DL-β-3-Oxindolylalanine (DL-Hydroxytryptophan)

1. SYNTHESIS

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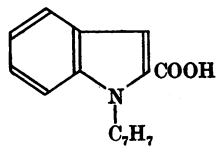
It is now generally assumed that the first series of reactions in the metabolism of tryptophan in mammals leads to kynurenine, the structure of which has been elucidated by Butenandt, Weidel, Weichert & Derjugin (1943). Kotake (1931, 1935), who, following its discovery by Matsuoka & Yoshimatsu (1925), carried out the first extensive investigations on kynurenine, suggested that β-oxindolylalanine (XVII) is an intermediate in the conversion of tryptophan to kynurenine and kynurenic acid; this hypothesis has been accepted by others (see Butenandt *et al.* 1943). β-Oxindolylalanine has not yet been found in mammalian tissues or excreta, but Wieland & Witkop (1940) obtained it from the hydrolysate of phalloidin, a toxic peptide from *Amanita phalloides*. Moreover, it was shown by Butenandt, Weidel & Becker (1940) that Wieland & Witkop's amino-acid was only slightly less active than kynurenine as precursor of the eye pigment of certain mutant strains of *Drosophila melanogaster*. It thus seemed likely that β-oxindolylalanine may also play a part in tryptophan metabolism in mammals, but no experiments to test this point have been carried out, largely owing to the fact that this amino-acid has so far been available in minute quantities only.

The occurrence of a tryptophan derivative containing a nuclear hydroxyl group in casein digests was reported by Abderhalden & Kempe (1907) and Abderhalden & Sickel (1924). Although these claims were later withdrawn (Abderhalden & Sickel, 1925), the earlier report stimulated Fischer & Smeykal (1923) to attempt the synthesis of oxindolylalanine. These authors condensed oxindole-3-aldehyde with hippuric acid, but obtained, instead of the expected azlactone, a substance which could not be converted to an amino-acid and which was suggested later by Horner (1941) to be an isatin derivative. Similar results were obtained by Gränacher & Mahal (1923) who attempted the condensation of oxindole-3-aldehyde with rhodanine. Julian, Pikl & Wantz (1935) condensed 1:3-dimethyloxindole with bromoacetal and converted the aldehyde obtained on hydrolysis by the Strecker method to β-(1:3-dimethyloxindolyl)alanine. This reaction, however, was unsuccessful with oxindole itself. Later Horner (1941) attempted the preparation of derivatives of oxindole suitably substituted in the 3-position; but no intermediate useful for the synthesis of the amino-acid was obtained. However, Witkop (1947) succeeded in oxidizing L-tryptophan with a mixture of acetic anhydride and hydrogen peroxide to a partially racemized oxindolylalanine. By comparison with the rotation of the material isolated from phalloidin, it was shown that the amino-acid obtained by Wieland & Witkop (1940) had the L-configuration. The method of Witkop (1947) gave apparently poor yields of pure product and does not lend itself, in our experience, to the preparation of large amounts of this amino-acid. We have therefore again attempted the synthesis of β-oxindolylalanine.

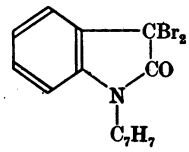
Various unsuccessful attempts at synthesis

It appeared that the mobility of the hydrogen atom in position 3 was the main reason why earlier attempts starting from 3-formyloxindole had failed. The latter reacts, as already emphasized by Gränacher & Mahal (1923), mainly in the enolic form. However, Julian *et al.* (1935) had reported that the sodium salt of 3-formyl-1-methyloxindole can be methylated to give a compound which has an *O*-methyl group in the 2-position and in which enolization is thus prevented. It was hoped to prepare the corresponding derivative of 1-benzyl-3-formyloxindole (V) which on reaction with hippuric acid might be expected to give an azlactone. Reduction of the intermediate *N*-benzyl compound might be expected to give the desired amino-acid. 1-Benzyloxindole (III) could not be prepared by the convenient Stollé synthesis, since treatment of *N*-benzylchloroacetanilide with aluminium chloride gives oxindole itself (see Stollé, 1930). III was therefore prepared by oxidation of 1-benzylindole-2-carboxylic acid (I) with hypobromite (Fischer & Hess, 1884; Colman, 1888; Michaelis, 1897). Good yields were obtained when this oxidation was carried out in a borate buffer pH 9·5–10·0. The bromine atoms in the resulting 3:3-*dibromo-oxindole* (II) show the same lability as in derivatives of bromo- or dibromo-malonate: they are readily reduced by iodide in acetic acid at room temperature. This method of debromination has been used for analytical purposes as well as for the preparative conversion of the dibromo-compound to 1-*benzyloxindole* (III). The

latter readily reacted with ethyl formate (Stollé, 1932) to give 1-benzyl-3-formyloxindole which from its reactions must be formulated as an enol (IV).



