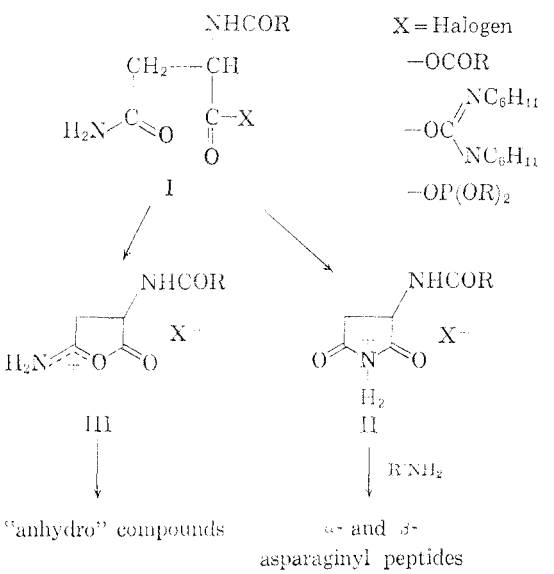
The Synthesis of Two Peptides Containing Methylene-L-asparagine

CHARLES H. STAMMER

Received October 31, 1960

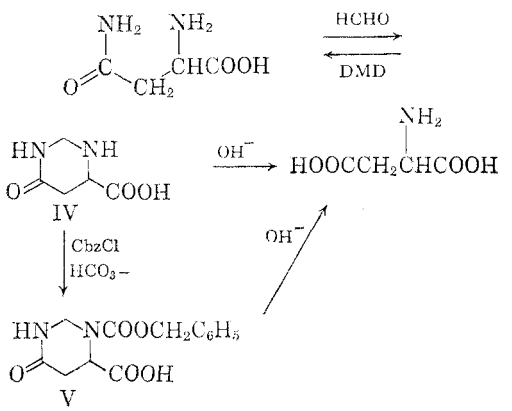
This paper describes attempts to develop a new synthesis of L-asparaginyl peptides using methylene-L-asparagine (IV).[1] Two carbobenzyloxy methylene-L-asparaginyl dipeptides were prepared and attempts to convert them to the corresponding L-asparaginyl peptides were made. Even though dimedone abstracted formaldehyde from IV giving L-asparagine in good yield, it converted methylene-L-asparaginyl peptides into complex mixtures which were not further investigated.

It is well known that the coupling of *N*-acylasparagines with amines is attended by low yields[2] and gives mixtures of products. The coupling reaction is most probably complicated by rearrangement of a reactive intermediate (I) formed from the *N*-acylasparagine and coupling agent. Rearrangement of I to the cyclic imide II followed by reaction with an amine might be expected to yield[3,4]



mixtures of α- and β-asparaginyl peptides. If I rearranged to III, "anhydro" compounds[5,6,7] might be the products. If, as here suggested, the difficulties are due to interaction of the carbamido group with the activated carboxyl, masking of the carbamido function might facilitate formation of the desired products. We hoped that the carbamido function of methylene-L-asparagine would be unable to interact with the activated carboxyl and that, once formed, methylene-L-asparaginyl peptides could be converted back to the corresponding asparaginyl peptides.

Methylene-L-asparagine (IV) was first isolated by Schiff in 1900[1] and its cyclic structure was established by the efforts of several workers.[8] We found that IV could be formed from L-asparagine and formaldehyde in either neutral or alkaline solution and crystallized in about 50% yield. The treatment of methylene-L-asparagine with dimedone[9] (DMD) in aqueous solution liberated[10] L-asparagine which was isolated and identified by its infrared spectrum. The reaction of IV with carbobenzyloxy chloride in alkaline solution gave *N*-carbobenzyloxymethylene-L-asparagine (V).

