17. *Amino-acids and Peptides. Part II.* The Constitution of Hypoglycin A.*

By E. V. Ellington, C. H. Hassall, J. R. Plimmer, and C. E. Seaforth.

The structure (I) is proposed for the natural product hypoglycin A. This is based on spectroscopic evidence, examination of the products of catalytic hydrogenation, and identification of a product of acid-hydrolysis as 2-amino-5-methyl-4-oxohexanoic acid.

We were prompted to investigate the toxic constituents of the fruit of *Blighia sapida* (ackee) by a possible connexion between these fruits and Jamaican vomiting sickness.[1] Two compounds, hypoglycin A and hypoglycin B, were then isolated: they were so named because they had the unusual property of lowering the level of blood sugar when administered, orally or by injection, to rats and other test animals.[2] We have earlier given a brief account [3] of our results for hypoglycin A and proposed the structure α-amino-β-(2-methylene*cyclo*propyl)propionic acid (I). Descriptions of independent investigations in six other laboratories appeared at about the same time.[4] In what follows a detailed account is given of the evidence on which our proposal is based.

Paper-chromatographic and paper-electrophoretic results suggested that hypoglycin A was homogeneous, and countercurrent-distribution studies extending over 2000 transfers indicated a single impurity, in an amount less than 1%. Analysis of hypoglycin A and its (mono-)2 : 4-dinitrophenyl derivative indicated the formula $C_7H_{11}O_2N$. Hypoglycin A is optically active. There is a prominent band at 889 cm.$^{-1}$ in the infrared absorption spectrum, but the ultraviolet absorption spectrum, in both acid and alkali, shows end-absorption and closely resembles that of *iso*leucine. This indication of the unconjugated group $CH_2{=}C\lesssim$ was supported by the observation of Anderson *et al.*[4a,†] that 0·97 mol. of formaldehyde was released when hypoglycin A was oxidised by periodic acid. When hypoglycin A is hydrogenated in presence of platinum in acid conditions, there is a rapid uptake of one mol. of hydrogen followed by a very slow uptake of a second mol. Paper chromatography of the product from the first stage of the hydrogenation led to the separation of two ninhydrin-sensitive components. The major constituent, dihydrohypoglycin A, $C_7H_{13}O_2N$, was obtained by ion-exchange chromatography and showed no infrared band near 889 cm.$^{-1}$. The paper chromatogram of the product from the second stage of hydrogenation indicated a trace of material with the same R_F value as dihydroglycin A, but the major fraction was separated by ion-exchange chromatography into two isomers, $C_7H_{15}O_2N$. One of this pair was identified as 2-amino-5-methylhexanoic acid. The second was not identified with certainty but, since it differs from 2-aminoheptanoic acid, is probably a mixture of the diastereoisomers of 2-amino-4-methylhexanoic acid.‡

Evidence that led to the formulation of the structure of hypoglycin A was provided by acid-hydrolysis. Two-dimensional paper chromatography of the mixture obtained by use of hydrochloric acid showed the presence of at least twelve new ninhydrin-sensitive compounds: it was this result that led to the preliminary and incorrect opinion that hypoglycin A was a peptide.[2,5] A major constituent was distinguished by the yellow

* Part I, Hassall and Reyle, *Biochem. J.*, 1955, **60**, 334.

† We are grateful to Dr. Anderson and his co-workers for making this information available before its publication.

‡ Further evidence indicating such a mixture has been obtained recently.[4d]

[1] Hassall and Reyle, *West Indian Med. J.*, 1955, **4**, 83.

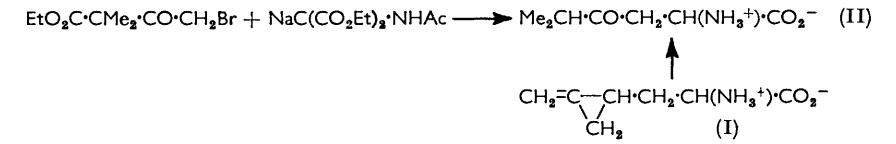
[2] Hassall, Reyle, and Feng, *Nature*, 1954, **173**, 356.

[3] Ellington, Hassall, and Plimmer, *Chem. and Ind.*, 1958, 329.

