uble in ether. Crystallization from ethyl acetate gave pale yellow needles, m. p. 124.4–125° dec.

Anal. Calcd. for $C_{18}H_{21}O_3N_3$: C, 66.04; H, 6.47. Found: C, 65.63; H, 6.63.

In order to test for the presence of 5,6-dimethoxy-1-hydrindone in the acylation experiment, a sample of the crude product was converted to the semicarbazone. The derivative was formed in 96% yield, and although the m. p. (115–120°) indicated some impurity the product was completely soluble in ether indicating the absence of the ether-insoluble semicarbazone of the dimethoxyhydrindone.

In order to discover if 3,4-dimethoxybenzylacetophenone is convertible to 5,6-dimethoxy-1-hydrindone, 1.00 g. of the former was treated with 1.53 g. of aluminum chloride in benzene for twelve hours at room temperature. The mixture was worked up in the usual way yielding 0.53 g. (53% recovery) of neutral material which was fairly pure starting ketone, m. p. 66–67.5°. The remainder of the material was phenolic and appeared in the potassium hydroxide washings. Methylation with dimethyl sulfate gave 0.46 g. (46%) of tan ketone, m. p. 67–68.5° undepressed on admixture with 3,4-dimethoxybenzylacetophenone.

Summary

The behavior of nine acids of varying susceptibility to ring closure has been studied in connection with a cyclization procedure involving an inverse Friedel–Crafts technique in which a solution of the acid chloride in benzene is added to a suspension of aluminum chloride in benzene. The yields of cyclic ketones were generally excellent, except in the case of 3,4-dimethoxyphenylpropionic acid which was shown to effect intermolecular acylation of the solvent giving 3,4-dimethoxybenzylacetophenone. This unusual behavior may be attributed to an inhibition of resonance by coördination of the catalyst with the methoxyl groups. The weaker coördinating agent, stannic chloride, effects intramolecular acylation giving the cyclic ketone.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

The Synthesis of DL-Threonine.* I. From α-Bromo-β-methoxy-*n*-butyric Acid and Derivatives

BY KARL PFISTER, 3RD., E. E. HOWE, C. A. ROBINSON,[1a] A. C. SHABICA,[1b] E. W. PIETRUSZA[1c] AND MAX TISHLER

Of all the known essential α-amino acids DL-threonine is the most difficult to isolate and to synthesize. The increasing demand for mixtures of the essential amino acids prompted us to evaluate the existing syntheses for DL-threonine with the view of developing a low cost process.

The best-known synthesis of DL-threonine consists in the conversion of crotonic acid into α-bromo-β-methoxy-*n*-butyric acid followed by amination and cleavage of the methoxy group. This sequence of reactions however is complicated by the formation of a mixture of two diastereoisomeric bromo acids, one of which leads to O-methyl-DL-threonine and the other to O-methyl-DL-allothreonine. Of the various reported methods for the preparation of α-bromo-β-methoxy-*n*-butyric acids and their simple derivatives all but one lead to mixtures too poor in the DL-threonine precursor to be useful in the synthesis of this amino acid.

Previous studies of the synthesis of DL-threonine have been handicapped by the facts that no simple separation from DL-allothreonine was known, and no method of analysis of mixtures of the diastereoisomeric amino acids had been developed. The recent description of a microbial assay for L-threonine[2] has greatly simplified the problem. With the aid of this analysis it has become possible to make direct comparisons of the various reported syntheses of DL-threonine and DL-allothreonine and to test the effect of experimental variables and modifications on the relative amounts of the two substances formed in each synthesis. The present paper reports the results of such studies, together with a new practical synthesis of DL-threonine developed in the course of the work.

Until recently the best recorded synthesis of DL-threonine was that of Carter and West.[3] In this method, crotonic acid is treated with mercuric acetate in methanol, and the addition product is brominated. The mixture of bromo acids is aminated, and the reaction products are separated by formylation and isolation of N-formyl-O-methyl-DL-threonine. The latter is converted into pure DL-threonine by boiling hydrobromic acid. The over-all yield of DL-threonine from crotonic acid is about 20%. The yield of DL-threonine from the bromo acid mixture is 30–33% of theory as determined by microbial assay.

