sucrose and polysaccharides would bring label into the HMP group of compounds, including UDPG, which are in rapid reversible equilibrium with each other, as evidenced by the zero slope for the percentage appearance curves.

The very rapid labeling of ATP in the dark might be brought about through the oxidative phosphorylating reaction of 3-phosphoglyceraldehyde, accounting for the possible early appearance of DIPGA labeled first in the number one phosphate. There are, of course, other routes by which ortho phosphate may appear in ATP. The negative slope of the percentage appearance curve may be taken to indicate that this reservoir is saturating more rapidly than any other so far observed by these methods.

The labeling of the phosphate of PGA in the dark occurs at an accelerating rate as evidenced by the positive slope of the percentage curve, and this is accounted for in such a scheme by the requirement of the prior labeling of ATP and fructose 1,6-diphosphate, followed by a slow step.

In the light, the negative slope of ATP again indicates its early saturation, but its lower level suggests that the labeled high energy phosphates so produced are in greater demand for their function in CO_2 fixation in photosynthesis,[27,28,38-40] leading to the production of PGA. This would account for the rapid labeling of PGA in the light.

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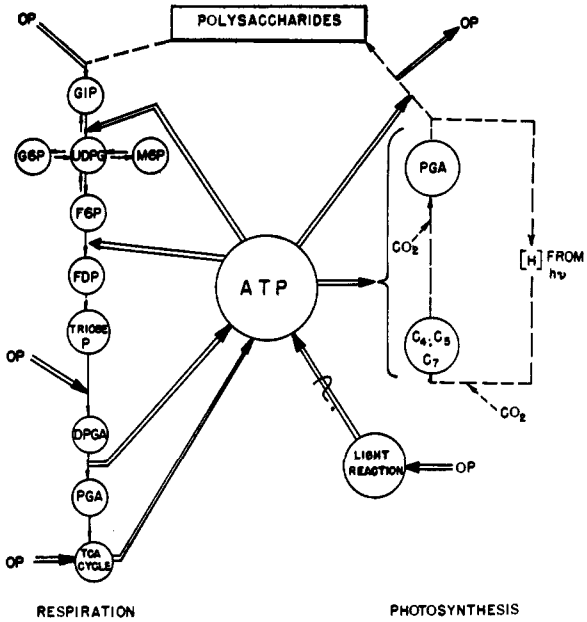

Fig. 4.—Schematic representation of the relationship between ortho phosphate and certain organic phosphates Double lines indicate phosphate transfer; dotted lines, a change in the carbon skeleton; single lines, other types of transformation. "TCA" denotes the tricarboxylic acid cycle and "Light Reaction" indicates methods of converting ortho phosphate to high energy phosphate in the form of ATP by means dependent upon photochemical reactions and not involving the reduction of CO_2 followed by reoxidation of the products.

(38) E. C. Wassink, J. F. G. M. Wintermans and J. E. Tjia, *Proc. Kon. Akad. Wetenschap*, **54c**, 41 (1951).
(39) W. Lindeman, *ibid.*, **54c**, 287 (1951).
(40) E. C. Wassink, J. E. Tjia and J. F. G. M. Wintermans, *ibid.*, **52**, 412 (1949).

[CONTRIBUTION FROM THE NATIONAL INSTITUTES OF HEALTH]

The Use of Various Aminomalonates in the Synthesis of α-Substituted Tryptophans

BY HENRY M. KISSMAN AND BERNHARD WITKOP

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Dibenzyl formaminomalonate (IV), carbobenzyloxyaminomalonate (V) and formaminopiperidinomethylmalonate (II) were prepared and tested in condensation reactions with 2-phenyl- and 2-carbethoxy-3-diethylaminoindoles (VIII and IX). XIII, the condensation product of V with IX, on catalytic debenzylation lost all three benzyl groups and gave 2-carbethoxytryptophan (XIX) in good yield. 2-Phenyltryptophan (XXI) and its ethyl ester (XXII) were prepared by four different routes. XV, the condensation product of IX with diethyl nitromalonate (VI), yielded, *via* XVII, 2-carbethoxytryptophan ethyl ester (XX) or the 4,5,6,7-tetrahydro-2-carbethoxytryptophan ethyl ester (XVIII) depending on the type of reduction.

The difficulties of obtaining the presumable primary breakdown product of tryptophan[1] would be expected to be smaller in a tryptophan derivative bearing a substituent in the α-position. In such a compound the well-known readiness with which β-hydroxy(or hydroperoxy)-indolenines undergo internal (or external) addition reactions, *e.g.*, with the alanine side chain, should be lessened or suppressed. 2-Phenyltryptophan (XXI) was synthesized in the hope of using the activating influence of the phenyl group for autoxidation experiments,[2] while 2-carbethoxytryptophan (XIX) was thought to be a suitable starting material for a β-hydroxyindolenine which could then lose the carbethoxy group with the same ease of saponification and decarboxylation as indoleninecarboxylic acid or α-picolinic acid.

2-Phenyltryptophan (XXI).—This amino acid and its ethyl ester (XXII) were obtained by the following four different routes: (i) The Mannich base VIII from 2-phenylindole (VII),[3] formaldehyde and diethylamine was condensed in the usual

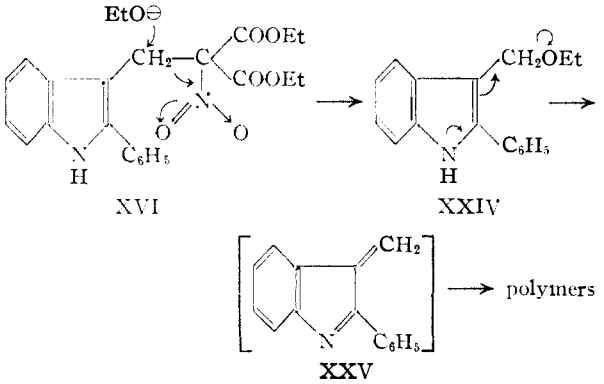
(1) A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952).
(2) B. Witkop and J. B. Patrick, THIS JOURNAL, **74**, 3855 (1952).
(3) H. Kissman, D. K. Farnsworth and B. Witkop, *ibid.*, **74**, 3948 (1952).

fashion with diethyl acetamidomalonate.[4] Basic hydrolysis of the condensation product XI (see Chart I) gave N-acetyl-2-phenyltryptophan yielding the free amino acid (XXI) on further acid hydrolysis.

(ii) The condensation product X from 2-phenylindole (VII) with diethyl piperidinomethylformaminomalonate (I)[5] on basic and acid hydrolysis gave directly 2-phenyltryptophan (XXI).

(iii) Dibenzyl malonate[6] was nitrosated, reduced with aluminum amalgam[7] and acylated with benzyl chlorocarbonate in benzene in the presence of potassium carbonate. Condensation of dibenzyl carbobenzyloxyaminomalonate (V) with the Mannich base (VIII) in xylene in the presence of sodium hydride led to the liberation of only about half of the calculated amount of diethylamine with the formation of the condensation product XII (25% yield). Catalytic debenzylation of XII in methanol with hydrogen and palladium-on-charcoal smoothly removed the three benzyl groups in one operation leading directly to the free amino acid (XXI) (91% yield).

