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15. *Preparation of* L-*Threonine. Interconversion of the Four Stereoisomeric α-Amino-β-hydroxybutyric Acids.*

By D. F. Elliott.

Resolution of *trans*-DL-2-phenyl-5-methyl-Δ^2-oxazoline-4-carboxylic acid with brucine affords the L-form (I) of the acid as a crystalline brucine salt. The D-form (IV) remaining in the mother-liquors was also converted into (I) by a stepwise inversion process. Almost all of the racemic acid was thus transformed into the L-form. Hydrolysis of (I) gave pure L-threonine in 22% overall yield from hippuric acid. A cycle of asymmetric transformations has been worked out whereby any one of the four stereoisomers of α-amino-β-hydroxybutyric acid may be prepared from (I). The configurations of the two *allo*threonines have been determined by reference to L-threonine.

In a previous paper (Elliott, *J.*, 1949, 589) it was shown that both stereoisomeric DL-forms of ethyl 2-phenyl-5-methyl-Δ^2-oxazoline-4-carboxylate gave, on hydrolysis with aqueous alkali, the *trans*-acid which is configurationally related to threonine. As a result of this discovery the overall yield of DL-threonine obtained in the synthesis from hippuric acid (Attenburrow, Elliott, and Penny, *J.*, 1948, 310) was considerably increased. It was apparent from the data accumulated in this work that it should be possible to evolve a cycle of asymmetric transformations for the interconversion of the four stereoisomers of α-amino-β-hydroxybutyric acid (cf. Elliott, *Nature*, 1948, **162**, 657). The conversion of D-threonine into its optical antipode was particularly desirable. Realisation of these possibilities depended on the asymmetry of the molecule being retained during the cyclisation of ethyl α-benzamido-β-hydroxybutyrate with thionyl chloride which was described in a previous paper (Elliott, 1949, *loc. cit.*). Owing to the presence of two asymmetric centres it was not possible to obtain unequivocal evidence on this point by working with the racemic substances. It could always be argued that the inversion

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took place through an optically inactive intermediate by destruction, followed by regeneration, of both centres of asymmetry.

Resolution of DL-*N*-formyl-*O*-methylthreonine has been described by West and Carter (*J. Biol. Chem.*, 1937, **119**, 109); this compound is not easily accessible from threonine itself, but only from crotonic acid. The main aim of the present investigation being to find a convenient synthesis of L-threonine from hippuric acid with the minimum number of steps it was desirable to resolve one of the intermediate compounds. This task proved to be exceedingly difficult. DL-*N*-Benzoylthreonine received a great deal of attention with complete lack of success. It gave non-crystalline salts with brucine, quinine, cinchonine, and ψ-ephedrine, whereas with 1-phenylethylamine it gave sparingly soluble diastereo-compounds in a variety of solvents. DL-N-*Formyl*-O-*benzoylthreonine* prepared from DL-*O*-benzoylthreonine in the usual way also gave a diastereo-compound with (−)-1-phenylethylamine. Mild alkaline hydrolysis of DL-*N*-formyl-*O*-benzoylthreonine gave DL-N-*formylthreonine* which was a very soluble compound and could not be resolved. Various other derivatives were tried without success. At this point the choice was becoming limited and it was decided to investigate the resolution of *trans*-DL-2-phenyl-5-methyl-Δ^2-oxazoline-4-carboxylic acid (VII). This compound was the last to be considered because of its instability; it was known to be sensitive to dilute mineral acid (Elliott, 1949, *loc. cit.*). Considerable time was devoted to the resolution of the oxazoline-carboxylic acid with (+)- and (−)-1-phenylethylamine because it would then have been possible to obtain both forms in a state of purity. Although partial success was achieved this resolution was abandoned when it was found that recrystallisation of the salts, even in anhydous solvents, resulted in fairly extensive decomposition. This was attributed to an attack on the cyclic imino-ether group by the primary amine to produce an open-chain amidine derivative (cf. Barber, Gregory, Slack, Stickings, and Woolman, *CPS.* 66, May 24th, 1944). It was obvious that only tertiary bases could be used with safety in this case. (−)-Ethyl-1-phenyldimethylamine (Snyder and Brewster, *J. Amer. Chem. Soc.*, 1949, **71**, 291) did not give a crystalline salt with the oxazoline-carboxylic acid.

