78. *A New Synthesis of* DL-*Threonine.*

By J. ATTENBURROW, D. F. ELLIOTT, and G. F. PENNY.

Condensation of sodium hippurate with acetic anhydride in the presence of β-picoline gave a high yield of 2-*phenyl*-4-(α-*hydroxyethylidene*)*oxazol*-5-*one* (I, R = Me), which was converted by boiling alcohol into ethyl α-benzamidoacetoacetate (II, R = CO_2Et). Hydrogenation of this compound to the corresponding hydroxy-ester was accomplished with Raney nickel catalyst, and from the resulting mixture of racemates DL-N-*benzoyl*allo*threonine ethyl ester* (VIII) was separated by virtue of its sparing solubility in ether. Pure DL-threonine was obtained from the part of the mixture of racemates that was soluble in ether.

Two methods of inverting the configuration at the β-carbon atom in the ester (VIII) have been found; in both instances a simultaneous ring closure to an oxazoline derivative occurred Hydrolysis of this oxazoline gave pure DL-threonine.

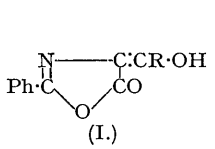
The total overall yield of DL-threonine from hippuric acid was about 30%, the greatest loss being incurred during the separation of the hydroxy-ester.

WHEN threonine was first isolated in the pure state from fibrin by McCoy, Meyer, and Rose (*J. Biol. Chem.*, 1935—1936, **112**, 283), α-amino-β-hydroxybutyric acid had already been synthesised (Burch, *J.*, 1930, 310; Abderhalden and Heyns, *Ber.*, 1934, **67**, 530), but its configuration was

unknown. A thorough investigation of the problem by Carter and his collaborators (*J. Biol. Chem.*, 1935—1936, **112**, 769; 1937, **117**, 1; 1937, **119**, 103, 109) culminated in a satisfactory laboratory-scale synthesis of pure DL-threonine from crotonic acid (Carter and West, *Org. Synth.*, 1940, **20**, 101). A simpler synthesis has been described by Adkins and Reeve (*J. Amer. Chem. Soc.*, 1938, **60**, 1328), but the purity of the product was not confirmed.

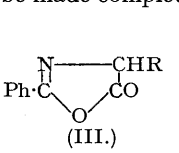
In connection with work relating to the synthesis of penicillin it seemed of interest to examine the action of acetic anhydride on sodium hippurate. It was found that when the substances were heated together for a few minutes at 100° there was produced in about 15% yield an unstable substance, $C_{11}H_9O_3N$, which was decomposed rapidly on continued heating of the reactants. Investigations on the structure of this compound were anticipated by workers at Oxford University, their results being made known to us in the form of a report to the Committee for Penicillin Synthesis (H. R. Bentley and Lady G. M. Robinson, C.P.S. 441, March 20th, 1945). In this report compounds prepared analogously from cinnamoylglycine were shown to be of the type (I), but with ·CH:CHPh instead of Ph. We found that the compound $C_{11}H_9O_3N$ was also a member of this group and it was therefore formulated as *2-phenyl-4-(1-hydroxyethylidene)oxazol-5-one* (I, R = Me). Details of our investigations were described in a report from these laboratories (C.P.S. 490, May 1st, 1945). The work now described formed a separate programme of research, but publication has been delayed because the initial experiments were carried out under a penicillin project.

On boiling with water the oxazolone (I, R = Me) rapidly dissolved with evolution of carbon dioxide and yielded benzamidoacetone (II, R = H), whereas with boiling alcohol a quantitative yield of ethyl α-benzamidoacetoacetate (II, R = CO_2Et) was obtained. On being heated under reduced pressure this β-keto-ester was partly reconverted into the oxazolone; although various conditions were tried, the conversion could never be made complete.

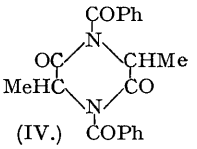


(I.)

$CH_3{\cdot}CO{\cdot}CHR{\cdot}NH{\cdot}COPh$ (II.)



(III.)



(IV.)

An attempt to prepare 4-acetyl-2-phenyl-4-methyloxazol-5-one by reaction of the sodium salt of benzoyl-α-alanine with acetic anhydride was not successful, the products consisting of *methyl 1-benzamidoethyl ketone* (II, R = Me), 2-phenyl-4-methyloxazol-5-one (III, R = Me), and a *compound*, $C_{18}H_{18}O_4N_2$, which may have been the diketopiperazine (IV).

The formation of an acylamido-ketone from benzoyl-α-alanine under base-catalysed conditions was not unexpected, in view of the work of Dakin and West (*J. Biol. Chem.*, 1928, **78**, 91, 745), who showed that hippuric acid, glycine, and many other amino-acids were converted into α-acetamido-ketones, with evolution of carbon dioxide, when heated with acetic anhydride and pyridine for several hours. Except for glycine and hippuric acid the reaction proceeded smoothly and the yields were good: $R{\cdot}CH(NH_2){\cdot}CO_2H \longrightarrow R{\cdot}CH(NHAc){\cdot}CO{\cdot}CH_3$. The oxazolone (I, R = Me) was also formed in the presence of a base under far less vigorous conditions, and it seemed possible, therefore, that oxazolones of type (I) were precursors of the acetamido-ketones prepared by Dakin and West and that the use of pyridine as a catalyst under mild conditions would lead to improved yields of the required oxazolone. Accordingly the effect of pyridine as a catalyst in the reaction between hippuric acid and acetic anhydride at room temperature was studied. It was found that the oxazolone (I, R = Me) was produced quite rapidly under these conditions, but simultaneous decomposition precluded good yields. An attempt to test the hypothesis that oxazolones exemplified by (I) were precursors of the acetamido-ketones described by Dakin and West (*loc. cit.*) gave inconclusive results. The oxazolone (I, R = Me) decomposed rapidly when heated with a mixture of acetic anhydride, pyridine, and some acetic acid, but only 50% of the theoretical amount of carbon dioxide was evolved and the ketonic product could not be obtained pure.

