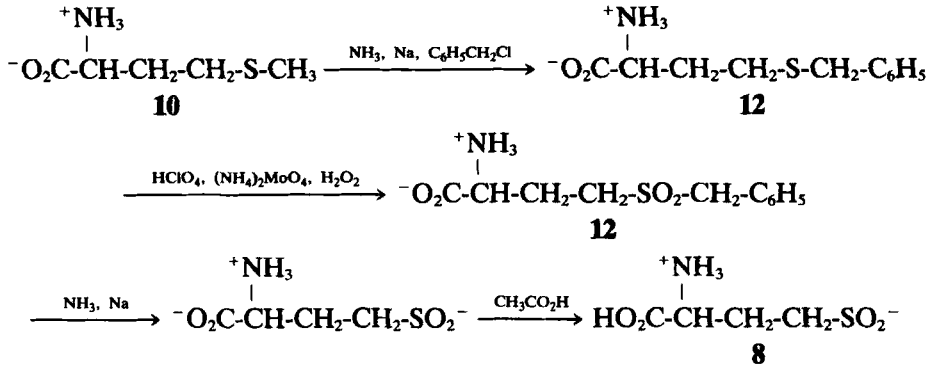
Water is added to the hot mixture (60 °C) until a homogeneous solution is obtained. Upon cooling small octahedral crystals separate out. The crystals are collected and dried over phosphorus pentoxide *in vacuo*. Yield of D-(−)-**2** 48%, $[\alpha]_D^{25}$ − 24° (c = 1, 1 N HCl).

## 3. SYNTHESES OF HOMOCYSTEINESULFINIC ACID



3.1. *L-(+)-Homocysteinesulfinic acid L-(+)-***8**[26]

A solution of L-(+)-homocystine **7** (1.34 g, 5 mmol) in 150 mL chloroform containing 65% perchloric acid (1.05 g, 10 mmol) and another solution of *p*-nitroperbenzoic acid[27] (2.79 g, 15 mmol) in 200 mL chloroform are prepared and cooled to 0 °C. The peracid solution is added in fractions to the homocystine solution, 90 mL acetonitrile added, and the mixture left at r.t. for 20 h. The solution is extracted three times with 125 mL distilled water. The aqueous layer is treated twice with 200 mL chloroform and concentrated to 20 mL. The concentrated solution is passed through a cation exchange column charged with 100 mL Dowex 50 ($H^+$ form), eluted with 200 mL distilled water and 200 mL 2 N aqueous ammonia, 50 mL fractions being collected. The product containing fractions are combined, treated with charcoal at pH 7, filtered, concentrated to dryness, and dissolved in 5 mL water. A column charged with 40 mL Dowex 1 ion exchanger is loaded with the crude homocysteinesulfinic acid, washed with water, and then eluted with 100 mL 0.5 N hydrochloric acid. Ten 4 mL fractions are collected. The fractions are evaporated *in vacuo* to leave a colorless powder. The powder is redissolved at 45 °C in the minimum volume of 0.05 N hydrochloric acid and five times this volume of absolute ethanol is added. Upon freezing colorless crystals of L-(+)-**8** are obtained, collected, and washed with absolute ethanol. Yield 25%, $[\alpha]_D^{22}$ + 30 ± 1.3° (c = 0.5, 1 N HCl), $[\alpha]_D^{22}$ + 30 ± 1.3° (c = 0.5, 1 N HCl), $[\alpha]_D^{22}$ + 11.6 ± 1.1° (c = 0.5, $H_2O$).



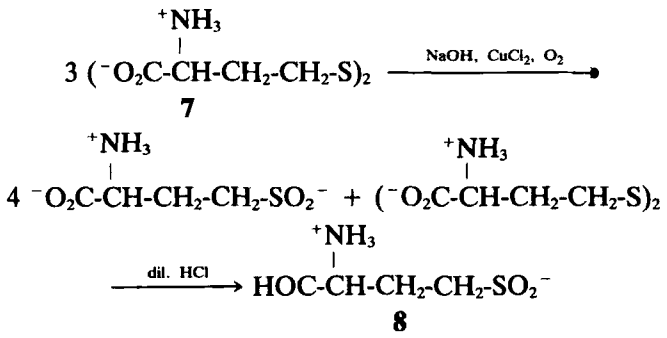

3.2. *L-(+)-Homocysteinesulfinic Acid L-(+)-***8**[21]

