16-Cyanoheroin. To 500 mg of **2b** dissolved in 4 mL of water was added a small excess of an aqueous solution of potassium cyanide. The product, which precipitated immediately, was filtered, washed with water, and air-dried to afford 450 mg (90%) of 16α- and 16β-cyanoheroin (note: this product eventually epimerized to 16β-cyanoheroin). ^{1}H NMR chemical shift data for both 16α- and 16β-cyanoheroin are given in Table I.

Registry No. 1, 86993-77-3; **2a**, 86993-78-4; **2b**, 86993-80-8; morphine *N*-oxide, 639-46-3; 16(*S*)-cyanocheroin, 87011-55-0; 16(*R*)-cyanoheroin, 87011-56-1; morphine, 57-27-2; heroin, 561-27-3.

Synthetic Studies on Sequences of Vitamin K Dependent Proteins. Gla-Arg-Gla Sequences

Daniel H. Craig, Karl A. Koehler, and Richard G. Hiskey*

The W. R. Kenan, Jr. Laboratories of Chemistry, The Univerwity of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Received March 8, 1983

The synthesis of peptides containing the Gla-Arg-Gla sequence is reported. Nuclear magnetic resonance studies suggest that intramolecular salt bridge interactions occur between the guanidino group of arginine and the γ-carboxyglutamic acid.

The sequenced proteins shown to contain γ-carboxyglutamic acid residues (Gla) contain a high degree of sequence homology within the Gla-rich region. In addition to containing 10–14 Gla residues and a small cystine loop, the sequences Gla-Arg-gla and/or Arg-Gla-Gla invariably occur. Direct spectroscopic studies of Eu^{3+}–Gla peptide and protein interactions[1,2] and theoretical calculations involving malonate/metal ion interactions[3] suggest that a single Gla residue will contribute one ligand to a divalent ion even though two side-chain carboxyl groups exist per Gla residue. We suspected that the Arg-Gla sequences might contribute to the overall stability of the secondary structure via salt bridges formed by side-chain interactions between the guanidino and carboxyl side chains in the presence or absence of divalent metal ions.

Lancelot et al.[4] have shown that carboxylate–guanidinium ion interactions occur in peptides containing Arg-Glu sequences. Salt bridge formation leads to a downfield shift of the N$^\epsilon$-proton resonance of arginine in the NMR spectrum of these peptides. Protonation of the carboxylate anion with strong acids such as trifluoroacetic acid or perchloric acid disrupted the interaction and resulted in an upfield shift of the same proton resonance. Hence, we suspected that the chemical shift of the N$^\epsilon$-proton of Arg should indicate hydrogen bonding between Arg and Gla side chains. In order to evaluate possible hydrogen bonding interactions of this sort and to develop synthetic methods that would yield Arg-Gla and Gla-Arg peptides, we undertook the synthesis of the peptides **16** and **17** representing the sequence 15–17 and 11–17 of bovine prothrombin.

Solutions of L-arginine can be prepared in DMF containing 1 equiv of either trifluoroacetic acid or methanesulfonic acid. Addition of 1 equiv of triethylamine liberates free arginine, which precipitates; however, precipitation can be prevented by using a weaker base such as *N*-methylmorpholine. Acylation of L-arginine with the *N*-succinimidoyl ester of *N*-(benzoyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid (**2**) provided *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginine (**3**) in 85% yield. The reaction was slow, requiring up to 48 h for completion (Scheme I).

L-Arginine solutions could also be prepared in DMF containing 1 equiv of *N*-hydroxybenzotriazole. Acylation of L-arginine under these conditions using the pentachlorophenyl ester of *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid (**4**) provided 81% of **3** in 24 h.

DiBello et al.[5] have shown that Z-AA-Arg-OH (AA = Phe, glu-γ-Bzl) dipeptides can be coupled to other amino acids or peptides by the method of Konig and Geiger[6] without racemization. Acylation of γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid α-methyl ester *p*-toluenesulfonate salt (**6**), generated from **5**, with the protected dipeptide acid **3** using DCC/HOBt provided the tripeptide **7** in 94% yield. When **7** was precipitated from the reaction by addition of ether, the peptide was obtained as the *N*-hydroxybenzotriazole salt. Alternatively, an aqueous sodium acetate wash of the reaction mixture in ethyl acetate