(iv) The condensation of the Mannich base (VIII) with diethyl nitromalonate (VI)[8] gave XIV which was cleaved by sodium ethoxide to the nitro ester XVI. This cleavage reaction was normally carried out in the presence of ether. When it was done in refluxing ethanol with sodium ethoxide, ethoxide ion apparently displaced diethyl nitromalonate forming 2-phenyl-3-ethoxymethylindole (XXIV), a sensitive compound



leading to polymeric material under a variety of conditions, probably *via* XXV. The similar conversion of gramine to 3-methoxymethylindole and 3-ethoxymethylindole under the action of methanolic or ethanolic potassium hydroxide in the presence of methyl iodide, previously thought to involve an intermediate hydrolysis,[9] proceeds by the attack of alkoxide ion on gramine methiodide[10] in an analogous fashion. Reduction of XVI either with palladium-on-charcoal in glacial acetic acid (40 lb., 70°) or with Raney nickel in ethanol at atmospheric pressure yielded the amino ester (XXII). No evidence for further hydrogenation has been observed in the 2-phenyl series. The attempted preparation of dibenzyl nitromalonate has not been successful yet.

2-Carbethoxytryptophan (XIX).—The condensation of the Mannich base (IX), prepared from 2-carbethoxyindole[11] by a Mannich reaction and previously obtained by a different route,[12,13] in contrast to VIII, could be smoothly condensed with dibenzyl carbobenzyloxyaminomalonate (V) to give the condensation product XIII in 89% yield. Hydrogenolysis of XIII removes all three benzyl groups easily. The intermediate malonic acid loses carbon dioxide readily. This mole of CO_2 simulates an uptake of only two moles of gas (instead of three) in the catalytic debenzylation reaction. 2-Carbethoxytryptophan (XIX) was obtained in 76% yield.

Dibenzyl formaminomalonate (IV) was prepared by two different routes: (i) By reductive acylation of the corresponding isonitrosomalonate with zinc and formic acid in poor yield.[14] (ii) By formylation of the previously mentioned dibenzyl aminomalonate with formic–acetic anhydride.[15] The Mannich reaction of (IV) with piperidine and formaldehyde[5] gave II in 75% yield. Attempted condensation reactions of II and IV with (IX) or (VII) were discontinued when difficulties were encountered, and XIX became easily accessible *via* XIII.

Diethyl nitromalonate (VI) was also employed in the synthesis of the liquid 2-carbethoxytryptophan ethyl ester (XX) *via* the intermediates XV and XVII. When in the reduction of XVII time and temperature were (inadvertently) increased, a solid compound, m.p. 87–87.5°, was obtained (yield 68%), the ultraviolet spectrum of which (Fig. 1) was markedly different from that of 2-carbethoxy- or 2-phenyltryptophan (Table I). The analysis showed this compound to be a tetrahydro-2-carbethoxytryptophan and the spectral comparison with 2-carbethoxy-3-methyl-4,5,6,7-tetrahydro-

(4) E. E. Howe, A. J. Zambito, H. R. Snyder and M. Tishler, THIS JOURNAL, **67**, 38 (1945).

(5) A. Butenandt, H. Hellmann and J. Renz, *Z. physiol. Chem.*, **284**, 175 (1949); H. Hellmann, *ibid.*, **284**, 163 (1949).

(6) H. J. Backer and C. J. Lolkema, *Rec. trav. chim.*, **57**, 1234 (1938).

(7) V. Cherchez, *Bull. soc. chim., France*, [4] **47**, 1279 (1930).

(8) D. I. Weisblat and D. A. Lyttle, THIS JOURNAL, **71**, 3079 (1949).

(9) J. Madinaveitia, *J. Chem. Soc.*, 1927 (1937).

(10) T. A. Geissman and A. Armin, THIS JOURNAL, **74**, 3917 (1952).

(11) *Cf.* W. J. Brehm and H. G. Lindwall, *J. Org. Chem.*, **15**, 685 (1950).

(12) B. Hegedüs, *Helv. Chim. Acta*, **29**, 1499 (1946).

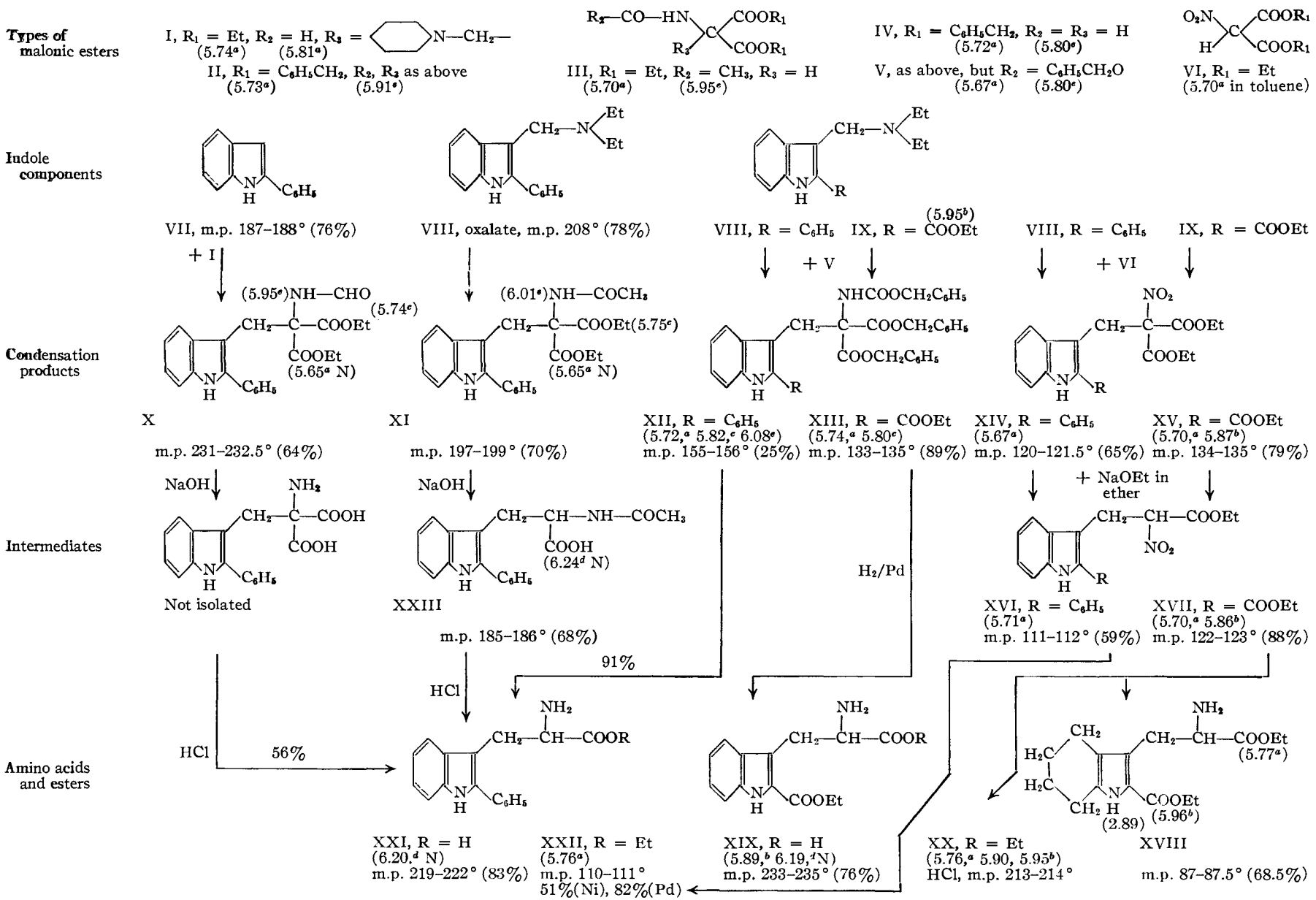
(13) V. Boekelheide and C. Ainsworth, THIS JOURNAL, **72**, 2134 (1950).

(14) *Cf.* A. Galat, *ibid.*, **69**, 965 (1947).