It was later found that the simultaneous decomposition of the oxazolone under the reaction conditions described was completely prevented by including one equivalent of sodium acetate in the reactants, or preferably by using sodium hippurate instead of hippuric acid. The catalytic properties of other bases in the pyridine series were also examined and some marked differences in activity were observed. It is noteworthy that dimethylaniline was found to be ineffective as a catalyst for the reaction under discussion, as it was for acetamido-ketone formation (Dakin and West, *loc. cit.*). β-Picoline was the most satisfactory catalyst, giving the highest yield and a clean product. The oxazolone was readily prepared on a substantial scale.

It is considered that the oxazolone (I, R = Me) is formed by a Claisen type of condensation between acetic anhydride and 2-phenyloxazol-5-one (III, R = H), which is in turn formed from hippuric acid or its sodium salt by the action of acetic anhydride. The high reactivity of the methylene group in 2-phenyloxazol-5-one under the catalytic influence of a tertiary base of the pyridine series was demonstrated by the reaction with acetic anhydride in the presence of pyridine to give a yield of the oxazolone (I, R = Me) higher than that obtained from sodium hippurate under the same conditions, by the reaction with propionyl chloride in the presence of β-picoline to give the homologous 2-*phenyl*-4-(1-*hydroxypropylidene*)*oxazol*-5-*one* (I, R = Et), and by the strongly exothermic reaction with benzaldehyde to give 2-phenyl-4-benzylideneoxazol-5-one. The compound (I, R = Et) was also prepared in good yield from sodium hippurate and propionic anhydride in the presence of β-picoline.

The most significant experiments with hippuric acid, sodium hippurate, and 2-phenyloxazol-5-one are summarised in the table and were carried out at room temperature, the use of heat giving less satisfactory results owing to the instability of the product. It will be seen that sodium hippurate gave a higher yield than an equimolecular mixture of sodium acetate and hippuric acid (compare Expts. 3, 4, and 5 with Expt. 6). This indicates that acetic acid has an inhibitory effect on this reaction, because the formation of 2-phenyloxazol-5-one from one molecule of hippuric acid would liberate two molecules of acetic acid, whereas formation of the oxazolone from one molecule of sodium hippurate would liberate one molecule of acetic acid and one of sodium acetate whatever course this particular step in the reaction may take.

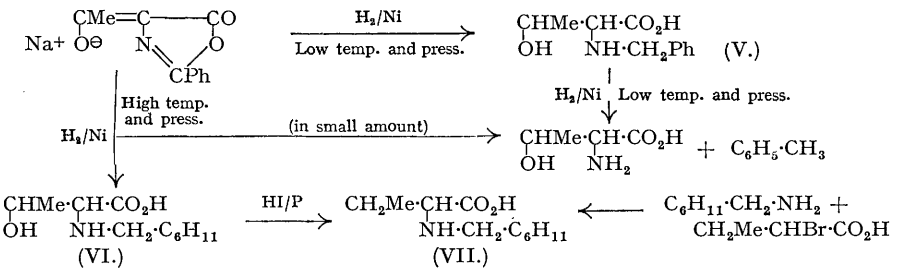
	Starting material and weight (g.).		Tertiary base, mols.		Ac_2O, mols.	NaOAc, mols.	Time, hrs.	Yield of (I), %.
1.	Hippuric acid,	7·2	Pyridine,	2	3	0	9	27·7
2.	,, ,,	7·2	,,	2	3	0	36	0
3.	,, ,,	17·9	,,	3	3	1	3	36·5
4.	,, ,,	17·9	,,	4	3	1	3	41·5
5.	,, ,,	17·9	,,	4	3	1	6	48
6.	Sodium hippurate,	20	,,	3	3	0	3	57·5
7.	2-Phenyloxazolone,	16·1	,,	2	2	1	3	76
8.	,, ,,	16·1	,,	2	2	0	1	34
9.	,, ,,	16·1	,,	2	2	1	1	48
10.	Sodium hippurate,	20	α-Picoline,	3	3	0	24	72 *
11.	,, ,,	20	β- ,,	3	3	0	$3\frac{1}{2}$	88
12.	,, ,,	20	γ- ,,	3	3	0	$3\frac{1}{2}$	87
13.	,, ,,	20	2 : 6-Lutidine,	3	3	0	24	18
14.	,, ,,	20	2 : 4 : 6-Collidine,	3	3	0	$3\frac{1}{2}$	73

* No signs of reaction after 6 hours.

Hydrogenation of the sodium salt of the oxazolone (I, R = Me) in aqueous solution could not be achieved with a platinum (Adams) catalyst or with small amounts of Raney nickel catalyst; in the presence of a large amount of the latter catalyst, 4 mols. of hydrogen were absorbed at a moderate rate and the products were α-amino-β-hydroxybutyric acid in 55% yield and toluene. When the hydrogenation was interrupted after absorption of 1 mol. of hydrogen a considerable amount of starting material was recovered and DL-N-*benzyl*allo*threonine* (V) was isolated in 63% yield (based on oxazolone used). The structure of this compound follows from the fact that the amino-acid absorbed 1 mol. of hydrogen on further hydrogenation and yielded almost pure DL-*allo*threonine, shown by microbiological assay to possess only 1·5% of the activity of pure DL-threonine. It is possible that one of the first steps in the hydrogenation is addition of hydrogen to the ·C:N· link in the oxazolone ring, followed by ring opening and further addition of hydrogen. A detailed mechanism cannot be proposed on the basis of these preliminary experiments.