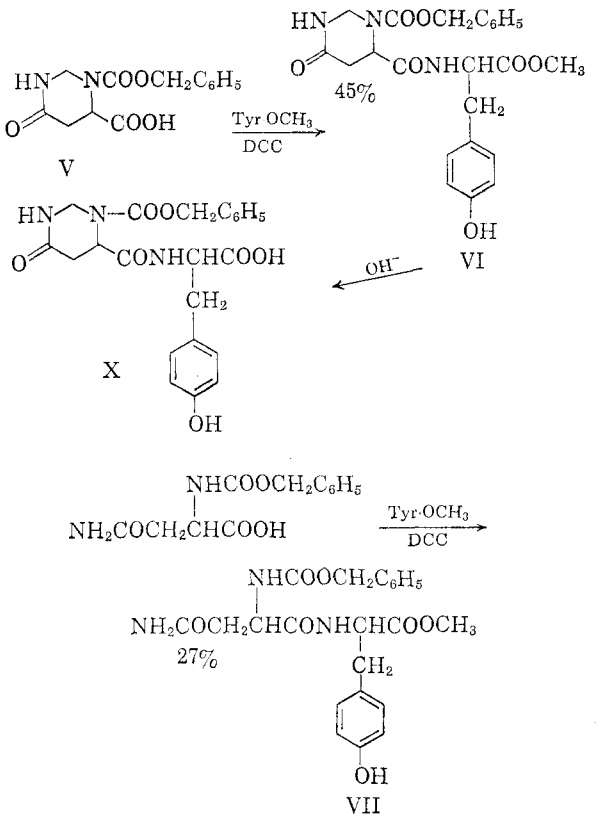


The formation of this *N*-acyl derivative confirms the ring structure of IV since the open-chain methylene-imino (CH_2=N—) form would not be expected to give such a derivative. The decomposition of V in 2*N* lithium hydroxide at room temperature required ten days while a solution of IV in 1*N* lithium hydroxide had reached constant optical rotation in twenty hours. Papergrams indicated that both IV and V were converted to aspartic acid by alkali.

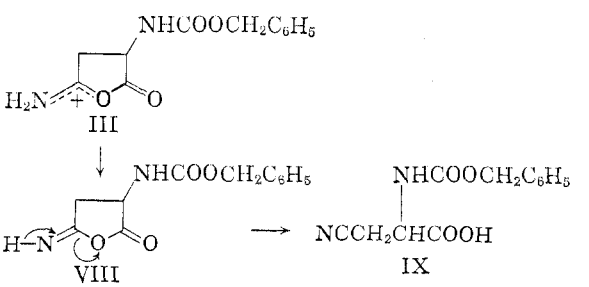
Two derived dipeptides were prepared from V. Tyrosine methyl ester reacted with V in the presence of *N*,*N*′-dicyclohexylcarbodiimide[11,12] (DCC) giving carbobenzyloxy methylene-L-asparaginyl-L-tyrosine methyl ester (VI) in 45% yield. Under the same reaction conditions, carbobenzyloxy-L-asparagine was coupled with tyrosine methyl ester and the carbobenzyloxy dipeptide ester (VII)

(1) H. Schiff, *Ann.*, **310**, 25 (1900).
(2) S. S. Leach and H. Lindley, *Australian J. Chem.*, **7**, 173 (1954).
(3) A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 259 (1955).
(4) E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954); *J. Am. Chem. Soc.*, **79**, 3767 (1957).
(5) C. Ressler, *J. Am. Chem. Soc.*, **78**, 5956 (1956).
(6) D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, *J. Am. Chem. Soc.*, **78**, 5954 (1956).
(7) P. G. Katsoyannis, D. T. Gish, and V. duVigneaud, *J. Am. Chem. Soc.*, **79**, 4516 (1957).
(8) D. French and J. T. Edsall, *Adv. in Prot. Chem.*, Vol. II, Academic Press, Inc., New York, N. Y., 1945, p. 306.
(9) D. Vorlander, *Z. Anal. Chem.*, **77**, 241 (1929).
(10) This ease of hydrolysis is consistent with the work of A. W. Titherly and G. E. K. Branch, *J. Chem. Soc.*, **103**, 330 (1913) on hexahydropyrimidine.
(11) H. G. Khorana, *Chem. Rev.*, **53**, 145 (1953).
(12) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

was obtained in 27% yield. Although the yield of dipeptide was considerably increased by the use of V, further improvement is still desirable.



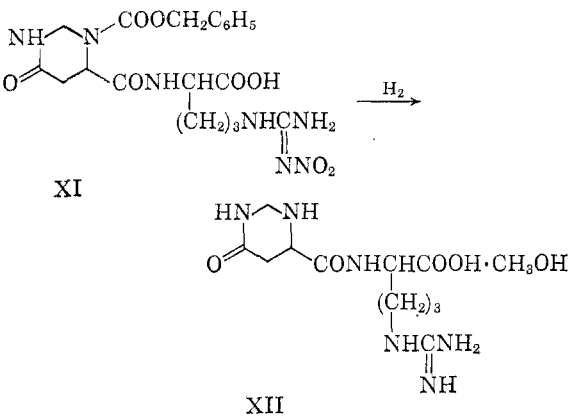
In order to investigate the possibility of participation by the carbamido group in the coupling reaction (as discussed earlier), carbobenzyloxy-L-asparagine was allowed to react with dicyclohexylcarbodiimide in the absence of tyrosine methyl ester. Dicyclohexylurea, in 92% yield, was precipitated and *N*-carbobenzyloxy-β-cyano-L-alanine[13] (IX) was obtained in 42% yield. The formation of IX may have occurred through intermediates such as III and VIII. Carbobenzyloxy-β-cyano-L-alanine (IX) thus becomes readily available and,



since it can be easily converted to asparagine with hydrogen bromide in acetic acid,[12] might be useful in asparaginyl peptide syntheses.