(15) Y. L. Goldfarb and L. M. Smorgonskii, *J. Gen. Chem. Russ.*, **12**, 255 (1942); *cf.* G. R. Clemo and G. A. Swan, *J. Chem. Soc.*, 603 (1945).

CHART I

The use of various amino- and nitromalonates in the synthesis of 2-substituted tryptophans. The figures in parentheses are infrared absorption bands of the important functional groups (taken in chloroform solution unless marked with N = Nujol) with the following assignment: [a] unconjugated carbethoxy or carbobenzyloxy group or groups; [b] conjugated carbethoxy group; [c] unconjugated carbethoxy or carbobenzyloxy possibly bonded; [d] carboxyl of amino acid; the carboxyl anion of amino acids is usually associated with a band at 6.35; *cf.* I. M. Klotz and D. M. Gruen, *J. Phys. Colloid Chem.*, **52**, 961 (1948); H. W. Thompson, D. L. Nicelson and N. L. Short, *Faraday Soc. Discussion*, 9, Spectroscopy and Molecular Structure, 1951; [e] formyl, acetyl or carbobenzyloxyamino group.


Types of malonic esters
I, R1 = Et, R2 = H, R3 = N—CH2— (5.74a) (5.81a)
II, R1 = C6H5CH2, R2, R3 as above (5.73a) (5.91a)
III, R1 = Et, R2 = CH3, R3 = H (5.70a) (5.95c)
IV, R1 = C6H5CH2, R2 = R3 = H (5.72a) (5.80e)
V, as above, but R2 = C6H5CH2O (5.67a) (5.80e)
VI, R1 = Et (5.70a in toluene)
Indole components
VII, m.p. 187–188° (76%)
VIII, oxalate, m.p. 208° (78%)
VIII, R = C6H5 IX, R = COOEt (5.95b)
VIII, R = C6H5 IX, R = COOEt
+ I
+ V
+ VI
Condensation products
X
XI
XII, R = C6H5 (5.72,a 5.82,c 6.08e) m.p. 155–156° (25%)
XIII, R = COOEt (5.74,a 5.80c) m.p. 133–135° (89%)
XIV, R = C6H5 (5.67a) m.p. 120–121.5° (65%)
XV, R = COOEt (5.70,a 5.87b) m.p. 134–135° (79%)
m.p. 231–232.5° (64%)
m.p. 197–199° (70%)
Intermediates
NaOH
Not isolated
XXIII
m.p. 185–186° (68%)
+ NaOEt in ether
H2/Pd
HCl
91%
HCl 56%
XVI, R = C6H5 (5.71a) m.p. 111–112° (59%)
XVII, R = COOEt (5.70,a 5.86b) m.p. 122–123° (88%)
Amino acids and esters
XXI, R = H (6.20,d N) m.p. 219–222° (83%)
XXII, R = Et (5.76a) m.p. 110–111° 51%(Ni), 82%(Pd)
XIX, R = H (5.89,b 6.19,f N) m.p. 233–235° (76%)
XX, R = Et (5.76,a 5.90, 5.95b) HCl, m.p. 213–214°
XVIII (5.77a) (2.89) (5.96b)
m.p. 87–87.5° (68.5%)

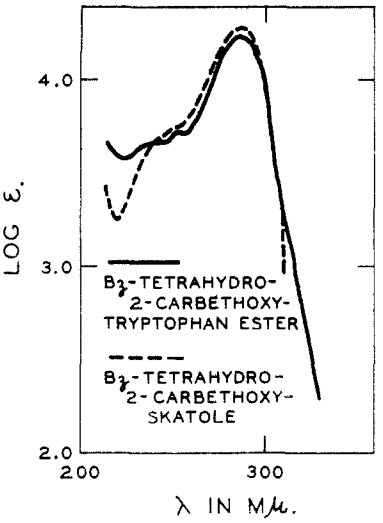


Fig. 1.—Ultraviolet absorption spectra in ethyl alcohol.

indole[16] (Fig. 1) proved it to be **2-carbethoxy-4,5,6,-7-tetrahydrotryptophan ethyl ester (XVIII)**. This reduction of the benzene part of the indole system was not observed in the 2-phenylindole series but has been reported for yobyrine,[17] tetrahydroisoyobyrine,[18] sempervirine,[19] cryptolepine[20a] and other indolic systems.[20b] XVIII would open a route to **4,5,6,7-tetrahydrotryptophan**, the biological assay of which might prove interesting.

TABLE I

	λmax I (log ε)	λmax II (log ε)
2-Phenyltryptophan (XXI)	306 (4.205)	227 (4.367)
2-Carbethoxytryptophan (XIX)	299 (4.108)	230 (4.215)
4,5,6,7-Tetrahydro-2-carbethoxytryptophan ethyl ester (XVIII)	288 (4.262)	.....
4,5,6,7-tetrahydro-2-carbethoxy-skatole	287 (4.301)	.....

Experimental[21]

Dibenzyl Aminomalonate.—Dibenzyl malonate was prepared according to the method of Backer and Lolkema[6] from benzyl alcohol and malonic acid[22]; b.p. 197–200° (1.5 mm.), $n^{20}D$ 1.5452.

The ester was nitrosated according to the general method of Cherchez[7] by adding to 17.04 g. (0.06 mole) of it in 10.8 g. of glacial acetic acid, dropwise over a period of 6 hours, a solution of 12.42 g. (0.18 mole) of sodium nitrite in 25 ml. of water. To the solution, which had been allowed to stand overnight, were added 30 ml. of water and 75 ml. of ether and the layers were separated. The water layer was extracted with three portions of 35 ml. each of ether and the combined ether layers were washed with 30 ml. of 5% sodium bicarbonate solution. The ethereal solution, dried briefly over magnesium sulfate, was concentrated to 50 ml. This solution was used in the subsequent step without further purification.[23]

The ether solution was added to 3 g. of amalgamated aluminum strips,[7] and the stirred mixture was allowed to reflux while 18 ml. of water were added dropwise over a period of 16 hours. The mixture was freed from unreacted aluminum and inorganic salts by filtration and the precipitate was washed with several portions of ether. The air-dried precipitate was then extracted with chloroform for 5 hours in a soxhlet apparatus. The chloroform solution was evaporated *in vacuo* and the residual oil was dissolved in ether and added to the original ethereal filtrate (total volume 150 ml.). This solution was washed consecutively with 20 ml. of water, 60 ml. of 10% sodium carbonate solution and 20 ml. of water. It was then added to 50 ml. of 6 *N* hydrochloric acid in a beaker. The white precipitate which formed was filtered off and washed with ether on the funnel. The filtrate and washings were discarded,[24] and the precipitate was added to a separatory funnel containing 50 ml. of saturated sodium carbonate solution and 70 ml. of ether. The aqueous layer was extracted several times with ether and the combined extracts (160 ml.) after having been washed with water were dried over magnesium sulfate. Removal of the solvent *in vacuo* left 6.41 g. of yellow oil which was pure enough for further operations (36% based on dibenzyl malonate). The amino ester could not be purified by distillation without extensive decomposition even at pressures as low as 8 microns.

A hydrochloride was prepared by adding a few drops of the substance to ethereal hydrogen chloride. The salt, which was not very soluble in benzene, ethyl acetate, or water, was recrystallized from chloroform; m.p. 143–144.5°.

Anal. Calcd. for $C_{17}H_{18}NO_4Cl$: C, 60.82; H, 5.40; N, 4.17. Found: C, 60.59; H, 5.36; N, 4.28.

A phenylurea derivative was prepared in hexane and recrystallized from hot ethyl acetate in which it was not very soluble; m.p. 188–190°.

Anal. Calcd. for $C_{24}H_{22}N_2O_5$: C, 68.88; H, 5.30; N, 6.70. Found: C, 69.14; H, 5.49; N, 6.63.

