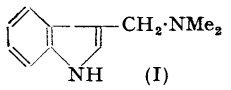
57. *New Syntheses of* DL-*Tryptophan. Part I. Syntheses from Ethyl α-Aceto-β-3-indolylpropionate and Ethyl α-Cyano-β-3-indolylpropionate.*

By D. O. HOLLAND and J. H. C. NAYLER.

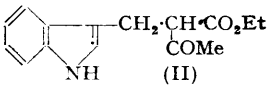
Three new syntheses of tryptophan are described, two of which start from ethyl α-aceto-β-3-indolylpropionate and the third from ethyl α-cyano-β-3-indolylpropionate.

NUMEROUS satisfactory syntheses of DL-tryptophan involve the condensation of gramine (I) or other 3-dialkylaminomethylindoles or their quaternary salts with such compounds as α-acylamino-acetoacetic, -cyanoacetic, or -malonic, nitroacetic, or nitromalonic esters, most of this work having been briefly reviewed by Rydon (*Ann. Reports*, 1949, **46**, 193; 1950, **47**, 175). Acetoacetic, cyanoacetic, and malonic ester undergo similar condensations (Snyder, Smith, and Stewart, *J. Amer. Chem. Soc.*, 1944, **66**, 200; Albertson, Archer, and Suter, U.S.P. 2,468,912) but the products, which lack a nitrogen-containing precursor of the amino-group, have not hitherto been utilised for the synthesis of tryptophan. The present paper records a number of attempts to convert condensation products of this second group into the amino-acid.

Ethyl α-aceto-β-3-indolylpropionate (II), previously obtained as a crude oil (Snyder, *et al.*; Albertson *et al.*, *locc. cit.*), has now been prepared more conveniently by treating gramine methosulphate with ethyl sodioacetoacetate in alcohol at room temperature, the gramine methosulphate being produced *in situ* (Albertson, Archer, and Suter, *J. Amer. Chem. Soc.*, 1945, **67**, 36). An excess of ethyl acetoacetate must be used, otherwise the crude product contains substantial amounts of ethyl αα-di-(3-indolylmethyl)acetoacetate (III). The latter was formed in high yield on treatment of the sodio-derivative of (II) with gramine methosulphate.



(I)



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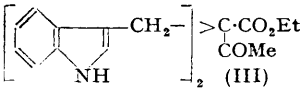


(III)

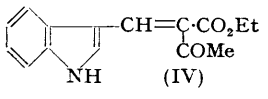
According to U.S.P. 2,468,912 hydrolysis of ethyl α-aceto-β-3-indolylpropionate (II) with hot concentrated sodium hydroxide solution gives β-3-indolylpropionic acid, but following

the prescribed method we obtained a high yield of 2-3′-indolylethyl methyl ketone and none of the acid.

We also examined the catalytic hydrogenation of ethyl α-3-indolylmethyleneacetoacetate (IV), formed by the condensation of ethyl α-ethoxymethyleneacetoacetate with indole. However, considerably more than the theoretical amount of hydrogen was absorbed and the oily product gave only a comparatively poor yield of 2-(3-indolyl)ethyl methyl ketone on hydrolysis.

Attempts to apply the Japp–Klingemann reaction using potassium benzenediazoate to ethyl α-aceto-β-3-indolylpropionate under essentially the conditions employed by Feofilaktov (*Compt. rend. Acad. Sci., U.R.S.S.*, 1939, **24**, 755) for the synthesis of a number of amino-acids were unsuccessful, yielding only tars and none of the required indole-3-pyruvic acid phenylhydrazone on subsequent hydrolysis. On the other hand, application of the Schmidt reaction with hydrazoic acid in the presence of concentrated sulphuric acid readily afforded *N*-acetyltryptophan ethyl ester in good yield. From impure specimens of ethyl α-aceto-β-3-indolylpropionate containing the disubstitution product (III), there was also obtained the corresponding ethyl αα-di-(3-indolylmethyl)aceturate (V). Hydrolysis of *N*-acetyltryptophan ester with aqueous sodium hydroxide at room temperature afforded *N*-acetyltryptophan, and prolonged heating gave the free amino-acid in 62% overall yield from indole.