The conversion of carbobenzyloxy methylene-L-asparaginyl-L-tyrosine methyl ester (VI) to the free dipeptide L-asparaginyl-L-tyrosine failed. The ester group was saponified in 0.5*N* sodium hydroxide giving the acid[14] X. Reductive removal of the carbobenzyloxy group gave a mixture of products which reacted with ninhydrin and diazotized sulfanilic acid. Treatment of this crude mixture with aqueous dimedone solution gave the dimedone-formaldehyde adduct in only 47% yield and a new mixture of peptides. The complexity of the mixture made further investigation impractical.

A second methylene-L-asparaginyl peptide was obtained when V was allowed to react through its mixed anhydride[15] with the sodium salt of nitro-L-arginine.[16] The crude dipeptide, obtained in 60% yield, afforded 45% of crystalline carbobenzyloxy methylene-L-asparaginyl-nitro-L-arginine (XI). Hydrogenation of XI in methanol gave crystalline



methylene-L-asparaginyl-L-arginine (XII) as its methanol adduct. When XII was treated with an aqueous dimedone solution, the peptide product showed three ninhydrin-positive components. Attempts to purify this mixture failed.

It is our hope that, even though our results are incomplete, others will be stimulated to use formaldehyde adducts of amino acids in peptide synthesis and that peptide methodology may benefit thereby.

EXPERIMENTAL

General. All melting points were taken on a Kofler Micro Hot Stage.

All paper chromatograms reported were done on 32-cm. Whatman No. 1 circles with a 1-cm. center hole.[17] The development of the chromatograms was carried out between two 12″ Pyrex pie plates. The eluting solvent mixture was

(13) (a) M. Zaoral and J. Rudinger, *Proc. Chem. Soc.*, 176 (1957); (b) *Coll. Czech. Chem. Comm.*, **24**, 1993 (1959).

(14) No racemization occurred during this hydrolysis since the optical rotation of the acid X checks that obtained from chymotrypsin hydrolysis of IX. Unpublished results.

(15) J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

(16) K. Hofmann, W. D. Peckham, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).

(17) E. Lederer and M. Lederer, *Chromatography*, Second Ed., Elsevier Publishing Co., New York, N. Y., 1957, p. 134.

contained in a 1″ × 1″ glass cup and was supplied to the paper through a paper wick which passed through the center hole of the circle into the cup. The developing solvent mixtures are designated as below:

BAW—butanol:acetic acid:water—4:1:5. The upper phase was used.
BAm—butanol:1.5*N* ammonium hydroxide—1:1. The upper phase was used.
MPW—methyl ethyl ketone:pyridine:water—4:1:1.6.

The compounds were located on the paper by means of ninhydrin (N), diazotized sulfanilic acid (P), or ultraviolet absorption (UV). A compound which has an R_f value of 0.5 in the MPW system and was located with ninhydrin reagent is reported as R_f^{MPW} 0.5(*N*). R_f's reported consecutively were run on the same sheet. Certain standards (L-asparagine and L-aspartic acid) were often run as reference points, since R_f values varied somewhat from sheet to sheet.

Unless otherwise specified, all analytical samples were dried at 52° and *ca.* 0.1 mm. pressure in the presence of phosphorus pentoxide or Drierite for 2 hr.

Methylene-L-asparagine (IV). (a) *In neutral solution.* One hundred grams of finely pulverized L-asparagine monohydrate was dissolved in 700 ml. of water maintained at 75° during a 1-hr. period. The solution was filtered and allowed to cool to *ca.* 45° when 54 g. (1 equivalent) of 37% aqueous formaldehyde was added. After 25 min., 2 l. of methanol was added slowly and the mixture was placed in a refrigerator for 2 hr. The crude methylene-L-asparagine, collected on a filter and washed with methanol, weighed 57.4 g. (59.5%) after drying in a vacuum oven at 50°. This material showed $[\alpha]_D^{25}$ −63.5° (*c*, 1.07 in water), R_f^{MPW} 0.28 (N, purple), 0.35 (N, brown), 0.40 (N, brown), m.p. 210–260° dec. A 5-g. sample of this material was recrystallized from 170 ml. of water (dissolves slowly) and 100 ml. of methanol at room temperature giving 3.0 g. of methylene-L-asparagine, $[\alpha]_D^{25}$ −73.5° (*c* 2.04 in water), R_f^{MPW} 0.50 (N, brown), m.p. 210–260° dec. infrared showed 3.1 μ (NH), 5.95–6.2 (C=O).