Dibenzyl Carbobenzyloxyaminomalonate (V).—To a stirred, refluxing solution of 5.26 g. (0.0176 mole) of dibenzyl aminomalonate in 30 ml. of dry benzene containing 2.76 g. (0.02 mole) of anhydrous potassium carbonate[25] was added dropwise over a period of 3 hours a toluene solution (5.33 g.) containing 0.0158 mole of benzyl chlorocarbonate. Refluxing was continued for one hour after completed addition. The reaction mixture was filtered while hot and the inorganic precipitate was thoroughly washed with 50 ml. of boiling benzene. The combined filtrates were allowed to stand in the refrigerator overnight and the resulting precipitate was collected by filtration. Partial evaporation of the filtrate yielded another portion of solid material. The combined solids were washed free of color with ether; 5.94 g. (86% based on benzyl chlorocarbonate). The material was recrystallized twice from chloroform–cyclohexane for analysis; fluffy white crystals, m.p. 111–112°.

Anal. Calcd. for $C_{25}H_{23}NO_6$: C, 69.27; H, 5.23; N, 3.23. Found: C, 69.08; H, 5.39; N, 3.26.

Dibenzyl Formaminomalonate (IV). A. By Reductive Acylation of Isonitrosomalonate.—Dibenzyl malonate (14.01 g., 0.05 mole) was nitrosated with sodium nitrite and glacial acetic acid as described above. The crude isonitroso compound (15.80 g.) was dissolved in 40 ml. of formic acid and was reduced with zinc dust according to the method of Galat.[14] Evaporation of the formic acid solution (total 150 ml.) to a small volume and addition of 180 ml. of ether to this solution gave rise to a white precipitate (zinc formate) which was removed by filtration. The ether solution was washed with water, dilute sodium bicarbonate solution, and water and was then dried over magnesium sulfate. The residue which remained after removal of the ether was dis-

(16) A. Treibs and D. Dinelli, *Ann.*, **517**, 152 (1935).

(17) P. Karrer and P. Waser, *Helv. Chim. Acta*, **32**, 409 (1949); A. J. P. Wibaut, v. Gastel and F. G. Buizer, *Rec. trav. chim.*, **68**, 497 (1949).

(18) M. M. Janot, J. Keufer and J. LeMen, *Bull. soc. chim., France*, 230 (1952).

(19) H. Schwarz and E. Schlittler, *Helv. Chim. Acta*, **34**, 629 (1951).

(20) (a) E. Gellért, Raymond-Hamet and E. Schlittler, *ibid.*, **34**, 642 (1951); (b) V. Boekelheide and Chu-Tsin Liu, THIS JOURNAL, **74**, 4920 (1952).

(21) All melting points are corrected, all boiling points are uncorrected. The microanalyses are from the Institutes' service analytical laboratory under the direction of Dr. William C. Alford.

(22) It is essential not to remove the water formed during the esterification. Two runs in which this was done by continuous azeotropic distillation with toluene suddenly polymerized with almost explosive violence to give a sticky highly viscous liquid which was not investigated.

(23) Attempts to isolate the nitroso compound failed because the substance could not be distilled without decomposition.

(24) Dibenzyl aminomalonate hydrochloride is relatively insoluble in water and the loss of compound sustained in this manner is balanced by the high state of purity of the remaining hydrochloride.

(25) M. Frankel, M. Harnik and Y. Levin, THIS JOURNAL, **74**, 3874 (1952).

tilled by evaporative distillation. A large amount of forerun (8.2 g., b.p. 150–155° at 25 μ) was identified as dibenzyl malonate by its infrared spectrum. Continued heating caused the sublimation of a white substance which weighed 1.8 g. (9.1% based on dibenzyl malonate) and which melted at 93–93.5° after three crystallizations from methyl acetate–cyclohexane.

Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.04; H, 5.24; N, 4.28. Found: C, 65.98; H, 5.18; N, 4.42.

B. By Direct Formylation of Aminomalonate.—To a solution of 4.65 g. (0.0157 mole) of dibenzyl aminomalonate (IX) (from 0.05 mole of dibenzyl malonate) in 70 ml. of dry benzene was added a formylation mixture[15] consisting of 3.4 ml. of 100% formic acid and 8.2 ml. of acetic anhydride which had previously been heated on a chloroform-bath for 3 hours. The reaction mixture stood at room temperature for 12 hours and was then carefully extracted with dilute sodium bicarbonate solution until no more carbon dioxide was evolved on mixing. Removal of the solvent from the dried solution (magnesium sulfate) *in vacuo* left a white solid which after one recrystallization from methyl acetate–cyclohexane melted at 93–94° and did not lower the melting point of the product obtained under (A) upon admixture; 3.77 g. (74%). The over-all yield based on dibenzyl malonate was 23%.

Dibenzyl N-Piperidinomethylformaminomalonate (II).—This was prepared from dibenzyl formaminomalonate (0.5 g.), piperidine (0.75 ml.) and 37% formalin (0.75 ml.) as described by Butenandt, *et al.*,[5] for the corresponding diethyl ester. There was obtained 0.476 g. (75%) of a white solid which melted at 86–88° after two recrystallizations from cyclohexane.

Anal. Calcd. for $C_{24}H_{28}N_2O_5$: C, 67.90; H, 6.65; N, 6.60. Found: C, 67.88; H, 6.72; N, 6.58.

3-Diethylaminomethyl-2-phenylindole (VIII).—To a cooled stirred mixture of diethylamine (5.5 g. 0.0753 mole) and 40% formalin (5.65 g., 0.0753 mole) in glacial acetic acid (20 ml.) and dioxane (20 ml.) was added 2-phenylindole (VII)[3] (7.26 g., 0.0377 mole) in small portions over a period of 5 hours. The dark solution was poured into 100 ml. of water containing 20 ml. of 6 *N* hydrochloric acid and the mixture was extracted with ether until that solvent was no longer colored (approximately 300 ml.). The ether solution containing some unreacted 2-phenylindole was discarded and the water layer in which was suspended a large amount of white solid material (3-diethylaminomethyl-2-phenylindole hydrochloride) was carefully saturated with solid sodium carbonate and was then extracted with 350 ml. of ether. Evaporation of the dried (magnesium sulfate) ethereal solution left 8.15 g. (78%) of a yellow, highly viscous material which could not be made to solidify. The material was pure enough for further reactions.

A yellow **picrate** was prepared in ethanol and melted after three recrystallizations from that solvent at 141–142° (dec.).

Anal. Calcd. for $C_{25}H_{25}N_5O_7$: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.07; H, 5.05; N, 13.60.

The oxalate was obtained by adding an ethereal solution of oxalic acid to the crude Mannich base in the same solvent. The precipitate was washed with ether and with water in which it was rather insoluble. It was purified by solution in the minimum amount of methanol with slight warming and addition of ether. After three such recrystallizations, the white crystalline substance melted at 206–208° with decomposition (color change to red) setting in at 187°. The oxalate is reasonably stable when kept in the dark and can be kept unchanged under these conditions for several months. The material turns red when exposed to the light.

Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C, 68.45; H, 6.57; N, 7.60. Found: C, 68.18; H, 6.61; N, 7.40.

The methosulfate was prepared according to the method of Schoepf[26] in tetrahydrofuran. The substance was washed with ether until free from color. Freshly prepared samples could be recrystallized from isopropyl alcohol (as soon as the compound started to become colored, it could no longer be recrystallized, since heating in any solvent caused it to turn into a deep violet gum) m.p. 125–125.5° (dec.). The material slowly turned pink on standing, especially when exposed to light.