L-(+)-Methionine **10** (50.0 g, 0.370 mol) is dissolved in 600 mL liquid ammonia and stirred mechanically. Sodium (23.3 g, 1.01 mol) is added in small pieces until a permanent blue color occurs, ammonium chloride (20.0 g, 0.374 mol) added slowly, and a vigorous reaction occurs. Benzyl chloride (40.0 mL, 0.384 mol) is added dropwise, the stirring stopped, and the ammonia allowed to evaporate. The residue is dissolved in 2.5 L water and brought to pH 4 with glacial acetic acid. The solution is boiled and filtered. The filtrate is set aside at 4 °C for 3 h, then the precipitate collected, washed with 150 mL water, 150 ethanol, and 50 mL diethyl ether, and thereafter dried in a desiccator over anhydrous calcium chloride. Yield 83%, m.p. 236 °C. The crude L-(+)-*S*-benzylhomocysteine **11** is recrystallized from boiling water (2.0 g in 200 mL) containing 10 mL 1 N hydrochloric acid with 91% recovery, m.p. 244–245 °C, $R_f$ 0.82 (Whatman paper No. 4 with *n*-butanol/acetic acid/water), $[\alpha]_D^{24}$ + 24.70° (c = 1, 1 N HCl). Lit.[28] $[\alpha]_D^{24}$ + 24.50°.

Ammonium molybdate (0.50 g, 3.8 mmol) is finely ground, suspended in 15 mL water, and 4 mL 60% w/v perchloric acid added dropwise. The mixture is boiled for 5 min and filtered. To the filtrate L-(+)-*S*-benzylhomocysteine **11** (12.8 g, 60 mmol) is added and the thick suspension cooled with an ice bath, and 38 mL (0.335 mol) 30% w/v hydrogen peroxide added dropwise with stirring. The suspension is left at r.t. for 14 h and the crystals collected, washed with 50 mL water, 50 mL ethanol and 10 mL diethyl ether, and dried in a desiccator over anhydrous calcium chloride. Yield 91% m.p. 227–229 °C. The dry crude L-(+)-*S*-benzylhomocysteine sulfone **12** is recrystallized from boiling water with 87% recover, m.p. 234–235 °C (dec.), $[\alpha]_D^{23}$ + 29.4° (c = 1, 5 N HCl).

L-(+)-*S*-Benzylhomocysteine sulfone **12** (5.00 g, 20 mmol) is dissolved in 400 mL liquid ammonia and sodium (0.99 g, 43 mmol) added with stirring until the blue color persists for 15 min. Excess sodium is destroyed with 0.5 mL glacial acetic acid and the ammonia allowed to evaporate. The residue is dissolved in 100 mL water and passed through a 2 × 40 cm cation exchange column charged with Dowex AG 50 X 8 ($H^+$ form), eluted with water, and the eluent collected until ninhydrin negative fractions appear. The eluent is concentrated to 10 mL

and upon cooling colorless crystals separate out. Ethanol (30 mL) is added and the mixture set aside overnight at 4 °C. The crystals of L-(+)-**8** are collected, washed with 5 mL cold ethanol and 3 mL diethyl ether, and dried in a desiccator over anhydrous calcium chloride and concentrated sulfuric acid. Yield 80%, $R_f$ 0.33 (Whatman paper No. 4 with *n*-butanol/acetic acid/water), $[\alpha]_D^{22}$ + 29.9° (c = 0.5, 1 N HCl), $[\alpha]_D^{22}$ + 11.8° (c = 0.5, $H_2O$).



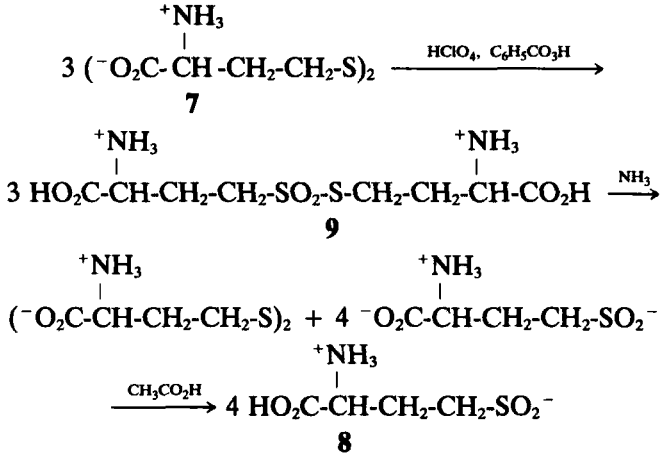
3.3. *L-(+)-Homocysteinesulfinic Acid L-(+)-***8**[29]