Attempts to alter the ratio of diastereoisomeric bromo acids formed by the bromination of α-acetoxymercuri-β-methoxy-*n*-butyric acid were

* This nomenclature is in accord with the rules approved by the American Chemical Society Committee on Nomenclature, Spelling and Pronunciation, as reported by Vickery, *J. Biol. Chem.*, **169**, 237 (1947) and Crane, *Chem. Eng. News*, **25**, 1363 (1947). Throughout this paper the small capital letter prefix will be used in the amino acid sense rather than in the carbohydrate sense. For the sake of brevity however, we have deleted the subscripts which refer to serine, the fundamental substance to which amino acids that bear structural resemblance to the carbohydrates can be formally related.

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(2) Stokes, Gunness, Dwyer and Caswell, *J. Biol. Chem.*, **160**, 35 (1945); Gunness, Dwyer and Stokes, *ibid.*, **163**, 159 (1946).

(3) (a) Carter and West, *Org. Syn.*, **20**, 101 (1940); *cf.* (b) Abderhalden and Heyns, *Ber.*, **67**, 530 (1934).

unsuccessful. Bromination in sunlight[3a] or in darkness, variation of the temperature or the reaction time did not significantly affect the ratio of isomers.

It is interesting that the same ratio of bromo acids is obtained when ethyl crotonate is methoxymercurated and the addition product is brominated as when the procedure of Abderhalden and Heyns[3b] is used. This fact was established by hydrolysis of the mixture of bromo esters to the free acids, amination of this mixture, cleaving the methoxy group and estimating microbially the L-threonine content of the mixture of isomers isolated from the cleavage.

Carter and Ney[4] also prepared a mixture of the bromo acids by treatment of ethyl α,β-dibromo-n-butyrate with two moles of sodium methoxide followed by saponification of the bromo esters. According to the authors, the mixed bromo acids on amination form predominantly DL-allothreonine. Microbial assay of the amination mixture (after cleavage of the methoxy group) indicated that DL-threonine is produced from the bromo acid mixture in 20–23% yield. The same results are obtained when ethyl α-bromocrotonate is substituted for ethyl α,β-dibromo-n-butyrate in the above sequence of reactions.

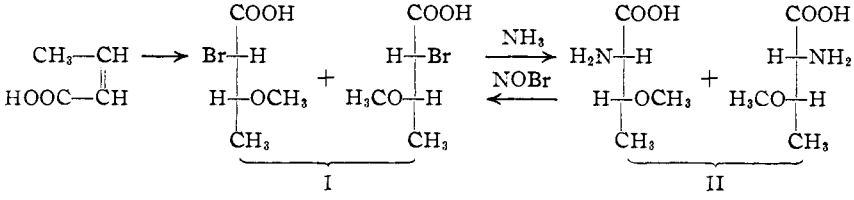
It was of interest to treat ethyl α-bromoisocrotonate with sodium methoxide since the unidirectional addition of methanol should produce a mixture of bromo acids richer in the precursor of DL-threonine.[5] The isolated mixture of amino acids, however, actually contained the same low proportion of DL-threonine as was found when ethyl α-bromocrotonate was the starting material. Both reactions presumably proceed through a common enolate ion.

The addition of the elements of methyl hypobromite to crotonic acid has been shown by West, Krummel and Carter[6] to yield essentially a pure bromo acid, which can be converted into DL-allothreonine. Microbial assays of the mixture resulting from amination of the crude bromo acid and subsequent cleavage indicated a maximum yield of 6–8% DL-threonine.

Since the addition of the elements of methyl hypobromite appears to be predominantly unidirectional, it was of interest to apply the same procedure to isocrotonic acid.[7]

Isocrotonic acid was converted in 80% yield into a crude α-bromo-β-methoxy-n-butyric acid, which on amination and hydrolysis formed DL-threonine in 52–55% yield as determined by microbial assay.[8] DL-Threonine was obtained in a 36% overall yield by isolation of O-methyl-DL-threonine as the N-formyl derivative followed by acid cleavage of the latter. From the mother liquors of the formylation step, a small amount of allothreonine was isolated.