Ethyl α-aceto-β-3-indolylpropionate (II) was also converted into tryptophan by way of β-3-indolyl-α-oximinopropionic acid (VI). This acid, first synthesised from indole-3-pyruvic acid and hydroxylamine by Bauguess and Berg (*J. Biol. Chem.*, 1934, **104**, 675), was readily prepared in moderate yield by the nitrosation of ethyl α-aceto-β-3-indolylpropionate (II) by one of the procedures described by Barry and Hartung (*J. Org. Chem.*, 1947, **12**, 460). The oximino-ester was hydrolysed *in situ* directly to the acid by brief heating with alkali, slow hydrolysis in the cold giving only a very crude product. Bauguess and Berg (*loc. cit.*) described the conversion of (VI) into tryptophan by hydrogenation in alcohol over Raney nickel at atmospheric temperature and pressure, but we found reduction to be more satisfactory in dilute aqueous ammonia at about 60°.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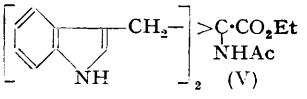
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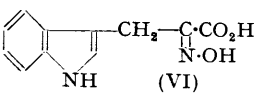
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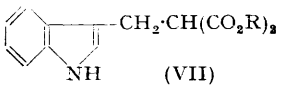
In view of the findings of Barry and Hartung (*loc. cit.*) it was expected that better yields of the oximino-acid (VI) might be obtained by nitrosation of diethyl (3-indolylmethyl)malonate (VII; R = Et) or the corresponding acid (VII; R = H). However, reaction of the ester with *n*-butyl nitrite in the presence of sodium ethoxide gave no oximino-acid, whilst reaction of either the ester or the acid in the presence of dry hydrogen chloride gave only tars.

Hofmann and Curtius reactions with ethyl α-cyano-β-3-indolylpropionate (VIII; R = Et) were next investigated. The amide from (VIII) with sodium hypobromite or hypochlorite gave neither the amino-nitrile nor the hydantoin (which is known). The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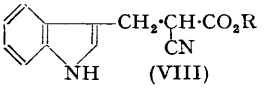
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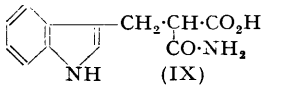
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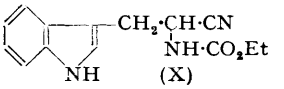
(X)

acid (VIII; R = H) was converted into the acid-amide (IX) but this with sodium hypobromite also gave no tryptophan. The failure of the Hofmann reaction in these two cases is not surprising in view of the known sensitiveness of the indole ring to hypohalites. By contrast, the Curtius reaction provided a satisfactory route from (VIII; R = Et) to tryptophan, hydrolysis of the urethane (X) being accomplished by prolonged heating with hydrochloric and acetic acids. The use of a mixture of hydrochloric and formic acids, which

has been reported by Gagnon and Boivin (*Canad. J. Res.*, 1948, **26**, *B*, 503) to be advantageous for other amino-acids, was unsuccessful in the present case, the mixture developing an intense purple colour and yielding much tar.

Experimental

M. p.s are uncorrected.

Ethyl α-Aceto-β-3-indolylpropionate (II).—To a solution of sodium (2·3 g.) in ethanol (80 ml.) were added ethyl acetoacetate (25·2 ml., 2 mols.) and gramine (17·4 g.). Methyl sulphate (19·2 ml.) was run in during 30 minutes with stirring and cooling to 10—20°, the gramine rapidly dissolving. After a further 4 hours' stirring at ordinary temperature the mixture was evaporated under reduced pressure and the sticky residue treated with chloroform (120 ml.) and water (80 ml.). When dissolution was complete the organic phase was washed with water and dried (Na_2SO_4), and chloroform and excess of ethyl acetoacetate were removed *in vacuo* to leave the crude product as an amber syrup (24·5 g., 95%). A specimen for analysis was obtained by distillation at 150°/0·0005 mm. (Found: C, 69·9; H, 6·5; N, 5·5. Calc. for $C_{15}H_{17}O_3N$: C, 69·5; H, 6·6; N, 5·4%).

