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THIOETHER DERIVATIVES OF CYSTEINE AND HOMOCYSTEINE[1]

MARVIN D. ARMSTRONG AND JOY D. LEWIS

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Several thioether derivatives of cysteine and homocysteine were prepared in the course of a study of the biological activity of certain compounds which are related structurally to the thioether amino acid, cystathionine. Since portions of three different homologous series of derivatives for each amino acid were needed, it was of interest to enlarge each series and to observe the variations in physical properties of the compounds with structural changes. This paper reports the methods used for the preparation and purification of these amino acid derivatives and observations made on the properties of the pure thioethers.

EXPERIMENTAL

L-*Cystine* from commercial sources was of satisfactory purity to use as a starting material for the preparation of the derivatives of cysteine; *S-benzyl*-D- or L-*homocysteine*, prepared by resolution of the racemic mixture by the method of du Vigneaud and Patterson (1), was used as starting material for the preparation of the optically active homocysteine compounds.

Three general methods of preparation were used in this work. Representative examples are given below for each method. Most of the compounds were prepared *via* method A, since it proved to be more convenient for use on a small scale; method B was avoided because of the danger that the optically active compounds would be racemized by the alkaline conditions necessary to effect the condensation. Method C was useful for the preparation of large amounts of S-benzylhomocysteine from methionine; S-benzylhomocysteine was preferable to methionine as a starting material for the derivatives of homocysteine.

A. S-Ethyl-L-*cysteine*. According to the method of du Vigneaud, Audrieth, and Loring (2), 12 g. of L-cystine was reduced with approximately 4.6 g. of sodium by the alternate additions of small portions of each to 500 ml. of liquid ammonia. Then 12 g. of ethyl bromide was added in one portion to the solution and stirring was continued until the ammonia had evaporated. The residue was left overnight in a vacuum desiccator over sulfuric acid and was then taken up in 80 ml. of cold water, treated with Norit, and filtered. The filtrate was warmed, adjusted to *p*H 5 by the addition of conc'd hydrochloric acid, and the crystals that separated after the solution cooled were collected. Yield, 8.7 g.; an additional 2.5 g. was obtained by reworking the mother liquors. Total yield, 11.2 g. (75%).

B. S-Butyl-L-*cysteine*. The procedure of Stoll and Seebeck (3) was followed; 15.8 g. of cysteine hydrochloride was dissolved in 225 ml. of 2 *N* sodium hydroxide and 180 ml. of ethanol. The stirred solution was cooled to 25°, 27.4 g. of *n*-butyl bromide was added, and stirring was continued overnight. Conc'd hydrochloric acid was added to bring the solution to *p*H 2, it was concentrated *in vacuo* to dryness, and the product was extracted with two portions of hot absolute ethanol. The combined ethanol extracts were concentrated to dryness, the residue was taken up in 300 ml. of water, and the solution was adjusted to *p*H 5. The product was collected, rinsed with cold water, then with ethanol, and was dried. Yield, 16.5 g. (93%).

C. S-Carboxymethyl-DL-*homocysteine*. This method follows the procedure reported by

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Dekker and Fruton (4) for the conversion of methionine to S-benzylhomocysteine. DL-Methionine (15 g.) and 9.8 g. of chloroacetic acid were dissolved in 200 ml. of conc'd hydrochloric acid and the resulting solution was refluxed 24 hours. It was then concentrated *in vacuo* to a sirup, and the sirup was dissolved in 100 ml. of warm water; this solution was adjusted to *p*H 4, and the crystalline product which separated was washed with water, with absolute ethanol, and was dried. Yield, 14.1 g. (73%).

The starting materials, yields, analyses, and physical constants of the cysteine derivatives are listed in Table I and of the homocysteine derivatives in Table II. Unless otherwise noted in the tables the compounds were prepared by method *A*. The same halogen compounds were used in the preparation of corresponding derivatives of the two amino

TABLE I

THIOETHER DERIVATIVES OF L-CYSTEINE

S-DERIVATIVE	HALOGEN COMPOUND	YIELD, %	M.P., °C.	Rotations		Analysis			
				$(\alpha)^{24}_D$	M°_D	N Calc'd	N Found	S Calc'd	S Found
Methyl	Iodide	75	247–248 d.[a]	−9.6°[b]	−12.9	10.36	10.21	23.72	23.60
Ethyl	Bromide	75	254–256 d.[c]	−6.2°[d]	−9.2	9.39	9.35	21.49	21.53
n-Propyl	Bromide	74	243–245 d.	−3.1°	−5.0	8.58	8.36	19.63	19.55
n-Butyl	Bromide	84	242–244 d.[e]	−0.2°[f]	−0.4	7.92	7.66	18.09	18.12
n-Amyl	Bromide	81	240–241 d.	+2.4°	+4.6	7.33	7.11	16.71	16.64
Benzyl	Chloride	76	222–225 d.[g]	−1.9°[h]	−4.0	6.63	6.46	15.18	14.63
β-Phenylethyl	Bromide	87	222–224 d.	+3.5°	+7.9	6.22	6.08	14.23	13.81
γ-Phenylpropyl	Bromide	81	222–224 d.	+4.5°	+10.8	5.85	5.88	13.39	13.49
Carboxymethyl*	Chloride	71	204–207 d.[i]	+0.5°	+0.9	7.82	7.82	17.89	17.88
β-Carboxyethyl	Chloride	55	227–230 d.[j]	−7.0°[k]	−13.5	7.25	7.06	16.59	16.33
γ-Carboxypropyl	Bromide	57	239–243 d.	−1.3°	−2.7	6.76	6.77	15.47	15.48
Carbamidomethyl	Chloride	85	188–190 d.	−6.0°	−10.7	15.72	15.20	18.00	17.98