Success was finally achieved with brucine as the resolving base. Fortunately only one of the diastereoisomeric salts crystallised and this was extremely well defined. It was quite stable in anhydrous solvents, possessed very satisfactory solubility properties, and was formed in high yield. Furthermore, the acid combined with the brucine was trans-L-2-*phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylic acid* (I), because it yielded L-threonine on acid hydrolysis. It then remained to test out the ideas concerning the interconversion of the stereoisomeric forms which were mentioned above. The brucine salt remaining in solution was decomposed with dilute alkali and chloroform, and the crude D-oxazolinecarboxylic acid (IV) converted in solution without isolation into *N*-benzoyl-D-threonine (V) through the *O*-benzoyl derivative. Polarimetric investigation revealed that not less than 70% of the crude product was the D-form. The benzoyl compound was esterified with diazomethane and cyclised with thionyl chloride at 0°. The oxazoline ester obtained had a high optical rotation; this ruled out the possibility that the crotonic acid derivative, $CHMe{:}C(NH{\cdot}CO{\cdot}Ph){\cdot}CO_2Me$ was an intermediate in the cyclisation. Although this had always been considered remote, it is the first time that definite evidence has been obtained on this point. Previous work has shown that the action of thionyl chloride on ethyl α-benzamido-β-hydroxybutyrate causes cyclisation with inversion at $C_{(\beta)}$. The oxazoline ester obtained from impure (V) must therefore have consisted of not less than 70% of the *cis*-D-form (VI), the remainder being the *cis*-L-form (III). According to experiments described in a previous paper (Elliott, 1949, *loc. cit.*) mild hydrolysis of this mixture with aqueous alkali should have caused mutarotation at $C_{(\alpha)}$, thus giving the corresponding *trans*-forms. This was not the case. A complex mixture resulted from which only a small quantity of the *trans*-L-form (I) was isolated as the brucine salt. DL-*N*-Benzoyl*allo*threonine was also isolated from this mixture. Clearly, incomplete mutarotation had occurred at $C_{(\alpha)}$. The earlier experiments referred to above had been carried out on the ethyl *cis*-DL-oxazoline ester, mutarotation appearing to be complete. Failure to obtain the same results with the corresponding optically active methyl ester was attributed to the much faster rate of hydrolysis of this compound. It seemed probable that mutarotation ceased as soon as the ester group had been removed.

In order to gain further information concerning the rate of this mutarotation under various conditions, experiments were conducted on *methyl* cis-L-2-*phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylate* (III) which was readily obtainable in pure form. Pure benzoyl-L-threonine (II) was prepared from the brucine salt of (I) and was esterified with diazomethane. The *benzoyl*-L-*threonine methyl* ester was cyclised with thionyl chloride to give the pure oxazoline ester

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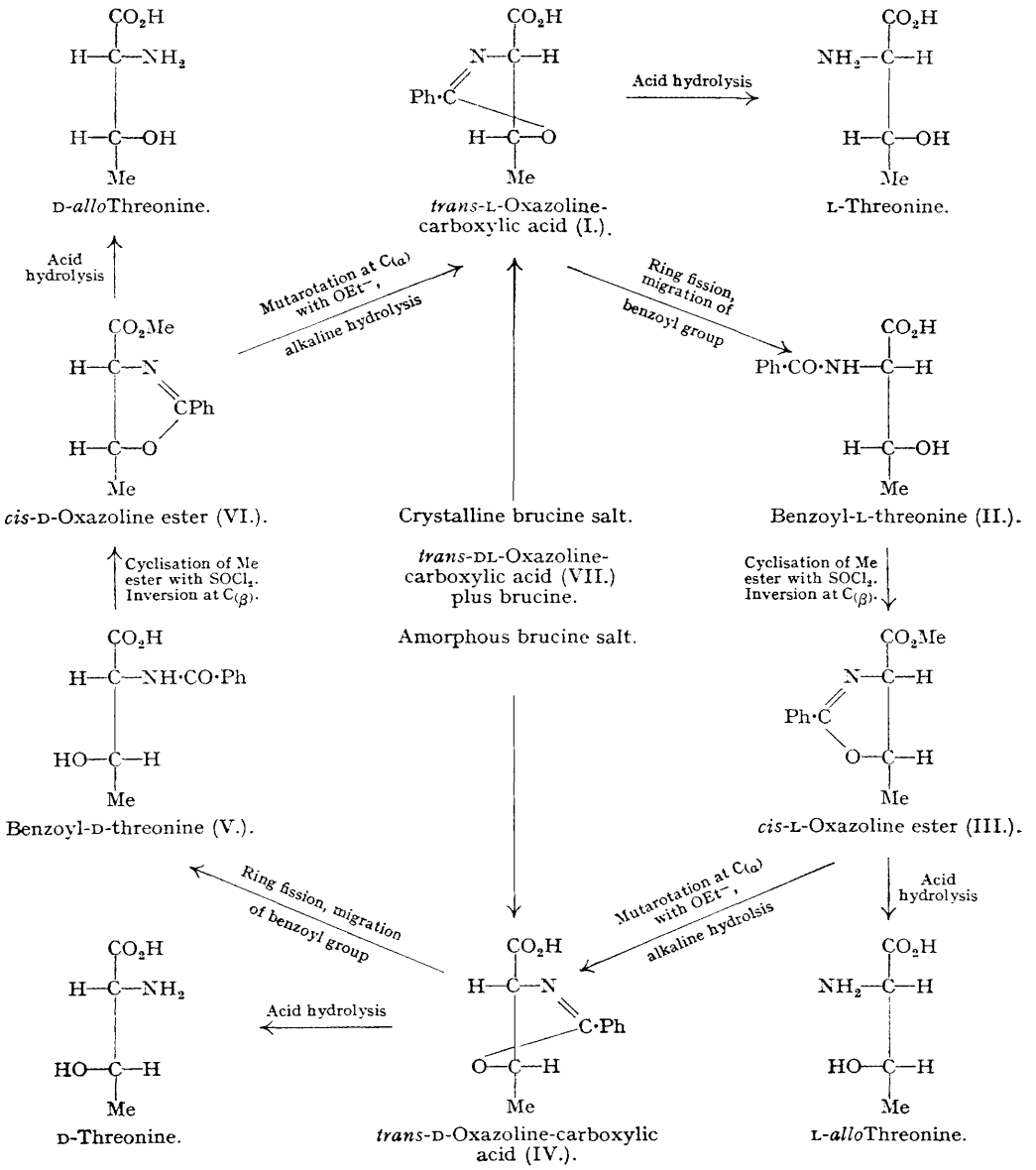
(III) in good yield. Mutarotation studies on this compound were carried out polarimetrically. In a mixture of equal parts of N-sodium hydroxide and alcohol mutarotation occurred to a much greater extent than with aqueous alkali, but conversion into (IV) was still not complete. This was the case even when the alkali was present in excess, showing that the free acid corresponding