(b) *In alkaline solution.* A solution prepared from 30.0 g. (200 mmoles) of L-asparagine monohydrate, 200 ml. of 1*N* lithium hydroxide, and 18 ml. of 37% aqueous formaldehyde was allowed to stand at room temperature. An hour after mixing, the observed optical rotation was −5.41° (1-dm tube). During the next 48 hr., the observed rotation changed to and became constant at −12.7°. The solution was acidified with 13 ml. of acetic acid and 500 ml. of absolute ethanol was added slowly. The cloudy solution was placed in a refrigerator overnight. The crystalline methylene-L-asparagine weighing 16.3 g. (53%) was collected on a filter and washed with ethanol and ether. The crude product showed: $[\alpha]_D^{25}$ −62.5° (*c*, 2.46 in water); $[\alpha]_D^{25}$ −103.5° (*c*, 7.25 in water containing 1 equivalent of sodium hydroxide); R_f^{MPW} 0.31 (N, purple), 0.44 (N, brown). L-Asparagine: R_f^{MPW} 0.30 (N, brown); L-aspartic acid: R_f^{MPW} 0.32 (N, purple). The crude product was dissolved in 350 ml. of water (required 2 hr. stirring at room temperature), filtered and the filtrate diluted with 350 ml. of methanol. After standing in the refrigerator overnight, the solution was filtered, giving 7.2 g. of crystalline methylene-L-asparagine, $[\alpha]_D^{25}$ −71.2° (*c*, 2.09 in water) and $[\alpha]_D^{25}$ −119° (*c*, 7.32 in water containing 1 equivalent of sodium hydroxide), R_f^{MPW} 0.50 (N, brown). L-Asparagine R_f^{MPW} 0.35 (N, brown); L-aspartic acid: R_f^{MPW} 0.40 (N, purple).

Anal. Calcd. for $C_5H_8N_2O_3$: C, 41.66; H, 5.59; N, 19.44. Found: C, 41.93; H, 5.66; N, 19.61.

Decomposition of methylene-L-asparagine (VI). (a) *In lithium hydroxide.* A solution of 254 mg. of IV in 25.0 ml. of 0.89*N* lithium hydroxide was allowed to stand at room temperature. The initial optical rotation ($[\alpha]_D^{25}$ in 1-dm. tube) of this solution was −109° and after 24 hr. it had become constant at −5.88°. The solution was acidified to *p*H 6 with 2 ml. of glacial acetic acid and lyophilized. Crystallization of the residue from 4 ml. of water and 7 ml. of ethanol gave 173 mg. of solid showing R_f^{BAW} 0.22 (N, blue), R^{MPW} 0.32 (N, blue); DL-aspartic acid: R_f^{BAW} 0.26 (N, blue), R_f^{MPW} 0.36 (N, blue); L-asparagine: R_f^{MPW} 0.29 (N, brown). The crystalline product contained inorganic salts and was not further characterized.

(b) *With dimedone.* To a warm solution of 560 mg. (4 mmoles) of dimedone in 75 ml. of water was added 288 mg. (2 mmoles) of IV. After standing overnight at room temperature, the solution was filtered. The dimedone-formaldehyde adduct weighed 524 mg. (89%). The filtrate was lyophilized and the residue, weighing 217 mg., was extracted three times with 5-ml. portions of hot ethanol. The insoluble material was dissolved in 2 ml. of hot water and 2 ml. of ethanol was added dropwise. The crystals were washed with ethanol and dried. The infrared of this product was identical with that of L-asparagine monohydrate.

Carbobenzyloxy methylene-L-asparagine (V). A solution of 5.0 g. (34.7 mmoles) of methylene-L-asparagine (IV) in 50 ml. of half-saturated potassium bicarbonate solution was stirred magnetically in a 250-ml. round bottomed flask. To this solution was added 4.9 ml. of carbobenzyloxy chloride and the mixture stirred at room temperature for 2.5 hr. The solution was washed with three 50-ml. portions of ether and acidified to *p*H 1.5 with concd. hydrochloric acid. The precipitated oil was extracted into 150 ml. of ethyl acetate. The extract was dried and evaporated to *ca.* 100 ml. and allowed to stand at room temperature. The crystalline carbobenzyloxy methylene-L-asparagine was collected on a filter and washed once with ethyl acetate. It weighed 5.7 g. (59%) and showed m.p. 135–138°, $[\alpha]_D^{25}$ −30° (*c*, 1.55 in water).

Anal. Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.01; H, 4.80; N, 9.78.

Decomposition of carbobenzyloxy methylene-L-asparagine (V). A solution of 2.78 g. (10 mmoles) of V in 25.0 ml. of 2.13*N* lithium hydroxide was allowed to stand at room temperature. The initial *observed* optical rotation was −7.24° and after 10 days the rotation had become constant at −0.28°. The solution was acidified with 3 ml. of acetic acid and lyophilized. The residue was extracted with three 50-ml. portions of hot ethanol and the insoluble solid, weighing 1.14 g., was collected on a filter and dried. It showed R_f^{MPW} 0.33 (N, blue); DL-aspartic acid, R_f^{MPW} 0.36 (N, blue); L-asparagine, R_f^{MPW} 0.29 (N, brown). The product even after crystallization from water and ethanol, contained inorganic salts and was not further characterized.

