tivity.[5,6] Although it appears that glutamine synthesis probably occurs by a stepwise reaction, there is no clear evidence for an intermediate. Recently, Black and Gray[7] reported evidence for β-L-aspartyl phosphate as the product of the enzymatic reaction of L-aspartate and ATP, a finding which renews interest in the possibility of a similar intermediate in glutamine synthesis.

We now report the enzymatic synthesis of hydroxamic acids from D-glutamate, and from both isomers of α-aminoadipate, using the enzyme obtained from peas,[4] as well as enzymes from pigeon liver[2] and sheep brain.[3] When ammonia was substituted for hydroxylamine, D-glutamine was formed, but at less than half the rate observed for D-glutamohydroxamic formation. There was no detectable amide formation from α-aminoadipate under the conditions employed (Table I).

TABLE I

Units—μM formed/mg. enzyme N. Digests contained 50 μM $MgCl_2$, 100 μM neutralized hydroxylamine hydrochloride or NH_4Cl, 50 μM glutamic acid or α-aminoadipic acid neutralized with tris, 25 μM β-mercaptoethanol, 10 μM sodium ATP, 50 μM imidazole buffer, *p*H 7.0. Incubated at 37° for 15 to 40 minutes, with sufficient pea enzyme to effect the synthesis of 2 to 4 μM of hydroxamic acid or amide with active substrates. Final volume, 1.0 ml.; *p*H 7.0. Values corrected by subtraction of blanks. The isomers of aspartic acid are not appreciably active in this system.

Substrate	Hydroxamic acid formed[8] (units/hour)	Amide formed, expressed as phosphate liberated[9] (units/hour)
L-Glutamate	407	428
D-Glutamate	385	171
L-α-Aminoadipate	19.8	0
D-α-Aminoadipate	24.6	0

The D-glutamate[10] employed in these experiments was obtained from the racemate by enzymatic resolution[11] or by the action of *Cl. welchii* decarboxylase.[12] The D-glutamine formed enzymatically was isolated in crystalline form ($[\alpha]^{26}D$ −6.5°), and was identified by paper chromatography, and by its failure to yield carbon dioxide with *Cl. welchii* decarboxylase. Both isomers of glutamine are deamidated by this preparation, but only L-glutamine yields carbon dioxide.

The fact that hydroxamic acids are formed from L- and D-glutamate at similar rates, while D-glutamine is formed considerably less rapidly, suggests the possibility of an initial activation of the glutamate which is of low optical specificity, followed by a more specific reaction with ammonia which becomes rate limiting in the case of D-glutamate. Such a limitation is not noted with hydroxylamine, which is known to react non-enzymatically with acyl phosphates, thiolesters, and certain other compounds. The apparent failure to synthesize the amides of the α-aminoadipate isomers is compatible with the view that the reaction of ammonia with an intermediate is relatively specific. On the other hand, racemic α-methylglutamic acid reacts in this system with both hydroxylamine and ammonia.[13,14]

It is of interest that study of the transferase reaction indicates a high degree of specificity (Table II).

TABLE II

For units, see Table I. Digests contained 50 μM $MgCl_2$, 1 μM sodium ADP, 50 μM glutamine or α-aminoadipamic acid, 100 μM neutralized hydroxylamine hydrochloride, 20 μM β-mercaptoethanol, 5 μM phosphate buffer *p*H 6.6. Incubated at 37° for 15 to 30 minutes, with sufficient pea enzyme to effect the formation of 0.5 to 1.0 μM of hydroxamic acid. Final volume, 1.0 ml.; *p*H 6.5. Values corrected by subtraction of blanks.

Substrate	Hydroxamic acid formed[8] (units/hour)
L-Glutamine	286
D-Glutamine[15]	3.93
L-α-Aminoadipamic acid[15]	0

While these findings might be interpreted as indirect evidence for an intermediate acyl phosphate similar to that described by Black and Gray,[7] we have been unable to obtain any evidence for a free phosphorylated product of the reaction of ATP and the glutamate or aminoadipate isomers.

The authors wish to thank Dr. Jesse P. Greenstein for generous samples of the isomers of glutamic and α-aminoadipic[16] acids.

(5) P. K. Stumpf and W. D. Loomis, *Arch. Biochem.*, **25**, 451 (1950).

(6) M. Schou, N. Grossowicz, A. Lajtha and H. Waelsch, *Nature*, **167**, 891 (1951).

(7) S. Black and N. Gray, THIS JOURNAL, in press. We thank the authors for making a copy of this paper available to us prior to publication.

(8) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(9) C. H. Fiske and Y. SubbaRow, *ibid.*, **66**, 375 (1925).