In an effort to devise more practicable hydrogenation conditions, the effect of using lese catalyst at high temperature and pressure was examined. Hydrogenation was much mors rapid, as would be expected, but under these conditions the main product was an amino-acid, $C_{11}H_{21}O_3N$, shown to be DL-α-cyclo*hexylmethylamino*-β-*hydroxybutyric acid* (VI). This was probably a mixture of stereoisomers, and to avoid difficulties in identification the asymmetry round the β-carbon atom was destroyed by reduction of the hydroxyl group with hydriodic acid and red phosphorus. The product, DL-α-cyclo*hexylmethylaminobutyric acid* (VII), formed in 60% yield, was converted into the *hydrochloride* and *ethyl ester hydrochloride*. *cyclo*Hexylmethylamine, prepared in good yield from cyano*cyclo*hexene by hydrogenation in alcoholic ammonia solution, was treated with α-bromobutyric acid in aqueous solution to give an authentic sample of α-*cyclo*hexylmethylaminobutyric acid for comparison.

This route did not give much promise of a useful synthesis of threonine and these experiments were discontinued.



Attention was then turned to the hydrogenation of ethyl α-benzamidoacetoacetate (II, R = CO_2Et). In alcoholic solution, with Raney nickel catalyst at elevated temperature and pressure, reduction took place readily. The resulting crude hydroxy-ester was hydrolysed directly with hydrobromic acid to give α-amino-β-hydroxybutyric acid in 64% yield from hippuric acid. When working on a large scale it was necessary to use impure materials and then it was frequently found that the oxazolone contained a detectable amount of an unknown sulphur compound; this must have been derived from the coal-tar base used as catalyst in the condensation. Fortunately the sulphur was readily removed by refluxing the alcoholic solution of the keto-ester with a small quantity of Raney nickel and filtering it before hydrogenation. Samples of amino-acid prepared in this way were found to contain 25—30% of DL-threonine and there was no significant change in the proportion of the required isomer when hydrogenations were carried out at various temperatures ranging from 80° to 160°.

Adkins and Reeve (*loc. cit.*) described a method of separating the amino-acids by a simple fractional crystallisation from water, and, although admitting that purity could only be checked by microbiological assay, they offered no proof that pure DL-threonine was obtained in this way: we were unable to obtain it pure by their method. Carter and West (*J. Biol. Chem.*, 1937, **117**, 1; **119**, 109) also report that fractional crystallisation of the amino-acids was impracticable.

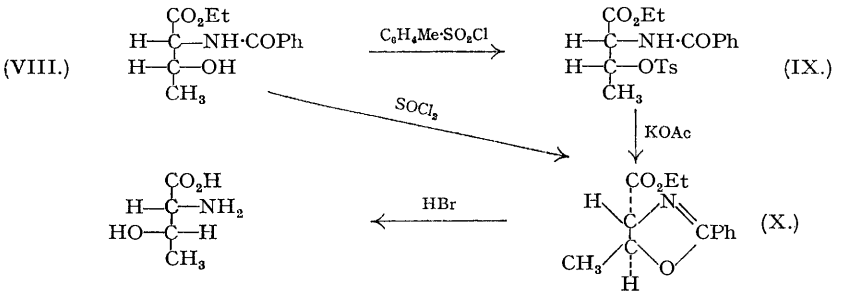
We found that the crude ethyl α-benzamido-β-hydroxybutyrate could be satisfactorily resolved into a solid and an oily ester with the aid of ether. The former was proved to be DL-N-*benzoyl*allo*threonine ethyl ester* (VIII), because it yielded on acid hydrolysis an amino-acid possessing no growth-promoting properties, and, on mild alkaline hydrolysis, pure DL-*N*-benzoyl*allo*threonine in 93% yield. The oily ester was hydrolysed with 1 equiv. of cold sodium hydroxide; the free acid was purified *via* the 2-phenylethylamine salt (Carter and Risser, *J. Biol. Chem.*, 1941, **139**, 255). The pure DL-*N*-benzoylthreonine so obtained was hydrolysed to DL-threonine in about 10% overall yield from hippuric acid.

It seemed desirable to find a convenient method of inverting the configuration of the hydroxyl group in the ester (VIII). This substance was converted into DL-N-*benzoyl*-O-p-*tosyl*allo*threonine ethyl ester* (IX) in 70% yield by reaction with *p*-tosyl chloride in dry pyridine. It appears that a portion of the ester (VIII) remained unattacked by the reagent even when an excess was used and the reaction time was increased considerably, but it is equally possible that a side reaction took place with formation of a labile compound, which was converted into (VIII) during the isolation procedure (compare Tipson, *J. Org. Chem.*, 1944, **9**, 235). The tosyl ester was obtained in two distinct crystal forms, according to the conditions of crystallisation. It was found possible to use the tosyl ester in the crude state. DL-*N*-Benzoyl*allo*threonine, as was expected, could not be converted into a tosyl derivative in the customary fashion, yielding instead 68% of 2-phenyl-4-ethylideneoxazol-5-one ("isomer I" of Carter, Handler, and Melville, *J. Biol. Chem.*, 1939, **129**, 359; see also Carter and Stevens, *ibid.*, 1940, **133**, 117).

The tosyl ester (IX) on treatment with potassium acetate in alcohol under the conditions described by Kenyon, Phillips, and Turley (*J.*, 1925, **127**, 399) for inversions of this type yielded a basic substance, forming a crystalline *picrate* and *hydrochloride* and the analytical data were in perfect agreement with the structure DL-trans-4-*carbethoxy*-2-*phenyl*-5-*methyl*-Δ^2-*oxazoline* (X). The configuration of this compound was demonstrated by hydrolysis with hydrobromic acid to pure DL-threonine.