(I)



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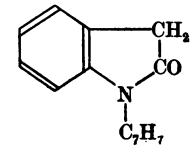

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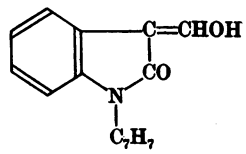

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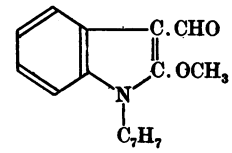
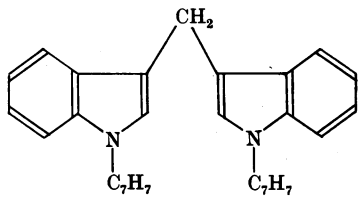


(V)

However, treatment of the sodium salt of (IV) with methyl iodide in acetone (Julian *et al.* 1935) produced an oil (possibly V) which could not be induced to crystallize. Attempts to condense the oil with hippuric acid under the usual conditions of the Erlenmeyer synthesis or with 2-phenyloxazolone in the presence of basic catalysts failed to give an azlactone.

fication of the method of Moe & Warner (1948) yielded (VIII) in a good yield. γ-Acetamido-γγ-dicarbethoxybutyraldehyde was condensed with benzylphenylhydrazine and the resulting hydrazone was cyclized. The ethyl α-acetamido-α-carbethoxy-β-(1-benzylindole-3) propionate obtained (VII) was hydrolysed first to α-*N*-acetyl-1-benzyltryptophan and then further to 1-benzyltryptophan. (In all names based on tryptophan, the atoms of the nucleus are numbered as in indole and those in the side chain lettered αβ as in alanine.)



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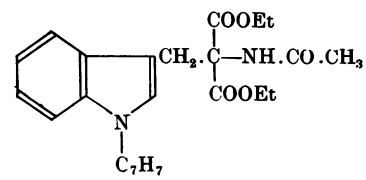

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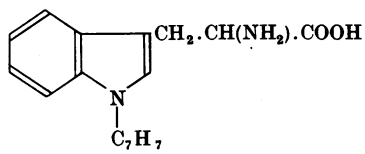

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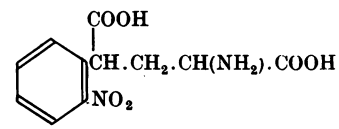


(IX)

We next attempted to oxidize a suitably substituted tryptophan derivative, since it appeared likely that the low yield of β-oxindolylalanine observed by Witkop was mainly due to the presence of a hydrogen atom in the 1-position of the indole nucleus. We therefore prepared DL-1-benzyltryptophan (VIII). The Mannich reaction with 1-benzylindole yielded mainly the methylene bis-compound (VI), whilst the reaction proceeded normally with 1-benzylindole-2-carboxylic acid. However, a modi-

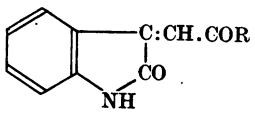
Oxidation of (VII) with hydrogen peroxide and acetic anhydride (peracetic acid) gave in small yield a crystalline product which apparently contained one more oxygen atom than would be required for an oxindole derivative. Its ultraviolet absorption spectrum was similar to those found with oxindole derivatives which had been exposed to autoxidation in alkaline solution (see Cornforth, Dalgliesh & Neuberger, 1951). The compound may be a dioxindole derivative. Oxidation experiments on α-*N*-

acetyl-1-benzyltryptophan using bromine were equally unsuccessful.

Having failed in these two approaches, we explored the possibility of synthesizing γ-(2-nitrophenyl)-glutamic acid (IX), reduction of which would have been followed by ring closure to oxindolylalanine. However, we were unable to obtain the desired nitro compound.

Synthesis of oxindolylalanine.

Lindwall & Maclennan (1932) showed that in the condensation of isatin with methyl ketones, 3-hydroxyoxindoles are formed first and these could easily be dehydrated to alkylideneoxindoles (X) which in turn may be reduced to alkyloxindoles (XI).



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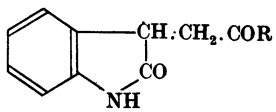
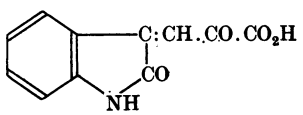


(XI)

We therefore attempted the condensation of isatin with ethyl pyruvate. When diethylamine was added to an equimolar mixture of these substances in ethanol, reaction was rapid and after acidification the deeply coloured 3-*isatylidenepyruvic acid* (XII) was isolated. The rapid hydrolysis of an ester grouping observed in this reaction is a little surprising, though α-keto acid esters are well known to hydrolyse in alkaline media very readily.