Although the pure bromo acid (m. p. 62–63°) convertible into DL-allothreonine has been prepared,[3b,6] the preparation of the pure α-bromo-β-methoxy-n-butyric acid (I) corresponding to DL-threonine has not been reported. We prepared this compound by treatment of O-methyl-DL-threonine (II) with nitrosyl bromide. This bromo acid is lower melting (m. p. 48–50°) than its diastereoisomer and was converted into DL-threonine in 75% yield (microbial assay) or into pure N-formyl-O-methyl-DL-threonine in 61% yield. DL-Allothreonine could not be detected in the mother liquors. The pure high-melting bromo acid is also prepared by treatment of O-methyl-DL-allothreonine with nitrosyl bromide and on amination forms DL-threonine in only 2% yield (microbial assay). On the basis of these data, the bromo acid obtained from isocrotonic acid consists of about 70% low-melting acid. The presence of the higher melting acid may be due to the ease of isomerization of isocrotonic acid into crotonic acid.[9]



A summary of the amount of DL-threonine formed from the various bromo acid mixtures is presented in Table I. The recorded yields were ascertained by microbial assays of solutions of the different reaction mixtures processed by a standardized procedure described in the Experimental Section.

TABLE I

Bromo acid mixture	Yield DL-threonine, %
West, Krummel and Carter[6]	
Crude, undistilled product (77.4% yield)	6–8
Recrystallized product (m. p. 59–61°)	2–3
Carter and Ney[4]	20–23
Carter and West[3a]	30–33
From isocrotonic acid	52–55
Pure low-melting bromo acid	75

In connection with the reaction between O-methyl-DL-threonine, II, and nitrosyl bromide and

(4) Carter and Ney, THIS JOURNAL, **64**, 1223 (1942).

(5) The authors are indebted to Dr. H. R. Snyder for this suggestion.

(6) West, Krummel and Carter, *J. Biol. Chem.*, **122**, 605 (1938).

(7) This synthesis was first suggested by Carter, *ibid.*, **112**, 769 (1936), but apparently was pursued no further.

(8) The bromo acid prepared by Carter and West's procedure[3a] on amination and cleavage gave DL-threonine in 30–33% yield as established by microbiological assays.

(9) A similar situation exists with the addition of the elements of methyl hypobromite to cinnamic and allocinnamic acids.[6] The former gave exclusively one isomer, whereas the latter gave predominantly a second isomer plus small amounts of the first.

TABLE II

DERIVATIVES OF α-BROMO-β-METHOXY-*n*-BUTYRIC ACID

Derivative	Yield, %[a]	M. p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
High-melting acid						
$-NH_2$[b]	69	106–107	30.03	30.48	5.14	5.18
$-N(CH_3)_2$	80	B. p. 101–104 (6–7 mm.)[c]	37.51	37.74	6.30	6.32
$-NHC_6H_5$	79	106–107	48.54	48.24	5.18	5.19
$-NC_5H_{10}$	68	41–43	45.46	45.68	6.87	7.13
Low-melting acid						
$-NHC_6H_5$	62	87–88	48.59	48.81	5.18	5.36
$-NC_5H_{10}$	79	B. p. 115 (2–3 mm.)[d]	45.46	45.22	6.87	6.64

[a] After recrystallization or distillation. [b] *Anal.* Calcd.: N, 7.15. Found: N, 7.34. [c] $n^{20}D$ 1.4915. [d] $n^{20}D$ 1.5091.

amination of the resulting low-melting bromo acid, I, evidently a Walden inversion occurs in neither step or in both steps of the cycle. If it is assumed that the carboxyl group does not participate through β-lactone formation,[10] and that the addition of the elements of methyl hypobromite to crotonic and isocrotonic acids occurs in a *trans* fashion; then the bromo acids have the same configuration as the derived amino acids. On this basis, the amination of our bromo acid takes place with retention of configuration. The O-methyl ethers of threonine and allothreonine are similar to valine and isoleucine, which have been shown to undergo no change in configuration when subjected to the action of nitrosyl bromide followed by amination of the bromo acids.[11] Other α-amino acids thus far examined undergo a change of configuration during the cycle of bromination and amination.[12]

A possible means of bringing about inversion during the amination step and thus utilizing the readily available high-melting bromo acid for the preparation of DL-threonine was suggested by the work of Fischer and Scheibler.[11a] These authors found that although the amination of dextrorotatory α-bromoisovaleric acid produced D-valine, conversion of this bromo acid into α-bromoisovalerylglycine followed by amination and hydrolysis of the amide linkage gave L-valine. Similarly Abderhalden and Zeisset[11b] obtained L-isoleucine by amination of (+)-β-bromo-β-methylvaleric acid but L-alloisoleucine on amination and hydrolysis of the glycine derivative of the same bromo acid.