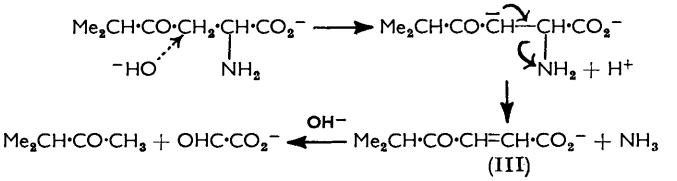
[4] (*a*) Anderson, Johnson, Nelson, Olson, Speeter, and Vavra, *ibid.*, p. 330; (*b*) Wilkinson, *ibid.*, p. 17; von Holt and Leppla, *Angew. Chem.*, 1958, **70**, 25; Renner, Jöhl, and Stoll, *Helv. Chim. Acta*, 1958, **41**, 589; (*c*) Carbon, Martin, and Swett, *J. Amer. Chem. Soc.*, 1958, **80**, 1002; (*d*) de Ropp, Van Meter, De Renzo, McKerns, Pidacks, Bell, Ullman, Safir, Fanshawe, and Davis, *ibid.*, p. 1004.

[5] von Holt and Leppla, *Bull. Soc. chim. belges*, 1956, **65**, 113.

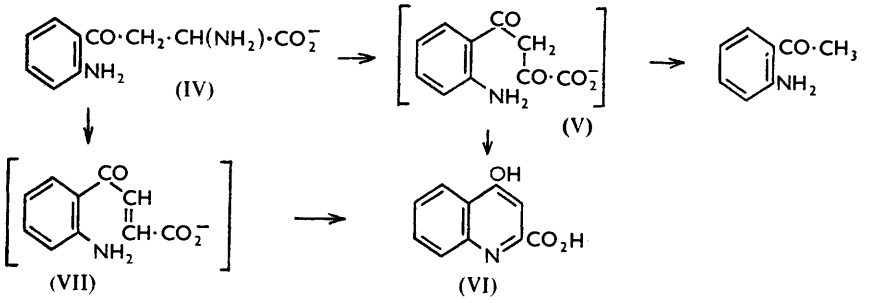
colour it gave with ninhydrin and has been identified as 2-amino-5-methyl-4-oxohexanoic acid (II). This constitution was suggested by hydrolysis with alkali to methyl *iso*propyl ketone and was confirmed by comparison with the amino-acid obtained when ethyl γ-bromo-αα-dimethyl-β-oxobutyrate was condensed with diethyl acetamidomalonate and then subjected to acid hydrolysis:



Hydrolysis of 2-amino-5-methyl-4-oxohexanoic acid with mild alkali (pH 8·5) liberates ammonia and affords 5-methyl-4-oxohex-2-enoic acid (III), whose structure is based on ultraviolet [6] and infrared spectra and conversion by concentrated alkali into methyl *iso*propyl ketone and glyoxylic acid:



These reactions are reminiscent of those suggested for the oxidation of kynurenine (IV) in alkaline conditions. In dilute alkali kynurenic acid (VI) is formed [7] but more concentrated alkali leads to *o*-aminoacetophenone.[8] The diketo-acid (V) has been proposed as an intermediate in both cases. As there is no direct evidence of the formation of compound



(V) we suggest, by analogy with the case of 2-amino-5-methyl-4-oxohexanoic acid, that the αβ-unsaturated acid (VII) is the intermediate in this degradation of kynurenine. There are good analogies for the cyclisation leading to a dihydroquinoline derivative,[9] for the oxidation of such a compound, by air, to kynurenic acid,[10] and, in triterpene chemistry [11] and elsewhere,[12] for the conversion of the αβ-unsaturated γ-keto-acids (III) and (VII) into methyl ketones during base-induced cleavage.