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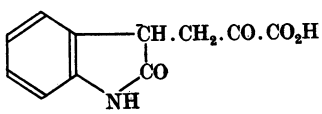
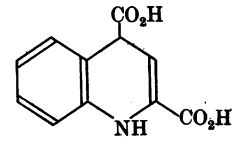


(XIII)

No indication of hydroxytryptophan formation was obtained when the acid (XII) was hydrogenated in the presence of ammonia, nor was any better result obtained when the addition of ammonia was withheld until one molecule of hydrogen had been absorbed. Attempts to isolate 3-oxindolylpyruvic acid (XIII) from the mixture obtained on catalytic hydrogenation of (XII) were defeated by the instability of the compound. It was, however, possible to prepare a *phenylhydrazone* of the α-keto acid. It is possible that the acid (XIII) rearranges easily to a dihydroquinoline (XIV) which polymerizes or undergoes dismutation. Reduction of the phenylhydrazones of acids (XII) and (XIII) either catalytically or with aluminium amalgam was tried, but was not promising.



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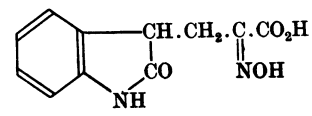

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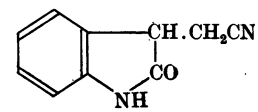
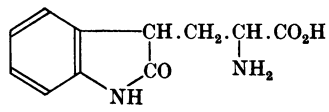


(XVI)

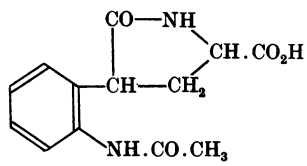
The next attempt was aimed at the oxime of (XIII). Reduction of isatylidenepyruvic acid (XII) with sodium dithionite to (XIII) occurred readily in aqueous solution; addition of hydroxylamine hydrochloride to the reaction mixture afforded the desired α-oximino-β-3-oxindolylpropionic acid (XV) in 55% yield on acidification. The success of this process is probably due to a masking of the keto group by the sodium bisulphite formed in the reduction, the pyruvic acid being thereby stabilized. On boiling with water the acid (XV) gave 3-oxindolylacetonitrile (XVI).

Reduction of the oximino group in the acid (XV) by aluminium amalgam did not proceed smoothly, but reduction with sodium amalgam in a slightly acid medium gave DL-oxindolylalanine (XVII) in 80–90% yield. The properties of the racemic amino-acid correspond closely with those given by Wieland & Witkop (1940) for the L-isomer. Optical resolution of the DL-compound presents difficulties which so far have not been overcome. The *N*-formyl, *N*-acetyl and *N*-benzoyl derivatives have been prepared by conventional methods. Among these only the acetyl derivative gave crystalline salts with brucine and (−)-α-phenylethylamine. Recrystallization of these salts gave no useful results. Fractions of different optical rotations were indeed obtained, but no controllable or progressive separation could be achieved.

When DL-hydroxytryptophan was boiled for a short time with dilute sodium or barium hydroxide and acetic anhydride was then added, a crystalline acid was isolated on acidification in a poor yield. This was found to be isomeric with the normal acetyl derivative and is probably the pyrrolidone (XVIII). The oxindole ring is opened on heating with alkali (Baeyer & Comstock, 1883) and β-oxindolylalanine behaves in an analogous manner (Wieland & Witkop, 1940). A crystalline brucine salt was obtained from this acid, but a preliminary attempt at optical resolution was unsuccessful.



(XVII)



(XVIII)

β-Oxindolylalanine can theoretically exist in two racemic forms, since, apart from the α-carbon atom of the side chain, C-3 of the oxindole nucleus is asymmetric. The optically active forms of dioxindole are rapidly racemized by a trace of alkali (McKenzie & Stewart, 1935), but no definite information is available on the mobility of the hydrogen atom in the 3-position of 3-alkyloxindoles. The behaviour of oxindolylalanine on crystallization can perhaps be attributed to the presence in solution of two diastereoisomers.

The ultraviolet absorption of oxindolylalanine and of related compounds is described in another paper (Cornforth *et al.* 1951). It should be mentioned here, however, that β-oxindolylalanine changes rapidly in alkaline solution and the value of the specific rotation given by Wieland & Witkop (1940) which was obtained in N-sodium hydroxide may have to be revised. The absorption data also suggest that the structure of the amino-acid is best represented by a lactam structure as in (XVII); the term hydroxytryptophan is therefore misleading and should be replaced by oxindolylalanine.

EXPERIMENTAL

All melting points are uncorrected.