A series of amide derivatives of the two diastereomeric forms of α-bromo-β-methoxy-*n*-butyric acid were prepared and aminated. The various amides studied are listed in Table II. As indicated in Table III, the amides behave toward amination differently from the free bromo acids. DL-Threonine was formed in significant yield from each of the amides of the higher melting bromo acid. The piperidide of this acid was studied most thoroughly; since it is readily isolated in a pure state and is converted into pure DL-threonine by amination, followed by cleavage of the methoxy group and hydrolysis of the amide linkage. The yields in the amination and in the subsequent steps are good, and the method constitutes a practical synthesis of DL-threonine.

TABLE III

AMINATION OF DERIVATIVES OF α-BROMO-β-METHOXY-*n*-BUTYRIC ACID

Derivative	% Yield DL-threonine[a]	% Yield isolated threonine-allo-threonine[b]	Microbial assay of product % DL-threonine	Over-all yield DL-threonine
High-melting acid				
–OH	6–8[c]	..	...	..
$-NH_2$[d]	46	47.2	72	34
$-N(CH_3)_2$	50.5	21.1	92	19.4
$-NHC_6H_5$	55	55.2	72	39.8
$-NC_5H_{10}$[e]	63.7	58.2	100	58.2
Low-melting acid				
–OH	74–76	..		
$-NHC_6H_5$	44.8	..		
$-NC_5H_{10}$	33.6	20.2	84	17
Carter-Ney acid				
–OH	24.6	..	...	..
$-OC_2H_5$	45.1	..	...	..

[a] These figures were obtained by microbial assay of aliquots of the solutions obtained after hydrolysis of the amination products. [b] These yields are for analytically pure DL-threonine-DL-allothreonine mixtures. [c] Same result obtained whether aminated with methanol–liquid ammonia or aqueous ammonia. [d] After eight hours of hydrolysis the % yield of DL-threonine was only 42.5%. [e] Aminated twenty-four hours at 75° instead of 100° as were all other aminations.

The formation of DL-threonine from the amides of the high melting bromo acid indicates that amination occurred with inversion, and that with the piperidide the inversion was complete or nearly so. Inversion also occurs when the amides of the low melting bromo acid are aminated although the degree of inversion has not been ascertained. The anilide and piperidide of the low melting bromo acid on amination gave DL-threonine in 44.8 and 33.8% yield, respectively (micro-

(10) Tarbell and Bartlett, THIS JOURNAL, **59**, 407 (1937).

(11) (a) Fischer and Scheibler, *Ber.*, **41**, 889 (1908); (b) Abderhalden and Zeisset, *Z. physiol. Chem.*, **200**, 179 (1931).

(12) See also Fischer and Warburg, *Ann.*, **340**, 168 (1905); Fischer and Schoeller, *ibid.*, **357**, 1 (1907); Fischer, *ibid.*, **381**, 123 (1911); Fischer, *Ber.*, **39**, 2893, 2929 (1906); Fischer and Carl, *ibid.*, **39**, 3996 (1906); Abderhalden and Chang, *Z. physiol. Chem.*, **77**, 471 (1912); Senter, Drew and Martin, *J. Chem. Soc.*, **113**, 151 (1918).

bial assay), whereas the parent acid forms DL-threonine in 75% yield.

The difference in behavior of the bromo amides from the bromo acids in the ammonia replacement reaction indicates that the carboxyl ion plays a role that is suppressed when ionization is blocked. The "neighboring group" effect[13] is probably involved.

It is interesting to note that the bromo acids corresponding to threonine, valine and isoleucine have a substituent on the β-carbon atoms. This fact may be responsible for the difference in amination behavior of these α-bromo acids as compared to others. Before the nature of the stereochemical transformation can be definitely described, a kinetic study will be required to determine the reaction order.