The solvated 2 : 4-*dinitrophenylhydrazone* (from ethyl acetate) had m. p. 129—131° (softening 127°) (Found: C, 57·0; H, 5·3, N, 14·3, 14·7. $C_{21}H_{21}O_6N_5,0{\cdot}5C_4H_8O_2$ requires C, 57·1; H, 5·2; N, 14·5%). Complete removal of solvent required 28 hours' heating at 100°/0·2 mm. and left an orange powder, m. p. 133—135° (Snyder, Smith, and Stewart, *loc. cit.*, give m. p. 133·5°) (Found: C, 57·3; H, 4·6; N, 16·2; loss, 9·1. Calc. for $C_{21}H_{21}O_6N_5$: C, 57·4; H, 4·8; N, 15·9; loss, 9·1%).

Ethyl αα-Di-(3-indolylmethyl)acetoacetate (III).—(*a*) When the preparation of ethyl α-aceto-β-3-indolylpropionate was carried out as described above but with only 1 mol. of ethyl acetoacetate a good yield of crude syrup resulted but this was found to be only very impure (II). Cautious dilution of its ethereal solution with light petroleum precipitated a pale solid (generally about 20% by wt. of the whole) which on recrystallisation from ether–light petroleum afforded *ethyl αα-di-(3-indolylmethyl)acetoacetate*, m. p. 143—144° (Found: C, 73·8; H, 6·4; N, 7·6. $C_{24}H_{24}O_3N_2$ requires C, 74·2; H, 6·2; N, 7·2%).

(*b*) Ethyl α-aceto-β-3-indolylpropionate (II) (6·47 g.) was dissolved in a solution of sodium (0·58 g.) in ethanol (20 ml.). Gramine (4·35 g.) was added and methyl sulphate (4·8 ml.) run in with stirring during 25 minutes. After a further 4 hours' stirring at ordinary temperature the mixture was evaporated *in vacuo* and the solid residue treated with chloroform (60 ml.) and water (35 ml.). Undissolved material, m. p. 141—145° (4·46 g.), was collected and the organic phase of the filtrate washed, dried, and evaporated *in vacuo*. The residual gum yielded a further quantity of solid (m. p. 136—139°) on trituration with toluene, the total yield of ethyl αα-di-(3-indolylmethyl)acetoacetate undepressed on admixture with material from (*a*) being 7·94 g. (82%). The 2 : 4-*dinitrophenylhydrazone* separated from ethyl acetate as an orange-yellow powder, m. p. 204—206° (decomp.) (Found: C, 63·2; H, 5·3; N, 14·4. $C_{30}H_{28}O_6N_6$ requires C, 63·4; H, 5·0; N, 14·8%).

Hydrolysis of Ethyl α-Aceto-β-3-indolylpropionate.—The propionate (II) (5·02 g.) was heated for 2 hours on the steam-bath with sodium hydroxide (18 g.) in water (33 ml.) as described in U.S.P. 2,468,912 but much oil remained undissolved. On cooling, the oil solidified and the resulting solid was collected and thoroughly washed with water to leave a fawn powder (3·42 g.), m. p. 87—89°. Thorough extraction of the acidified filtrate with ether failed to yield β-3-indolylpropionic acid. Recrystallisation of the above-mentioned neutral solid from light petroleum gave needles of 2-3′-*indolylethyl methyl ketone*, m. p. 93° (Found: C, 76·4; H, 6·7; N, 7·7. $C_{12}H_{13}ON$ requires C, 77·0; H, 7·0; N, 7·5%). The 2 : 4-*dinitrophenylhydrazone* crystallised from dioxan–alcohol in small red needles, m. p. 209—210° (Found: C, 58·5; H, 4·8; N, 19·0. $C_{18}H_{17}O_4N_5$ requires C, 58·8; H, 4·7; N, 19·1%).

Ethyl α-3-Indolylmethyleneacetoacetate (IV).—A solution of ethyl α-ethoxymethyleneacetoacetate (Claisen, *Annalen*, 1897, **297**, 16) (39·0 g.) and indole (24·5 g.) in acetic acid (80 ml.) containing acetic anhydride (20 ml.) was heated on the steam-bath for 2 hours and the solvents were removed *in vacuo*. The residual sticky yellow-brown solid was washed with ice-cold ether to leave a yellow powder, m. p. 132—134°, concentration of the ethereal washings affording a small second crop. The combined solids (32·5 g.) crystallised first from ethanol and then from benzene, to give *ethyl α-3-indolylmethyleneacetoacetate* as yellow needles, m. p. 135—136° (slow heating from 115°) (Found: C, 70·0; H, 5·8; N, 5·35. $C_{15}H_{15}O_3N$ requires C, 70·0; H, 5·9; N, 5·45%).