Preparation of 1-*benzyl*-3-*formyloxindole*

NN-*Benzylphenylhydrazine.* Benzylation of phenylhydrazine in liquid NH_3 (Audrieth, Weisiger & Carter, 1941) or in benzene (Crowther, Mann & Purdie, 1943), with sodium and sodamide, respectively, yield besides the required material other products in small quantities. One of these was *NN'*-dibenzyl-*N*-phenylhydrazine hydrochloride, crystallized from ethanol, m.p. 195°; Franzen & Kraft (1911) give m.p. 186°. (Found: C, 74·0, H, 6·7; N, 8·6. Calc. for $C_{20}H_{20}N_2.HCl$; C, 74·0; H, 6·5; N, 8·6%.) The second by-product had m.p. 110° and was the benzylphenylhydrazone of benzaldehyde. (Found: C, 84·4; H, 6·7; N, 10·2. Calc. for $C_{20}H_{18}N_2$: C, 83·9, H, 6·3; N, 9·8%.) This compound has previously been produced by oxidation of dibenzylphenylhydrazine; in the present case autoxidation presumably occurred during the working up. The most convenient method, however, for the preparation of benzylphenylhydrazine was found to be that of Minunni (1892), which consists of heating carefully a mixture of 2 equiv. of phenylhydrazine with 1 equiv. of benzyl chloride. The benzylphenylhydrazine was distilled at 137–141°/0·8 mm., and then converted into the hydrochloride which is stable.

1-*Benzylindole*-2-*carboxylic acid.* This compound was prepared from the benzylphenylhydrazone of pyruvic acid as described by Antrick (1885). Cyclization was performed by heating the hydrazone (133 g.) which had been dissolved in ethanol (200 ml.) with 3N-HCl (1·5 l.) at 90° for 20 min. The solid formed on cooling was collected and dissolved in N-NaOH (1 equiv.). Insoluble material was filtered off and the alkaline solution was extracted with ether. On acidification the acid crystallized out; recrystallized from 50% (v/v) acetic acid, the m.p. was 194°. Yield 50% of theory.

3:3-*Dibromo*-1-*benzyloxindole.* A solution of 1-benzylindole-2-carboxylic acid (40 g.) in 0·45N-NaOH (250 ml.) was mixed with 0·2N-borate pH 9·6 (1 l.). To the cooled and mechanically stirred solution was added in ten equal portions over 30 min. a cooled solution (1 l.) of NaOBr (80 g. Br_2 dissolved in N-NaOH with cooling). The pH was kept between 9·5 and 10·0 by addition of N-acetic acid. After standing for 1 hr. at room temperature, the solid was collected, washed with water and dried. Yield was 80%. The material after successive recrystallization from acetic acid and ethanol had m.p. 158–159°. (Found: C, 47·3; H, 3·1; N, 3·8. $C_{15}H_{11}ONBr_2$ requires C, 47·3; H, 2·9; N, 3·7%.) The two Br atoms are readily replaced by H; 38·1 mg. were dissolved in acetic acid and KI (100 mg.) was added. After standing at 37° for 1·5 hr. the liberated I_2 was titrated with 0·1N-$Na_2S_2O_3$. 4·1 ml. were consumed; $C_{15}H_{11}ONBr_2$ requires 4·0 ml.

1-*Benzyloxindole.* To a solution of the dibromo compound (40 g.) in boiling ethanol (750 ml.) was added Zn dust (40 g.) (treated successively with 5% (w/v) $HgCl_2$, water and ethanol) and conc. HCl (100 ml.). Reaction started at once and the solution boiled. After 0·5 hr. more Zn (30 g.) and conc. HCl (30 ml.) were added and the solution boiled under reflux for 1·5 hr. The solution was concentrated under reduced pressure to dryness and the residue dissolved by addition of ether and water. The ethereal layer was separated and the aqueous layer was repeatedly extracted with ether. The combined ethereal solutions were dried and the solvent removed. The remaining oil was distilled at 150–155°/0·3 mm. The distillate soon crystallized; the m.p. after recrystallization from a small quantity of light petroleum (b.p. 100–120°) was 65°. Yield was 75%. On repeated crystallization from this solvent or from aqueous ethanol the m.p. was raised to 67°. (Found: C, 80·3; H, 5·8; N, 6·5. $C_{15}H_{13}ON$ requires C, 80·7; H, 5·7; N, 6·3%.) 1-*Benzyloxindole* was also prepared and in slightly better yield by reduction of the dibromo-indole with KI in acetic acid.