(26) C. Schoepf and J. Thesing, *Angew. Chem.*, **63**, 377 (1951); J. Thesing and F. Sebülde, *Ber.*, **85**, 324 (1952); *cf.* ref. 9.

Anal. Calcd. for $C_{21}H_{28}N_2O_4S \cdot H_2O$: C, 59.69; H, 7.15; N, 6.63. Found: C, 59.17; H, 7.09; N, 6.74.

Ethyl α-Acetylamino-α-carbethoxy-β-(2-phenyl-3-indolyl)-propionate (XI).—To a solution of 8.94 g. (0.0325 mole) of 3-diethylaminomethyl-2-phenylindole in 100 ml. of dry xylene was added 6.98 g. (0.0325 mole) of ethyl acetamidomalonate and 0.4 g. of powdered sodium hydroxide. The mixture was stirred and refluxed while a stream of nitrogen was allowed to bubble through it until the escaping gas no longer contained diethylamine (6 hours). The hot solution was filtered and was allowed to stand in the refrigerator overnight. The precipitate which had formed during that time was filtered off and washed with a small amount of cold xylene; 9.6 g. (70%). The substance was recrystallized from benzene and once from dilute methanol; m.p. 197–199°.

Anal. Calcd. for $C_{24}H_{26}N_2O_5$: C, 68.22; H, 6.20; N, 6.63. Found: C, 68.36; H, 6.46; N, 6.86.

2-Phenyl-N-acetyltryptophan (XXIII).—To a hot suspension of 1.08 g. (2.6 mole) of ethyl α-acetylamino-α-carbethoxy-β-(2-phenyl-3-indolyl)-propionate (XI) in 40 ml. of water containing 1 g. of sodium hydroxide was added enough ethanol (approx. 15 cc.) to form a homogeneous solution which was refluxed for three hours. Ethanol was then removed by distillation, and the remaining solution was allowed to boil for another two hours. The cooled solution was filtered and extracted once with 40 cc. of ether; the colored ether extract was discarded. The aqueous layer was brought to *p*H 4 through the addition of 3 *N* hydrochloric acid and the precipitate which appeared was extracted with several portions of ether (180 ml.). The combined extracts were dried over magnesium sulfate and the solvent evaporated *in vacuo*. This left 0.79 g. of a gummy solid, presumably the N-acetylmalonic acid, which was soluble in most organic solvents but which changed on being boiled in benzene to a white solid which was no longer soluble in ether. Washing with that solvent left 0.57 g. (68%) of material which melted at 185–186° after two recrystallizations from benzene containing a small amount of methanol.

Anal. Calcd. for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.92; H, 5.82; N, 8.67.

Diethyl 2-Phenylskatylformaminomalonate (X).—This compound was prepared from 4.92 g. (0.0254 mole) of 2-phenylindole and 7.65 g. (0.025 mole) of diethyl piperidinomethylformaminomalonate.[5] There was obtained 6.62 g. (64%) of a white solid which melted at 231–232.5° (sintering above 220°) after two recrystallizations from ethyl acetate–benzene.

Anal. Calcd. for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.70; H, 5.66; N, 6.72.

Condensation Product (XII) of 3-Diethylaminomethyl-2-phenylindole (VIII) with Dibenzyl Carbobenzyloxyaminomalonate (V).—The condensation was carried out with 0.839 g. (0.003 mole) of 3-diethylaminomethyl-2-phenylindole (VIII) and an equivalent amount of V in 50 ml. of xylene as described for the corresponding reaction with 2-carbethoxy-3-diethylaminomethylindole (IX). The evolution of diethylamine stopped after 12 hours of refluxing but only about 50% of the theoretical amount of the amine had been given off by that time. The reaction mixture was worked up as described for XIII. This left 1.5 g. of a brown oily residue which was still contaminated with xylene. It was dissolved in 12 ml. of benzene and was chromatographed on an alumina column (1.8 × 11 cm.). Elution with benzene resulted in the isolation of 0.2 g. of a white substance which after recrystallization from benzene–hexane melted at 111–112°. This was identified as starting material (V) by mixed melting point and infrared absorption spectrum. Further elution of the column with 50% chloroform–benzene yielded 0.5 g. of a white substance which after three recrystallizations from methanol melted at 155–156° (25.3% yield based on the Mannich base). In subsequent experiments, this substance could be obtained without chromatography by seeding a methanol mixture containing the crude reaction product.

Anal. Calcd. for $C_{40}H_{34}N_2O_6$: C, 75.22; H, 5.37; N, 4.39. Found: C, 75.07; H, 5.26; N, 4.40.

DL-2-Phenyltryptophan (XXI). A.—A suspension of 0.6493 g. (1.95 mmoles) of 2-phenyl-N$^{\alpha}$-acetyltryptophan (XXIII) in 30 ml. of 3 *N* hydrochloric acid was refluxed for

five hours. The somewhat discolored solution was treated with Darco, filtered and was allowed to cool. It was then extracted with two 20-ml. portions of ether and the ether extracts were discarded. The water layer was brought to *p*H 6 by the addition of concentrated ammonium hydroxide and was allowed to stand in the refrigerator overnight. The precipitate was collected by centrifugation, washed with a little cold water (the substance was rather insoluble in water) and dried in a desiccator; 0.47 g. (83%). It was recrystallized from methanol–benzene and melted with decomposition at 219–222° (sintering above 185°). The substance gave a positive ninhydrin reaction.

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.83; H, 5.75; N, 10.00. Calcd. for $C_{17}H_{16}N_2O_2 \cdot H_2O$: C, 68.43; H, 6.08; N, 9.40. Calcd. for $C_{17}H_{16}N_2O_2 \cdot 2H_2O$: C, 64.53; H, 6.38; N, 8.85. Found: after drying at 100° *in vacuo:* C, 68.41; H, 5.94; N, 9.85; (monohydrate). After exposure to air for several hours: C, 64.84; H, 6.03; (dihydrate).

The Nα-2,4-dinitrophenyl derivative was prepared[27] by dissolving 19 mg. of the amino acid in 5% ethanol (1 ml.) and shaking for 15 hours with 32 mg. of 2,4-dinitrofluorobenzene and 35 mg. of sodium carbonate. The mixture was freed from ethanol by boiling, 2 ml. of water was added and the solution was extracted with several portions of ether (total 15 ml.) to remove excess reagent. The aqueous layer was made acid with a few drops of 3 *N* hydrochloric acid and the resulting precipitate was collected and washed with water. After drying in a desiccator (15 mg.), it was recrystallized from ethanol; m.p. 201–205° (dec.).

Anal. Calcd. for $C_{23}H_{18}N_4O_6 \cdot H_2O$: C, 59.47; H, 4.34; N, 12.07. Found: C, 59.69; H, 4.83; N, 11.90.

The *p*-toluenesulfonamide was synthesized by shaking a solution of 0.1 g. of the amino acid in 2 ml. of 2 *N* sodium hydroxide with 0.2 g. of freshly crystallized *p*-toluenesulfonyl chloride in 5 ml. of ether for 48 hours. The mixture was extracted with ether and was then made acid with 6 *N* hydrochloric acid and extracted again with ether. Evaporation after drying, of this second ether extract left a dark colored oil which solidified after being agitated with hexane–ether. The solid was recrystallized from methyl acetate–cyclohexane; m.p. 180–181.5°, white, slightly birefringent, crystals (0.0892 g.).

Anal. Calcd. for $C_{24}H_{22}N_2O_4S$: C, 66.33; H, 5.11; N, 6.45. Found: C, 66.35; H, 5.14; N, 6.47.