Table I. Chemical Shift Data (ppm) for 16 in the Presence and Absence of Cesium Carbonate/Me_2SO-d_6

resonance	no Cs_2CO_3	1 equiv of Cs_2CO_3
Gla γ-H	3.35	3.18
Arg δ-H	3.10	3.10
Arg N$^\epsilon$-H	7.57	7.83

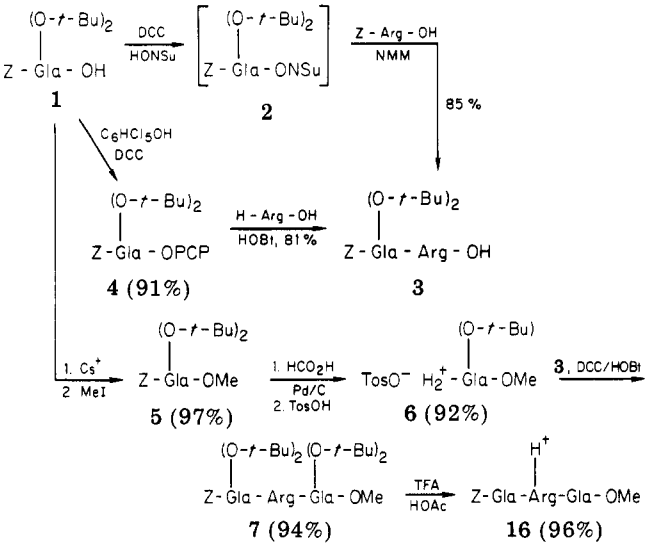
Table II. Concentration Dependence of Chemical Shift Data (ppm) for 16 in the Presence of Cesium Carbonate/Me_2SO-d_6

	various concentrations of 16, M				
resonance	5×10^{-2}	2.5×10^{-2}	1×10^{-2}	5×10^{-3}	1×10^{-3}
Gla γ-H	3.18	3.12	3.10	3.10	3.10
Arg δ-H	3.10	3.10	3.08	3.10	3.10
Arg N$^\epsilon$-H	7.83	7.83	7.85	7.88	7.83

(1) Sarasua, M. M.; Scott, M. E.; Helpern, J. A.; Ten Kortenaar, P. B. W.; Boggs, N. T., III; Pedersen, L. G.; Koehler, K. A.; Hiskey, R. G. *J. Am. Chem. Soc.* **1980**, *102*, 3404.
(2) Marsh, H. C.; Sarasua, M. M.; Madar, D. A.; Hiskey, R. G.; Koehler, K. A. *J. Biol. Chem.* **1981**, *256*, 7863.
(3) Gottschalk, K. E.; Hiskey, R. G.; Pedersen, L. G.; Koehler, K. A. *J. Mol. Struct.* **1982**, *185*, 155.
(4) Lancelot, G.; Mayer, R.; Helene, C. *J. Am. Chem. Soc.* **1979**, *101*, 1569. See also: Mayer, R.; Lancelot, G. *J. Am. Chem. Soc.* **1981**, *103*, 4738.
(5) DiBello, C.; Mariago, A.; Buso, O.; Lucchiari, A. *Tetrahedron Lett.* **1977**, 1135.
(6) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

0022-3263/83/1948-3954$01.50/0

Scheme I



provided the acetate salt of **7** in which form it was characterized. In our earlier studies we utilized 90% TFA-H_2O to remove the γ-*tert*-butyl esters. Careful analysis of the reaction mixture using ninhydrin suggested partial cleavage of the *N*-benzyloxycarbonyl group had also occurred under these conditions. With a modification of the method of Klausner and Bodansky[7] employing a 50:50 mixture of TFA-HOAc, the *tert*-butyl ester groups were removed without the appearance of ninhydrin-positive sideproducts to yield the acid **16**.

The synthesis of the heptapeptide **15** involved the 3 + 4 coupling of **13** and **14**. The product **15** was obtained in 65% yield by using DCC/HOBt as the coupling agent. Treatment of **15** with a 50:50 mixture of TFA/HOAc afforded **17**, in good yield (Scheme II).