Reduction of Ethyl α-3-*Indolylmethyleneacetoacetate.*—A solution of the ester (IV) (15·0 g.) in alcohol (600 ml.) was hydrogenated at ordinary temperature and pressure over Adams's platinum oxide (0·4 g. added in two lots). Uptake of hydrogen ceased after about 24 hours, approx. 1·5 mols. having been consumed. After removal of the catalyst the pale filtrate and washings were evaporated to leave an amber-coloured syrup of impure ethyl α-aceto-β-3-indolylpropionate (II). The product was characterised as the 2 : 4-dinitrophenylhydrazone and by hydrolysis to 2-3′-indolylethyl methyl ketone, but the yields of these derivatives were not good.

Schmidt Reaction on Ethyl α-*Aceto*-β-3-*indolylpropionate.*—Ethyl α-aceto-β-3-indolylpropionate (14·5 g.) in dry chloroform (60 ml.) was treated with hydrazoic acid in chloroform (46·6 ml. of 5·14% w/v solution), and the mixture was added dropwise during 1 hour to a vigorously stirred mixture of concentrated sulphuric acid (40 ml.) and chloroform (40 ml.) kept at −5° to 0°. The mixture was then stirred for 30 minutes at the same temperature and then poured on about 300 g. of ice. The aqueous phase was extracted with chloroform (2 × 100 ml.). The combined chloroform solutions were washed, dried (Na_2SO_4), and evaporated *in vacuo* and the residual red gum was triturated with a little ether, to yield crude *N*-acetyltryptophan ethyl ester as a cream-coloured powder (12·7 g., 83%), m. p. 130—133°. A specimen recrystallised from 50% aqueous alcohol formed plates, m. p. 131·5—132·5° (Albertson, Tullar, King, Fishburn, and Archer, *J. Amer. Chem. Soc.*, 1948, **70**, 1150, give m. p. 133·5—135·5°) (Found : C, 65·5; H, 6·9; N, 10·5. Calc. for $C_{15}H_{18}O_3N_2$: C, 65·7; H, 6·6; 10·2%).

In one early experiment a specimen of ethyl α-aceto-β-3-indolylpropionate prepared from gramine methosulphate and only 1 mol. of ethyl acetoacetate and consequently containing a substantial amount of ethyl αα-di-(3-indolylmethyl)acetoacetate (III), gave, in addition to the normal product, *ethyl* αα-*di*-(3-*indolylmethyl*)*aceturate* (V), separated therefrom by reason of its insolubility in hot 50% aqueous alcohol and crystallising from acetone–water or acetonitrile in needles, m. p. 244—245° (Found : C, 71·6; H, 6·4; N, 10·4. $C_{24}H_{25}O_3N_3$ requires C, 71·5; H, 6·3; N, 10·4%).

Hydrolysis of N-*Acetyltryptophan Ethyl Ester.*—(*a*) A suspension of *N*-acetyltryptophan ethyl ester (1 g.) in 10% aqueous sodium hydroxide (5 ml.) was shaken overnight at room temperature and the solution acidified, to yield crude *N*-acetyltryptophan (0·79 g.), m. p. 201—203°, raised to 206° by recrystallisation from dilute aqueous alcohol (Found : C, 63·2; H, 5·6; N, 11·6. Calc. for $C_{13}H_{14}O_3N_2$: C, 63·4; H, 5·7; N, 11·4%).

(*b*) *N*-Acetyltryptophan ethyl ester (10 g.) was refluxed for 21 hours with 10% aqueous sodium hydroxide (100 ml.) and the resulting hot orange solution decolorised with charcoal, treated with acetic acid (35 ml.), and stored in the refrigerator for 48 hours. The solid was collected, washed with water, dried, and recrystallised from acetic acid, a little insoluble matter being discarded. Further purification was effected by dissolution in hot 33% aqueous alcohol (190 ml.) containing sodium hydroxide (2·5 g.) and addition of acetic acid to pH 5—6, pure tryptophan separating on cooling in plates (6·26 g., 84%), m. p. 285—287° (decomp.) [control m. p. 283—287° (decomp.)] not raised by further crystallisation from 33% aqueous alcohol (Found : C, 64·8; H, 6·0; N, 13·7. Calc. for $C_{11}H_{12}O_2N_2$: C, 64·7; H, 5·9; N, 13·7%).