B.—A suspension of diethyl 2-phenylskatylformaminomalonate (X) (1.23 g., 3 mmole) in 25 ml. of 1 *N* sodium hydroxide was refluxed with stirring for 4 hours. The cooled mixture was filtered free from undissolved material (0.18 g., not identified) and the precipitate was washed with water (10 ml.). The filtrate was acidified with 6 *N* hydrochloric acid and enough 1,2-dimethoxyethane was added to bring about a homogeneous solution. The mixture was allowed to reflux for 3.5 hours after which time the condenser was removed and a few ml. of solvent was allowed to distil out in order to remove the dimethoxyethane. The dark solution was treated with Darco and was allowed to cool. It was brought to *p*H 5–6 with sodium bicarbonte and was extracted with several portions of *n*-butyl alcohol (50 ml. total). Evaporation of the solvent *in vacuo* left 1.12 g. of a tan solid which was washed with ether. This substance was quite hygroscopic and turned to a dirty, green gum on exposure to air. It was purified by adding it to hot water containing enough ethanol to effect solution. The mixture was treated with Norit and then freed from most of the ethanol by boiling. This caused the precipitation of a grayish-white substance (0.58 g., 56%) which melted at 212–218°. It could be further purified by crystallization from acetone–water (1:9). This did not affect the decomposition point but removed most of the color. The material did not lower the decomposition point of the product obtained under (A) when the latter was also purified from an aqueous solvent. **The *p*-toluenesulfonamide and the ethyl ester** (see below) were identical with the derivatives obtained from product A. This identity was further confirmed by the infrared absorption spectra and by the behavior of products A and B on paper chromatography (see below).

C.—A solution of 0.46 g. (0.72 mmole) of compound XII in 30 ml. of methanol containing 0.1 g. of 10% palladium-on-charcoal catalyst was reduced with hydrogen at atmospheric pressure. The hydrogen uptake stopped after 20 minutes (29 ml. of gas was taken up; calcd. 17.2 ml. per mole). The solution was freed from the catalyst by filtration and the latter was washed with methanol. Evaporation of the combined filtrates left 0.34 g. of residue which was crystallized from ethanol–benzene and finally from aqueous ethanol; (0.183 g. 91%). The melting point (210–216° dec.) and the infrared spectrum indicated that the substance was 2-phenyltryptophan. **The Nα-*p*-toluenesulfonamide** derivative prepared as under (B) melted at 179–181° and did not depress the melting point of the compound obtained there.

Ethyl α-Carbethoxy-α-nitro-β-(3-indolyl-2-phenyl)-propionate (XIV).—The addition of 3-diethylaminomethyl-2-phenylindole (VIII) to ethyl nitromalonate (VI) was adapted from the procedure of Weisblat and Lyttle.[8] To a solution of I (3.76 g., 0.0135 mole) in 100 ml. of anhydrous toluene was added 20.9 g. of a toluene solution containing 0.0135 mole of ethyl nitromalonate and the mixture was allowed to reflux under a stream of nitrogen until the escaping gas no longer contained diethylamine (6 hours). The cooled solution was then washed with 20 ml. each of water, 0.3 *N* hydrochloric acid, 0.3 *N* sodium hydroxide, and water and was dried over magnesium sulfate. Evaporation of the solvent *in vacuo* left 5.35 g. of oily residue which solidified when treated with pentane containing a small amount of ether. It was recrystallized twice from cyclohexane–chloroform; yellow crystals, m.p. 120–121.5°, 3.59 g. (65%).

Anal. Calcd. for $C_{22}H_{22}N_2O_6$: C, 64.37; H, 5.41; N, 6.82. Found: C, 64.20; H, 5.59; N, 6.70.

Ethyl α-Nitro-β-(3-indolyl-2-phenyl)-propionate (XVI).—To a stirred suspension of 1 g. (2.44 mmoles) of XIV in 30 ml. of anhydrous ether was added dropwise over a period of 2 hours a solution of 0.056 g. of sodium in 10 ml. of absolute ethanol. The solution was allowed to reflux gently for 3 hours after completed addition. It then stood at room temperature for 48 hours. To the mixture was added 100 ml. of ether and the solution was extracted twice with 20 ml. of water and three times with 15 ml. of 0.3 *N* sodium hydroxide. The dried ethereal layer yielded 0.12 g. of starting material on evaporation. The aqueous layer was made acid with 3 *N* hydrochloric acid and extracted with several portions of ether (120 ml.). The combined extracts were treated with Darco, dried over magnesium sulfate and evaporated. The orange residue was taken up in cyclohexane containing enough benzene to effect solution and was treated with a small amount of diatomaceous earth. The resulting light yellow solution gave rise to a slightly yellow, fluffy substance which after one more recrystallization from the same solvent pair weighed 0.428 g. (59% based on unrecovered starting material) and melted at 111–112°.

Anal. Calcd. for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: C, 67.22; H, 5.32; N, 8.41.

2-Phenyl-3-ethoxymethylindole (XXIV).—To a stirred, refluxing solution of 43.51 g. (0.106 mole) of the diester XIV in 1000 ml. of absolute ethanol was added dropwise a solution of 2.44 g. of sodium in 160 ml. of ethanol over a period of 3 hours. Refluxing was continued for 4 hours after completed addition. The reaction mixture was evaporated *in vacuo* and the residue was taken up in ether (500 ml.) and extracted with water (50 ml.) and 1 *N* sodium hydroxide (200 ml.). The organic layer was dried with magnesium sulfate and evaporated under reduced pressure to leave 26.2 g. of colored oily residue. Treatment with hot cyclohexane caused partial solidification. The colored solid was removed mechanically and was chromatographed on alumina from a 50% benzene–cyclohexane solution. Elution with 5% chloroform in benzene brought down 3.46 g. of a light yellow substance which could be crystallized from cyclohexane–ether and which melted at 112–113°.

Anal. Calcd. for $C_{17}H_{17}NO$ (XXV): C, 81.23; H, 6.82; N, 5.58. Found: C, 81.06; H, 6.21; N, 5.14.

Elution of the column with pure chloroform yielded several amorphous, yellow substances which melted unsharply above 200°. These were not further investigated. An attempt to re-chromatograph the compound melting at 112–113° on alumina resulted in partial decomposition and the formation of high melting amorphous substances which again were not further investigated.

The original aqueous alkaline extract was worked up as

(27) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

described above. This yielded 3.18 g. of XVI, m.p. 110–112° (8.9%).

Ethyl-DL-2-phenyltryptophan (XXII). A.—The nitro ester (XVI) (1 g., 2.96 mmole) was added to a suspension of 1 g. of palladium-on-charcoal catalyst (10%) in 60 ml. of glacial acetic acid. The mixture was hydrogenated in a Parr shaker at approximately 70° and under a pressure of 40 lb. for 4 hours. The filtered solution was freed from most of the solvent by evaporation *in vacuo* and the remainder (10 ml.) was added to 20 ml. of 3 *N* hydrochloric acid. The solution was extracted with 60 ml. of ether (these extracts were discarded) after which it was saturated with sodium carbonate while being cooled in ice. The yellow oil which formed was extracted with ether (180 ml.) and the extract after washing with water (10 ml.) and drying over magnesium sulfate was evaporated under reduced pressure to leave an oily residue which crystallized after having been agitated for some time with hexane containing a small amount of benzene. The material was recrystallized twice from that solvent pair; it melted at 110–111° (0.753 g., 82.6%).