The formation of the keto-acid (II) on acid hydrolysis suggests that the terminal methylene group in hypoglycin A forms part of an allylic system which is associated with a ring structure. A van Slyke estimation on hypoglycin A indicates a free amino-group

[6] Raymond, *J. Amer. Chem. Soc.*, 1950, **72**, 4304.
[7] Butenandt, Weidel, Weichert, and von Derjugin, *Z. physiol. Chem.*, 1943, **279**, 27.
[8] Kotake and Kiyokawa, *ibid.*, 1931, **195**, 147; Kotake, *J. Chem. Soc. Japan*, 1940, **61**, 511.
[9] Fischer and Kuzel, *Ber.*, 1883, **16**, 163; Mannich and Dannehl, *Ber.*, 1938, **71**, 1899.
[10] Johnson and Buell, *J. Amer. Chem. Soc.*, 1952, **74**, 4517.
[11] Barton, de Mayo, and Orr, *J.*, 1958, 2240; Arigoni, Viterbo, Dünnenberger, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1954, **37**, 2306.
[12] von Pechmann, *Ber.*, 1882, **15**, 891; Dixon, Gregory, and Wiggins, *J.*, 1949, 2139.

and the infrared absorption spectra of this compound and its reduction products contain maxima in the region of 3100, 1630, and 1510 (NH_3^+) and 1580 cm.$^{-1}$ (CO_2^-) which are characteristic of α-amino-acids. These observations, together with fission of the ring by hydrogenation to yield a mixture of α-amino-methylhexanoic acids, lead unambiguously to the structure (I) for hypoglycin A. The results reported by other laboratories, after completion of this work, support this structure. Racemic hypoglycin A has been synthesised.[4c]

Experimental

M. p.s were determined by means of a Kofler block. Rotation measurements employed aqueous solutions at 28—30° except where other solvents are specified. Ultraviolet spectra were determined on a Beckman spectrophotometer, model D.U. Infrared spectra were measured with potassium bromide discs unless otherwise stated. We are grateful to Dr. S. M. Nagy, Massachusetts Institute of Technology, and Dr. H. E. Hallam, University College, Swansea, for the determinations of infrared spectra. Amberlite ion-exchange resins were treated before use according to the makers' instructions.[13] Paper chromatography generally employed Whatman No. 1 paper in glass tanks. Papers were equilibrated at 30° ± 2° for 48 hr. before the eluting solvent was added. Microanalyses were carried out by Dr. F. Pascher, Bonn, Germany.

Purification of Hypoglycin A.—There was variation in the purity of different batches of hypoglycin A prepared by ion-exchange.[14] The crude product, after three recrystallisations from ethanol–water, generally contained one major contaminant, R_F 0·48 [butan-1-ol–acetic acid–water (4 : 1 : 5)]. Chromatography on a powdered cellulose column yielded hypoglycin A which was used for all subsequent investigations. This preparation has $[\alpha]_D^{26}$ +11° ± 1° (*c* 1·00), $[\alpha]_D^{26}$ +49·0° ± 1° (*c* 1·00 in water at pH 2) [Found: C, 59·2; H, 7·8; O, 22·8; N, 10·0; N (van Slyke), 10·7. Calc. for $C_7H_{11}O_2N$: C, 59·6; H, 7·9; O, 22·7; N, 9·9%], $\nu_{max.}$ 890 (>C=CH_2), 1760 (>C=CH_2 overtone), 1023 (*cyclo*propane-CH_2), 3100, 1630, 1510 (NH_3^+), and 1580 cm.$^{-1}$ (CO_2^-).

Homogeneity. The preparation of hypoglycin A gave a single spot when used for paper chromatography with the following solvents (R_F in parentheses; proportions are v/v): 1 : 1 : 1 Benzyl alcohol–water–butan-1-ol (0·29); 2 : 5 : 7 *tert.*-butyl alcohol–butan-1-ol–2N-ammonia (0·38); 2 : 2 : 1 ethyl methyl ketone–propan-2-ol–water (0·52); 4 : 1 : 5 butan-1-ol–acetic acid–water (0·59); 2 : 3 *tert.*-butyl alcohol–2N-ammonia (0·85); 3 : 2 ethyl acetate–water (0·90).

Hypoglycin A (42 μg.) was submitted to paper electrophoresis at 4·5 milliamp. and 90 v. It moved as a single spot on strips of Whatman No. 1 paper dipping into phosphate buffers at pH 4·0, 7·0, or 8·0.