β-3-*Indolyl*-α-*oximinopropionic Acid.*—(*a*) Ethyl α-aceto-β-3-indolylpropionate (4·32 g.) was dissolved in a solution of sodium (0·38 g.) in dry ethanol (16 ml.) and to the stirred solution a mixture of *n*-butyl nitrite (1·9 ml.) and ethanol (8 ml.) was added dropwise during 30 minutes at −5° to 0°. After a further 3 hours at 0° the dark solution was poured on crushed ice (about 100 g.) containing 5 ml. of concentrated hydrochloric acid, and the red-brown gum was extracted into ether. After being washed with a little water the ethereal solution was extracted with 10% aqueous sodium hydroxide (3 × 10 ml.), and the red alkaline solution heated on the steam-bath for 15 minutes. The cooled solution was acidified below 5°, the crude oximino-acid separating as a light brown powder. Partial purification was effected by dissolution in sodium hydrogen carbonate solution, filtration from a little insoluble material, and reprecipitation with acid, to give a fawn-coloured powder (2·16 g., 59%), m. p. 144° (decomp.). This material was suitable for reduction to tryptophan, but for analysis a specimen was further purified by repeated recrystallisation from ether–light petroleum, to m. p. 154° (decomp.) (Found : C, 60·5; H, 4·7; N, 12·4. Calc. for $C_{11}H_{10}O_3N_2$: C, 60·5; H, 4·6; N, 12·8%). Bauguess and Berg (*loc. cit.*) give m. p. indef., >175°.

Other methods of nitrosation (cf. Barry and Hartung, *loc. cit.*) failed.

(*b*) A solution of indole-3-pyruvic acid (0·14 g.), sodium hydrogen carbonate (0·8 g.), and hydroxylamine hydrochloride (0·25 g.) in water (8 ml.) was kept at ordinary temperature for 18 hours, then filtered, and the filtrate acidified. The precipitate was almost pure β-3-indolyl-α-

oximinopropionic acid, m. p. 153° (decomp.) (0·13 g.). Recrystallisation from ether–light petroleum gave very pale fawn-coloured crystals, m. p. 155° (decomp.) not depressed on admixture with the product from (*a*) (Found : N, 12·9%).

Reduction of β-3-*Indolyl-α-oximinopropionic Acid.*—A solution of the oximino-acid (0·5 g.) in water (50 ml.) containing aqueous ammonia (*d* 0·88, 2 ml.) was hydrogenated at 60—65°/approx. 1 atm. over Raney nickel (about 0·5 g.), uptake of hydrogen ceasing after 4 hours when approx. 1·8 mols. had been consumed. Filtration and evaporation *in vacuo* gave crude tryptophan as a nearly white powder (0·34 g., 72%), m. p. 261—264° (decomp.), raised to 279—280° (decomp.) [control, m. p. 279—280° (decomp.)] by recrystallisation from acetic acid and then from 33% aqueous alcohol.

*Ethyl α-Cyano-*β*-3-indolylpropionate* (VIII; R = Et).—This ester was obtained almost quantitatively as a crude red oil by the action of gramine methiodide on ethyl sodiocyanoacetate as described by Snyder, Smith, and Stewart (*loc. cit.*) except that xylene was substituted for butyl ether as solvent.

*α-Cyano-*β*-3-indolylpropionamide.*—A solution of crude α-cyano-ester (24·7 g.) in methanol (300 ml.) was saturated with ammonia at 0° and set aside in a stoppered vessel at room temperature for 4 days. Concentration *in vacuo* to about 80 ml. and cooling to 0° gave a first crop (7·62 g.) of white powder, m. p. 170—172°; evaporation of the filtrate and trituration of the residual syrup with ether yielded a sticky solid which on recrystallisation from aqueous alcohol gave a further 2·04 g., m. p. 169—170°. Further crystallisation from acetic acid or aqueous alcohol gave pure *α-cyano-*β*-3-indolylpropionamide*, plates, m. p. 173—174° (Found: C, 67·7; H, 5·5; N, 20·1. $C_{12}H_{11}ON_3$ requires C, 67·6; H, 5·2; N, 19·7%).