Carbobenzyloxy methylene-L-asparaginyl-L-tyrosine methyl ester (VI). A solution of 5.85 g. (30 mmoles) of L-tyrosine methyl ester[18] and 6.18 g. (30 mmoles) of *N,N'*-dicyclohexylcarbodiimide in 50 ml. of dry dimethylformamide was prepared in a 250-ml. Erlenmeyer flask provided with a magnetic stirrer and immersed in an ice bath. A solution of 8.34 g. (30 mmoles) of carbobenzyloxy methylene-L-asparagine in 30 ml. of dry dimethylformamide was added to the stirred amine-carbodiimide solution over a 1-hr. period. After being stirred overnight at room temperature, the reaction mixture was cooled in ice and the precipitated *N,N'*-dicyclohexylurea, weighing 4.55 g. (68%), was collected on a filter. The filtrate was evaporated to dryness and the residue was dissolved in 200 ml. of ethyl acetate. The solution was washed with two 30-ml. portions of 1*N* hydrochloric acid and two 30-ml. portions of saturated potassium bicarbonate solution. The organic phase was dried and evaporated giving 12.9 g. of a crystalline residue. Recrystallization of the crude product from 100 ml. of ethyl acetate gave 6.2 g. (45.5%) of carbobenzyloxy methylene-L-asparaginyl-L-tyrosine methyl ester, m.p. 163–165°, $[\alpha]_D^{24}$ −45.7° (*c*, 1.05 in methanol). A sample recrystallized from 1:3 isopropyl alcohol-water showed m.p. 163–165°, $[\alpha]_D^{25}$ −48° (*c*, 1.0 in methanol), R_f^{MPW} 1.0 (P) R_f^{BAW} 0.89 (P).

Anal. Calcd. for $C_{23}H_{25}N_3O_7$: C, 60.65; H, 5.53; N, 9.23. Found: C, 60.96; H, 5.60; N, 9.62.

(18) H. Schwarz and F. M. Bumpus, *J. Am. Chem. Soc.*, 81, 890 (1959).

Carbobenzyloxy methylene-L-asparaginyl-L-tyrosine (X). A solution of 228 mg. (0.50 mmole) of carbobenzyloxy methylene-L-asparaginyl-L-tyrosine methyl ester in 2.0 ml. of 0.5*N* sodium hydroxide was allowed to stand at room temperature for 2 hr. (At intervals of 0, 15, 35, 60, and 120 min., 5–10 μl. of the reaction mixture was applied to a 32-cm. Whatman No. 1 circle and this sheet was developed in MPW. The spots developed by diazotized sulfanilic acid showed that after 15 min., the saponification was complete and that after 2 hr., the methylene-L-asparaginyl ring had not been destroyed by the alkali present.) The reaction mixture was acidified with 1.0 ml. of 1.0*N* hydrochloric acid and the separated oil extracted into three 3-ml. portions of ethyl acetate. After drying, the organic layer was evaporated giving 228 mg. of crude product. This material was dissolved in 1 ml. of hot isopropyl alcohol, 3 ml. of water added and after 3 hr. at room temperature, the solution was placed in a refrigerator. The crystalline carbobenzyloxy methylene-L-asparaginyl-L-tyrosine, weighing 108 mg. (50%), was collected by centrifugation and washed with 1:4 isopropyl alcohol–water solution. When dry, the product showed m.p. 118–122°, $[\alpha]_D^{25}$ −25.5° (*c*, 1.06 in pyridine), R_f^{MPW} 0.88 (P, R^{BAm} 0.31 (P). A sample again recrystallized from 1:3 isopropyl alcohol–water melted at 119–122°.

Anal. Calcd. for $C_{22}H_{23}N_3O_7$: C, 59.86; H, 5.25; N, 9.52. Found: C, 60.11; H, 5.53; N, 9.64.

Attempted conversion of X *to* L-*asparaginyl*-L-*tyrosine.* (a) *Reduction.* A solution of 1.2 g. of X in 20 ml. of methanol was hydrogenated overnight at 40 p.s.i. and room temperature using 1.2 g. of 10% palladium-on-carbon as catalyst. The catalyst was filtered and extracted with four 10-ml. portions of hot methanol, four 10-ml. portions of hot water, and four 10-ml. portions of 10% pyridine in hot water. The combined extracts weighed 742 mg. and showed numerous components when paper chromatographed.