L-(+)-Homocystine **7** (1.34 g, 5 mmol) is dissolved in 150 mL 0.1 N sodium hydroxide and 5 mL 0.15 N copper(II) chloride added. The mixture is incubated with stirring for 3 h at 38 °C on a thermostated water bath. Then the solution is acidified to pH 2 with 2 N hydrochloric acid, filtered, and concentrated to 20 mL in a flash evaporator at 70 °C. The concentrate is loaded on a 2 × 28 cm cation exchange column charged with Dowex 50 W X 8 (200–400 mesh) in the $H^+$ form. The column is washed with 100 mL water and eluted with 2 N aqueous ammonia, the sulfinic acid appearing in the 150 mL before the alkaline front and the 150 mL after it. The eluate is reduced to 20 mL by flash evaporation and the slight yellow solution treated with charcoal. The concentrate is loaded on a 2 × 13 cm ion exchange column charged with Dowex 1 X 8 (200–400 mesh) in the $HCOO^-$ form. The column is washed with water and the sulfinic acid eluted with 0.5 N hydrochloric acid in fractions of 10 mL. The fractions containing homocysteinesulfinic acid are flash evaporated at 70 °C, leaving an oily residue which is dissolved in 4 mL water, taken to dryness, redissolved, and dried (for the removal of residual hydrogen chloride). The oily residue is diluted with 20 mL water and loaded on a 2 × 28 cm cation exchange column charged with Dowex 50 W X 8 (200–400 mesh) in the $H^+$ form. The column is eluted with water and fractions of 10 mL are collected, the homocysteinesulfinic acid appearing in fractions no. 7 to 27. These fractions are dried by flash evaporation and the solid residue dissolved in the minimum volume of 0.01 N hydrochloric acid at 70 °C. Five times this volume of absolute ethanol is added and the resulting solution kept overnight at −20 °C. The colorless precipitated L-(+)-**8** is collected, washed with ethanol, and dried with diethyl ether. Yield of L-(+)-homocysteinesulfinic acid 40%, $R_f$ (Whatman paper No. 1) with saturated aqueous

phenol: 0.27; with saturated aqueous collidine/lutidine: 0.23; with *n*-butanol/acetic acid/water (4:1:5, upper phase): 0.10; with *n*-butanol/ethanol/water (4:1:5, upper phase): 0.05, m.p. 175 °C, $pK_a$ 1.66, 2.60.[22] $^1H$ NMR: δ 2.0–2.85 (m, 4H), 4.13 (t, 1H), IR.[28]

### 3.4. *DL-Homocysteinesulfinic Acid DL-***8**[26,29]

The preceding procedure, carried out with DL-homocystine, yields DL-**8** with the same data as for L-(+)-**8** except for the m.p. which is 177–80 °C.




### 3.5. *DL-Homocysteinesulfinic Acid DL-***8**[30]

A mixture of the DL- and *meso*-form of homocystine (1.34 g, 5 mmol) is added to 25 mL cold acetonitrile. Perchloric acid (62%, 0.8 mL, 5 mmol) is added with cooling, followed by a solution of acetic anhydride (1.7 mL, 17 mmol) in 30 mL acetonitrile. The mixture is then filtered to remove undissolved homocysteine. Perbenzoic acid[25] (1.66 g, 12 mmol) in 15 mL chloroform is added and the solution increased to a volume of 100 mL and allowed to stand 1 h at r.t. The mixture is extracted twice with 25 mL 1 N hydrochloric acid, the aqueous phases combined, and extracted four times with 5 mL chloroform. The aqueous layer is brought to pH 4 with 8 N aqueous ammonia and allowed to stand overnight at 0 °C. The solution is then warmed to r.t. and made strongly alkaline with concentrated aqueous ammonia. After 1 h the solution is evaporated under reduced pressure while being kept below 40 °C. The residue is dissolved in 30 mL water, filtered, evaporated again, and dissolved in 50 mL water. Further purification takes place on a column charged with 100 mL anion exchanger Dowex 1 ($CH_3COO^-$ form). The column is loaded with the crude sulfinic acid, washed with water, and eluted with 6 N acetic acid. The eluate is collected in 250 mL fractions, the homocysteinesulfinic acid appearing in the 750–1500 mL range. The

fractions are concentrated and crystallized with a mixture of water/ethanol/diethyl ether to form colorless needles. Yield of DL-**8** 50%, m.p. 178 °C.

### 3.6. *D-(−)-Homocysteinesulfinic acid D-(−)-***8**[31]

No explicit information about the synthesis of D-(−)-**8** is available, but this compound is mentioned in a number of papers.[31–35] This work relating to D-(−)-**8** has been carried out with DL-**8** in order to compare the biochemistry of the D-form with that of the L-form.

## 4. SYNTHESIS OF HOMOHYPOTAURINE

$$3\ (H_3N^+\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}S)_2 \xrightarrow{HCl,\ KI,\ H_2O_2}$$

**13**

$$3\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}{}^+NH_3 \xrightarrow{NaOH}$$

**14**

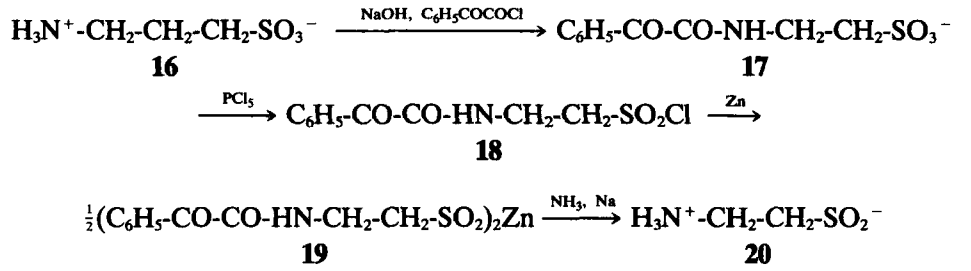
$$(H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}S)_2 + 4\ H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_2^- \xrightarrow{H_2O} 4\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_2^-$$