1-Benzyloxindole-3-aldehyde. *N*-Benzyloxindole (13·74 g.) and ethyl formate (5·55 g.) dissolved in warm ethanol (60 ml.) were added to a hot solution of 2·1 N-NaOEt in ethanol (36 ml.). The mixture, which solidified at once, was allowed to stand for 10 min. and then cooled to 0°. The Na salt was decomposed by addition with shaking of N-HCl (100 ml.) and water (300 ml.). The solid was filtered and dried. Yield was 90%. Successive recrystallization from $CHCl_3$-light petroleum (b.p. 30–40°) and benzene gave material of m.p. 175°. (Found: C, 76·1; H, 5·4; N, 5·6. $C_{16}H_{13}O_2N$ requires C, 76·5; H, 5·2; N, 5·9%.) The aldehyde gives a blue colour with ethanolic $FeCl_3$; it is soluble in ethanol and $CHCl_3$ and moderately soluble in benzene. The *phenylhydrazone* was prepared by boiling a solution of equimolar amounts of the aldehyde and phenylhydrazine acetate in ethanol and had m.p. 186–187°. (Found: C, 77·4; H, 5·3; N, 12·5. $C_{22}H_{19}ON_3$ requires C, 77·4; H, 5·6; N, 12·3%.)

Attempted preparation of 1-benzyl-2-methoxyindole-3-aldehyde. To a solution of 1-benzyloxindole-3-aldehyde (11·166 g.) in a mixture of ethanol (150 ml.) and dry ether (75 ml.) was added 1·75 N-sodium ethoxide (26·5 ml.). After cooling the mixture to −10° the precipitate which was formed was filtered off and washed with cold ethanol and dry ether. The Na salt was dried by heating for 1 hr. at 100°/0·1 mm. It was then suspended in dry acetone (250 ml.) containing methyl iodide (30 g.) and the mixture boiled under reflux for 8 hr. The clear solution, which did not give a $FeCl_3$ reaction, was concentrated to low bulk; no solid was obtained. Water was added and the oil extracted with ether. On drying the ethereal solution and removal of the solvent, an oil was obtained which could not be induced to crystallize. Heating of the oil with hippuric acid (1 equiv.), acetic anhydride (4 equiv.), and anhydrous sodium acetate (0·5 equiv.) did not apparently yield an azlactone. Treatment of the oil with 2-phenyloxazolone in ethanol in the presence of β-picoline or pyridine was equally unsuccessful.

The Mannich reaction with 1-benzylindole derivatives

Mannich reaction with 1-benzylindole. 1-Benzylindole (Antrick, 1885) (2 g.) was submitted to the Mannich reaction using the procedure of Snyder & Eliel (1948). After completion of the reaction, the mixture was made alkaline, extracted with ether and the ether solution extracted with dilute acid. The aqueous extract on addition of NaOH and extraction with ether eventually gave an oil in poor yield which solidified on standing. Recrystallization from light petroleum (b.p. 40–60°) gave *1-benzyl-3-dimethylaminomethylindole*, m.p. 52–54°. (Found: N, 10·4. $C_{18}H_{20}N_2$ requires N, 10·6%.) The base gave a *picrate*, dark-yellow prisms from ethanol, m.p. 122°. (Found: N, 14·1. $C_{18}H_{20}N_2.C_6H_3O_7N_3$ requires N, 14·2%.) The original ether extract on evaporation gave as the main product of the reaction a pinkish solid. Recrystallization from a large volume of methanol gave colourless crystals, m.p. 137°, identified as *3:3′-methylene-bis(1-benzylindole)*. (Found: C, 87·0; H, 6·2; N, 6·9. $C_{31}H_{26}N_2$ requires C, 87·3; H, 6·1; N, 6·6%.)

Mannich reaction with 1-benzylindole-2-carboxylic acid. To a cooled solution of 3·2 ml. dimethylamine (33% aqueous solution) and glacial acetic acid (5 ml.) was added 0·9 ml. 40% formaldehyde solution. After chilling, the indole derivative (2·5 g.) was added and the mixture shaken. As no apparent reaction took place, a further 2 ml. acetic acid were added and the mixture heated on a boiling-water bath for 1·5 hr., diluted with water and extracted with ether. The aqueous solution deposited crystals of the Mannich base which were recrystallized from methanol; m.p. 211° (decomp.). (Found: N, 8·9. $C_{19}H_{20}O_2N_2$ requires 9·2%.) The compound owing to its zwitterionic structure is fairly soluble in water and does not form an insoluble picrate.

1-Benzyltryptophan

Benzylphenylhydrazone of ethyl α-acetamido-δ-aldehydo-α-carbethoxybutyrate. To ethanol (65 ml.) containing a small amount of Na was added ethyl acetamidomalonate (15·2 g.). To the cooled solution acrolein (4·5 g.) was added slowly with shaking. The acrolein had been prepared immediately before the experiment by hydrolysis of acrolein diacetate with the calculated amount of water containing a trace of mineral acid. The mixture was set aside in the dark for 1 week, and then treated with benzylphenylhydrazine (13·9 g.) and glacial acetic acid (2 ml.), heated to boiling, diluted with water (15 ml.), filtered and left in the dark for 18 hr. The *hydrazone* separated in the form of white needles, m.p. 127–128° (22·5 g.; 62% yield based on acrolein). On recrystallization from ethanol m.p. was 131°. (Found: C, 66·3; H, 6·6; N, 9·3. $C_{25}H_{31}O_5N_3$ requires C, 66·2; H, 6·8; N, 9·3%.) The product became coloured if exposed to light.