(b) *Dimedone treatment.* To a hot solution of 600 mg. of dimedone in 50 ml. of water was added 619 mg. of the reduction product obtained above. After heating the mixture 1.5 hr. on a steam bath, it was placed in the refrigerator. The dimedone-formaldehyde adduct, weighing 274 mg. (47%), was filtered and the filtrate was lyophilized. The residue was was extracted with three 50-ml. portions of hot ethyl acetate leaving 466 mg. (79%) of insolubles which showed multiple spots in both MPW and BAW systems. After trituration with hot isopropyl alcohol, the product weighed 287 mg. and showed R_f^{MPW} 0.30, 0.42, 0.60, 0.79 (N + P). Elemental analysis gave values inconsistent with L-asparaginyl-L-tyrosine.

Carbobenzyloxy-L-asparaginyl-L-tyrosine methyl ester. A solution of 2.66 g. (10 mmoles) of carbobenzyloxy-L-asparagine[19] in 27 ml. of dry dimethylformamide was added during 15 min. to an ice-cold magnetically stirred solution of 2.06 g. (10 mmoles) of *N,N'*-dicyclohexylcarbodiimide and 1.95 g. (10 mmoles) of L-tyrosine methyl ester in 12 ml. of dry dimethylformamide. After *ca.* 20 hr. at room temperature, the mixture was cooled and the precipitated dicyclohexylurea weighing 2.0 g. (89%), was collected on a filter. The filtrate was evaporated to dryness and the residue dissolved in a warm mixture of 100 ml. of ethyl acetate and 25 ml. of butanol. Two 25-ml. washings with 2.5*N* hydrochloric acid removed 0.5 g. of L-tyrosine methyl ester, m.p. 133–136°, B_f^{BAm} 0.86 (P), and two 25-ml. washings with half-saturated potassium bicarbonate solution removed 0.8 g. of acidic components, R_f^{BAm} 0.13 (P), 0.36 (P), from the solution. The organic layer was dried and evaporated giving 2.9 g. of crystalline product. Recrystallization of the above solid from 85 ml. of methyl ethyl ketone gave a first crop weighing 0.9 g., m.p. 188–194°; and a second crop weighing 0.33 g., m.p. 165–190°. The total crude yield of carbobenzyloxy-L-asparaginyl-L-tyrosine methyl ester was thus 1.23 g. (27%). The first crop was recrystallized from a mixture of 40 ml. of methyl ethyl ketone and 10 ml. isopropyl alcohol. This product, 479 mg., m.p. 197–199°, $[\alpha]_D^{25}$ 1.96° (*c*, 1.02 in methanol), was submitted for analysis.

Anal. Calcd. for $C_{22}H_{25}N_3O_7$: C, 59.58; H, 5.68; N, 9.48. Found: C, 59.48; H, 5.72; N, 9.40.

Carbobenzyloxy-β-cyano-L-alanine. A solution of 2.66 g. (10 mmoles) of carbobenzyloxy-L-asparagine in 27 ml. of dry dimethylformamide was added during 15 min. to an ice-cold magnetically stirred solution of 2.06 g. (10 mmoles) of *N,N'*-dicyclohexylcarbodiimide in 12 ml. of dry dimethylformamide. *N,N'*-dicyclohexylurea began precipitating within 10 min. After *ca.* 20 hr. at room temperature, a solution of 1.95 g. (10 mmoles) of L-tyrosine methyl ester[18] in 10 ml. of warm dimethylformamide was added to the reaction mixture in which solid dicyclohexylurea was present. After another 4 hr. at room temperature, the mixture was filtered giving 2.07 g. (92%) of dicyclohexylurea. The filtrate was evaporated to dryness and the residue was dissolved in 100 ml. of ethyl acetate. Extraction of this solution with two 25-ml. portions of half-saturated potassium bicarbonate solution removed 2.0 g. of acidic products. Further extraction with two 25-ml. portions of 2.5*N* hydrochloric acid removed 0.8 g. of L-tyrosine methyl ester, R_f^{BAm} 0.87 (P), from the solution. The remaining ethyl acetate solution was evaporated to dryness giving 0.5 g. of neutral material which showed R_f^{BAm} 0.95 (P) and gave some crystals, m.p. 200–210°, after solution in methanol. The neutral product may be impure carbobenzyloxy-L-asparaginyl-L-tyrosine methyl ester. The 2.0 g. of acidic products were crystallized from 25 ml. of ethylene dichloride. The product obtained weighed 1.1 g. (42%) and melted at 126–128°. Another crystallization from 60 ml. of ethylene dichloride gave 880 mg. of carbobenzyloxy-β-cyano-L-alanine, m.p. 126–128°,[20] $[\alpha]_D^{21}$ −19° (*c*, 1.26 in methanol). Its infrared spectrum showed a 4.43 μ band characteristic of a cyano function.