Countercurrent distribution was carried out in an 80-tube apparatus, with *tert.*-butyl alcohol–ethyl acetate–water (25 : 50 : 52 v/v). Hypoglycin A (604 mg.) was dissolved in 40 c.c. of the bottom phase and distributed in four tubes. The weight-distribution curve determined after 150 transfers could not be distinguished from the theoretical curve for a single component. In an experiment involving 2000 transfers (kindly carried out on our behalf by members of the Research Division, The Upjohn Company, Kalamazoo, U.S.A.), comparison of the experimental and the theoretical curve indicated that the preparation of hypoglycin A was contaminated with less than 1% of an impurity that moved more slowly in this solvent system [*K* (hypoglycin A) 0·231].

N-2 : 4-*Dinitrophenylhypoglycin A.*—This was prepared in the usual way with 1-fluoro-2 : 4-dinitrobenzene. The product was amorphous even after purification by chromatography on columns of Celite or powdered cellulose. Paper chromatography [using *tert.*-butyl alcohol–phenol–phosphate buffer, pH 5·91 (1 : 1 : 1 v/v)] indicated that the main product (R_F 0·65) was contaminated with a trace of impurity (R_F 0·35). This preparation (1·40 g.) was distributed in 3 × 10 c.c. fractions in the lower phase of the system chloroform–methanol–0·1N-hydrochloric acid (2 : 2 : 1 v/v) and submitted to 585 transfers (with recycling) in an 80-tube countercurrent distribution apparatus. Measurement of optical density at 350 mμ and comparison with a theoretical distribution curve showed that the main fraction (*K* 0·059) was pure. This fraction was accumulated by evaporating the solvent from the appropriate tubes. The

[13] Bulletins No.'s M-3-47, IE-25-56, Rohm and Haas Coy., U.S.A.
[14] Hassall and Reyle, *Biochem. J.*, 1955, **60**, 334.

amorphous *product* was sublimed at 150—180°/0·2 mm. It has $\lambda_{max.}$ 360 mμ (ε 15,500) [Found: C, 51·3; H, 4·7; N, 13·0; O, 30·8%; *M* (Battersby and Craig [15]), 304. $C_{13}H_{13}O_6N_3$ requires C, 50·8; H, 4·3; N, 13·7; O, 31·2%; *M*, 307].

Hydrolysis of Hypoglycin A with Hydrochloric acid.—Hypoglycin A (10 mg.) was heated with 11N-hydrochloric acid at 150° in a sealed tube for periods varying from 3 to 148 hr. The residue obtained when the reaction mixture was filtered through Celite and evaporated to dryness was extracted with water. The solution was evaporated and the process was repeated until all traces of free hydrochloric acid were removed. The product, in 10% aqueous propan-2-ol (0·2 c.c.), was applied to Whatman No. 52 paper so that the spot contained 42 μg. of amino-nitrogen. Two-dimensional paper chromatography with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) in one direction and phenol–*m*-cresol–borate buffer (pH 9·3) in the other [16] led to an increasingly complex pattern of spots as the length of the degradation period increased up to 24 hr. Longer periods led to diminished intensity of some of the spots (see Table).

Two-dimensional paper chromatography of 24 *hr. hydrolysis mixture.*

R_F (phenol etc.)	R_F (butan-1-ol etc.)	Colour with ninhydrin	Intensity *	R_F (phenol etc.)	R_F (butan-1-ol etc.)	Colour with ninhydrin	Intensity *
0·03	0·16	Purple	3	0·72	0·59	Purple	1
0·05	0·22	Purple	4	0·73	0·35	Purple	5
0·16	0·16	Purple	3	0·70	0·47	Yellow	4
0·23	0·36	Purple	4	0·92	0·27	Brown-yellow	2
0·33	0·24	Purple	2				
0·65	0·29	Purple and yellow	4	0·93	0·33	Brown-yellow	2
0·72	0·45	Yellow	1				

* Decreasing intensity from 1 to 5.