In this scheme the prefixes D- or L- refer to the configuration at $C_{(\alpha)}$ as in all amino-acids, but the projection formulæ are those conventionally used for carbohydrates.

to (III) did not undergo mutarotation. In alcoholic sodium ethoxide solution at room temperature mutarotation was immeasurably fast; after addition of water to the solution, followed by short refluxing to hydrolyse the ester group, the rotation of the solution revealed that about 95% conversion into the *trans*-D form (IV) had occurred.

Application of these experimental conditions to the impure *cis*-D-oxazoline ester (VI), obtained from the amorphous brucine salt as described above, gave the desired result. The

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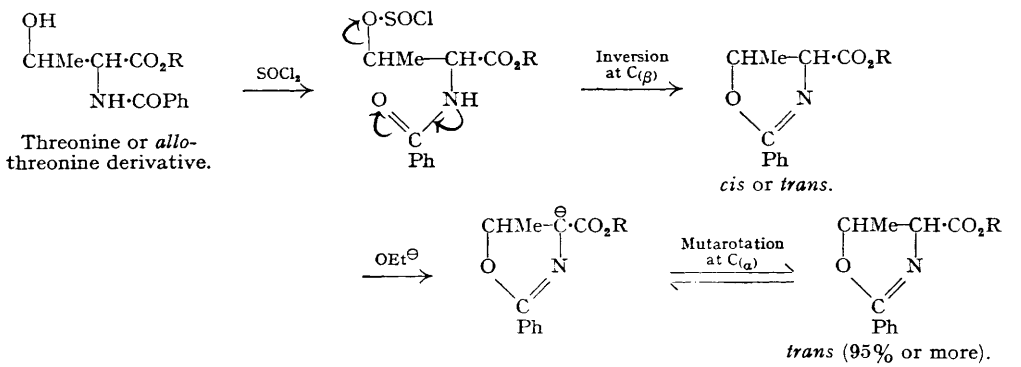
yield of (I) isolated as the brucine salt from the product was approximately that expected from the specific rotation of the crude benzoyl-D-threonine. The total yield of brucine salt obtained from the resolution of the oxazoline-carboxylic acid and from the inversion of the D-form was about 75% (based on the utilisation of both forms) of slightly impure material. It was recrystallised twice with a loss of 10—15%. The overall yield of L-threonine from hippuric acid was 22%; this could have been raised by re-utilisation of the mother-liquors, but for ordinary preparative work this seemed hardly necessary. Recently, Pfister, Robinson, Shabica, and Tishler (*J. Amer. Chem. Soc.*, 1949, **71**, 1101) have described a synthesis of DL-threonine in good overall yield from acetoacetic ester, and in a later paper from the same laboratories (Zambito, Peretz, and Howe, *ibid.*, p. 2541) resolution through the *p*-nitrobenzoyl derivative is reported. Although it was known that the resolution of DL-*p*-nitrobenzoylserine had been accomplished by Fischer and Jacobs (*Ber.*, 1906, **39**, 2942) this method of resolving DL-threonine was not investigated in this laboratory because it would have added extra stages to the synthesis. Fortunately, the present investigation was greatly simplified by the successful resolution of the oxazoline-carboxylic acid, which lies directly on the synthetic pathway from hippuric acid. It must be admitted that the oxazoline-carboxylic acid is not an ideal substance for resolution because of its instability, but by careful attention to experimental detail this disadvantage can be overcome.