It has been shown by Bergmann and Miekeley (*Z. physiol. Chem.*, 1924, **140**, 128) that the methyl ester of *N*-benzoylserine is readily converted into the corresponding oxazoline by the action of thionyl chloride and it seemed worth while to determine the configuration of the

product obtained from the ester (VIII) by this method. It was found that a smooth and quantitative inversion at the β-carbon atom took place as well as the expected ring closure

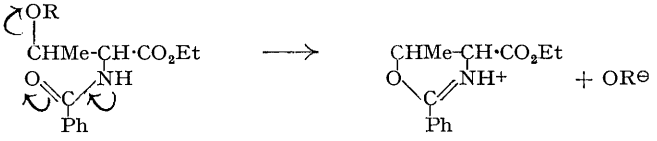


to give the *trans*-oxazoline (X), identical in all respects with the sample prepared from the tosyl derivative. The purity of the product was also confirmed by hydrolysis and microbiological assay of the amino-acid produced.

In order to show that inversion could also be carried out on a threonine derivative, a sample of crude ethyl α-benzamido-β-hydroxybutyrate, which was found to contain 33% of the DL-threonine derivative by microbiological assay of the amino-acid produced on hydrolysis, was treated with thionyl chloride to give a mixture of *cis*- and *trans*-oxazolines. The amino-acid produced on hydrolysis of this mixture was found to contain 70% of DL-threonine.

The hydrochloride of the oxazoline (X) rapidly absorbed water from the atmosphere and was converted into DL-O-*benzoylthreonine ethyl ester hydrochloride*. DL-2-Phenyl-Δ^2-oxazoline-4-carboxylic acid undergoes a similar ring fission to give DL-*O*-benzoylserine (Bergmann and Miekeley, *loc. cit.*).

It is considered that inversion and ring formation take place simultaneously in the above examples and the following mechanism [1] is proposed :



in which anionoid attack is carried out by the enolisable amide group. In the case of cyclisation with thionyl chloride, transient formation of an ester containing the group –O·SOCl is possible (Dostrovsky, Hughes, and Ingold, *J.*, 1946, 188).

Microbiological assays, using *Strept. fæcalis*, were carried out in these laboratories using the method of Stokes, Gunness, Dwyer, and Caswell (*J. Biol. Chem.*, 1945, **160**, 35). A standard sample of pure DL-threonine was prepared by hydrolysis of a repeatedly recrystallised specimen of DL-*N*-formyl-*O*-methylthreonine (Carter and West, *Org. Synth.*, 1940, **20**, 101). DL-*allo*-Threonine was found to have no effect on the growth of *Strept. fæcalis* in the presence or absence of DL-threonine. Ammonium bromide, a likely contaminant in crude samples of amino-acid, was also without effect on the growth of the organism over a wide range of concentrations.

Experimental.

(M. p.s are corrected, unless otherwise stated.)

Sodium hippurate was prepared by dropwise addition of sodium (57·5 g.) dissolved in ethyl alcohol (800 ml.) to a boiling solution of hippuric acid (447·5 g.) in ethyl alcohol (2 l.) with stirring. The thick paste was filtered off and the mother liquors were evaporated to dryness to obtain a further small crop. The combined solids were dried first at 100° and then at 150°. The yield was 488 g. (97·5%). The substance was preserved in a tightly stoppered bottle because of its hygroscopic nature.

2-Phenyl-4-(1-hydroxyethylidene)oxazol-5-one.—Sodium hippurate (878 g.), β-picoline (1330 ml.), and acetic anhydride (1310 ml.) were placed in a 3½-gallon glazed-earthenware jar with 144 5/8″ glass balls, and the jar was closed with a calcium chloride tube in a rubber bung. The jar was turned on a roller mill for 2 hours. After 20 mins. the mixture had attained a temperature of about 40° and then gradually cooled. The dark green solution was separated, and the excess acetic anhydride decomposed by stirring with ethyl alcohol (750 ml.) at 30—35° with water cooling. Distilled water (7·5 l.) was added, and the solution acidified to Congo-red with dilute hydrochloric acid (1 : 1; 4·5 l.) while being

[1] The authors are indebted to Dr. J. W. Cornforth of the National Institute for Medical Research for this suggestion.

vigorously stirred. The green solid was filtered off and washed with distilled water (5 l.). The oxazolone was then dissolved in N-sodium hydroxide solution (4·5 l.) at 80°, charcoal (50 g.) was added, and the mixture stirred for 15 mins. The filtrate was cooled to room temperature and acidified (Congo-red) with dilute hydrochloric acid (1 : 1; *ca.* 1 l.) with vigorous stirring in order to obtain a more easily filterable product. The yellowish-pink product was dried in a desiccator; yield 739 g. (83·4%). The slightly impure 2-*phenyl*-4-(1-*hydroxyethylidene*)*oxazol*-5-*one* so obtained had m. p. 194—195° (decomp.) and was pure enough for further work. After sodium fusion the substance gave a weak positive test for sulphur.

The experiments given in the table were generally carried out in a similar manner, the yields quoted being for the crude products. Those experiments in which hippuric acid or 2-phenyloxazolone were used without addition of sodium acetate gave homogeneous solutions which were merely allowed to stand, though here it was found preferable to add sodium acetate immediately before working up to prevent slight losses in yield due to decomposition of the oxazolone by the alcohol used to remove excess acetic anhydride.

The oxazolone used for hydrogenations in aqueous solution, described below, was purified by chromatography of the sodium salt on alumina in acetone solution. The sodium salt was adsorbed on the alumina in 95% acetone and pigment removed by washing with 95% acetone. The column was then developed with 80% acetone. The oxazolone, purified in this way, formed yellow flattened needles from pure ethyl acetate, m. p. 198—199° (decomp.) (Found : C, 64·95; H, 4·6; N, 6·8. $C_{11}H_9O_3N$ requires C, 65·0; H, 4·5; N, 6·9%).