Isolation of (−)-2-*amino*-5-*methyl*-4-*oxohexanoic acid.* Hypoglycin A (568 mg.) was hydrolysed under reflux with constant-boiling hydrochloric acid (50 c.c.) for 84 hr. The residue which remained after removal of the hydrochloric acid was dissolved in water (2 c.c.), filtered, and introduced on a column (75 × 2·7 cm.) of the ion-exchange resin Amberlite I.R.-4B (150—200 mesh). This resin had been washed successively with 0·1N-hydrochloric acid, excess of 0·2M-sodium acetate, and glass-distilled water. The column was eluted with distilled water (24 c.c./hr.); 10 c.c. fractions were collected and examined by paper chromatography. Fractions 23—40 (250 mg.) contained two compounds, R_F 0·38, 0·58 [butan-1-ol–acetic acid–water (4 : 1 : 5)] which gave yellow and purple colours, respectively, with ninhydrin. The individual components were obtained by paper chromatography of a band of the mixture on sheets of Whatman No. 52 paper. The *compound* with R_F 0·38 was eluted from the paper with water. Evaporation gave a residue which recrystallised from 92% ethanol as needles, m. p. 187—192°, $[\alpha]_D^{28}$ −5·9° ± 0·2° (*c* 2·7), $\lambda_{max.}$ 278 mμ (ε 32·5) in ethanolic 0·1N-hydrochloric acid [Found: C, 53·2; H, 8·5; N, 8·6; O, 30·2; N (van Slyke), 8·5%; *M* (Rast), 169. $C_7H_{13}O_3N$ requires C, 52·8; H, 8·2; N, 8·8; O, 30·2%; *M*, 159], $\nu_{max.}$ 1710 (CO), 3100, 1600, 1515, 1405 cm.$^{-1}$ (α-amino-acids).

Unlike the accompanying product the compound of m. p. 187—192° did not give a precipitate with Brady's reagent. When 15 mg. were heated in 1·5N-sodium hydroxide so that volatile material passed into aqueous 2 : 4-dinitrophenylhydrazine sulphate, methyl *iso*propyl ketone dinitrophenylhydrazone (12 mg.) was obtained (Found: C, 49·4; H, 5·4. Calc. for $C_{11}H_{14}O_4N_4$: C, 49·6; H, 5·3%), m. p. and mixed m. p. 119°. This and the authentic hydrazone gave identical infrared spectra.

(±)-2-*Amino*-5-*methyl*-4-*oxohexanoic Acid* (With Dr. R. F. Curtis).—*Ethyl γ-bromo-αα-dimethyl-β-oxobutyrate* was prepared by the procedure of Conrad [17] and Scheibler and Schmidt.[18] They did not record any constant. The product, n_D^{29} 1·4622, b. p. 119—122°/9 mm., decomposed when kept for several hours [Found: C, 39·2; H, 5·9; Br, 34·0; O, 20·6%; *M* (Rast), 230. $C_8H_{13}O_3Br$ requires C, 40·0; H, 5·5; Br, 33·8; O, 20·3%; *M*, 237].

Diethyl acetamidomalonate (5·8 g.) was dissolved in ethanolic sodium ethoxide (20 c.c.,

[15] Battersby and Craig, *J. Amer. Chem. Soc.*, 1952, **74**, 4023.
[16] Levy and Chung, *Analyt. Chem.*, 1953, **25**, 396.
[17] Conrad, *Ber.*, 1897, **30**, 857.
[18] Scheibler and Schmidt, *Ber.*, 1921, **54**, 144.

from 0·6 g. of sodium) and kept at room temperature under anhydrous conditions for 16 hr. Ethanol was removed under reduced pressure. Ethyl γ-bromo-αα-dimethyl-β-oxobutyrate (6·2 g.) and dry benzene (45 c.c.) were added to the residue. After the mixture had been refluxed for 48 hr., sodium bromide was removed by filtration and the benzene was removed under reduced pressure, to give a red oil (9·2 g.). A portion of this red oil (7·33 g.) was subjected to short-path distillation. The forerun (2·8 g.) with b. p. up to 125°/0·9 mm. was rejected. *Triethyl 1-acetamido-4-methyl-4-oxopentane-1 : 1 : 4-tricarboxylate* was collected (2·15 g.); it had b. p. 125—130°/0·9 mm. and crystallised. Recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave prisms, m. p. 63—64° (Found: C, 55·2; H, 7·2; N, 3·7; O, 34·1; OEt, 33·5. $C_{17}H_{27}O_8N$ requires C, 54·7; H, 7·3; N, 3·8; O, 34·3; OEt, 36·2%).