Experimental

Amination of Bromo Acid Mixtures.—For purposes of comparison, the various bromo acid mixtures were prepared as described in the literature. Amination, hydrolysis and microbial assay as described below gave the values reported in Table I.

Five grams of bromo acid mixture in 55 cc. of concd. ammonium hydroxide was heated at 100° for two hours. This solution was concentrated to dryness under reduced pressure, and the residue was redissolved in water and again concentrated. The residue was hydrolyzed by refluxing for two hours with 17.5 cc. of 40% hydrobromic acid. A solution for microbial assay was prepared by concentrating to dryness, dissolving in water and again concentrating to remove excess acid, adjusting *p*H to neutral, filtering and diluting.

α-Bromoisocrotonic Acid.—The method of Michael and Schulthess[14] was employed for preparing α-bromoisocrotonic acid, m. p. 92–93°, in 54% yield from crotonic acid. α-Bromoisocrotonic acid is reported to melt at 92° and α-bromocrotonic acid at 106.5°.

Ethyl α-Bromoisocrotonate.—α-Bromoisocrotonic acid was esterified according to the method of Auwers and Harres[15] with ethanol in the presence of sulfuric acid. Twice distilled product boiling at 72–73.5° (12 mm.) was obtained in 57% yield. A sample of the ester on hydrolysis gave pure α-bromoisocrotonic acid, which indicated that no isomerization had occurred.

Ethyl α-Bromo-β-methoxybutyrate.—To a vigorously agitated solution of 110 cc. of methanol containing 1.4 g. (0.03 mole) of sodium methoxide, 43 g. (0.223 mole) of ethyl α-bromoisocrotonate was slowly added while the temperature was held below 25°. The reaction mixture was agitated for an additional two hours and then poured into 250 cc. of ice water containing 5-cc. of concentrated hydrochloric acid. The ester layer was separated, and the aqueous phase was extracted with three 60-cc. portions of ether. The combined ester layer and ether extracts were washed once with a saturated solution of calcium chloride and dried with sodium sulfate. The ether was removed by evaporation, and the ester was distilled at reduced pressure. A product boiling at 85–87° (18 mm.) was obtained in 79% yield (39.3 g.).

This ester after saponification, amination, and cleavage with hydrobromic acid gave in 31% yield a mixture of DL-threonine and DL-allothreonine in an 18:82 ratio (microbial assay).

Isocrotonic Acid.—This material was prepared according to the procedure of Michael and Schulthess[14] by action of phosphorus pentachloride on acetoacetic ester to produce β-chlorocrotonic (18–20% yield) and β-chloroisocrotonic (29–30% yield) acid, which are separable by steam distillation. Reduction of the latter with sodium amalgam gave isocrotonic acid in 50% yield after purification as the sodium salt and distillation. Isocrotonic acid was also prepared by conversion of β-chlorocrotonic acid into tetrolic acid[16] (20–30% yield) followed by hydrogenation with palladium on Norit (75% yield).[17] All isocrotonic acid used was carefully separated from crotonic acid via the sodium salts and then distilled, b.p. 70–71° (16–17 mm.), m. p. 12–15°.

α-Bromo-β-methoxy-n-butyric Acid from Isocrotonic Acid.—A run was made exactly as described by West, Krummel and Carter,[6] but isocrotonic was substituted for crotonic acid. The yield of crude α-bromo-β-methoxy-*n*-butyric acid obtained as an oil by concentrating the ether solution to constant weight was 79.8% of theory. When crotonic acid was used, the crude product resulted in nearly identical yield (77.4%).

Pure Low-melting α-Bromo-β-methoxy-n-butyric Acid from O-Methyl-DL-threonine.—Pure N-formyl-O-methyl-DL-threonine[3a] (100 g., 0.621 mole) was refluxed for three hours with 1 l. of 1 *N* hydrobromic acid. The resulting solution of O-methyl-DL-threonine was concentrated under reduced pressure, and the gummy residue was dissolved in 200 cc. of water and again concentrated to dryness. The residue was dissolved in 1240 cc. of 2.5 *N* sulfuric acid containing 250 g. of potassium bromide. The solution was cooled to 0° and stirred while 65.5 g. of sodium nitrite was added in small portions over a period of one and one-half hours. The solution was stirred for an hour at 0°, then heated to 25° and stirred another hour. Ether extraction followed by washing with water, drying over sodium sulfate and concentrating gave α-bromo-β-methoxy-*n*-butyric acid (113.9 g., 93.7%). Fractional distillation yielded chiefly material (101 g.) that distilled at 126–128° (6 mm.) and on cooling gave a solid of m. p. 48–50°.