B.—A solution of the nitro ester (XVI) (0.32 g., 0.945 mmole) in 15 ml. of absolute ethanol containing approximately 0.3 g. of Raney nickel was reduced under atmospheric pressure. After the calculated amount of hydrogen (68.1 ml.) had been taken up (65 hours), the solution was filtered with the aid of Celite and the filtrate was freed from solvent under vacuum. The residue was dissolved in 50 ml. of ether and the reaction product was extracted with two 20-ml. portions of 3 *N* hydrochloric acid. The acid extracts were then made alkaline with sodium carbonate and the solution was extracted with ether (80 ml.). Evaporation of the ether extracts which had been dried over magnesium sulfate left 0.15 g. (51%) of oily residue which solidified when agitated with pentane, and melted at 110–111° after one recrystallization from hexane containing a small amount of benzene. This substance was shown to be identical with the product under (A) by mixed melting point, ultraviolet and infrared spectra.

(C).—A solution of 2 g. (0.0071 mole) of DL-2-phenyltryptophan (XXI) in 80 ml. of absolute ethanol was saturated with dry hydrogen chloride and was allowed to reflux for 8 hours. The dark solution was filtered and evaporated *in vacuo* to leave an oily, colored residue which was dissolved in 30 ml. of water and treated once with Darco in the cold. The filtered solution was saturated with sodium carbonate and the resulting oil was worked up as indicated under (A) or (B). There was obtained 1.4 g. of white solid (64.2%), m.p. 110–111°, which did not depress the melting point of the compound obtained under (A) or (B).

Anal. Calcd. for $C_{19}H_{20}N_2O_2$: C, 73.99; H, 6.54; N, 9.09. Found: C, 73.86; H, 6.63; N, 8.98.

A **picrate** was prepared in ether and recrystallized from ethanol; orange crystals which melted at 223–228° (dec.) with a definite change in crystal structure at 138°.

Anal. Calcd. for $C_{25}H_{23}N_5O_9$: C, 55.86; H, 4.31. Found: C, 55.83; H, 4.32.

2-Carbethoxy-3-diethylaminomethylindole (IX).—To a mixture of 3.8 g. (0.046 mole) of 40% formalin, 3.36 g. (0.046 mole) of diethylamine and 10 g. of glacial acetic acid was added 4.35 g. (0.023 mole) of 2-carbethoxyindole.[11] The mixture was stirred and heated for 6 hours on the steam-bath and was then diluted with 60 ml. of 3 *N* hydrochloric acid. The non-homogeneous mixture was extracted with several portions of ether. The ether extracts were washed with 10% sodium carbonate solution and were dried over magnesium sulfate. Evaporation of the solvent left a residue which melted at 119–121° (after crystallization from cyclohexane) and weighed 0.9 g. It was identified as starting material. The acidic aqueous layer was treated with Darco and was saturated with sodium carbonate. The white precipitate which formed was extracted into 170 ml. of ether and the extracts were washed with water and dried over magnesium sulfate. Evaporation of the solvent left 4.24 g. (68%) of a white solid which melted at 112–114° after one recrystallization from chloroform–cyclohexane (lit. 104°[11]; 114–115°[12]).

A yellow **picrate** was prepared in ether and recrystallized from ethyl acetate–cyclohexane; m.p. 159–160°.

Anal. Calcd. for $C_{16}H_{22}N_2O_2 \cdot C_6H_3N_3O_7$: C, 52.48; H, 5.01. Found: C, 52.82; H, 5.29.

Condensation Product (XIII) of 2-Carbethoxy-3-diethylaminomethylindole (IX) and Dibenzyl Carbobenzyloxyaminomalonate (V).—Dibenzyl carbobenzyloxyaminomalonate (V) (1.3 g., 3 mmole) and 2-carbethoxy-3-diethylaminomethylindole (IX) (0.72 g., 3 mmole) were dissolved in 40 ml. of dry xylene containing a trace of sodium hydride. The mixture was refluxed while a stream of dry nitrogen was bubbling through it. The escaping gas was passed through an aqueous solution of boric acid containing a few drops of Kjeldahl indicator. Periodic titrations of the boric acid solution with 0.3 *N* hydrochloric acid showed that the theoretical amount of diethylamine had been given off after a reaction period of 3.5 hours. The reaction mixture was allowed to cool under nitrogen and was then washed with 20 ml. of each, water, 2 *N* hydrochloric acid, and water. Evaporation of the solvent *in vacuo* after drying of the solution over magnesium sulfate left 2.56 g. of a yellow semi-solid which could be freed from traces of xylene and color by washing with cold methanol in which it was almost insoluble. The substance was recrystallized from benzene–cyclohexane to give fluffy white crystals which melted at 133–135°, 1.68 g. (89%).

Anal. Calcd. for $C_{37}H_{34}N_2O_8$: C, 70.01; H, 5.40; N, 4.42. Found: C, 69.87; H, 5.52; N, 4.47.

2-Carbethoxytryptophan (XIX).—A slow stream of hydrogen was allowed to bubble through a suspension of 0.587 g. (0.9 mmole) of XIII and 0.5 g. of paladium-on-charcoal catalyst (10%) in 30 ml. of dry methanol by means of a sintered glass tube. The escaping gas was led through a trap containing a saturated solution of barium hydroxide in water (45 ml.). The hydrogenation was allowed to continue for 3 hours when the barium carbonate which had formed during that time was collected and washed by centrifugation with CO_2-free water and with methanol. After drying in a desiccator over calcium chloride this material weighed 0.2313 g. and corresponded to 1.26 moles of CO_2 per mole of reacting substance. The reaction mixture was freed from the catalyst by filtration and the latter was washed with 40 ml. of methanol. The combined filtrates were allowed to evaporated *in vacuo* to leave a yellow oil (0.34 g.) which was completely soluble in methanol and benzene and probably represented the malonic acid. The substance was dissolved in 20 ml. of benzene and the solution was taken to dryness on the steam-bath. This treatment was repeated two times; there was obtained a yellow-white substance which could be freed from color by washing with methanol, in which it was no longer soluble. The dried material weighed 0.21 g. (76%) and was recrystallized from 50% aqueous ethanol; small, white needles, m.p. 233–235° (dec.).

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.85; H, 5.84; N, 10.14. Calcd. for $C_{14}H_{16}N_2O_4 \cdot {}^1/_2H_2O$: C, 58.93; H, 6.01; N, 9.82. Found (sample dried *in vacuo* at 100°): C, 60.62; H, 6.00; N, 9.84. Found (sample dried as above but exposed to laboratory air for some time before analysis): C, 59.58; H, 5.62.

Freshly prepared 2-carbethoxytryptophan is soluble in water, becomes less soluble on standing, and can then be recrystallized from ethanol–water (1:1). The amino acid dried in a desiccator loses 0.7% water on drying at 110° *in vacuo* and takes this same amount of water up again on exposure to air; this material then shows m.p. 217–220°. The Ehrlich reaction with *p*-dimethylaminobenzaldehyde and hydrochloric acid is negative in the cold, on warming and on addition of sodium nitrite.[28]

For preparative purposes the reduction was normally carried out in an atmospheric hydrogenation apparatus. Under these conditions, the gas uptake seemed to cease after the absorption of two equivalents of hydrogen (approximately 40 minutes) and this was attributed to the evolution of one equivalent of carbon dioxide. The reaction was allowed to proceed for another hour and was then worked up as described above, with approximately the same results.

(28) Whereas J. D. Dutcher and A. Kjaer, THIS JOURNAL, **73**, 4139 (1951), state that a carboxyl or carboxamide in the 2-position of indoles in contrast to a 2-carbethoxy group does not interfere with the Ehrlich test, W. O. Kermack, W. H. Perkin and R. Robinson, *J. Chem. Soc.*, **119**, 1622 (1919), find a positive Ehrlich test for methyl 2-carbomethoxy-3-indolylacetate. In the pyrrole series it is stated that α- or β-carbomethoxy groups do not hinder the Ehrlich reaction but are eliminated: H. Fischer and H. Orth, "Chemie des Pyrrols," Vol. I, Leipzig, 1934, p. 66.