Anal. Calcd. for $C_{12}H_{12}N_2O_4$:C, 58.06; H, 4.87; N, 11.29. Found: C, 57.61; H, 4.91; N, 11.37.

Carbobenzyloxy methylene-L-asparaginyl-nitro-L-arginine. A solution of 22.2 g. (80 mmoles) of carbobenzyloxy methylene-L-asparagine (V) in 300 ml. of 1:1 tetrahydrofuran-dioxane mixture was stirred mechanically in a 200-ml. three-necked round bottomed flask immersed in an ice-methanol cooling bath. To this was added successively 12.3 ml. (80 mmoles) of triethylamine and a solution of 11.9 ml. (80 mmoles) of isobutylchlorocarbonate. Twenty minutes later, an ice-cold solution of 21.9 g. (100 mmoles) of nitro-L-arginine[16] and 15.1 g. (110 mmoles) of triethylamine in 300 ml. of water was added to the mixed anhydride solution over a 5-min. period. The reaction mixture was stirred for 4 hr., while the temperature was allowed to rise to 25°. The mixture was concentrated *in vacuo* to remove the organic solvents, and the resulting slurry was acidified with concentrated hydrochloric acid. This mixture was extracted with one 200-ml. and two 100-ml. portions of 1:1 butanol–ethyl acetate solution. These combined extracts were evaporated to dryness *in vacuo* and the residue was triturated with three portions of hot ethyl acetate. The dry crude carbobenzyloxy methylene-L-asparaginyl-nitro-L-arginine weighed 26.4 g., (60%), R_f^{BAm} 0.23, 0.35, 0.49 (UV).

Ten grams of the above crude product was dissolved in 75 ml. of hot 1:1 ethanol-water mixture. After 1 week at room temperature, the solution afforded 4.5 g. of crystalline carbobenzyloxy methylene-L-asparaginyl-nitro-L-arginine, m.p. 199–202°. Dilution of the mother liquor with water gave only an oily precipitate. A small sample, recrystallized for analysis from 3:7 ethanol-water, melted at 202–204° and showed R_f^{MPW} 0.82 (UV), R_f^{BAW} 0.70 (UV), R_f^{BAm} 0.20 (UV), $[\alpha]_D^{25}$ −27.5° (*c*, 2.18 in 0.1*N* sodium hydroxide).

Anal. Calcd. for $C_{19}H_{25}N_7O_8$: C, 47.59; H, 5.26; N, 20.45. Found: C, 47.72; H, 4.95; N, 20.75.

Methylene-L-asparaginyl-L-arginine (XII). A solution of 2.5 g. of carbobenzyloxy methylene-L-asparaginyl-nitro-L-

(19) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(20) The authors of reference 12 report m.p. 133–134°.

arginine (XI) in 60 ml. of warm 5:1 methanol-water mixture was cooled to room temperature and hydrogenated overnight at room temperature and 40 p.s.i. using 2.5 g. of 10% palladium-on-carbon catalyst. After removal of the catalyst, the solvent was evaporated *in vacuo* and the residue was dissolved in 50 ml. of water and the solution lyophilized. The residue, weighing 1.30 g., was dissolved in 12 ml. of hot methanol whereupon crystallization occurred. The crystalline methylene-L-asparaginyl-L-arginine·CH_3OH (XII) weighed 1.13 g. (72%) and showed m.p. 160–165° dec., R_f^{MPW} 0.29 (N). A 250-mg. sample of this product was dissolved in 1 ml. of water and diluted with 1 ml. of isopropyl alcohol. Seeding and addition of 3 ml. of methanol gave 177 mg. of crystalline material, m.p. 163–165°, $[\alpha]_D^{23}$ −31° (*c*, 1 in 0.1*N* hydrochloric acid). A sample was dried at 78° for 2 hr. at *ca.* 0.1 mm. over Drierite for analysis. NMR spectrum on this sample showed the presence of 1 mole of methanol.

Anal. Calcd. for $C_{12}H_{24}N_6O_5$: C, 43.36; H, 7.28; N, 25.29. Found: C, 43.16; H, 7.26; N, 25.40.

Attempted conversion of XII *to* L-*asparaginyl*-L-*arginine.* A solution of 600 mg. of dimedone was prepared in 50 ml. of hot water and a solution of 600 mg. of XII in *ca.* 3 ml. of water was added to it. The mixture was heated 1.5 hr. and put in the refrigerator. The dimedoneformaldehyde adduct, weighing 413 mg. (71%), was collected on a filter and the filtrate was lyophilized. The residue, after extraction with three 50-ml. portions of hot ethyl acetate, weighed 698 mg. and showed R_f^{MPW} 0.29–0.38 (N); L-arginine·HCl, R_f^{MPW} 0.32–0.48 (N). Further precipitations from methanol–ethyl acetate gave multicomponent products which were not further characterized.