Cyclization of the hydrazone. A mixture of the crude hydrazone (18·5 g.), water (120 ml.) and conc. H_2SO_4 (5·6 ml.) was refluxed with vigorous stirring for 3 hr. On standing a reddish gummy precipitate separated. The mixture was diluted with water, extracted with ether, and the ethereal solution, after re-extraction with water, was dried and concentrated. The residue was taken up in ethanol and the solution filtered. From this solution separated 14·7 g. crystalline material, m.p. 120–122°. Recrystallization from ethanol gave *ethyl α-acetamido-α-carbethoxy-β(1-benzylindole-3)propionate* of m.p. 123°. (Found: C, 68·9; H, 6·9; N, 6·2. $C_{25}H_{28}O_5N_2$ requires C, 68·8; H, 6·6; N, 6·4%.)

α-N-Acetyl-1-benzyltryptophan. The ester (4·9 g.) was refluxed with 2·5 N-NaOH (22 ml.) for 3 hr. The solution was cooled and acidified with 2 N-HCl (35 ml.). After standing at 0° the *malonic acid* (4·65 g.) was filtered off and dried. Melting point was 127° (decomp.). (Found: N, 6·9. $C_{21}H_{20}O_5N_2$ requires N, 6·7%.) The acid was decarboxylated by refluxing for 2 hr. with water (30 ml.) containing enough ethanol to give a clear solution. The solution was filtered and cooled. The *acetamido acid* first separated as an oil which later crystallized in the form of needles. Yield was 2·9 g. The material had, after recrystallization from 50% ethanol, m.p. 156°. (Found: C, 70·8; H, 5·8. $C_{20}H_{20}O_3N_2$ requires C, 71·4; H, 5·9%.)

1-Benzyltryptophan. The acetyl derivative (2·0 g.) was refluxed with 2 N-NaOH (10 ml.) for 24 hr. The solution was acidified with acetic acid. On standing at 0° the amino-acid crystallized; yield was 1·4 g. It was recrystallized from aqueous ethanol and separated as *monohydrate*, m.p. 214° (decomp.). (Found: C, 69·7; H, 6·3. $C_{18}H_{18}O_2N_2.H_2O$ requires C, 69·3; H, 6·4%.) Drying to constant weight at 100–180° at 60 mm. over $Mg(ClO_4)_2$ gives the anhydrous amino-acid. (Found: C, 73·1; H, 6·2; N, 9·7. $C_{18}H_{18}O_2N_2$ requires C, 73·5; H, 6·1; N, 9·5%.)

Oxidation experiments with H_2O_2. Most of these experiments were carried out with (1-benzylindolyl)-acetamidomalonate. If more than 1 equiv. H_2O_2 was used, only brown

gummy or amorphous products were obtained. When 1 equiv. H_2O_2 was employed, a crystalline substance was obtained which had a composition which indicated that two atoms of O had entered the molecule, much of the starting material being recovered.

To an ice-cold solution of the acetamidomalonate (6·5 g.) in acetic anhydride (25 ml.) was added 1·52 ml. 9·85 M-H_2O_2 (1 equiv.). After 10 min. the flask was removed from the ice bath and the temperature allowed to rise to 20°. The flask was then cooled again and allowed to warm to 20°, the cycle being repeated several times. After standing at 0° for 18 hr. the mixture was poured on to ice and after decomposition of the anhydride the product was filtered off, washed with water, taken up in hot ethanol and allowed to cool to 0°. A small amount of crystalline material was isolated, m.p. 200°. The filtrate on dilution with water gave successive crops of material with m.p. 105–110°. These were crystallized and identified as starting material. The material which separated first was recrystallized twice from ethanol to give needles of m.p. 201°. Analysis showed that two atoms of O had been taken up. (Found: C, 64·7; H, 6·2; N, 6·0. The original material with addition of one O atom, $C_{25}H_{28}O_6N_2$ requires C, 66·4; H, 6·2; N, 6·2%; with addition of two O atoms, $C_{25}H_{28}O_7N_2$ requires C, 64·1; H, 6·0; N, 6·0%.) The absorption spectrum showed a maximum at 248·5 mμ. and a subsidiary peak at 304 mμ. The absorption curve corresponded closely with that of oxindole which had been allowed to oxidize in alkaline solution and was then acidified; this showed a maximum at 242·5 mμ. and a subsidiary peak at 303 mμ.

Oxidation experiments with hypobromite. These were carried out in borate buffer as described above for benzylindolecarboxylic acid. Only brown amorphous products were obtained, from which no crystalline compounds could be isolated either directly or after treatment with NaI in acetic acid.