Benzamidoacetone.—The oxazolone (1 g.) was refluxed for 1 hour with water (50 ml.). The solid rapidly dissolved with effervescence (carbon dioxide) and gave a colourless solution. After evaporation to small bulk under reduced pressure, the ketone separated as colourless plates (yield 93%) and had m. p. 84° (Found : C, 67·7; H, 6·2; N, 7·7. Calc. for $C_{10}H_{11}O_2N$: C, 67·8; H, 6·3; N, 7·9%); Gabriel (*Ber.*, 1910, **43**, 1285) gives m. p. 85°. The 2 : 4-*dinitrophenylhydrazone* formed long golden needles from alcohol, m. p. 214—215° (Found : C, 53·9; H, 4·2; N, 19·4. $C_{16}H_{15}O_5N_5$ requires C, 53·8; H, 4·2; N, 19·6%).

Ethyl α-Benzamidoacetoacetate.—The oxazolone (10 g.) was refluxed for 2 hours with absolute alcohol (100 ml.); it slowly dissolved, and the solution was evaporated to dryness under reduced pressure. Crude ethyl α-benzamidoacetoacetate (yield quantitative) remained as a brown oil which gave a deep port-wine colour with aqueous-alcoholic ferric chloride. At 150°/10^{-4} mm. the ester distilled as a colourless oil; at the same time partial reconversion into the oxazolone took place, and this appeared in the distillate as a yellow precipitate (about 5—10% of the total). The oxazolone was removed by shaking the distillate with sodium hydrogen carbonate solution and ether, and the pure ester obtained by evaporation of the ether in a vacuum. This purified ester on redistillation behaved as before. The *semicarbazone* of the ester, formed in quantitative yield in aqueous solution, had m. p. 172—173° (decomp., uncorr.) (Found : C, 54·9; H, 5·7; N, 17·6; OEt, 14·3. $C_{14}H_{18}O_4N_4$ requires C, 54·9; H, 5·9; N, 18·3; OEt, 14·7%).

Reaction of the Sodium Salt of Benzoyl-α-alanine with Acetic Anhydride.—The dry sodium salt (5 g.) was heated for 5 mins. at 100° with acetic anhydride (7·5 ml.). The solution became pale yellow and effervesced slightly. The mixture was rapidly cooled and diluted with dry ether (200 ml.) and, after thorough shaking, the white solid was filtered off and washed with dry ether. This solid contained no nitrogen and was probably sodium acetate (Found : Na, 26·4. Calc. : Na, 28·0%). The filtrate was evaporated in a vacuum, and the residue distilled, giving first a colourless oil, b. p. 62—63°/0·01 mm. (1·5 g.) (Found : C, 68·4; H, 5·6; N, 8·4. Calc. for $C_{10}H_9O_2N$: C, 68·5; H, 5·2; N, 8·0%). That this compound was 2-phenyl-4-methyloxazol-5-one was confirmed by hydrolysis to benzoyl-α-alanine, m. p. 162—164° (uncorr.), identical with an authentic specimen. The second fraction had b. p. 116—130°/0·01—0·03 mm. and was a pale yellow oil (1 g.). The 2 : 4-*dinitrophenylhydrazone* of *methyl* 1-*benzamidoethyl ketone*, prepared in 6N-hydrochloric acid from this fraction, formed pale yellow needles, m. p. 199—200° (uncorr.) (Found : C, 55·3; H, 4·8; N, 19·4. $C_{17}H_{17}O_5N_5$ requires C, 55·0; H, 4·6; N, 18·9%). This dinitrophenylhydrazone could conceivably have arisen also from 4-acetyl-2-phenyl-4-methyloxazol-5-one, if present in the high-boiling fraction, by ring fission and loss of carbon dioxide. That none of the required oxazolone was present was shown by the fact that the 2 : 4-dinitrophenylhydrazone prepared in alcoholic sulphuric acid was the same as the one prepared in aqueous solution and also by the following experiment. The high-boiling fraction (400 mg.) was refluxed for 2 hours with 2N-sulphuric acid (30 ml.), water (20 ml.), and phenylhydrazine (3 g.). The brown precipitate had m. p. 241—242° (decomp., uncorr.) after crystallisation from glacial acetic acid and gave no depression in m. p. with an authentic specimen of diacetyl phenylosazone. A negligible amount of carbon dioxide was produced during this reaction.

In a second experiment in which heating at 100° was continued for 1 min. and the reaction mixture worked up as before, the residue in the distilling flask solidified after removal of the first fraction. It was removed, rubbed with cold ether, and filtered off; after crystallisation from alcohol, the *compound* had m. p. 225—226° (decomp., uncorr.) (Found : C, 68·8; H, 5·2; N, 8·0. $C_{20}H_{18}O_4N_2$ requires C, 68·6; H, 5·2; N, 8·0%). The ethereal filtrate yielded the same high-boiling fraction as in the first experiment.

2-*Phenyl*-4-(1-*hydroxypropylidene*)*oxazol*-5-*one.*—(a) Sodium hippurate was brought into reaction with propionic anhydride in much the same manner as with acetic anhydride to give a 76% yield. After crystallisation from pure ethyl acetate the 2-*phenyl*-4-(1-*hydroxypropylidene*)*oxazol*-5-*one* was obtained as clusters of pale yellow needles, m. p. 194—195° (decomp.), identical with the product described below.

(b) 2-Phenyloxazol-5-one (8 g.), dissolved in β-picoline (15 ml.), was cooled to 0° and propionyl chloride (4·1 g.) was added dropwise with vigorous shaking and continued cooling. After addition of ice and dilute hydrochloric acid, the oxazolone was filtered off and dried; yield 3·6 g. (34%). The purified substance had m. p. 194—195° as before (Found : C, 66·7; H, 5·1; N, 6·5. $C_{12}H_{11}O_3N$ requires C, 66·35; H, 5·1; N, 6·45%).