*α-Cyano-*β*-3-indolylpropionic Acid* (VIII; R = H).—Crude ethyl α-cyano-β-3-indolylpropionate (11·65 g.) was refluxed for 1 hour with one equiv. of aqueous alcoholic potassium hydroxide. The solution was then evaporated *in vacuo*, the residue dissolved in hot water, decolorised with charcoal, and the cooled light red filtrate acidified with hydrochloric acid. The red oil which separated was removed in ether and the dried extracts were evaporated to a syrup which was dissolved in boiling chloroform (100 ml.) and concentrated to about 30 ml. On cooling, *α-cyano-*β*-3-indolylpropionic acid* (3·78 g.) was obtained as colourless needles, m. p. 142—144°, after further crystallisation from chloroform (Found : C, 67·2; H, 4·8; N, 13·3. $C_{12}H_{10}O_2N_2$ requires C, 67·3; H, 4·7; N, 13·1%).

*α-Carbamyl-*β*-3-indolylpropionic Acid* (IX).—A solution of the α-cyano-acid (1 g.) in concentrated sulphuric acid (7 ml.) was set aside overnight at room temperature, then poured on crushed ice, and the sticky precipitate extracted with ethyl acetate (3 × 50 ml.). The dried extracts were concentrated to about 30 ml. and diluted with chloroform (70 ml.) to yield, after storage in the refrigerator overnight, 0·99 g. of crude pink solid, m. p. 151° (decomp.) (softening and shrinking from 144°). *α-Carbamyl-*β*-3-indolylpropionic acid* was purified only with some difficulty, by dissolution in hot ethyl acetate (charcoal), concentration to a small volume, dilution with chloroform, and refrigeration. It formed a colourless microcrystalline powder, m. p. 151° (decomp.) (Found : C, 62·5; H, 5·4; N, 12·6. $C_{12}H_{12}O_3N_2$ requires C, 62·0; H, 5·2; N, 12·1%).

*α-Cyano-*β*-3-indolylpropionhydrazide.*—A mixture of crude ethyl α-cyano-β-3-indolylpropionate (15·84 g.), 85% hydrazine hydrate (4·1 g.), and ethanol (20 ml.) was heated gently to effect solution and then refluxed for 1 hour. The clear orange solution was decanted from a trace of gum and kept in the refrigerator for 2 days. A colourless solid (7·44 g.) slowly separated. Concentration of the filtrate gave a small second crop, the total yield of solid, m. p. 144—147°, being 7·92 g. (53%). The *hydrazide* crystallised from alcohol in needles, m. p. 149—150° (Found : C, 62·9; H, 5·3; N, 24·8. $C_{12}H_{12}ON_4$ requires C, 63·1; H, 5·3; N, 24·6%).

Ethyl 1-*Cyano-*2-3′-*indolylethylcarbamate* (X).—Sodium nitrite (2·08 g.) in water (10 ml.) was added during 5 minutes with vigorous stirring to a cooled mixture of ether (100 ml.) and a solution of the hydrazide (4·56 g.) in 5N-hydrochloric acid (40 ml.), at −10° to 0°. The layers were separated immediately and the ethereal solution was washed with water and aqueous sodium hydrogen carbonate and quickly dried ($CaCl_2$). Ethanol (100 ml.) was added and the ether was distilled off through a short column. The residual alcoholic solution was refluxed for 1 hour to complete the conversion of the azide into the urethane and then evaporated *in vacuo* to a brown gum. Trituration with toluene (cooling) yielded a crude light brown solid (3·55 g., 69%), m. p. 109—113°, crystallisation from aqueous alcohol (charcoal) affording needles of the *urethane* (X), m. p. 123—124° (Found : C, 65·3; H, 6·1; N, 16·5. $C_{14}H_{15}O_2N_3$ requires C, 65·3; H, 5·9; N, 16·3%).

Hydrolysis of the Urethane (X).—A solution of the urethane (1 g.) in acetic acid (8 ml.) and

concentrated hydrochloric acid (8 ml.) was refluxed for 72 hours, then evaporated *in vacuo*. The residue was dissolved in a little water, and the evaporation repeated twice more to remove the excess of acid. The residue was then dissolved in water (15 ml.) and heated with triethylamine (5 ml.) on the steam-bath for 30 mins., and the solution was evaporated to dryness *in vacuo*. The resulting sticky solid was thoroughly washed with alcohol to remove triethylamine hydrochloride, leaving crude tryptophan as a light brown powder (0·57 g., 67%), m. p. 273—275° (decomp.), which was purified as previously described.

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