Synthesis of β-oxindolylalanine

3-*Isatylidenepyruvic acid.* A mixture of isatin (29·4 g.), ethyl pyruvate (22 ml.) and ethanol (60 ml.) was stirred rapidly and cooled in an ice bath. When the temperature had fallen to 10°, diethylamine (20 ml.) was added during 9 min.; the temperature rose to 15–18°. After a further 3 min. unreacted isatin was removed by rapid filtration, and washed with a little 25% (v/v) ethanol. To the filtrate 2 N-HCl (180 ml.; previously chilled) was added immediately. Crystallization was induced by scratching and was encouraged to proceed as rapidly as possible by stirring and shaking. If the solution became turbid a little more ethanol was added. After about 1 hr. the crystals were collected, washed with 25% (v/v) ethanol, and air-dried. The yield was 16–20 g. It was found unprofitable to work up the mother liquors.

The product is a dihydrate. It is unstable in solution and recrystallization is wasteful; hence we used it directly for further work. A sample crystallized from aqueous dioxan in lustrous plum-coloured prisms. 3-*Isatylidenepyruvic acid* is easily soluble in ethanol, acetone and dioxan; sparingly in water and ether. The Na salt separates readily from aqueous solution, especially when excess alkali is present. The acid gives pale-yellow solutions in aqueous $NaHSO_3$ or H_2SO_3. It decomposes at about 210° without melting. (Found for air-dried material: C, 53·2; H, 4·7; loss of weight after drying at 80°/15 mm., 14·2, 14·5. $C_{11}H_7O_4N.2H_2O$ requires C, 52·2; H, 4·3; $2H_2O$, 14·2%. Found after drying at 80°: C, 60·8; H, 3·2. $C_{11}H_7O_4N$ requires C, 60·8; H, 3·2%.) The *phenylhydrazone*, prepared in aqueous acetic acid, crystallized from much ethanol in starlike aggregates of red needles, m.p. 191·5–193° (decomp.). (Found: C, 66·2; H, 4·4; N, 13·9. $C_{17}H_{13}O_3N_3$ requires C, 66·4; H, 4·2; N, 13·7%.)

3-*Oxindolylpyruvic acid phenylhydrazone.* This product was obtained in poor yield by two methods: (*a*) isatylidenepyruvic acid was shaken in aqueous suspension with PtO_2 and H_2 until somewhat more than 1 mol. of H_2 had been absorbed; the almost colourless solution was treated with phenylhydrazine acetate; (*b*) isatylidenepyruvic acid was reduced with $Na_2S_2O_4$ as described below, and the solution treated with phenylhydrazine. The *phenylhydrazone* crystallized from ethanol in pale-yellow needles, m.p. 190–191° (decomp.). (Found: C, 66·1; H, 5·6. $C_{17}H_{15}O_3N_3$ requires C, 66·0; H, 4·9%.)

α-*Oximino-β-(3-oxindolyl)propionic acid.* 3-Isatylidenepyruvic acid (23·2 g.) was stirred with water and cooled in ice during the addition (5 min.) of $Na_2S_2O_4$ (15·9 g.). Stirring was continued until the dark-coloured acid had disappeared. The solution was then boiled for 4 min., frothing being countered by use of a roomy flask and addition when necessary of a little ether. The hot solution was treated with charcoal and filtered; hydroxylamine hydrochloride (12 g.) was dissolved in the cooled filtrate. 2 N-NaOH was now added to neutralize the solution and then to keep it neutral as oximation proceeded; after about 1 hr. the solution was acidified to congo red and kept at 0°. The crystalline precipitate was collected next day and dried *in vacuo*. The yield was 13·8 g. It was recrystallized (with about 75% recovery) by dissolving in boiling methanol (10 ml./g.), treating with charcoal, filtering and adding an equal volume of water. The *acid* (XV) separated in small, colourless needles, m.p. 172–173° (decomp.; variable with rate of heating) after drying *in vacuo*. It tended to crystallize as a trihydrate. (Found: for an air-dried specimen: loss at 80°, 18·7, 18·8. $C_{11}H_{10}O_4N_2.3H_2O$ requires $3H_2O$, 18·7%). This became anhydrous in a vacuum desiccator. (Found for vacuum-dried material: C, 56·2; H, 4·8; $C_{11}H_{10}O_4N_2$ requires C, 56·2; H, 4·3%.)

3-*Oxindolylacetonitrile.* The oximino acid (XV) (1·75 g.) was refluxed for 1·5 hr. in 25 ml. water. After cooling, the crystals (1·15 g.) were collected; the mother liquor was only faintly acid. Recrystallization from water and then ethanol gave the *nitrile*, colourless rhombic prisms m.p. 162–165°. (Found: C, 69·3; H, 4·7; N, 16·1. $C_{10}H_8ON_2$ requires C, 69·8; H, 4·6; N, 16·3%.)