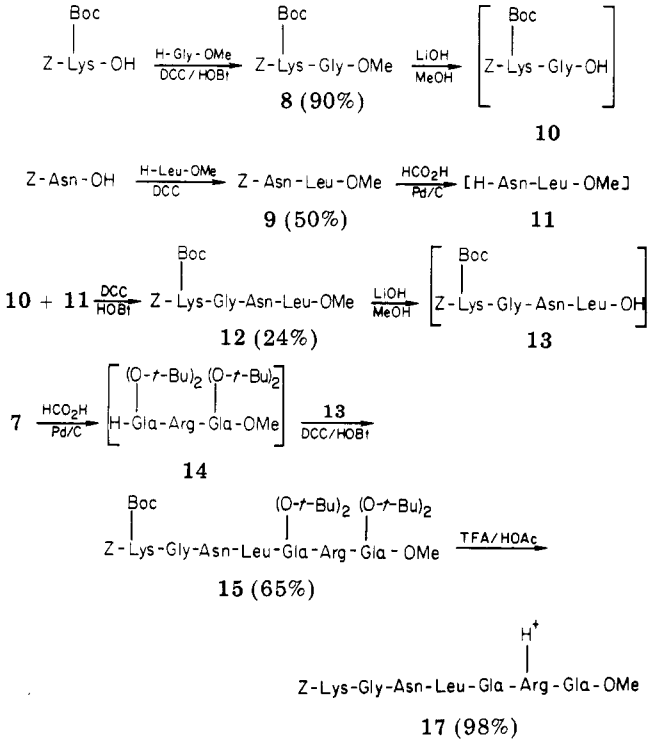
The 250-MHz proton NMR spectrum of the tripeptide derivative **16** was examined in MeSO-d_6 and compared to the spectrum of **16** in Me_2SO-d_6 containing 1 equiv of cesium carbonate. The chemical shift data for the unionized form of **16** and the mono salt form of **16** are given in Table I; Table II reports the concentration dependence of the indicated resonance signals for the mono salt form of **16**.

As suggested by the work of Lancelot et al.,[4] the resonance signal of the N^{ϵ}–H of arginine in the ionized form of **16** moved downfield as the result of ionization of 1 equiv of carboxyl group. The corresponding upfield movement of the γ-H resonance of both Gla residues indicated that the negative charge resulting from the ionization is equilibrated among the four side-chain carboxyl groups. The chemical shift of the N^{ϵ}-H resonance is independent of concentration throughout the range reported in Table II, suggesting that the interaction is intramolecular. Lancelot et al. observed similar effects in their study of *N*-acetyl-L-arginyl-L-glutamic α-*N*-ethyl amide. Thus we conclude that Arg-Gla sequences very likely contribute to secondary structure stabilization by formation of type-2 salt bridge interactions between the arginyl and γ-carboxyglutamyl side chains.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Amino acid analyses were obtained on a Phoenix Model V6-6000 amino acid analyzer and are not corrected for destruction during hydrolysis. Unless otherwise stated the products were dried in vacuo over phosphorus pentoxide and sodium hydroxide pellets.

Scheme II



N-Benzyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid α-*N'*-Succinimidoyl Ester (2). To a solution of 4.37 g (10 mmol) of **1** and 1.15 g (10 mmol) of *N*-hydroxysuccinimide in 50 mL of ethyl acetate was added 2.06 g (10 mmol) of dicyclohexylcarbodiimide, and the resulting solution was stirred for 2 h at 0 °C and then at room temperature overnight. After filtration to remove *N,N'*-dicyclohexylurea, the solution was evaporated in vacuo to yield an oil, which was directly coupled to arginine as described below.

N-Benzoyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginine (3). Method A. The oil obtained from **2** was dissolved in 10 mL of DMF to which 1.74 g (10 mmol) of L-arginine, 1.14 g (10 mmol) of trifluoroacetic acid, and 1.01 g (10 mmol) of *N*-methylmorphine were added, and the reaction was allowed to stir at room temperature for 48 h. Addition of 100 mL of a 50:50 mixture of ethyl acetate and H_2O with vigorous stirring followed by separation of the phases, extraction of the organic phase with 50 mL of H_2O, drying of the ethyl acetate layer with $MgSO_4$, and evaporation in vacuo, yielded 5.19 g (85%) of **3**: mp 92–95 °C; $[\alpha]_D^{23}$ –6.63° (*c* 1.07, MeOH).

Anal. Calcd for $C_{28}H_{45}N_5O_{10}$: C, 54.98; H, 7.41; N, 11.45. Found: C, 55.00; H, 7.44; N, 11.42.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Arg_{1.00}$ $Glu_{1.02}$.