A second portion of the red oil (1·03 g.) was kept at 80° for 16 hr. in concentrated hydrochloric acid (10 c.c.). A dark precipitate was removed. Evaporation of the filtrate gave a residue (0·35 g.), a sample (200 mg.) of which was placed on a column (56 × 1·7 cm.) of the ion-exchange resin Amberlite CG-45, Type I. The column was washed with glass-distilled water; 10 c.c. fractions of the eluate were collected. Glycine was eluted completely in fractions 8—20. *2-Amino-5-methyl-4-oxohexanoic acid* (115 mg.) was obtained pure from fractions 25—120. It crystallised from water–ethanol (4 : 1) as plates, m. p. 204° (decomp.) (Found: C, 52·9; H, 8·1; O, 30·3; N, 8·7%; *M*, 157. $C_7H_{13}O_3N$ requires C, 52·8; H, 8·2; O, 30·2; N, 8·8%; *M*, 159). The synthetic amino-acid and the natural optically active isomer gave identical R_F values in: butan-1-ol–acetic acid–water (4 : 1 : 5), R_F 0·39; acetone–water–butan-1-ol–pyridine (14 : 3 : 6 : 3), R_F 0·60; pyridine–methanol–water (10 : 77 : 20), R_F 0·20. However, these α-amino-acids gave a mixed m. p. 180° (decomp.). Their infrared absorption spectra showed minor differences.[19]

Racemisation of (−)-2-Amino-5-methyl-4-oxohexanoic Acid.—This was done by heating the optically active amino-acid (30 mg.) in glacial acetic acid (0·5 c.c.) with acetic anhydride (0·3 c.c.) for 1·5 hr. on a water-bath. Evaporation gave a viscous residue which was refluxed with 2·5N-hydrochloric acid (0·6 c.c.) for 1 hr. The residue obtained on evaporation was dissolved in water (3 c.c.) and purified by means of Amberlite CG-45, Type I. The resulting (±)-acid crystallised from water–ethanol (4 : 1) as plates, m. p. and mixed m. p. 204° (correct infrared spectrum).

Hydrolysis of (±)-2-Amino-5-methyl-4-oxohexanoic Acid with Sodium Hydrogen Carbonate.—(±)-2-Amino-5-methyl-4-oxohexanoic acid (140 mg.) and sodium hydrogen carbonate (360 mg.), dissolved in water (15 c.c.), were kept at 70—80° for 10 min. Ammonia was evolved. The solution was cooled and extracted with ether to remove any methyl *iso*propyl ketone. The aqueous layer was acidified with sulphuric acid and repeatedly extracted with ether. Evaporation of this extract gave white crystals (18 mg.) which sublimed as needles, m. p. 83°, $\lambda_{max.}$ 226 mμ (ε 15,000), $\lambda_{infl.}$ 320 mμ (ε 40) (Found: C, 58·8; H, 7·2; O, 33·9. $C_7H_{10}O_3$ requires C, 59·2; H, 7·0; O, 33·8%), $\nu_{max.}$ 1706 (CO_2^-), 1681 (conjugated C:O), and 1626 cm.$^{-1}$ (C=C). This product decolorised neutral permanganate at room temperature but gave no colour with ferric chloride solution. It was readily decomposed in excess of sodium hydroxide solution into methyl *iso*propyl ketone (2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 119°) and glyoxylic acid, which was identified by its reducing properties, by conversion into glycollic acid, and by its indole–sulphuric acid colour reaction.

These properties indicate that this product is *5-methyl-4-oxohex-2-enoic acid* (III).

Dihydrohypoglycin A.—Hydrogenation of hypoglycin A in glacial acetic acid in presence of platinum oxide led to an uptake of 1·05 mol. of hydrogen in 8 min. Paper chromatography of the product with butan-1-ol–acetic acid–water (4 : 1 : 5) showed the separation of two ninhydrin-sensitive spots, R_F 0·64 (major component) and 0·74. The mixture was applied to a column (24 × 1 cm.) of Amberlite CG-45, Type I. The column was eluted with water (10 c.c./hr.). Fractions (3 c.c.) were collected. Fractions 2—15 contained only the major component (R_F 0·64; 50 mg.). This, *dihydrohypoglycin A*, recrystallised from water–ethanol as plates, m. p. 201—209 (decomp.) (Found: C, 58·5; H, 8·9; O, 22·5; N, 9·8; *C*-Me, 10·9. $C_7H_{13}O_2N$ requires C, 58·7; H, 9·1; N, 9·8; O, 22·4; 1*C*-Me, 10·5%), $\nu_{max.}$ 1587 (CO_2^-), 1515, 3100 (NH_3^+), 2100 (methyl*cyclo*propyl-), 1020 cm.$^{-1}$ (*cyclo*propane-CH_2) and maxima characteristic of α-amino-acids. It was degraded with 10N-hydrochloric acid in a sealed tube at 100° for 6 hr., the reaction mixture being shown by two-dimensional paper chromatography (see above) to contain at least seven new ninhydrin-sensitive compounds.