DL-α-*Amino-β-(3-oxindolyl)propionic acid.* (DL-*Hydroxytryptophan.*) The recrystallized anhydrous oximino-acid (XV) (5·1 g.) was stirred with 100 ml. water and cooled in ice during the gradual addition (about 0·5 hr.) of 2·3% sodium amalgam (118 g.; freshly prepared). Conc. HCl was added *pari passu* to keep the reaction mixture slightly acid. When the amalgam had reacted, a few drops of the liquor were treated with 2:4-dinitrophenylhydrazine in 2 N-HCl: if any precipitate appeared on warming, more amalgam and acid were added. The filtered and neutralized aqueous solution was evaporated rapidly at low pressure to small bulk. Crystallization was usually spontaneous and was more rapid if the solution was kept hot for a time. After keeping overnight at 0° the crystals were collected (4·2–4·8 g.). This product had m.p. 241–242° (decomp.). Recrystallization was best effected by taking up quickly in

a relatively large volume of O_2-free hot water, filtering (if necessary, after addition of charcoal), and concentrating to small volume at low pressure with a N_2 leak. The *amino-acid* (XVII) separated very slowly in small plates forming a hard white crust; m.p. 244–245° (decomp.). (Found: C, 59·6, 59·9; H, 5·7, 5·8; N, 13·0, 12·7, 12·9. $C_{11}H_{12}O_3N_2$ requires C, 60·0; H, 5·5, N, 12·7%.) The colours with ninhydrin and Millon's reagent, and the precipitation with mercuric and silver ions, were as described by Wieland & Witkop (1940) for the L-amino-acid.

β-Oxindolyl-α-formamidopropionic acid. DL-Oxindolylalanine (2 g.) was dissolved in 15 ml. anhydrous formic acid (treated with P_2O_5) and acetic anhydride (5 ml.) was gradually added with stirring at 58°. The solution was allowed to stand at 18–20° for 0·5 hr. and then poured into water (150 ml.). The aqueous solution was extracted with three portions of ethyl acetate (total 500 ml.). The ethyl acetate solution was dried and concentrated to dryness. The resulting oil slowly crystallized in the form of plates. Yield was 1·15 g. The material was recrystallized from water (5 ml.) and had m.p. 172°. (Found: C, 58·2; H, 5·3; N, 13·2. $C_{12}H_{12}O_4N_2$ requires C, 58·1; H, 4·8; N, 13·2%.)

The acetyl derivative was obtained by dissolving the amino-acid (0·5 g.) in N-NaOH (2·4 ml.) and adding acetic anhydride (1·5 ml.). When all anhydride had reacted the mixture was evaporated at low pressure. The residue was taken up in water and again concentrated; 0·24 ml. of conc. HCl was added and evaporation continued to dryness in a vacuum over KOH. The crystalline residue was washed with water; yield was 0·55 g., m.p. 208–210°. Recrystallization from water gave DL-*α-acetamido-β-(3-oxindolyl)propionic acid*, in colourless prisms, m.p. 209–210°. (Found: C, 59·6; H, 5·9; N, 10·7; equiv. 264. $C_{13}H_{14}O_4N_2$ requires C, 59·5; H, 5·4; N, 10·7%; mol.wt. 262.) The acid gave a yellow-brown colour with Millon's reagent.

DL-*α-Benzamido-β-(3-oxindolyl)propionic acid.* This was obtained by shaking the amino-acid (0·5 g.) with 1·3 N-$NaHCO_3$ (1·8 ml.) and benzoyl chloride (0·9 ml.). The solution was acidified and the precipitate extracted, after drying, with light petroleum to remove benzoic acid. Recrystallization from acetone and aqueous acetone gave colourless clustered rods, m.p. 153–154° after drying at 110°. (Found: N, 8·3. $C_{18}H_{16}O_4N_2$ requires N, 8·6%.)

3-(2′-Acetamidophenyl)-2-pyrrolidone-5-carboxylic acid (XVIII). DL-Oxindolylalanine (110 mg.) was refluxed with N-NaOH (1·5 ml.) for 6 min. The cooled solution was treated with acetic anhydride (0·4 ml.). Isolation of the product was carried out as described for the 'normal' acetyl derivative (above). The new *acid* (25 mg.; m.p. 227–228°) crystallized from water in rosettes of needles, m.p. 230°. (Found: C, 59·8; H, 5·7; equiv. 261. $C_{13}H_{14}O_4N_2$ requires C, 59·5; H, 5·4%; mol.wt. 262.) It gave no colour with Millon's reagent.

SUMMARY

Various possible routes for the synthesis of 'β-oxindolylalanine have been explored. A satisfactory synthesis in three stages from isatin has been worked out.

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