N-Benzyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid Pentachlorophenyl Ester (4). To a solution of 4.37 g (10 mmol) of **1** and 2.70 g (10.1 mmol) of pentachlorophenol in 25 mL of ethyl acetate at 0 °C was added 2.06 g (10 mmol) of dicyclohexylcarbodiimide, and the mixture was stirred overnight, while warming to room temperature. The solution was filtered and evaporated in vacuo to an oil. The oil was taken up in a minimum of hot 2-propanol and stored at –20 °C overnight. The white solid was filtered and air-dried, yielding 6.24 g (91%) of **4**: mp 106–107 °C; $[\alpha]_D^{23}$ –16.23° (*c* 1.03, EtOAc).

Anal. Calcd for $C_{28}H_{30}NO_8Cl_5$: C, 49.04; H, 4.41; N, 2.04; Cl; 25.84. Found: C,49.04; H, 4.45; N, 2.03; Cl, 25.77.

N-Benzyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginine (3). Method B. To a solution of 0.135 g

(7) Klausner, Y. S.; Bodandsky, M. *Bioorg. Chem.* **1973**, *2*, 354.
(8) Boggs, N. T., III; Goldsmith, B.; Gawley, R. E.; Koehler, K. A.; Hiskey, R. G. *J. Org. Chem.* **1979**, *44*, 2262.

of *N*-hydroxybenzotriazole (1 mmol) and 0.685 g (1 mmol) of **4** in 1 mL of DMF was added 0.174 g (1 mmol) of L-arginine, and the mixture was stirred at room temperature for 2 h. The reaction mixture was treated with 5 mL of 10% aqueous potassium bicarbonate with vigorous stirring for an additional 2 h at room temperature, afterwhich time 5 mL of H_2O and 10 mL of ethyl acetate were added. The organic layer was separated and re-extracted with 10 mL of H_2O. Evaporation in vacuo of the ethyl acetate yielded 0.495 (81%) of **3** identical in all respects with the material obtained by method A.

N-Benzyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid α-Methyl Ester (5). A solution containing 4.37 g (10 mmol) of **1** and 1.625 g (5 mmol) of cesium carbonate in 100 mL of MeOH was evaporated to dryness. The solid was dissolved in 25 mL of DMF, and 2.0 g (13 mmol) of MeI was added. The reaction was stirred at room temperature for 2 h, 200 mL of a 50:50 mixture of ethyl acetate and 5% aqueous $Na_2S_2O_3$ were added, and the aqueous phase was separated. The ethyl acetate layer was washed once with 100 mL of H_2O, dried over $MgSO_4$, and evaporated in vacuo, yielding 4.4 g (97%) of **5**: mp 72–75 °C; $[\alpha]_D^{23}$ −16.36° (*c* 0.935, MeOH).

Anal. Calcd for $C_{23}H_{33}NO_8$: C, 61.18; H, 7.,36; N, 3.10. Found: C, 61.10; H, 7.37; N, 3.09.

γ,γ-Di-*tert*-butyl-L-γ-carboxyglutamic Acid α-Methyl Ester *p*-Toluenesulfonate Salt (6). A solution containing 2.25 g (5 mmol) of **5** in 50 mL of MeOH was treated with 1 g of 90% HCOOH and stirred at room temperature for 1 h with 0.2 g of 5% Pd/C. To this suspension was added 0.95 g (5 mmol) of *p*-toluenesulfonic acid monohydrate. After filtration the solution was evaporated to dryness with toluene. The resulting oil was taken up in 10 mL of toluene, and 10 mL of petroleum ether was added. After 24 h at −20 °C, the solvent was removed by decantation, and the solid was dried in vacuo yielding 4.5 g (92%) of the salt **6**: mp 95–96 °C; $[\alpha]_D^{23}$ +24.4° (*c* 1.14, MeOH).

Anal. Calcd for $C_{22}H_{35}NO_9S$: C, 53.97; H, 7.20; N, 2.86; S, 6.55. Found: C, 53.81; H, 7.15; N, 2.84; S, 6.51.

N-Benzyloxycarbonyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid α-Methyl Ester Acetate Salt (7). To 0.593 g (1 mmol) of **3**, 0.489 g (1 mmol) of **6**, and 0.135 g (1 mmol) of *N*-hydroxybenzotriazole in 10 mL of acetonitrile at 0 °C was added 0.206 g (1 mmol) of dicyclohexylcarbodiimide. After being stirred for 24 h at 4 °C, the solution was filtered and the acetonitrile was removed in vacuo to yield an oil, which was taken up in 10 mL of ethyl acetate. The ethyl acetate was washed once with 10 mL of 10% aqueous sodium acetate, and once with 10 mL of H_2O and concentrated in vacuo to yield 0.90 g (94%) of **7**: mp 80–85 °C; $[\alpha]_D^{23}$ −13.57° (*c* 0.56, EtOAc).