[19] Brockmann and Musso, *Chem. Ber.*, 1956, **89**, 241.

Tetrahydrohypoglycin A.—Dihydrohypoglycin A (250 mg.) in acetic acid (7 c.c.) containing 11N-hydrochloric acid (0·3 c.c.) was hydrogenated at 1 atm. in the presence of platinum oxide (184 mg.) for 30 hr. (uptake 0·95 mol.). Paper chromatography of the product distinguished two purple ninhydrin-sensitive spots, R_F 0·64 and 0·74 (major component) respectively. The hydrogenation mixture was applied to a column (29 × 1·7 cm.) of Amberlite resin CG-45, Type I. The column was eluted with water which was collected in 2 c.c. fractions. Fractions 110—260 gave the major component (126 mg.), (−)-2-*amino*-5-*methylhexanoic acid*, which gave a single spot on paper chromatography. It crystallised from water as plates, m. p. 220—225° (decomp.) (Found: C, 57·6; H, 10·1; O, 21·9; N, 9·4. $C_7H_{15}O_2N$ requires C, 57·9; H, 10·4; O, 22·0; N, 9·6%), $\nu_{max.}$ 3100, 1515 (NH_3^+), 1580 cm.$^{-1}$ (CO_2^-), and others characteristic of α-amino-acids.

The infrared absorption spectrum of racemised material was almost indistinguishable from that of a sample of (±)-2-*amino*-5-*methylhexanoic acid*, m. p. 215—225° (Found: C, 57·9; H, 10·4; N, 9·7%), prepared by the general procedure of Snyder, Shekleton, and Lewis.[20] Both these spectra differed markedly from that of (±)-2-aminoheptanoic acid,[21] m. p. 223° (decomp.), which was also prepared by the procedure of Snyder *et al.*[20] (Found: C, 57·8; H, 10·3; N, 9·6. Calc. for $C_7H_{15}O_2N$: C, 57·9; H, 10·4; N, 9·6%).

Ninhydrin degradation of the major component of tetrahydrohypoglycin A, by the procedure of Virtanen and Rautanen,[22] yielded a volatile aldehyde which was identified through the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 91°, as *iso*hexanal. The infrared absorption spectra of the derivative, from the natural material and the authentic aldehyde were identical.

The minor component (Found: C, 57·8; H, 10·4; N, 9·7. Calc. for $C_7H_{15}O_2N$: C, 57·9; H, 10·4; N, 9·7%) from the hydrogenation mixture was racemised by acetic anhydride by the procedure outlined above. The infrared absorption spectrum differed markedly from that of (±)-2-aminoheptanoic acid and 2-amino-5-methylhexanoic acid. The minor component and the last-mentioned two amino-acids gave identical R_F values on paper chromatography in conventional solvent systems.

We are grateful for maintenance grants from the Government of Jamaica (to E. V. E.) and Eli Lilly and Co. (to J. R. P.). One of us (C. E. S.) is indebted to the University College of the West Indies for an Overseas Postgraduate Scholarship. We are indebted to members of the Research Division of the Upjohn Company, Kalamazoo, for making available unpublished information.

Department of Chemistry, University College of Swansea.
University College of the West Indies, Jamaica. [*Received, August 13th*, 1958.]

[20] Snyder, Shekleton, and Lewis, *J. Amer. Chem. Soc.*, 1945, **67**, 310.
[21] Helms, *Ber.*, 1875, **8**, 1167.
[22] Virtanen and Rautanen, *Biochem. J.*, 1947, **41**, 101.