The *trans*-L-oxazoline-carboxylic acid (I), which can be obtained absolutely pure as the brucine salt, is the key substance from which all four stereoisomers of α-amino-β-hydroxybutyric acid were prepared as shown in the reaction scheme. The yields were good at every stage. It has been shown (Elliott, 1949, *loc. cit.*) that the ethyl ester hydrochlorides of threonine or *allo*threonine are converted into the corresponding oxazoline esters with retention of configuration at both asymmetric centres on treatment with benzimino ethyl ether. Thus it is possible to leave and re-enter the main pathway (I to VI) at will; all four stereoisomers are therefore easily interconvertible. This cycle of interconversions establishes the configurations of the *allo*threonines relative to L-threonine. L-*allo*Threonine was dextrorotatory in water and much more so in N-HCl. This increase in positive rotation on conversion into the cation is in agreement with Clough's rule concerning naturally occurring amino-acids (Clough, *J.*, 1915, **107**, 1509; 1918, **113**, 526). The preparation of D- and L-*allo*threonine was first carried out by West and Carter (*J. Biol. Chem.*, 1938, **122**, 611). The specific rotations of these substances prepared by the present method are in good agreement with those recorded by West and Carter.

The asymmetric transformations described in this paper can be explained satisfactorily by the reaction mechanisms previously proposed by the author which are summarised below :



The term mutarotation has been applied to the inversion of configuration which occurs when the *cis*-oxazoline esters are brought into contact with ethoxide ions. It is believed that this expression correctly describes the phenomenon, because the final product is probably an equilibrium mixture containing a small but definite amount of the *cis*-isomer. The evidence from the polarimetric study is that at least 95% of the *trans*-form was present after equilibration and hydrolysis. No figures were available for the rotation of the corresponding *cis*-oxazoline-carboxylic acid, but as judged by the figures for the rotation of the methyl esters the sign would have been opposite to that for the *trans*-form. The fact that the optical rotation did not reach the theoretical figure after equilibration could be explained by decomposition to optically

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inactive substances, or to the presence of a very small quantity of the *cis*-form. The remarkable stereochemical specificity of this reaction must be attributed to powerful steric forces.

Experimental.

(M. p.s are uncorrected.)

DL-N-*Formyl*-O-*benzoylthreonine*.—Ethyl DL-*trans*-2-phenyl-5-methyl-Δ^2-oxazoline-4-carboxylate (2·3 g.) was refluxed with 0·5N-sodium hydroxide (22 ml.) until dissolved, and then cooled to room temperature, and 5N-hydrochloric acid (4·2 ml., 2 equivs.) was added. After 4 hours hydrated sodium acetate (1·4 g.) was added with stirring. The main bulk of the *O*-benzoylthreonine separated rapidly. It was filtered off; the mother-liquors were evaporated to obtain a second crop and were finally evaporated to dryness under reduced pressure. The solids were combined, mixed with 90% formic acid (10 ml.), and warmed to 45°, and acetic anhydride (3 ml.) was added dropwise with shaking. The solution was then heated to 80° for 15 minutes and evaporated to dryness under reduced pressure. The residue was lixiviated with cold water to remove sodium chloride and dried at 100°. DL-N-*Formyl*-O-*benzoylthreonine* (1·9 g., 76%) formed irregular masses of prisms (from hot water), m. p. 172—173° (Found : C, 57·3; H, 5·25; N, 5·85. $C_{12}H_{13}O_5N$ requires C, 57·4; H, 5·2; N, 5·6%).

The salt with (+)-1-phenylethylamine had m. p. 154—155°. The *N*-formyl-*O*-benzoylthreonine liberated on addition of acid to an aqueous solution of this salt was optically inactive.

DL-N-*Formylthreonine*.—The corresponding *O*-benzoyl compound (1·9 g.) was suspended in water (10 ml.), and 5N-sodium hydroxide added dropwise until the acid was neutralised. An additional 1·65 ml. of 5N-sodium hydroxide was then added and the solution set aside 30 minutes at room temperature The solution was then treated with 5N-hydrochloric acid (3·1 ml.), the benzoic acid was filtered off, and the filtrate extracted thoroughly with light petroleum. The aqueous layer was evaporated to dryness under reduced pressure, the residue was extracted with warm ethanol (20 ml.) and filtered from sodium chloride, and the filtrate again evaporated to dryness. The residue was dissolved in dry dioxan, a small amount of resinous material was filtered off, and the solvent removed under reduced pressure. The residual oil was dissolved in a small quantity of anhydrous ethanol, and anhydrous ether was added until a cloudiness was produced. On storage at 0° DL-N-*formylthreonine* (0·4 g., 36%) separated in heavy prisms, m. p. 104°. It was recrystallised from ethanol–ether and then had m. p. 105—106°, which changed to 125—126° during storage for several weeks in a closed tube (Found : C, 41·0; H, 6·7; N, 9·3. $C_5H_9O_4N$ requires C, 40·8; H, 6·2; N, 9·5%). Owing to the unsatisfactory yield of this substance, attempts at resolution were abandoned after a few unsuccessful experiments.