Anal. Calcd for $C_{45}H_{72}N_6O_{16}$: C, 56.71; H, 7.61; N, 8.82. Found: C, 56.45; H, 7.61; N, 8.81.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Arg_{1.00}$ $Glu_{2.02}$.

N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysylglycine Methyl Ester (8). To a solution of 0.561 g (1 mmol) of *N*-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine dicyclohexylamine salt, 0.125 g (1 mmol) of glycine methyl ester hydrochloride, and 0.135 g (1 mmol) of *N*-hydroxybenzotriazole in 10 mL of DMF at 0 °C was added 0.206 g (1 mmol) of dicyclohexylcarbodiimide, and the reaction mixture was allowed to warm to room temperature overnight. The solution was filtered and concentrated in vacuo to an oil. The oil was dissolved in 10 mL of ethyl acetate and extracted sequentially with 10 mL of 5% aqueous sodium bicarbonate, 10 mL of H_2O, 20 mL of aqueous citric acid, and 10 mL of H_2O, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting oil was triturated with H_2O to yield 4 g (90%) of the ester **8**: mp 68–72 °C; $[\alpha]_D^{23}$ −13.1° (*c* 1.04, MeOH) [lit.[9] mp 86–90 °C, $[\alpha]_D^{24}$ −13.5 (*c* 1.0, MeOH)].

Anal. Calcd for $C_{22}H_{33}H_3O_7$: C, 58.52; H, 7.36; N, 9.31. Found: C, 58.58; H, 7.37; N, 9.30.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Gly_{1.00}$ $Lys_{1.02}$.

N-Benzyloxycarbonyl-L-asparaginyl-L-leucine Methyl Ester (9). To a solution of 2.66 g (10 mmol) of *N*-benzyloxycarbonyl-L-asparagine,[10] 1.81 g (10 mmol) of L-leucine methyl ester hydrochloride, and 1.35 g (10 mmol) of *N*-hydroxybenzotriazole in 50 mL of DMF at 0 °C was added 2.06 g (10 mmol) of dicyclohexylcarbodiimide. The reaction mixture was stirred and allowed to warm to room temperature overnight. The solution was filtered, and 100 mL of 10% aqueous sodium bicarbonate was added with stirring for 1 h. The solid that formed was filtered and sequentially washed with 50 mL of 20% aqueous citric acid, 50 mL of H_2O, 50 mL of 10% aqueous sodium bicarbonate, and 50 mL of H_2O and then dried in vacuo to yield 1.96 g (50%) of **9**: mp 173–175 °C; $[\alpha]_D^{23}$ −27.8° (*c* 1.03, MeOH) [lit.[11] mp 177–178 °C].

Anal. Calcd for $C_{19}H_{27}N_3O_6 \cdot {}^1/_2\ H_2O$: C, 56.70; H, 7.01; N, 10.44. Found: C, 56.75; H, 7.03; N, 10.43.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Asp_{1.00}$ $Leu_{0.99}$.

N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysylglycyl-L-asparaginyl-L-leucine Methyl Ester (12). The oils **10** and **11** were combined in 50 mL of DMF to which 1.35 g (10 mmol) of *N*- hydroxybenzotriazole and 2.06 g (10 mmol) of dicyclohexylcarbodiimide had been added. The reaction mixture was stirred at room temperature overnight. After filtration, 200 mL of a 50:50 mixture of ethyl acetate and 10% aqueous sodium bicarbonate were added with vigorous stirring. The phases were allowed to separate, and the solid appearing at the interface of the two layers was collected via filtration and dried, yielding 1.5 g (24%) of **12**: mp 184–186 °C; $[\alpha]_D^{23}$ −14.9° (*c* 1.01, DMF).

Anal. Calcd for $C_{32}H_{50}N_6O_{10}$: C, 56.62; H, 7.42; N, 12.38. Found: C, 56.68; H, 7.45; N, 12.30.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Asp_{1.01}$ $Gly_{0.99}$ $Leu_{0.99}$ $Lys_{1.00}$.