(10) The D-glutamate and D-glutamine employed contained less than 0.1% of their respective enantiomorphs, *cf.* A. Meister, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *ibid.*, **192**, 535 (1951).

(11) V. E. Price, J. B. Gilbert, and J. P. Greenstein, *ibid.*, **179**, 1169 (1949).

(12) M. M. Camien, L. E. McClure and M. S. Dunn, *Arch. Biochem.*, **28**, 220 (1950).

(13) B. M. Braganca, J. H. Quastel and R. Schucher, *Arch. Biochem. and Biophys.*, **41**, 478 (1952).

(14) N. Lichtenstein, H. E. Ross and P. P. Cohen, *Nature*, **171**, 45 (1953); *J. Biol. Chem.*, **201**, 117 (1953).

(15) Preparation and properties to be reported.

(16) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, THIS JOURNAL, **75**, 1994 (1953).

NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH — LEON LEVINTOW
BETHESDA, MARYLAND — ALTON MEISTER

RECEIVED MAY 16, 1953

THE SYNTHESIS OF UROPORPHYRIN I

Sir:

The structure proposed for uroporphyrin I by Hans Fischer[1] has been confirmed by synthesis.

The Pyrrole A[2] was brominated to give the crystalline methene B which, when fused with methylsuccinic acid for six hours at 118° cf [3], gave porphin-1,3,5,7-tetraacetic acid-2,4,6,8-tetrapropionic acid as the octamethyl ester m.p. 290–292°[4] (5.7%). Analysis gave no indication of partial decarboxylation [Calcd. for $C_{48}H_{54}O_{16}N_4$: C, 61.14; H, 5.77; N, 5.94; OCH_3, 26.33; C–CH_3, 0.0. Found: C, 61.00; H, 5.85; N, 5.76; OCH_3, 26.38; C-CH_3, 0.0.] In analogous cases, this method has given type I porphyrins exclusively. Here, the type was confirmed by partial decarboxylation to coproporphyrin I[5] obtained as the tetramethyl ester m.p.

(1) H. Fischer and H. Orth, "Chemie des Pyrrols," Akademische Verlag, Leipzig, 1937, Band II/I, p. 504 ff.

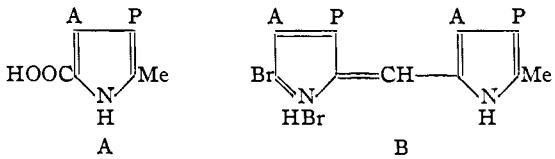
(2) S. F. MacDonald, *J. Chem. Soc.*, 4184 (1952).

(3) H. Fischer and H. Zischler, *Z. physiol. Chem.*, **245**, 123 (1937).

(4) M.p.s. (hot stage) are corrected.

(5) H. Fischer and W. Zerweck, *Z. physiol. Chem.*, **137**, 242 (1924).

252–253°. This m.p. was not depressed by an authentic synthetic specimen, and the identity was confirmed by solubility, crystal form, visible and infrared spectra.



A = CH_2·COOH
P = CH_2CH_2·COOH

The m.p. of the synthetic porphin-1,3,5,7-tetraacetic acid-2,4,6,8-tetrapropionic acid octamethyl ester was not depressed by the purest uroporphyrin I methyl ester,[6] and their identity was confirmed by comparison of their visible spectra, infrared spectra, and X-ray powder photographs.

DIVISION OF PURE CHEMISTRY
THE NATIONAL RESEARCH COUNCIL S. F. MACDONALD
OTTAWA, CANADA R. J. STEDMAN[7]

RECEIVED MAY 21, 1953

(6) C. Rimington and P. A. Miles, *Biochem. J. (London)*, **50**, 202 (1951).

(7) National Research Council of Canada Postdoctoral Fellow.

A METHOD FOR THE SYNTHESIS OF CYCLIC POLYPEPTIDES

Sir:

The recognition of the cyclic nature of several of the antibiotic polypeptides has focused attention on the paucity of methods for the synthesis of cyclic peptides with rings larger than those of diketopiperazines. It is the purpose of this communication to report a procedure for the synthesis of such "cyclopeptides" by the catalytic hydrogenolysis of carbobenzoxypeptide azides. The method is exemplified by the synthesis of cyclo-DL-phenylalanylglycylglycine.