DL-trans-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylic Acid* (VII).—This compound may be prepared from the corresponding pure ethyl *trans*-oxazoline ester (obtained from DL-threonine ethyl ester hydrochloride and benzimino ethyl ether), in which case the yield was 87%, or from the mixture of *cis*- and and *trans*-oxazoline esters obtained in the synthesis of DL-threonine from hippuric acid, as follows. The mixture of esters obtained as described by Elliott (1949, *loc. cit.*) was distilled and the fraction of b. p. 110—130°/0·05 mm. collected. The yield of purified product was 75% calculated on crude ethyl α-benzamido-β-hydroxybutyrate. The ester mixture (46·6 g.) was added to N-sodium hydroxide (220 ml.); after addition of ethanol (220 ml.) the solution was refluxed for 15 minutes and then evaporated to 150—200 ml. under reduced pressure. The solution was cooled to 0° in an alcohol–solid carbon dioxide bath and efficiently stirred whilst 5N-hydrochloric acid (*ca.* 44 ml.) was added dropwise, the temperature being maintained at 0° throughout; the addition of the acid required 20—30 minutes. Local excess of acid was carefully avoided. The pH of the solution was tested from time to time by means of Johnson's universal indicator paper, and when it had fallen to 3·5—4·0 the addition of acid was stopped. Generally, about 90% of the theoretical amount of acid was required. The oxazolinecarboxylic acid usually began to crystallise before the addition of hydrochloric acid was complete; when it was delayed, the solution was seeded and the sides of the vessel were scratched. After the addition of hydrochloric acid was complete the solution was stirred for 15 minutes at 0° with occasional scratching of the vessel to complete the crystallisation. The acid was filtered by suction, washed with a small quantity of ice-water, and dried as quickly as possible over sodium hydroxide and concentrated sulphuric acid in an efficient vacuum. The acid appeared to be quite stable when dry. The yield was 28·5 g. (71%) and the m. p. 134—137°. This material was pure enough for further work.

trans-L-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylic Acid* (I).—(a) *Resolution of the* DL-*acid*. The acid (28·4 g.) was added to a solution of anhydrous brucine (54·5 g.) in dry ethanol (110 ml.), and the mixture heated until all or most of the solid had dissolved. Anhydrous ethyl acetate (250 ml.; distilled over phosphoric oxide) was then added and heating continued if necessary. After a further addition of dry ethyl acetate (300 ml.) the solution was kept at 0° with exclusion of moisture for 4 days. The crop of crystalline solid was filtered off and washed with a little anhydrous ethyl acetate. The mother-liquors and washings were evaporated to dryness under reduced pressure at a temperature not exceeding 45°. The residue was dissolved in a mixture of anhydrous ethanol (16 ml.) and anhydrous ethyl acetate (80 ml.) and kept at 0° for 3 days. The second crop was treated as before. The total yield of slightly impure brucine salt was 35·6 g. (82%). The m. p. of the salt was variable; melting was generally over a 2° range between the limits 128° and 140°. Crystallisation of the salt from aqueous methanol or ethanol gave a salt, m. p. 162—164°, but some decomposition (of unknown nature) to an oily substance also occurred. Crystallisation of the salt from anhydrous ethanol, on the other hand, gave an excellent recovery of crystalline material. The m. p. was again somewhat variable : in 3 separate experiments, 128°, 131—132°, and 132—133°. It was established that there was no difference in the purity of the three samples, by decomposition, as described below, to L-threonine which in every case had the correct specific rotation. The impure brucine salt (35·6 g.) was crystallised twice from anhydrous ethanol (180 ml. and 160 ml. respectively). The yield of pure salt was 31 g. (70%). Analysis showed that the pure salt contained one molecule of alcohol of crystallisation (Found : C, 66·2; H, 6·7; N, 6·7. $C_{34}H_{37}O_7N_3,C_2H_5OH$ requires C, 67·0; H, 6·7; N, 6·5%). $[\alpha]_D^{25}$ was +13·6° (*c*, 6 in chloroform).