**15**

### 4.1. *Homohypotaurine* **15**[36]

Homocystamine dihydrobromide **13**[36,37] (3.40 g, 1 mmol) or dihydrochloride **13**[36,37] (2.50 g, 1 mmol) is dissolved in 10 mL 0.1 N hydrochloric acid. Potassium iodide (20 mg) is added, then dropwise 2.3 mL (20 mmol) 30% hydrogen peroxide. The solution turns yellow and slightly hot. After 0.5 h the solution is kept for 3 min on a boiling water bath which causes decolorization. After cooling to r.t. the pH is brought to 12–13 with 40% aqueous sodium hydroxide and the mixture containing **14** is left for 0.5 h at r.t. The solution is loaded on a 25 × 2 cm cation exchange column charged with Dowex 50 X 8 in the $H^+$ form. The column is washed with water and eluted with 1 N aqueous ammonia. The five 10 mL **15** containing fractions are evaporated to dryness. A small amount of absolute ethanol is added to the oily residue with subsequent evaporation to dryness and this procedure repeated until crystalline **15** is obtained. Crude yield 66%. Recrystallization from ethanol/diethyl ether yields pure **15**, m.p. 188–190 °C, $^1$H NMR: δ 1.90 (m, 2H), 2.38 (m, 2H), 3.08 (t, 2H), IR. Jollés-Bergeret has purified homohypotaurine from *Clostridium welchii* and recorded an IR spectrum.[38]

## 5. SYNTHESES OF HYPOTAURINE

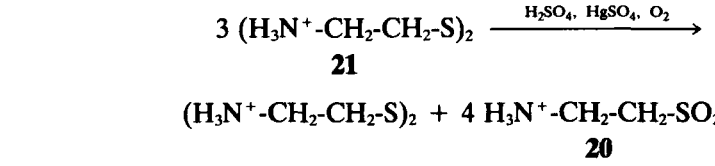


5.1. *Hypotaurine* **20**[39]

Taurine **16** (40.0 g, 0.320 mol) is dissolved in 200 mL water and 80 mL 4 N sodium hydroxide and, with stirring, 2-oxo-2-phenylethanoyl chloride[40] (57.0 g, 0.450 mol) is added in small portions over 3 to 4 h. The mixture is kept mildly alkaline by addition of 300 mL 10% sodium hydrogen carbonate and then of 45 mL 4 N sodium hydroxide, the final pH being 7. The remaining 2-oxo-2-phenylethanoyl chloride is removed by extraction with diethyl ether. The aqueous solution is evaporated to dryness at 35–50 °C. The remaining solid **17** is thoroughly dried under reduced pressure over phosphorus pentoxide and then mixed with 300 mL anhydrous diethyl ether. Phosphorus pentachloride (70 g, 0.336 mol) is added in small portions with stirring. The mixture is cooled to r.t. over a few hours. Then ice water is added whereupon the sulfonyl chloride **18** crystallizes immediately. The crystals are collected and dried in a desiccator over phosphorus pentoxide. The 2-[(2-phenyl-2-oxoethanoyl)amino]ethanesulfonyl chloride **18** is recrystallized from benzene/petroleum ether. Fine crystals melting at 50–51 °C are obtained.

2-[(2-Phenyl-2-oxoethanoyl)amino]ethanesulfonyl chloride **18** (68.0 g, 0.245 mol) is dissolved in 300 mL absolute methanol and zinc (16.5 g, 0.252 mol) is added with stirring and the reaction continued 3–4 h until most of the zinc has been consumed. A colorless precipitate of **19** is formed, collected, and recrystallized from methanol. Yield of zinc bis{(2-phenyl-2-oxoethanoyl)amino] ethanesulfinate]} trihydrate 82%, $R_f$ 0.79 (Whatman paper No. 1, *n*-butanol/formic acid/water, 15:3:2).