2-*Phenyl*-4-*benzylideneoxazol*-5-*one.*—2-Phenyloxazol-5-one (4 g.) was mixed with β-picoline (5 ml.)

and benzaldehyde (2·6 ml.). The solution became hot after a few minutes and then solidified to a crystalline mass. After 3 hours, ice and excess of hydrochloric acid were added, and the solid was filtered off, washed with a small quantity of alcohol, and crystallised from benzene (yield 3·8 g.; 64%). The pale yellow needles had m. p. 166° and gave no depression in m. p. with an authentic specimen.

Hydrogenations of the Sodium Salt of the Oxazolone (I, R = Me).—(a) *α-Amino-β-hydroxybutyric acid.* The pure oxazolone (2 g.) was dissolved in 0·5 N-sodium hydroxide (20 ml.), Raney nickel (4 g.) added, and the solution shaken with hydrogen at room temperature and pressure. Absorption of hydrogen ceased after 20 hours when 4 mols. had been taken up. After filtration, N-hydrochloric acid (22 ml.) was added, and the solution evaporated to dryness under reduced pressure. The residue was warmed with alcohol (50 ml.) and filtered from sodium chloride, and aniline (3 ml.) was added to the filtrate which was allowed to stand overnight in the refrigerator. The amino-acid was filtered off, washed with alcohol, and dried at 100°; yield 650 mg. (54·5%), m. p. 234—235°, raised by crystallisation from 80% alcohol to 238—239°. Microbiological assay showed that this mixture contained about 7% of *dl*-threonine. A mixed m. p. with authentic α-amino-β-hydroxybutyric acid (Adkins and Reeve, *loc. cit.*; m. p. 230°) was 232°. It gave a deep blue colour with " ninhydrin " reagent (Found : C, 40·5; H, 7·4; N, 11·65. Calc. for $C_4H_9O_3N$: C, 40·3; H, 7·6; N, 11·8%). The use of less catalyst in this hydrogenation did not give satisfactory results.

(b) DL-N-*Benzyl*allo*threonine.* The oxazolone (10 g.) was dissolved in 0·5N-sodium hydroxide (110 ml.) and hydrogenated as before in the presence of Raney nickel (20 g.). After absorption of 1 mol. of hydrogen, the catalyst was removed and the filtrate acidified with concentrated hydrochloric acid (12·5 ml.). Unchanged starting material (6 g.) was removed, and the filtrate evaporated to small bulk in a vacuum. A small quantity of unidentified solid was filtered off and the evaporation continued to dryness. The isolation of the *amino-acid* then followed the procedure given in (a). The yield was 2·6 g. (63% on oxazolone used). It crystallised from water in tufts of long needles, m. p. 238° (decomp.), and gave no colour with " ninhydrin " reagent (Found : C, 63·0; H, 7·2; N, 6·7. $C_{11}H_{15}O_3N$ requires C, 63·2; H, 7·2; N, 6·7%).

The amino-acid (500 mg.) in a mixture of water (20 ml.) and N-sodium hydroxide (3 ml.) was hydrogenated as before in the presence of Raney nickel (1 g.). Absorption ceased after 12 hours when 1 mol. of hydrogen had been taken up. Slightly impure DL-*allo*threonine was isolated in the usual way in 70% yield and had m. p. 240—241° (decomp.) (Found : C, 40·4; H, 7·7; N, 11·5%).

(c) α-cyclo*Hexylmethylamino-β-hydroxybutyric acid.* The oxazolone (20 g.) was dissolved in 0·5N-sodium hydroxide (200 ml.) and hydrogenated at 85—90°/110 atm. in the presence of Raney nickel (20 g.). Absorption was complete in 2 hours. The product was worked up in the usual way and gave 7·5 g. of crude amino-acid, m. p. 232—238°. The solid was heated on the water-bath for an hour with water (15 ml.) and then cooled. After being filtered off, the solid was washed with water and dried at 100°; yield 4·2 g., m. p. 254° (decomp.). The filtrate was treated with hot alcohol (100 ml.). α-Amino-β-hydroxybutyric acid (3 g.) separated on cooling and had m. p. 231—232° (Found : C, 40·5; H, 7·6; N, 11·3%). Microbiological assay showed 19% of DL-threonine. The insoluble solid gave no colour with " ninhydrin " reagent. After several crystallisations from glacial acetic acid the α-cyclo*hexylmethylamino-β-hydroxybutyric acid* had m. p. 270° (decomp.) (Found : C, 61·3, 61·5; H, 9·8, 9·8; N, 6·3, 6·3. $C_{11}H_{21}O_3N$ requires C, 61·35; H, 9·8; N, 6·5%). In another experiment on the same scale carried out at 100—110°/125 atm. in the presence of Raney nickel (5 g.) the product consisted almost entirely of α-*cyclo*hexylmethylamino-β-hydroxybutyric acid. The toluene produced in one of these experiments was separated from the hydrogenation mixture by steam-distillation and had b. p. 110°. It was converted into *o*-(*p*'-toluoyl)benzoic acid, m. p. 139°, by the procedure of Underwood and Walsh (*J. Amer. Chem. Soc.*, 1935, **57**, 940) (Found : C, 74·8; H, 4·9. Calc. for $C_{15}H_{12}O_3$: C, 75·0; H, 5·0%).