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(b) *Inversion of the* D-*isomer.* The mother-liquors from the resolution and from the crystallisation of the salt were combined and evaporated to dryness under reduced pressure below 45°. The residual frothy mass was shaken with N-sodium hydroxide (100 ml.) and chloroform (200 ml.) until completely dissolved. The aqueous layer was extracted twice more with chloroform, the chloroform extracts were combined and washed once with water, and the two aqueous layers combined. To the aqueous solution was then added 5N-hydrochloric acid (*ca.* 38 ml.) with stirring to pH 1 (glass electrode). This caused ring fission of the D-oxazoline-carboxylic acid to D-*O*-benzoylthreonine. After 4 hours at room temperature 5N-sodium hydroxide (*ca.* 36 ml.) was added to the solution with stirring until alkalinity to phenophthalein was attained. The *O*-benzoyl-D-threonine separated as a crystalline solid halfway through the neutralisation and then redissolved. The solution of crude *N*-benzoyl-D-threonine was acidified (Congo-red) with concentrated hydrochloric acid and extracted 6 times with an equal volume of ethyl acetate. The combined ethyl acetate extracts were shaken with a small quantity of anhydrous sodium sulphate, filtered, and evaporated to small bulk under reduced pressure. The benzoyl compound rapidly crystallised. It was filtered off and washed with a little ethyl acetate. The mother-liquors were evaporated to dryness under reduced pressure and kept over concentrated sulphuric acid and sodium hydroxide in a vacuum desiccator until quite solid. The two crops of solid (18 g., 89%) were mixed and ground together in a mortar. $[\alpha]_D^{21}$ was −12·75° (*c*, 1·7 in water).

The solid was suspended in ether (200 ml.) and esterified by careful addition, with shaking, of an ethereal solution of diazomethane in slight excess. On evaporation of the ether the crude methyl ester remained as a syrup which rapidly solidified; it was dried in a vacuum desiccator over concentrated sulphuric acid. The yield was 18·55 g. (97%).

The powdered methyl ester was added in small portions, with stirring, to thionyl chloride (53 ml.; purified by distillation over quinoline and linseed oil) which was cooled to 0—5°. The temperature was not allowed to rise above 5° during the addition. The mixture was allowed to stand overnight at 0°; excess of thionyl chloride was then removed under diminished pressure below 35°. The residue, which frequently crystallised, was dissolved in dry chloroform and without delay the solution was poured slowly into sodium carbonate solution (10%; 250 ml.) with efficient stirring. If necessary, addition of solid sodium carbonate was made to prevent the solution becoming acid. The chloroform layer was separated, the aqueous layer was extracted once more with chloroform, and the combined chloroform extracts were washed with water, dried and distilled. The product (14·3 g., 83·5%) had b. p. 110—115°/0·03 mm., $[\alpha]_D^{25}$ −36·7° (*c*, 4·5 in ethanol). This was a mixture of the two methyl *cis*-oxazoline esters (III) and (VI), containing about 70% of the latter.

The mixture was dissolved in dry ethanol (69 ml.), and to it was added a solution of sodium (1·58 g.; 5% excess over 1 mol.) in dry ethanol (69 ml.). After the solution had been kept for 5 minutes at room temperature, water (138 ml.) was added and the mixture refluxed for 15 minutes. The solution was evaporated under reduced pressure to about 50 ml. and cooled to 0°, and 5N-hydrochloric acid (13·7 ml.; exactly equivalent to the sodium used) added dropwise with stirring, with the precautions referred to above in the isolation of the DL-oxazolinecarboxylic acid from its sodium salt. The product (11·55 g., 86·5%), m. p. 151—154° with previous softening, was a mixture of the *trans*-oxazolinecarboxylic acids (IV) and (I) containing about 70% of the latter. The acid was added to a solution of anhydrous brucine (22·2 g.) in anhydrous ethanol (160 ml.) and heated until dissolved. The crystalline brucine salt (29·1 g., 72% calc. on the methyl oxazoline ester), m. p. 126—128°, separated on cooling. It was recrystallised twice from absolute ethanol (*ca.* 5 parts), giving 24·9 g. of pure salt, m. p. 127—129°.

The total yield of pure brucine salt from (*a*) and (*b*) was 55·9 g. (62·5%, calc. on the oxazoline-carboxylic acid).

The salt (27·4 g.) was decomposed with N-sodium hydroxide (50 ml.) and chloroform in the usual way, and the aqueous layer cooled to 0° and treated with 5N-hydrochloric acid (10 ml.) with the usual precautions. trans-L-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylic acid* separated in small hexagonal plates (7·1 g., 78%), m. p. 162—163°, $[\alpha]_D^{27}$ +109·8° (*c*, 10 in 1:1 ethanol–N-aqueous sodium hydroxide), +108·2° (*c*, 6·25 in the same solvent) (Found: N, 6·7. $C_{11}H_{11}O_3N$ requires N, 6·8%).