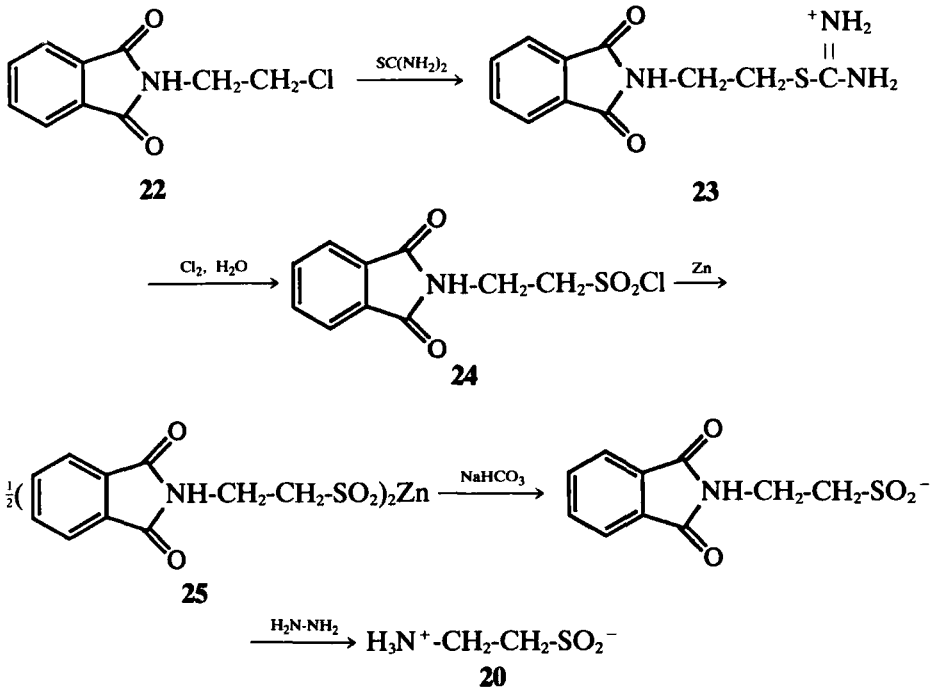
Zinc bis{[(2-phenyl-2-oxoethanoyl)amino]ethanesulfinate]} trihydrate **19** (20.0 g, 33 mmol) is added with stirring to 600–700 mL liquid ammonia and sodium added in small pieces until a permanent blue color appears. The stirring is continued for 1 h and ammonium chloride added until the blue color disappears. The ammonia is then allowed to evaporate and the free acid obtained by passing the water diluted solution (3 L) through a 100 × 2 cm ion exchange column charged with Dowex 50. Upon elution of the column with aqueous ammonia 200 mL eluent is collected which is treated with charcoal. The filtrate is evaporated to near dryness in a hydrogen atmosphere. Solid **20** is obtained by the addition of ethanol, yield 88%, and recrystallized from aqueous ethanol, $R_f$ 0.63 (Whatman paper No. 1, phenol/water, 4:1). Additional $R_f$ values have been reported.[39]




5.2. *Hypotaurine* **3**[41]

Mercury(II) sulfate (1.05 g, 3.35 mmol), dissolved in 7 mL 2.25 N sulfuric acid, is added to a stirred solution of cystamine dihydrochloride **21** (100 mg, 0.44 mmol) in 3 mL sulfuric acid at r.t. Ethanol (2 mL) is added and the precipitated mercury(II) thiolate removed by centrifugation. The volume of the supernatant is reduced by freeze drying and this solution cleared by centrifugation. Hypotaurine **20** is isolated by ion exchange chromatography on a 10 × 0.6 cm Dowex 50 column. The column is successively preeluted with 2 N sodium hydroxide, 1 N hydrochloric acid, and 0.2 N aqueous ammonia. Hypotaurine is eluted with 0.2 N aqueous ammonia and appears in a 2 to 3 mL range after elution of 50 to 100 mL. The yield at this stage is 56 to 92 %. Crystalline **3** is obtained by freeze drying of the eluate and crystallization from water/ethanol/diethyl ether, $R_f$ 0.25 (ethanol/isopropanol).[42]





5.3. *Hypotaurine* **20**[43]

2-(Phthalimido)-1-chloroethane[44] **22** (1.045 g, 5 mmol) and thiourea (0.380 g, 5 mmol) in 2 mL 90–95 °C absolute ethanol (too much ethanol impedes the condensation reaction) is allowed to react for 48 h. The precipitated *S*-(2-phthalimidoethyl)isothiouronium hydrochloride **23** is crystallized and recrystallized from ethanol. The crude product is dissolved in 25 mL distilled water and chlorine added with stirring at 0 °C. The precipitate is filtered and dissolved in dichloromethane, filtered, evaporated to dryness, sublimed, and recrystallized from dichloromethane. The 2-phthalimido-1-ethanesulfonyl chloride **24** is reduced with zinc (5.1 g, 78 mmol) in anhydrous methanol and stirred for 5 h. The methanol is distilled off and water added to the residual **25**. The mixture is treated with sodium hydrogen carbonate, stirred for 2 h, and filtered. Hydrazine hydrate (0.2 mL) is added to the filtrate and the mixture allowed to stand at r.t. for 48 h. The solution is acidified and allowed to stand for 24 h at 0 °C. Then the phthalyl hydrazide is filtered off, the filtrate evaporated to dryness, dissolved in the minimum volume of 20 N hydrochloric acid, and eluted from a 20 × 2 cm ion exchange column charged with Dowex 50 with 700–800 mL 20 N hydrochloric acid and then 450–500 mL water. The hypotaurine **20** is found in the 100 to 150 mL range upon subsequent elution with 1 N aqueous ammonia. Yield 70–75%, $R_f$ 0.60 [Whatman paper No. 1, phenol/water (4:1)].