* Prepared by method *B*. [a] M.p. 248° d. (7). [b] $(\alpha)^{20}_D$ −32° (*c*, 1, water) (7). [c] M.p. 226–228° (8), 228–230° (9), 260° (10). [d] $(\alpha)^{24}_D$ −3.8° (*c*, 2.8, *N* HCl) (10). [e] M.p. 230° d. (3). [f] $(\alpha)^{20}_D$ +9.0° (*c*, 1, water) (3). The conditions reported for this value must be an error; we observed $(\alpha)^{24}_D$ −13.6° (*c*, 0.5, water) and $(\alpha)^{24}_D$ +9.7° (*c*, 1, *N* NaOH). The compound is not soluble to the extent of 1% in water at ordinary temperatures. [g] M.p. 215–216° d. (10). [h] $(\alpha)^{24}_D$ −0.3° (*c*, 3, *N* HCl) (10). [i] M.p. 191–192° (11) [j] M.p. 214–216° (12). [k] $(\alpha)^{16}_D$ −8.19° (*c*, ?, *N* HCl) (12).

acids and the yields of the optically active derivatives of homocysteine obtained were approximately the same as those noted for the racemic ones.

DISCUSSION

All of the derivatives are white crystalline compounds which can be recrystallized from water. It was convenient to add a slight amount of hydrochloric acid to aid in dissolving the more insoluble compounds and to crystallize them by adjusting the hot solution to *p*H 5 by the dropwise addition of conc'd ammonium hydroxide. The more soluble compounds, particularly the carbamidomethyl derivatives, were recrystallized by dissolving them in the minimum amount of hot water, adding 1–2 volumes of hot absolute ethanol, and allowing the hot solution to cool slowly.

They were all rendered disulfide free by the treatment of an ammoniacal solu-

TABLE II
THIOETHER DERIVATIVES OF HOMOCYSTEINE

S-DERIVATIVE	DL-HOMOCYSTEINE						L-HOMOCYSTEINE				
	YIELD, %	M.P., °C.	Analysis				M.P., °C.	Rotations		Analysis	
			N Calc'd	N Found	S Calc'd	S Found		$(\alpha)^{24}_D$	M°_D	N Found	S Found
Methyl[n]	—	267–269 d.[a]	—	—	—	—	277–279 d.[b]	+23.4°[c]	+34.8	—	—
Ethyl	78	257–260 d.[d]	8.58	8.67	19.63	19.90	272–274 d.	+25.1°[e]	+40.9	8.40	19.74
n-Propyl	75	250–252 d.[f]	7.92	7.98	18.09	18.46	261–263 d.	+24.5°	+43.4	8.20	18.58
n-Butyl	78	244–246 d.[g]	7.33	7.29	16.71	17.15	253–255 d.	+22.8°	+43.6	7.10	16.72
n-Amyl	81	241–243 d.[h]	6.82	6.62	15.62	15.30	248–250 d.	+21.9°	+44.9	6.84	15.59
Benzyl	48[o]	244–246 d.[i]	6.22	6.02	14.23	13.71	244–246 d.[j]	+24.5°[k]	+55.1	—	—
β-Phenylethyl	68	221–223 d.	5.85	5.81	13.39	13.37	221–224 d.	+22.7°[p]	+54.3	5.87	13.44
γ-Phenylpropyl	83	232–234 d.	5.53	5.61	12.66	12.81	231–234 d.	+15.0°[p,r]	+38.0	5.66	12.36
Carboxymethyl[q]	65	224–226 d.[l]	7.25	6.95	16.59	16.71	232–234 d.	+21.2°	+40.9	7.06	16.05
β-Carboxyethyl[q]	67	227–230 d.[m]	6.76	6.53	15.47	15.66	245–247 d.	+23.1°	+47.8	7.05	15.73
γ-Carboxypropyl	63	208–210 d.	6.33	6.09	14.49	14.49	225–227 d.	+17.6°	+39.0	6.36	14.14
Carbamidomethyl	56	224–225 d.	14.57	14.50	16.68	16.84	234–235 d.	+21.0°[p]	+40.4	14.90	16.83