α-cyclo*Hexylmethylaminobutyric Acid.*—(a) α-*cyclo*Hexylmethylamino-β-hydroxybutyric acid (1 g.) was heated in a sealed tube with hydriodic acid (*d* 1·94; 7 ml.) and red phosphorus (0·17 g.) at 160° for 6 hours. After dilution to 200 ml. the solution was extracted three times with ether and then evaporated to dryness under reduced pressure. The residual syrup was dissolved in boiling water (50 ml.), a crystal of sodium sulphite was added, and then 2N-ammonia to faint alkalinity (litmus). After cooling, the *amino-acid* (600 mg., 65%) was filtered off, washed with water and alcohol, and then crystallised from glacial acetic acid. It formed fine needles, m. p. 300—302° (decomp. and sublimation; sealed tube) (Found : C, 66·4; H, 10·4; N, 7·2. $C_{11}H_{21}O_2N$ requires C, 66·3; H, 10·6; N, 7·0%). The amino-acid (200 mg.) was suspended in dry alcohol (2·5 ml.) and dry hydrogen chloride passed in at room temperature to saturation. The solid rapidly dissolved. After standing overnight, the solvent was removed in a vacuum and the residue triturated with ether. The *hydrochloride* was filtered off, washed with ether, and crystallised from ethanol–ether, and had m. p. 211—213° (decomp.) (Found : Cl, 15·4. $C_{11}H_{22}O_2NCl$ requires Cl, 15·0%). The *ethyl ester hydrochloride* separated slowly from the ether washings but was preferably prepared by passing dry hydrogen chloride into a suspension of the amino-acid (200 mg.) in boiling alcohol (2·5 ml.) for 3 hours. The amino-acid was then completely esterified. The product, isolated in a similar way, formed fine needles from ethanol–ether and had m. p. 118—120° (Found : Cl, 13·4; OEt, 17·2. $C_{13}H_{26}O_2NCl$ requires Cl, 13·4; OEt, 17·1%).

(b) Cyano*cyclo*hexene (Ruzicka and Brugger, *Helv. Chim. Acta*, 1926, **9**, 399) was prepared from *cyclo*hexanone by the method used by Qudrat-i-Khuda and Ghosh (*J. Indian Chem. Soc.*, 1940, **17**, 19) for the 4-methyl homologue. The nitrile (21 g.), mixed with alcoholic ammonia (200 ml., saturated at room temp.) and Raney nickel (5 g.), was hydrogenated at 100—110°/125 atm. The reduction was complete in 30 mins., and after filtration of the catalyst the product was distilled. *cyclo*Hexylmethylamine distilled as a colourless oil, b. p. 160—162° (yield 16·2 g., 73%), giving a benzoyl derivative, m. p. 106—106·5°, and *N-cyclo*hexylmethylurea, m. p. 173·5° (Ruzicka and Brugger, *loc. cit.*, give m. p. 105—106° for the benzoyl derivative and m. p. 170—171° for the urea derivative of the amine prepared by chemical reduction of the nitrile).

α-Bromobutyric acid (2 g.), water (50 ml.), and the amine (5 g.) were mixed and heated on the steam-bath for 3 hours with occasional vigorous shaking. After cooling and neutralisation (litmus) with 2N-sulphuric acid, the amino-acid (1·6 g., 67%) was filtered off and purified as in (a). It had the same

m. p. as the specimen described above, and the hydrochloride (Found : Cl, 15·3; N, 5·9. Calc. for $C_{11}H_{22}O_2NCl$: Cl, 15·0; N, 5·9%) and ethyl ester hydrochloride (Found : Cl, 13·3; OEt, 17·1%) were also identical in m. p. and mixed m. p. with the samples described in (a).

DL-*Threonine.*—(1) *Hydrogenation of the keto-ester* (II, R = CO_2Et) *and separation of the isomeric products.* The oxazolone (220 g.) was refluxed for one hour with absolute alcohol (880 ml.); Raney nickel (15 g.) was then added, and the refluxing continued for 30 mins. The solution was filtered and hydrogenated at 100°/75—100 atm. in the presence of Raney nickel (50 g.). This required 1—3 hours. After removal of the catalyst the solution was evaporated to dryness in a vacuum to give 265 g. (97%) of crude hydroxy-ester. Hydrolysis of the crude substance so prepared with hydrobromic acid gave α-amino-β-hydroxybutyric acid in 64% yield from hippuric acid. The ester distilled at 100—110° (bath temp.)/10^{-6} mm. as a pale yellow oil which partly crystallised. Crystals obtained in this way were used to " seed " the crude ester as follows.

The ester (265 g.) was dissolved in dry ether (1·1 l.), " seeded ", and set aside at 0° for 3 days. The solid was filtered off and washed with absolute ether (220 ml.), the yield being about 160 g., m. p. 65—85°. It was crystallised first from a mixture of ethyl acetate (400 ml.) and light petroleum (b. p. 40—60°; 500 ml.) and then from a mixture of ethyl acetate (500 ml.) and light petroleum (b. p. 40—60°; 1 l.). The yield of DL-N-*benzoyl*allo*threonine ethyl ester* was 104 g. (38% based on the oxazolone), m. p 98·5—101·5° (Found : C, 62·2; H, 6·5. $C_{13}H_{17}O_4N$ requires C, 62·1; H, 6·8%). Microbiological assay of the DL-*allo*threonine produced on hydrolysis showed that less than 2% of DL-threonine was present. Mild alkaline hydrolysis by the method described below gave 93% of DL-*N*-benzoyl*allo*threonine, m. p. 177°; 2-phenylethylamine salt, m. p. 150—153°. These m. p.s are in good agreement with those recorded (West and Carter, *J. Biol. Chem.*, 1937, **119**, 109; Carter and Risser, *loc. cit.*).

The ethereal filtrate from the separation of the solid ester was evaporated to dryness under reduced pressure, and the yellow oil (108 g.) hydrolysed by shaking it with N-sodium hydroxide (430 ml.). The solution was filtered, neutralised with 1 equiv. of 5N-hydrochloric acid, treated with charcoal, filtered again, and evaporated to dryness under reduced pressure. The residue was dissolved in boiling alcohol (400 ml.) and filtered from sodium chloride, and 2-phenylethylamine (53 g.) added. The crystalline solid which separated on cooling was filtered off and recrystallised from alcohol (225 ml.). The m. p. of the pure 2-phenylethylamine salt was 161—162° (Carter and Risser, *loc. cit.*, give m. p. 159—162°) and the yield was 66 g. (18% based on the oxazolone). The salt was decomposed by dissolving it in water (100 ml.) at 60° and adding 2N-hydrochloric acid (144 ml.). After cooling in the refrigerator, the pure DL-*N*-benzoylthreonine (32 g.; 13% based on oxazolone) was filtered off and washed with ice-water. It had m. p. 145·5—146·5°. Hydrolysis with hydrobromic acid in the usual manner (see below) gave pure DL-threonine in about 12% yield from the oxazolone.