$$6\ H_2N\text{-}CH_2\text{-}CH_2\text{-}SH \xrightarrow{HCl,\ H_2O_2,\ KI}$$

**26**

$$3\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SO_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}{}^+NH_3 \xrightarrow{NaOH}$$

**27**

$$(H_2N\text{-}CH_2\text{-}CH_2\text{-}S)_2 + 4\ H_2N\text{-}CH_2\text{-}CH_2\text{-}SO_2^- \xrightarrow{H_2O} 4\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SO_2^-$$

**20**

5.4. *Hypotaurine* **20**[45]

Cysteamine **26** (3.08 g, 40 mmol) is dissolved in 25 mL 2 N hydrochloric acid and 6 mL (53 mmol) 30% hydrogen peroxide and 50 mg potassium iodide is added with shaking. The solution turns hot and a strong iodine color appears. After 15 min the oxidation is complete and the mixture containing **27** is placed on a boiling water bath. The solution is cooled to r.t. over 45 min and 20 mL 2 N sodium hydroxide added. The solution is left to stand for 10 min and then loaded on a 2.5 × 30 cm cation exchange column charged with Dowex 50 X 4 (200–400 mesh) in the $H^+$ form. The column is washed with distilled water (ca. 250 mL) until the eluent is neutral and then eluted with 1 N aqueous ammonia, the eluate being collected in 10 mL fractions until 6–8 fractions after the alkaline front. Hypotaurine **20** appears generally in fractions no. 3–5, the first of these appearing before the alkaline front. Hypotaurine is identified with the perman-

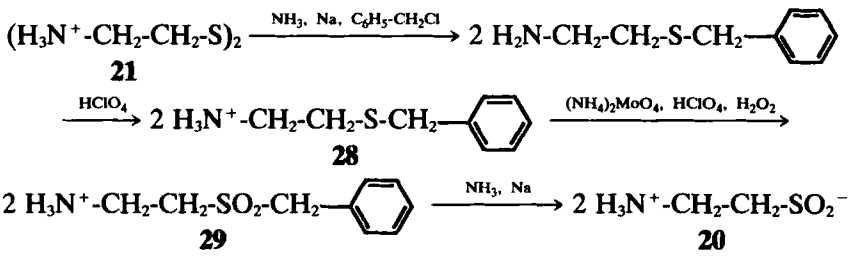
ganate test.[a] The permanganate positive fractions are combined and passed through a 2.5 × 20 cm ion exchange column charged with Amberlite IRC (40–80 mesh) in the $H^+$ form to remove ammonia and traces of cystamine. The eluent is collected in 10 mL fractions until 25 fractions are obtained and tested for **3**. The **3** containing fractions are pooled and evaporated under reduced pressure on a boiling water bath. Colorless or pale yellow semicrystals of **3** are obtained. Yield 45%, $R_f$ 0.65 (Whatman paper No. 4, saturated aqueous phenol).

$$\underset{\textbf{27}}{H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SO_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}{}^+NH_3} \xrightarrow{\text{Amberlite IRA}}$$

$$(H_2N\text{-}CH_2\text{-}CH_2\text{-}S)_2 + 4\ H_2N\text{-}CH_2\text{-}CH_2\text{-}SO_2^- \xrightarrow{H_2O} \underset{\textbf{20}}{4\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SO_2^-}$$

5.5. *Hypotaurine* **20**[46]

2-Aminoethyl 2-aminoethanethiolsulfonate dihydrochloride **27**[47] (2.58 g, 10 mmol) in 20 mL water is added during 30 min to a stirred suspension of 50 mL (0.041 equiv.) Amberlite IRA-400 in the $OH^-$ form in 100 mL water at 20 °C. The suspension is transferred to a separatory funnel, drained, and washed with 100 mL water. The aqueous washings are discarded and the resin bed washed with 100 mL 10% acetic acid (flow 10 mL/min). Evaporation of the aqueous acetic acid under reduced pressure and repeated addition and evaporation of absolute ethanol gives crystalline **20** which is triturated with ethanol and collected by filtration. Yield 73%, m.p. 180–180.5 °C.




[a]The elution can easily be followed by dropping the eluate into a small volume of potassium permanganate. A mixture of 10 mL 0.1 N potassium permanganate and 90 mL 1 N sulfuric acid is prepared. To 0.5 mL of this solution two drops of the eluent is added. Immediate decolorization indicates the presence of hypotaurine.