Anal. Calcd. for $C_5H_9O_3Br$: C, 30.48; H, 4.61. Found: C, 30.25; H, 4.65.

N-Formyl-O-methyl-DL-threonine.—The bromo acid (45.0 g., 0.228 mole) from isocrotonic acid and 495 cc. of concentrated aqueous ammonia were heated at 100° in an autoclave for two hours. Exactly 10% of the resulting solution was concentrated to dryness; the residue was dissolved in water and again concentrated to dryness. This residue was refluxed for two hours with 15.5 cc. of 40% hydrobromic acid and the resulting solution concentrated to dryness as described above. The residue was again taken up in water and the solution diluted to 100 cc. for microbial assay. Found: 14.1 mg. of DL-threonine/cc. or a total of 14.1 g. or 51.8%. The remaining 90% of the amination mixture was concentrated to dryness, diluted with 30 cc. of water and again concentrated to dryness. A solution of the solid white residue in 101 cc. of 88% formic acid at 45° was treated dropwise with 33.8 cc. of acetic anhydride. The addition took fifteen minutes, and the temperature rose to 60°. After fifteen minutes at 60°, concentration gave a residue, which was dissolved in 40 cc. of water and again concentrated to dryness. The residue was dissolved in 35 cc. of hot water and cooled to 0° for several hours. Filtration gave crude N-formyl-O-methyl-DL-threonine of m. p. 164–168° in 51.8% yield. Recrystallization of this material from water gave N-formyl-O-methyl-DL-threonine of m. p. 176–177.5° in two crops totaling 40.8% yield from the bromo acid.

The combined mother liquors were concentrated, and the residue dissolved in 84 cc. of 40% hydrobromic acid was refluxed for two hours. The mixture was concentrated to dryness, and the dried residue was extracted with 200 cc. of ethyl alcohol. The alcoholic solution was made alkaline with concentrated ammonia, and a small precipitate of inorganic material was filtered. On standing overnight at 0°, the filtrate deposited crystalline material (8.1 g.), which was recrystallized from 10 cc. of hot water and 23 cc. of alcohol. This product (3.4 g.) was again

(13) Defined and studied by S. Winstein and his students. The more recent papers are Winstein and Seymour, THIS JOURNAL, **68**, 119 (1946); Winstein and Grunwald, *ibid.*, **68**, 536 (1946).

(14) Michael and Schulthess, *J. prakt. Chem.*, [2] **46**, 236 (1892).

(15) Auwers and Harres, *Z. physik. Chem.*, **A143**, 1 (1929).

(16) Deprez, *Bull. soc. chim.*, [3] **11**, 392 (1894).

(17) Paul and Schiedewitz, *Ber.*, **63**, 766 (1930).

recrystallized from water–alcohol to give a mixture of DL-threonine and DL-allothreonine melting at 213–214° (2.4 g., 9.8% yield).

Anal. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62. Found: C, 40.19; H, 7.59.

Since bioassay showed this material was 60% DL-threonine, the isolated yield of DL-allothreonine was 3.9%.

When the pure low melting (m. p. 48–50°) α-bromo-β-methoxy-*n*-butyric acid was subjected to the above amination steps, the yield of DL-threonine (microbial assay) was 75%. Application of the formylation step gave N-formyl-O-methyl-DL-threonine melting at 176–178° (no recrystallization) in 60.3% yield. When the mother liquors of the formylation step were hydrolyzed with hydrobromic acid, more pure DL-threonine was isolated (9% yield). No DL-allothreonine could be detected.

Amides of α-Bromo-β-methoxy-*n*-butyric Acid.—These derivatives were prepared essentially by the procedure described below for the piperidide of the high-melting bromo acid. Analyses and some physical properties of these derivatives are listed in Table II. The high melting acid employed in this work was the crude acid obtained by the procedure of West, Krummel and Carter,[6] whereas the low-melting acid was prepared from O-methyl-DL-threonine by the nitrosyl bromide reaction (b. p. 126–128° (6 mm.). The solid amides were recrystallized from benzene or Skelly-solve D.