(2, a) *Walden inversion of the tosyl derivative* (IX). DL-*N*-Benzoyl*allo*threonine ethyl ester (240 g.) was dissolved in dry pyridine (850 ml.) and the mixture cooled to − 10°. *p*-Tosyl chloride (192 g., recryst.) was added, and the mixture shaken until the acid chloride had dissolved. The solution was kept at 0° for 3½ hours, and then poured, with stirring, on a mixture of concentrated hydrochloric acid (1·2 l.) and crushed ice (2·4 kg.). When solid, the product was filtered off, washed with 2N-hydrochloric acid (2 l.), then water (2 l.), and dried in a desiccator. The yield was 276 g. and the unused DL-*N*-benzoyl-*allo*threonine ethyl ester was recovered quantitatively from the washings by extraction with chloroform. DL-N-*Benzoyl*-O-p-*tosyl*allo*threonine ethyl ester* separated from light petroleum in fine needles, m. p. 82·5—83° (Found : N, 3·1; S, 8·2; OEt, 11·2. $C_{20}H_{23}O_6NS$ requires N, 3·5; S, 7·9; OEt, 11·1%), but when crystallised from ethyl acetate–light petroleum (b. p. 40—60°) it had m. p. 148·5—151·5° (Found : N, 3·4; S, 7·5; OEt, 10·1%). No difference in chemical behaviour, including the Walden inversion, could be detected in these samples.

The crude tosyl derivative (276 g.), fused and powdered potassium acetate (138 g.), and anhydrous ethyl alcohol (950 ml.) were mixed and refluxed for 2½ hours. The solution was evaporated and diluted with water (1·5 l.). The yellow oil was separated by ether extraction (3 × 500 ml.), and the ether dried and evaporated. The residual oil weighed 150 g. (36% based on the oxazolone). DL-trans-4-*Carbethoxy*-2-*phenyl*-5-*methyl*-Δ²-*oxazoline* distilled as a colourless oil at 100°/10^{-5} mm. (Found : C, 67·1; H, 6·5; N, 6·1; OEt, 17·8. $C_{13}H_{15}O_3N$ requires C, 66·9; H, 6·5; N, 6·0; OEt, 19·3%). The *picrate* had m. p. 127·5° (Found : C, 49·6; H, 3·6; N, 12·2. $C_{19}H_{18}O_{10}N_4$ requires C, 49·4; H, 3·9; N, 12·1%). The *hydrochloride* formed colourless, tetragonal crystals, m. p. 117·5—118°, from methanol–ether, and rapidly absorbed moisture from the air, becoming opaque; accurate analytical figures were not obtained for it (Found : C, 57·1; H, 6·4; N, 4·9; Cl, 12·15. $C_{13}H_{16}O_3NCl$ requires C, 57·9; H, 6·0; N, 5·2; Cl, 13·15%).

(2, b) *Walden inversion of the ester* (VIII). DL-*N*-Benzoyl*allo*threonine ethyl ester (20 g.) was mixed with pure thionyl chloride (50 ml.). A vigorous evolution of gas took place and after one hour at room temperature, ether was added until a cloudiness was produced and the solution was treated with solid sodium hydrogen carbonate until there was no further effervescence. After filtration, washing of the residue with ether, and evaporation, a yellow oil (16 g.; 33% based on the oxazolone) remained. A sample was distilled in a high vacuum for analysis (Found : C, 67·05; H, 6·4; N, 6·2; OEt, 19·0%). The picrate had m. p. 127·5°, and the hydrochloride m. p. 118°. These derivatives gave no depression in a mixed m. p. determination with those described under (2, a). The identity of the two preparations of the oxazoline was confirmed by microbiological assay of the amino-acid produced on hydrolysis with hydrobromic acid. Both were found to be pure DL-threonine. A specimen of the oxazoline hydrochloride which had been standing in the laboratory atmosphere for a few days was crystallised from ethanol–ether and had m. p. 167°. This was found to be DL-O-*benzoylthreonine ethyl ester hydrochloride* (Found : C, 54·7; H, 6·4; N, 4·6; Cl, 11·9; OEt, 15·3. $C_{13}H_{18}O_4NCl$ requires C, 54·3; H, 6·3; N, 4·9; Cl, 12·3; OEt, 15·7%). The configuration of this substance was confirmed by microbiological assay of the amino-acid produced on hydrolysis. The hydrochloride, on treatment with aqueous sodium hydrogen carbonate, gave an oil, yielding a *picrate* as bright yellow needles, m. p. 140·5° (Found : C, 48·0; H, 4·25; N, 11·3. $C_{19}H_{20}O_{11}N_4$ requires C, 47·5; H, 4·2; N, 11·7%).

(3) *Hydrolysis.* The crude oxazoline ester (150 g.) was refluxed for 4 hours with 23% hydrobromic

acid (750 ml.) and cooled, the benzoic acid filtered off, and the residue evaporated to dryness under reduced pressure. The syrupy residue was dissolved in hot alcohol (750 ml.), and concentrated ammonia added to the hot solution in slight excess. After standing for 16 hours, the crude amino-acid (74 g.) was filtered off and recrystallised from a mixture of water (100 ml.) and ethyl alcohol (400 ml.). The yield of pure DL-threonine was 56 g. (26% based on the oxazolone). Other hydrolyses, mentioned above, were performed in a similar way.

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