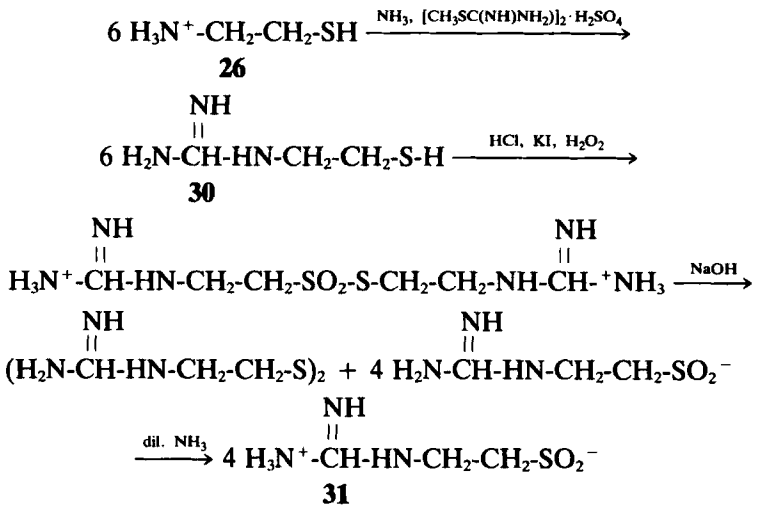
5.6. *Hypotaurine* **20**[21]

Cystamine dihydrochloride **21** (22.52 g, 0.100 mol) is dissolved in liquid ammonia and small pieces of sodium (9.66 g, 0.420 mol) added with mechanical stirring until a blue color persists for 20 min. Benzyl chloride (25.32 g, 0.200 mol) is added in 2 mL portions during 15 min. The stirring is discontinued and the remaining ammonia removed under reduced pressure at 50 °C. The residue is dissolved in 150 mL water and extracted with 600 mL diethyl ether. The diethyl ether extract is evaporated to leave an oil which is emulsified with 15 mL water and neutralized with 43.5 mL (0.130 mol) 30% w/v perchloric acid. The mixture is filtered, the salt washed with water, and dried in a desiccator over anhydrous calcium chloride and concentrated sufluric acid. Yield 93%, m.p. 156–159 °C. The dry *S*-benzylcysteamine perchlorate **28** is recrystallized from boiling water, 89% recovery, m.p. 158–159 °C, $R_f$ 0.86 (Whatman paper No. 4 with *n*-butanol/acetic acid/water).

Ammonium molybdate (0.60 g, 4.05 mmol) is finely ground, suspended in 25 mL water, 5 mL 60% w/v perchloric acid added, and the solution boiled for 5 min. The mixture is filtered and to the filtrate *S*-benzylcysteamine perchlorate **28** (30.0 g, 0.116 mol) is added with cooling in an ice bath. Hydrogen peroxide (30% w/v, 45 mL, 0.400 mol) is added dropwise to the thick suspension and crystallization occurs during 1 h in the ice bath. Water (50 mL) is added and the mixture kept overnight at 4 °C. The colorless crystals are filtered off, washed with 100 mL water, 100 mL ethanol, and 50 mL diethyl ether, and then dried in a desiccator over anhydrous calcium chloride. The dry *S*-benzylcysteamine sulfone perchlorate **29** (yield 86%, m.p. 216–217 °C) is recrystallized from hot water (2.0 g in 10 mL) with 88% recovery, m.p. 224–228 °C, $R_f$ 0.72 (Whatman paper No. 4 with *n*-butanol/acetic acid/water).

*S*-Benzylcysteamine sulfone perchlorate **29** (20.0 g, 67 mmol) is dissolved in 450 mL liquid ammonia and reduced with small pieces of sodium (3.22 g, 0.140 mol) until a persistent blue color appears. Excess sodium is destroyed with 1 mL glacial acetic acid and the ammonia allowed to evaporate, the last traces under reduced pressure. The colorless residue is redissolved in 150 mL water and loaded on a 4 × 41 cm cation exchange column charged with Dowex AG 50 X 8 (200–400 mesh) in the $H^+$ form. Elution with 310 mL water removes the perchloric acid whereafter the elution continues with 830 mL 2 N aqueous ammonia. The alkaline eluent is collected and concentrated under reduced pressure, leaving ca. 10 mL of an oil which partly crystallizes upon cooling. Methanol (20 mL) is added, the crystals of **20** collected, washed with 6 mL methanol, 5 mL diethyl ether, and dried under reduced pressure over anhydrous calcium chloride and sulfuric acid. Yield 81%, $R_f$ 0.34 (Whatman paper No. 4 with *n*-butanol/acetic acid/water), m.p. 180–181 °C, $pK_a$ 2.16, 9.56.[22]

## 6. SYNTHESIS OF HYPOTAUROCYANAMINE



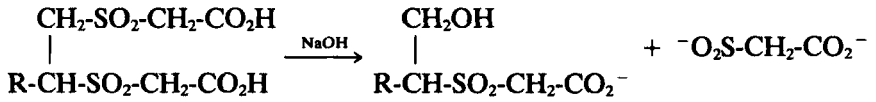

6.1. *Hypotaurocyanamine* **26**[48]