Ethyl α-Carbethoxy-α-nitro-β-(2-carbethoxy-3-indolyl)-propionate (XV).—This compound was prepared from 4.24 g. (0.0155 mole) of 2-carbethoxy-3-diethylaminomethylindole (IX) and an equivalent amount of ethyl nitromalonate (VI) in toluene as described for the reaction of this nitro ester (VI) with (VII). The reaction was complete after 5 hours and the product was isolated by evaporating the toluene solution to a small volume *in vacuo* followed by cooling. The light yellow solid weighed 4.98 g. (79%) after washing with ether to remove the color. It melted at 134–135° after three recrystallizations from cyclohexane (needles).

Anal. Calcd. for $C_{19}H_{22}N_2O_8$: C, 56.15; H, 5.46; N, 6.89. Found: C, 56.04; H, 5.29; N, 6.83.

Ethyl α-Nitro-β-(2-carbethoxy-3-indolyl)-propionate (XVII).—To a suspension of 4.06 g. (0.01 mole) of (XV) in 75 ml. of anhydrous ether was added dropwise a solution of 0.23 g. (0.01 mole) of sodium in 15 ml. of absolute ethanol.[8] The stirred solution was allowed to reflux during the addition and for eight hours thereafter. The white precipitate which had formed during that time was filtered off. Addition of hexane (50 ml.) to the filtrate caused the formation of more precipitate. The combined solids were washed with a little dry ether and were added to a separatory funnel containing 70 ml. of 3 *N* hydrochloric acid and 120 ml. of ether. The solid went into solution on shaking and the aqueous layer was separated and extracted with several portions of ether. The combined organic extracts (320 ml.) were dried over magnesium sulfate and the solvent was removed *in vacuo*. This left 2.93 g. (88%) of solid residue which was recrystallized several times from chloroform–cyclohexane; white crystals, m.p. 122–123°.

Anal. Calcd. for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.42; H, 5.31; N, 8.23.

Ethyl-2-carbethoxytryptophan (XX). A.—The nitro ester (XVII) (0.3512 g.) was hydrogenated in 25 ml. of glacial acetic acid with 0.35 g. of 10% palladium-on-charcoal catalyst under 38 lb. of hydrogen pressure and at a temperature of 70°. The reaction was allowed to proceed for 2 hours and the reaction mixture was worked up as described for ethyl-2-phenyltryptophan. There was obtained 0.303 g. (94%) of a colorless oil which could not be made to crystallize.

A hydrochloride was prepared with ethereal hydrogen chloride. It melted at 213–214° after two crystallizations from benzene–methanol.

Anal. Calcd. for $C_{16}H_{20}N_2O_4Cl$: C, 56.38; H, 6.21; N, 8.22. Found: C, 56.35; H, 6.37; N, 8.24.

A yellow **picrate** was prepared with ethereal picric acid and was recrystallized twice from methanol; m.p. 220–222° (dec.).

Anal. Calcd. for $C_{22}H_{23}N_5O_{11}$: C, 49.53; H, 4.34; N, 13.13. Found: C, 49.68; H, 4.46; N, 13.13.

Ethyl-Nα-acetyl-2-carbethoxytryptophan.—The oily ester XX obtained above (0.2428 g.) was dissolved in 10 ml. of acetic anhydride. To the cooled solution was added 1 ml. of dry pyridine and the mixture was stirred and kept in an ice-bath for 1 hour. It was then allowed to stand at room temperature overnight. The solution was poured on ice and was allowed to stand for 1 hour. It was then made alkaline with solid sodium carbonate and was extracted with ether. The ethereal solution was in turn washed with 3 *N* hydrochloric acid (30 ml.) and a saturated solution of cadmium chloride (20 ml.) to remove traces of pyridine. Evaporation of the dried ether solution *in vacuo* left 0.304 g. of a solid residue which was recrystallized from hot cyclohexane containing just enough methyl acetate to effect solution; 0.253 g. (91%), m.p. 154–155°.

Anal. Calcd. for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.38; H, 6.44; N, 7.89.

B.—The nitro ester XVII (0.3343 g., 1 mmole) was reduced in 20 ml. of absolute ethanol at atmospheric pressure in the presence of Raney nickel catalyst[29] (approximately 0.3 g.). The calculated uptake of hydrogen (72 ml.) was reached only after a period of 80 hours. After the reaction mixture had been freed from the catalyst by filtration it was evaporated under reduced pressure and the residue was dissolved in ether. The ethereal solution was washed with 3 *N* hydrochloric acid and then with water. It was dried over magnesium sulfate and freed from ether *in vacuo*. This left 0.25 g. (82%) of a slightly yellow oil. Chromatography of this substance on Florisil[30] from 50% benzene–cyclohexane produced traces of a yellow material which came off the column with 10% chloroform in benzene and as the major fraction a colorless gummy substance which was eluted with 5% methanol in chloroform. This substance could not be made to crystallize. **Its hydrochloride, picrate and Nα-acetyl** derivative were shown to be identical with the same derivatives obtained under (A) by mixed melting points.

Ethyl-2-carbethoxy-bz-tetrahydrotryptophan (XVIII).—The nitro ester XVII (1.97 g., 5.9 mmoles) was hydrogenated in glacial acetic acid as described under (A) except that the temperature was raised to 80° and the reduction time increased to 6 hours. The product was worked up as before and this time there was obtained a white solid which could be recrystallized from cyclohexane–methylene chloride; m.p. 87–87.5°, 1.24 g. (68.5%).

Anal. Calcd. for $C_{16}H_{24}N_2O_4$: C, 62.31; H, 7.84; N, 9.09. Found: C, 62.02; H, 7.85; N, 8.95.

A hydrochloride was prepared in ether and recrystallized from benzene–methanol; m.p. 212–214°. Admixture of ethyl-2-carbethoxytryptophan hydrochloride caused a slight depression in the melting point (206–212°).

Anal. Calcd. for $C_{16}H_{24}N_2O_4 \cdot HCl$: C, 55.72; H, 7.31; N, 8.12. Found: C, 55.28; H, 7.34; N, 8.03.

A picrate was prepared with ethereal picric acid and was recrystallized from a mixture of chloroform–methanol with enough carbon tetrachloride to start crystallization. The yellow substance melted at 216–219° (dec.) with strong sintering at 134° and from 183° to the decomposition point.

Anal. Calcd. for $C_{22}H_{27}N_5O_{11}$: C, 49.15; H, 5.07; N, 13.03. Found: C, 49.03; H, 4.97; N, 12.85.

Ethyl-2-carbethoxy-3-methyl-bz-tetrahydroindole.—This compound was prepared from cyclohexanone and ethyl isonitrosoacetoacetate according to Treibs and Dinelli.[16] After two crystallizations from hexane, it melted as reported at 110°.

TABLE II

R_f VALUES

Compound	80% aq. pyridine	70% aq. pyridine
Tryptophan	0.51	0.49
2-Carbethoxytryptophan (XIX)	.88	.54
2-Phenyltryptophan (XXI)	.71	.59

Paper Chromatograms.—The three amino acids, DL-tryptophan, DL-2-phenyltryptophan and DL-2-carbethoxytryptophan were chromatographed ascendingly on Whatman #1 filter paper in the solvents shown in the table. The chromatograms were developed by dipping the air-dried sheets into a solution of 0.5 g. of ninhydrin and 2 ml. of pyridine in 250 ml. of acetone. They were then dried in an oven. The zones were measured from the leading edge for R_f value determination.

BETHESDA 14, MARYLAND

(29) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).

(30) Floridin Company, Warren, Pennsylvania.