[a] M.p. 281° d. (14). [b] M.p. 283° d. (15). [c] $(\alpha)^{18}_D$ +23.4° (c, 5, 6 N HCl) (16); $(\alpha)^{24}_D$ +20.7° (c, 1, 0.2 N HCl) (4). [d] M.p. 272–284° d. (17). [e] $(\alpha)^{23}_D$ +20.1° (c, 1, N HCl) (18). [f] M.p. 249° (19). [g] M.p. 254° (19). [h] M.p. 250–252° (19). [i] M.p. 240–250° d. (1). [j] M.p. 243–244° d. (4); M.p. 241–244° (20). [k] $(\alpha)^{24}_D$ +23.7° (c, 1, N HCl) (20); $(\alpha)^{24}_D$ +27.2° (c, 1, N HCl) (4). [l] M.p. 218–222° (21). [m] M.p. 221–222° (21). [n] Methionone was resolved using the procedure of Windus and Marvel (13). [o] Prepared by Method C. [p] The D forms of these compounds were prepared. The values included in this table are opposite in sign to that actually observed for the D-isomers. [q] The optically active forms of these compounds were prepared by method C. The yields were 78% for the carboxymethyl and 53% for the carboxyethyl derivatives. [r] (c, 0.5% in 1 N HCl).

tion with sodium cyanide as was done by Anslow, Simmonds, and du Vigneaud (5) in their purification of cystathionine. Some concern was felt when it was noted that many of the derivatives of cysteine had very small numerical values for their rotations when they were measured in acid solution, since derivatives of cysteine and serine are known to racemize easily in alkaline solution. Measurement of the rotations of these compounds in water or in alkaline solutions gave much larger numerical rotations, however, and it was firmly established that, once freed of cystine, no further change in their rotation could be observed after repeated treatments with ammonia and sodium cyanide.

The derivatives of both cysteine and homocysteine decompose above 200°; the decomposition points are characteristic when measured under controlled conditions, *i.e.*, heating the melting point block rapidly to a temperature about 20° less than the melting point and raising the temperature 5°/minute thereafter until decomposition occurs. The decomposition points of most of the optically active derivatives of homocysteine are several degrees higher than those of the corresponding inactive compounds, indicating that the inactive compounds are probably racemic mixtures rather than racemic compounds.

The alkyl derivatives of both cysteine and homocysteine all have characteristic odors when pure; their odors are reminiscent of the corresponding alkyl mercaptans. The carboxyalkyl and carbamidomethyl derivatives are odorless. Both carbamidomethyl compounds have a faintly sweet taste similar to that of glutamine, which they structurally resemble, while the carboxyalkyl derivatives of both amino acids have an acidic taste. The alkyl derivatives of homocysteine have only slight tastes; the D-forms are slightly sweet and the L-forms are either slightly bitter or tasteless immediately upon tasting and may develop a very slight sulfide-like taste after a few minutes.

The tastes of the alkyl derivatives of cysteine are quite characteristic and present a distinct phenomenon. The compounds have no strong taste immediately when they are placed on the tongue, but an intense sulfury, and characteristic taste develops within one or two minutes and in some cases the taste persists to a marked degree for as long as 12 hours. This is particularly true of β-phenylethyl- and γ-phenylpropyl-L-cysteine. There is no immediate taste from these compounds but after about a minute an intense mercaptan taste develops in the mouth, and the breath possesses a similar odor. As little as 1 milligram of γ-phenylpropyl-L-cysteine may be tasted in this way for as long as 12 hours. It is interesting to note that the odor of both β-phenylethyl- and γ-phenylpropyl-L-cysteine manifests itself as a taste in the back of the palate rather than as an odor. Since it has been reported that methyl-, ethyl-, propyl-, and butyl-L-cysteine are all acted upon by an enzyme to yield the corresponding alkyl mercaptans (6), it is interesting to conjecture that a similar enzyme system located in the tissues of the mouth may effect a slow splitting of these alkyl cysteines to mercaptans, which then exert a strong taste and may even be detected in the exhaled breath.

The group of 24 closely related amino acid derivatives reported here represents the greatest number of optically active amino acids yet prepared whose optical rotations have been measured under comparable conditions. It was hoped that some regularity in the observed rotations would be noted which could be

related to a structural change. The rotation of each compound was measured for a 1% solution in 1 N hydrochloric acid; these conditions offer many advantages for the measurement of rotations for characterization of amino acid derivatives.

One observation that can be made in the case of derivatives of both cysteine and homocysteine is that there does not appear to be any advantage in the use of molar rotations rather than specific rotations for relating structure to optical rotation. This is especially marked in the series of homocysteine thioethers where the specific rotations of all the derivatives so far studied are not only positive in sign, but have approximately the same numerical value. If the γ-phenylpropyl- and γ-carboxypropyl derivatives are omitted, the remaining ten compounds, varying in molecular weight from 149 to 239, all have specific rotations of $+23° \pm 2°$.

A different behavior is seen with the derivatives of cysteine. The carboxyalkyl derivatives show no regularity, but a regular change toward a more positive rotation is seen with increasing chain length for both the alkyl and the ω-phenylalkyl thioethers. A discussion of the possible significance of the regularities and irregularities in the rotation of the cysteine derivatives and the constancy of the rotations of the homocysteine derivatives will be made in a future communication.

The microanalyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

SUMMARY

Several thioether derivatives of the amino acids cysteine and homocysteine have been prepared. Methods for their synthesis and purification are described and their physical properties are reported.

SALT LAKE CITY, UTAH

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