Cysteamine hydrochloride **26** (1.43 g, 12.5 mmol) is dissolved in 10 mL deionized and nitrogen saturated water. Concentrated aqueous ammonia (2 mL) and *S*-methylisothiouronium hydrogen sulfate (1.90 g, 6.83 mmol) is added. The solution is left for 30 min at r.t. and then evaporated to dryness. The residual **30** is dissolved in the minimum volume of water and loaded on a 45 × 1.5 cm ion exchange column charged with Dowex 5 X 2 and preequilibrated with deionized, nitrogen saturated water. The thiol is attached to the resin by elution of the column with $10^{-4}$ M EDTA and $10^{-3}$ M dithiothreitol. The thiol is eluted with 0.5 N aqueous ammonia and collected in 5 mL fractions. The product containing fractions are mixed, evaporated, and freeze dried. Yield of crude (2-mercaptoethyl)guanidine **30** 86%.

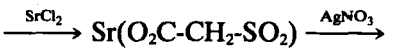
(2-Mercaptoethyl)guanidine **30** (1.19 g, 10 mmol) is dissolved in 6 mL 2 N hydrochloric acid. Potassium iodide (12 mg) and 1.44 mL (13 mmol) 30 % hydrogen peroxide is added. After a few minutes a brown color appears due to the formation of iodine. After 15 min standing the mixture is placed for 2 min on a water bath and then cooled to r.t. Then 5 mL 2 N sodium hydroxide is added and the reaction allowed to proceed for 10 min. The reaction mixture is purified on a 47 × 1.1 cm ion exchange column charged with Dowex 50 X 2. The column is preeluted with deionized, nitrogen saturated water and washed with the above-mentioned EDTA and dithiothreitol solutions. A solution of 0.5 N aqueous ammonia elutes the sulfinic acid. The **31** containing fractions are concentrated to dryness and the residue recrystallized three times from deionized, nitrogen saturated water to yield 45% **31**, m.p. 187–188 °C.

## 7. SYNTHESIS OF SULFINOACETIC ACID

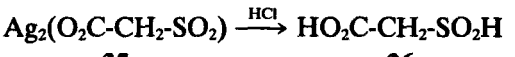
R = H, $CH_3$



**32, 33**



**34**



**35** **36**

7.1. *Sulfinoacetic Acid* **36**[49,50]

1,2-Bis(carboxymethylsulfonyl)ethane **32**[49] (10.0 g, 36 mmol) or 1,2-bis(carboxymethylsulfonyl)propane **33**[49] (10.5 g, 36 mmol) is suspended in 140 mL water. Addition of 1.5 equivalents 4 N sodium hydroxide leads to a clear solution. After 24 h the solution is neutralized with hydrochloric acid and strontium chloride (10 g, 63 mmol) in the minimum volume of water is added. Acetone (260 mL) is added, first dropwise and under vigorous shaking until the solution becomes turbid, then the rest during 1 h, and the suspension let stand overnight. The precipitated **34** is filtered off and recrystallized twice (1 g salt in 30 mL water) with 90% recovery.

The sulfinoacetic acid strontium salt **34** [$Sr(O_2CCH_2SO_2)$] (8.4 g, 40 mmol) is treated with a silver nitrate solution at 30–40 °C to give the silver salt of sulfinoacetic acid **35** [$(Ag_2(O_2CCH_2SO_2)$], yield 93%. The free acid **36** is obtained by treatment of a suspension of the silver salt in acetone with the calculated amount of anhydrous gaseous hydrogen chloride at −70 °C. For the removal of silver chloride the mixture is filtered *in vacuo*. To avoid decomposition sodium hydroxide can be added in order to convert the acid to its sodium salt.

## 8. RADIOLABELED NATURALLY OCCURRING SULFINIC ACIDS

Six radiolabeled naturally occurring sulfinic acids have been synthesized, three labeled cysteinesulfinic acids and three labeled hypotaurines. Cysteinesulfinic acids: [3-$^{14}$C]-L-(−)-cysteinesulfinic acid [84888-70-0],[51,52] [1-$^{14}$C]-L-(−)-cysteinesulfinic acid [13270-19-4],[51,53,54] and [$^{35}$S]-L-(−)-cysteinesulfinic acid [80251-78-1].[2,34,54,55] Hypotaurines: [1-$^{14}$C]-hypotaurine [84888-77-7],[51] [2,2-$^{3}H_2$]-hypotaurine [79315-35-8],[56] and [$^{35}$S]-hypotaurine [2742-26-9].[35,41,43,57]

The radiolabeled sulfinic acids have been used to elucidate the metabolism of cysteinesulfinic in mice[2,51,57] and the mechanism of the transamination of hypo-

taurine and taurine in mammalian tissue and microorganisms.[56] Much attention has also focused on the bioconversion of hypotaurine to taurine.[36,41,43]

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