Acknowledgment. The author expresses gratitude to Mr. R. N. Boos and associates for microanalyses and Mr. B. Arison for NMR spectra.

MERCK SHARP & DOHME RESEARCH LABORATORIES
DIVISION OF MERCK & CO., INC.
RAHWAY, N. J.

New Synthesis of 18-Hydroxy-17-methoxy-15,16,17,18,19,20-hexadehydroyohimbane Hydrochloride

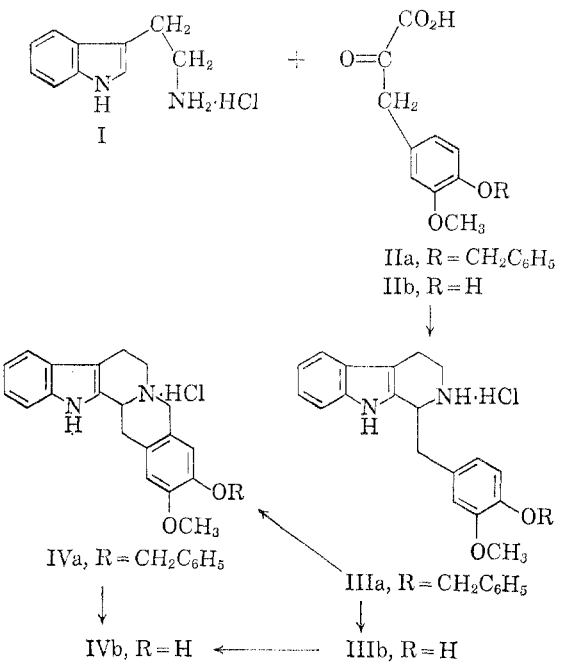
JAMES H. SHORT, MORRIS FREIFELDER, AND GEORGE R. STONE

Received November 28, 1960

Synthesis of 18-hydroxy-17-methoxy-15,16,17,-18,19,20-hexadehydroyohimbane hydrochloride (IVb), which contains the pentacyclic ring system of reserpine, deserpidine, and related compounds, was accomplished as early as 1938.[1] Since then, other investigators[2–4] have reported the preparation of this substance and related compounds.

Preparation of IVb is usually accomplished by condensing tryptamine (I) with 4-hydroxy-3-methoxyphenylpyruvic acid (IIb). The product, 1 - (4 - hydroxy - 3 - methoxybenzyl) - 1,2,3,4-tetrahydro-β-carboline hydrochloride (IIIb), is then cyclized with formaldehyde to obtain IVb. The preparation of IIIb by this method, while satisfactory on a small scale, did not lend itself to large scale work. The difficulty appeared to be the lack of stability of IIb. Douglas and Gulland[5] have previously commented on the instability of IIb.

As we desired a large quantity of IVb, it was necessary to find a suitable modification of this sequence. Covering the phenolic function with a group which could be removed later suggested itself, and it seemed likely that the benzyl group might satisfactorily serve this function.



Vanillin, therefore, was converted to its *O*-benzyl derivative, and then into an azlactone. Hydrolysis of the latter with barium hydroxide gave the desired 4-benzyloxy-3-methoxyphenylpyruvic acid (IIa).

Tryptamine (I) and IIa gave 1-(4-benzyloxy-3-methoxybenzyl) - 1,2,3,4 - tetrahydro - β - carboline hydrochloride (IIIa) in good yields, irrespective of the quantities used. Debenzylation of IIIa led to IIIb and the latter was cyclized to the desired IVb, or IIIa could first be cyclized to IVa, and then debenzylated to give IVb.

EXPERIMENTAL[6]

1-(4-Benzyloxy-3-methoxybenzyl)-1,2,3,4-tetrahydro-β-carboline hydrochloride (IIIa).[7] A mixture of 65.5 g. (0.33 mole) of tryptamine hydrochloride[8] and 100 g. (0.33 mole) of

(1) G. Hahn and A. Hansel, *Ber.*, **71**, 2195 (1938).
(2) W. Logemann *et al.*, *Ber.*, **88**, 1952 (1955); **89**, 1043 (1956).
(3) M. Onda and M. Kawanishi, *J. Pharm. Soc. Japan*, **76**, 966 (1956).
(4) T. Nogradi, *Monatsh. Chem.*, **88**, 1093 (1957).
(5) R. Douglas and J. Gulland, *J. Chem. Soc.*, **134**, 2893 (1931).
(6) Microanalyses were carried out by Mr. Elmer Shelberg and his staff of the Abbott Microanalytical Laboratory.
(7) The preferred Chemical Abstracts name for this substance is 1-(4-benzyloxy-3-methoxybenzyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole hydrochloride.
(8) M. Freifelder, *J. Am. Chem. Soc.*, **82**, 2386 (1960).