α-Bromo-β-methoxy-*n*-butyric Acid Piperidide.—A mixture of 29.6 g. (0.15 mole) of high melting α-bromo-β-methoxy-*n*-butyric acid, 150 cc. benzene and 2 drops of pyridine was treated with 13.5 cc. of thionyl chloride and then refluxed for one and one-half hours. The mixture was concentrated under reduced pressure until 50 cc. of benzene was removed. After addition of 100 cc. of benzene, the solution was cooled to 5° and added dropwise at 4–5° over a period of one and one-fourth hours to an ice-cooled solution of 32.5 cc. (0.33 mole) of piperidine in 100 cc. of benzene. After removal of the ice-bath the mixture was stirred one-half hour before the piperidine hydrochloride was filtered.

The benzene solution was extracted with three 50-cc. portions of water and then concentrated under reduced pressure to constant weight, 36.1 g. (91.2%). The yellow oil crystallized on standing at 5° but became sticky at room temperature. The crude product was recrystallized from 20 cc. of Skelly-solve D, refrigerated overnight and filtered on a pre-cooled Buchner funnel. After being washed with a small amount of ice-cold Skelly-solve and dried, the product weighed 26.8 g. (67.7%), m. p. 41–43°. This piperidide is soluble in all the common solvents except water.

Amination of the Bromo Amides.—The amination of the bromo amides was carried out by the procedure described below for the piperidide of the high-melting bromo acid except that the reaction temperature was 100°. In every case, Volhard titration of the completed reaction mixture showed that over 98% of the organic bromine had been converted into bromide ion. A hydrolysis time of fifteen hours was found to give DL-threonine in maximum yields.

DL-Threonine.—A solution of 13.2 g. (0.05 mole) of the piperidide of the high-melting bromo acid in 60 cc. of methanol and 30 cc. of liquid ammonia was heated at 75° for twenty-four hours in a glass-lined bomb.

The solution was concentrated under reduced pressure and the residue refluxed with 40 cc. of 40% hydrobromic acid for fifteen hours. After the reaction mixture was concentrated to dryness water was added and again removed. This operation was repeated after which the residue was dissolved in 40 cc. of water and made alkaline with concentrated ammonium hydroxide solution. Piperidine was removed by extraction with ether and a sample of the aqueous solution taken for bioassay. Found: 63.7% of theoretical yield of DL-threonine.

The remaining solution was made acid to congo red with concentrated hydrochloric acid and concentrated to dryness. The residue was extracted with three 25-cc. portions of boiling isopropyl alcohol. After cooling a little more inorganic material precipitated in the isopropyl alcohol extract and was removed by filtration. Aniline (7.8 cc.) was added to the warm isopropyl alcohol solution, and the resulting slurry was stirred overnight at room temperature. The white product was filtered, washed with isopropyl alcohol and ether, and dried at 65°; yield, 3.8 g. (74.8%). The crude product was recrystallized by the addition of 45 cc. of isopropyl alcohol to a vigorously agitated solution in 19 cc. of hot water. After being chilled overnight DL-threonine was obtained (2.96 g., 58.2% from the piperidide).

Anal. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.03; H, 7.70; N, 11.96.

Microbial assay indicated this material was 100% pure, while solubility analysis showed a purity of over 97%.

Acknowledgment.—We are indebted to Mr. R. N. Boos for the microanalytical data and to Dr. J. L. Stokes for the microbial assays.

Summary

1. An evaluation of the previously described α-bromo-β-methoxy-*n*-butyric acid mixtures for the preparation of DL-threonine has been made.

2. DL-Threonine has been prepared from isocrotonic acid in substantially higher yields than from crotonic acid through the corresponding α-bromo-β-methoxy-*n*-butyric acids.

3. Evidence indicating that a Walden inversion does not occur during the amination of α-bromo-β-methoxy-*n*-butyric acid is presented. Amination of the amides of the bromo acid takes place with inversion.

4. A practical synthesis of DL-threonine from the piperidide of the readily available, high-melting α-bromo-β-methoxy-*n*-butyric acid is described.

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