N-*Benzoyl*-L-*threonine* (II).—The corresponding *trans*-L-oxazoline-carboxylic acid was dissolved in 1 equivalent of N-hydrochloric acid at room temperature, and after 4 hours at room temperature the solution was back-titrated with alkali etc., as described above for the preparation of crude benzoyl-D-threonine. Benzoyl-L-threonine was formed in almost theoretical yield. Recrystallised from ethyl acetate it had m. p. 146—148°, $[\alpha]_D^{25}$ +25·8 (*c*, 1·4 in water). West and Carter (1937, *loc. cit.*) recorded $[\alpha]_D^{26}$ +25·1 (in water). *Benzoyl*-L-*threonine methyl ester*, prepared (93%) in the usual way with diazomethane and crystallised from ether, had m. p. 96°, $[\alpha]_D^{26}$ +23·2° (*c*, 5·4 in ethanol) (Found: N, 6·1. $C_{12}H_{15}O_4N$ requires N, 5·9%).

Methyl cis-L-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylate* (III).—Benzoyl-L-threonine methyl ester (7 g.) was cyclised with thionyl chloride (20 ml.) at 0° as described above for crude benzoyl-D-threonine methyl ester. The yield of *oxazoline* ester, b. p. 110—115°/0·01 mm., was 6 g. (93%). It solidified on storage to a colourless solid which was crystallised from light petroleum (b. p. 40—60°), forming dense prisms, m. p. 74—75°, $[\alpha]_D^{27}$ +69·2° (*c*, 8·5 in ethanol) (Found: N, 6·7. $C_{12}H_{13}O_3N$ requires N, 6·4%).

Polarimetric Investigations of the Mutarotation and Hydrolysis of Methyl cis-L-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylate*.—Aqueous alcoholic sodium hydroxide was prepared by diluting N-sodium hydroxide (50 ml.) to 100 ml. with ethanol. The oxazoline ester (1·095 g., 0·005 mol.) was dissolved in the sodium hydroxide solution (11 ml.). Unfortunately the rotation could not be measured at once because of a cloudiness which developed. After centrifugation in a stoppered tube the solution was placed in a 1-dm. polarimeter tube. Zero time was arbitrarily chosen as the instant when all the oxazoline ester had dissolved in the sodium hydroxide solution. The following readings were obtained at 23° (zero of instrument 360°) (the second place of decimals was not considered significant in this rough experiment):

After 8 mins.	352·2°	After boiling for 2 mins.	352·0°
After 51·5 mins.	352·2	After boiling for 15 mins.	352·0

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It was known that hydrolysis of the ester group would certainly be complete after 15 minutes' boiling. The final rotation of the solution should have been $-10{\cdot}2°$ instead of $-8{\cdot}0°$ if complete conversion into the *trans*-isomer had occurred. From the very small change in rotation that took place after the first 8 minutes it seemed probable that hydrolysis was complete in this time. To test whether further mutarotation took place after hydrolysis in the presence of excess of alkali, a portion of the above solution was diluted with the 0·5N-aqueous-alcoholic sodium hydroxide and the rotation of the solution measured before and after 15 minutes' boiling. There was no change in the rotation of the solution, which had the expected value when allowance was made for the dilution.

The oxazoline ester (1·095 g.) was dissolved in anhydrous ethanol (10 ml.) containing exactly 1 equivalent of sodium ethoxide. Polarimeter readings were taken in a 1-dm. tube at 24—25°. After 4 minutes from zero time which was arbitrarily chosen as before, the reading was 350·8° and no further change took place on storage for one hour. The ester in solution was hydrolysed by addition of water (10 ml.), followed by boiling for 15 minutes. The rotation of the solution was then $-5{\cdot}0°$. For complete conversion into the *trans*-isomer it should have been $-5{\cdot}6°$. From the rotation of the methyl *cis*-L-oxazoline ester it seemed probable that the *cis*-L-oxazolinecarboxylic acid would possess a high rotation opposite in sign to that of the *trans*-L-oxazolinecarboxylic acid. On the basis of this assumption the extent of the conversion was approx. 95%.

L-allo*Threonine*.—The methyl *cis*-L-oxazoline ester (2 g.) was refluxed 5 hours with 6N-hydrochloric acid (20 ml.), the solution was cooled, extracted with ether to remove benzoic acid, and evaporated to dryness under reduced pressure. The residual syrup was dissolved in hot ethanol (20 ml.) and excess of pure pyridine added. On cooling L-*allo*threonine separated as dense prisms (1·05 g., 96%). It was recrystallised from 50% aqueous ethanol and had m. p. 273—274° (decomp.), $[\alpha]_D^{24}$ $+9{\cdot}3°$ (*c*, 3·8 in water), and $[\alpha]_D^{27}$ $+32{\cdot}5°$ (*c*, 8·2 in N-hydrochloric acid) (Found: N, 11·7. Calc. for $C_4H_9O_3N$: N, 11·8%). West and Carter (1938, *loc. cit.*) recorded $[\alpha]_D^{26}$ $+9{\cdot}6°$ (in water) for their dextrorotatory isomer.

trans-D-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylic Acid* (IV).—The methyl *cis*-L-oxazoline ester (III) was treated with sodium ethoxide, etc., as described above for the crude D-isomer. The *trans*-D-acid had m. p. 163—164° and $[\alpha]_D^{23}$ $-106{\cdot}1°$ (*c*, 5·4 in 1 : 1 ethanol–N-aqueous sodium hydroxide).