Carbobenzoxy-DL-phenylalanylglycylglycine hydrazide, m.p., 170–171° (*Anal.* Calcd. for $C_{21}H_{25}O_5N_5$: N, 16.4. Found: N, 16.0 (Dumas)), was converted to the azide in the usual manner. A solution of the azide (from 0.54 g. of the hydrazide) in 500 ml. of dry ethyl acetate was introduced slowly (at room temperature over a period of 26 hours) into 750 ml. of dry ethyl acetate containing *ca.* 2 g. of palladium black,[1] hydrogen being bubbled through the solution After 29 hours, the catalyst was removed by filtration, the solution was allowed to stand at room temperature overnight, and it was then concentrated *in vacuo* to a small volume. The resulting solid product was collected, washed with ethyl acetate, and reprecipitated from ethanol with ether; yield, 0.1 g. The cyclic tripeptide decomposed at 177–179° (*Anal.* Calcd. for $C_{13}H_{15}O_3N_3$: C, 59.8; H, 5.8; N, 16.1. Found: C, 60.0; H, 6.0; N, 15.6 (Dumas)). A determination of the molecular weight by the method of Cottrell[2] gave a value of 250, as compared with the theoretical value of 261. The Van Slyke nitrous acid method gave a value of 0.045 per cent. NH_2-N, indicating the absence of free amino groups. The product gave no color with ninhydrin (3 mg. heated with 2 ml. of 0.25 per cent. solution in 1:1 pyridine–water). Hydrolysis with 6 *N* hydrochloric acid at 100° for 24 hours, followed by chromatographic examination of the hydrolysate, gave a molar ratio of glycine to phenylalanine of 1.9:1. The cyclic peptide is soluble in methanol, ethanol, glacial acetic acid, and hot water; it is sparingly soluble in ethyl acetate, ether, cold water, or aqueous acid or alkali. When a suspension of the product in aqueous picric acid-sodium carbonate is heated, a permanent orange-red color is produced. 2,5-Diketopiperazine also gives a positive reaction,[3] but the color fades on standing. The infrared spectrum of the cyclic tripeptide differs from that of glycycl-L-phenylalanine anhydride.

While this work was in progress, the report of Boissonas and Schuman[4] appeared on the preparation of a cyclic peptide from D-leucylglycylglycine by treatment with ethyl chloroformate in dimethylformamide; no quantitative analytical data were presented, however, to establish the identity of the product with the desired cyclopeptide.

(1) C. A. Dekker and J. S. Fruton, *Methods in Medical Research*, **3**, 280 (1950).

(2) F. G. Cottrell, THIS JOURNAL, **41**, 721 (1919).

(3) E. Abderhalden and E. Komm, *Z. physiol. Chem.*, **139**, 181 (1924).

(4) R. A. Boissonas and I. Schuman, *Helv. Chim. Acta*, **35**, 2229 (1952).

DEPARTMENT OF BIOCHEMISTRY
YALE UNIVERSITY MILTON WINITZ
NEW HAVEN, CONN. JOSEPH S. FRUTON

RECEIVED MAY 7, 1953

EVIDENCE FOR COBALT HYDROCARBONYL AS THE HYDROFORMYLATION CATALYST

Sir:

It has been postulated[1] and indirect evidence has been presented[2,3] that cobalt hydrocarbonyl, a strong acid, is the catalyst for the reactions that occur under hydroformylation conditions (90–200° and 100–300 atmospheres of synthesis gas (mixtures of hydrogen and carbon monoxide) in the presence of cobalt). However, the presence of the hydrocarbonyl, $HCo(CO)_4$, during or after the reaction has been difficult to demonstrate. This communication presents experimental work which shows that (a) cobalt hydrocarbonyl is formed under hydroformylation conditions, and (b) pure hydrocarbonyl in the absence of carbon monoxide and hydrogen reacts with certain substrates at room temperature and 1 atmosphere to give products that are also secured from the same substrates under hydroformylation conditions.

(a) Treatment of a solution of dicobalt octacarbonyl in pyridine with synthesis gas at 120° and 230 atmospheres resulted in the conversion of all of the cobalt to the pyridinium salt of cobalt hydrocarbonyl, $[C_5H_5NH]^+[Co(CO)_4]^-$. The same salt was obtained by adding pure cobalt hydrocarbonyl to pyridine at room temperature.

(b) The products from experiments involving

(1) (a) O. Roelen, Office of Tech. Services, U. S. Dept. Commerce, PB 81383; (b) H. Adkins and G. Krsek, THIS JOURNAL, **70**, 383 (1948); (c) I. Wender and M. Orchin, U. S. Bureau of Mines Rep. of Investigations 4270 (1948).

(2) I. Wender, S. Metlin and M. Orchin, THIS JOURNAL, **73**, 5704 (1951).

(3) I. Wender, H. Greenfield, S. Metlin and M. Orchin, *ibid.*, **74**, 4079 (1952).