N-*Benzoyl*-D-*threonine* (V).—The methyl *cis*-L-oxazoline ester (III) (7·9 g.) was treated with sodium ethoxide, etc., as described above except that the oxazoline-carboxylic acid was not isolated but was converted in solution into *N*-benzoyl-D-threonine in the usual way (see above). The product was recrystallised from ethyl acetate. The pure material (6 g., 75%) had m. p. 145—146° and $[\alpha]_D^{28}$ $-25{\cdot}1°$ (*c*, 1·5 in water) (Found: N, 6·0. Calc. for $C_{11}H_{13}O_4N$: N, 6·3%). West and Carter (1937, *loc. cit.*) recorded $[\alpha]_D^{26}$ $-25{\cdot}5°$ (in water) for this compound. *Benzoyl*-D-*threonine methyl ester*, prepared with diazomethane in the usual way, had m. p. 96°, $[\alpha]_D^{26}$ $-23{\cdot}3°$ (*c*, 6 in ethanol) (Found: N, 5·9. $C_{12}H_{15}O_4N$ requires N, 5·9%).

D-*Threonine*.—In this case the amino-acid was prepared from benzoyl-D-threonine and not from (IV) as shown in the chart. The benzoyl compound (2 g.) was hydrolysed as in the preparation of L-*allo*threonine. D-Threonine (yield, 0·95 g., 89%) formed aggregates of hexagonal plates (from 80% ethanol), m. p. 264° (decomp.), $[\alpha]_D^{25}$ $+28{\cdot}4°$ (*c*, 4·4 in water) (Found: N, 11·5. Calc. for $C_4H_9O_3N$: 11·8%). West and Carter (1937, *loc. cit.*) gave $[\alpha]_D^{26}$ $+28{\cdot}4°$ (in water).

Methyl cis-D-2-*Phenyl*-5-*methyl*-Δ^2-*oxazoline*-4-*carboxylate* (VI).—This *ester* was prepared from benzoyl-D-threonine methyl ester, as described for the L-isomer, in 88% yield. It had m. p. 75—76°, $[\alpha]_D^{25}$ $-68{\cdot}9°$ (*c*, 8·4 in ethanol) (Found: N, 6·6. $C_{12}H_{13}O_3N$ requires N, 6·4%).

D-allo*Threonine*.—This substance was prepared from (VI) in the usual way, in 94% yield. It had m. p. 272—273° (decomp.), $[\alpha]_D^{25}$ $-9{\cdot}1°$ (*c*, 3·9 in water) (Found: N, 11·7. Calc. for $C_4H_9O_3N$: N, 11·8%). West and Carter (1938, *loc. cit.*) gave $[\alpha]_D^{26}$ $-9{\cdot}11°$ (in water).

L-*Threonine*.—The pure brucine salt of the *trans*-L-oxazolinecarboxylic acid (I) (109 g.) was decomposed with N-sodium hydroxide (200 ml.) and chloroform in the usual way. To the aqueous layer was then added 10N-hydrochloric acid (440 ml.), the solution was diluted to 700 ml., and the mixture was refluxed for 5 hours. After cooling, benzoic acid was removed by one extraction with ether, and the aqueous layer was evaporated to dryness under diminished pressure. The residue was extracted with hot ethanol (99%; 400 ml.), the solution was filtered from sodium chloride, and pyridine (25 ml.) added to the filtrate. After 24 hours at 0° the L-threonine {17 g., 85%; $[\alpha]_D^{28}$ $-27{\cdot}45°$ (*c*, 2·8 in water)} was collected and washed with absolute alcohol. It was crystallised from 80% ethanol and formed hexagonal plates, m. p. 262—263° (decomp.), $[\alpha]_D^{27}$ $-28{\cdot}3°$ (*c*, 6 in water), $[\alpha]_D^{21}$ $-28{\cdot}5°$ (*c*, 2·4 in water) (Found: N, 11·7. Calc. for $C_4H_9O_3N$: N, 11·8%). West and Carter (1937, *loc. cit.*) recorded $[\alpha]_D^{26}$ $-28{\cdot}3°$ (in water). The yield of pure amino-acid was 16 g. (80% calc. on the brucine salt.).

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