N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysylglycyl-L-asparaginyl-L-leucine Lithium Salt (13). A 0.0678-g (0.1 mmol) sample of **12** was dissolved in 3 mL of 80% *tert*-butyl alcohol/H_2O containing 0.0042 g (0.1 mmol) of lithium hydroxide, and the solution was evaporated in vacuo. The resulting oil was coupled directly to **14**.

γ,γ-Di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid α-Methyl Ester *p*-Toluenesulfonate Salt (14). A 0.053 g (0.1 mmol) sample of **7** was dissolved in 2 mL of MeOH containing 0.1 g of 90% HCOOH and treated with 0.05 of 5% Pd/C for 1 h at room temperature. One equivalent of p-toluenesulfonic acid was added and the solution filtered. The solvent was removed azeotropically with toluene, and the resulting oil was directly used in the next step.

N-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysylglycyl-L-asparaginyl-L-leucyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginyl-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic Acid α-Methyl Ester *p*-Toluenesulfonate Salt (15). The oils **13** and **14** were combined in 2 mL of DMF to which 0.0135 g (0.1 mmol) of *N*-hydroxybenzotriazole and 0.0206 g (0.1 mmol) of dicyclohexylcarbodiimide were added, and the reaction mixture was stirred overnight. The solution was filtered and concentrated in vacuo, yielding an oil, which was triturated with acetone resulting in 0.102 g (65%) of **15**: mp 110–114 °C; $[\alpha]_D^{23}$ −11.4° (*c* 0.66, MeOH).

Anal. Calcd for $C_{73}H_{116}N_{12}O_{24}S$: C, 55.57; H, 7.41; N, 10.65. Found: C, 56:02; H, 7.40; N, 10.46.

Amino acid analysis after hydrolysis in sealed tubes with 6 N HCl at 110 °C for 24 h showed $Arg_{1.02}$ $Asp_{1.02}$ $Glu_{1.98}$ $Gly_{0.99}$ $Leu_{0.97}$ $Lys_{1.00}$.

N-Benzyloxycarbonyl-L-γ-carboxyglutamyl-L-arginyl-L-γ-carboxyglutamic Acid α-Methyl Ester Trifluoroacetate Salt (16). A 0.0953-g (0.1 mmol) sample of **7** was dissolved in 2 mL of a 50:50 mixture of glacial acetic acid and trifluoroacetic acid and allowed to stand at room temperature for 30 h. The solution was evaporated in vacuo and the resulting oil was triturated with ethyl ether to yield 0.075 g (96%) of the acid **16**: mp

(9) Ten Kortenaar, P. B. W.; Wilkerson, W. W.; Boggs, N. T., III; Madar, D. A.; Koehler, K. A.; Hiskey, R. G. *Int. J. Pept. Protein Res.* **1980**, *16*, 440.

(10) Bergmann, M.; Zervas, L. Chem. *Ber.* **1932**, 65, 1192.

(11) Woodward, R. B.; Olofson, R. A.; Mayer, H. *Tetrahedron, Suppl.* **1966**, *8* (Part I), 321.

90–95 °C; dec; $[\alpha]_D^{23}$ −16.4° (c 0.64, MeOH).

Anal. Calcd for $C_{29}H_{37}N_6O_{16}F_3$: C, 44.50; H, 4.77; N, 10.74. Found: C, 44.72; H, 4.93; N, 10.65.

N^{α}-Benzyloxycarbonyl-L-lysylglycyl-L-asparaginyl-L-leucyl-L-γ-carboxyglutamyl-L-arginyl-L-γ-carboxyglutamic Acid α-Methyl Ester p-Toluenesulfonate Trifluoroacetate Mixed Salt (17). A 0.025-g (0.016 mmol) sample of **15** was dissolved in 1 mL of a 50:50 mixture of glacial acetic acid and trifluoroacetic acid and allowed to stand at room temperature for 36 h. The solution was evaporated in vacuo and triturated with ethyl ether to yield 0.021 g (98%) of **17**: mp 63–65 °C.

Anal. Calcd for $C_{54}N_{77}O_{24}SF_3$: C, 47.43; H, 5.68; N, 12.29. Found: C, 47.82; H, 5.75; N, 12.03.

^{1}H NMR Studies on the Carboxylate–Guanidino Interaction of 16. A 0.95-g (0.025 mmol) sample of **16** was dissolved in 0.5 mL of Me_2SO-d_6 and the proton NMR spectrum recorded. Homonuclear decoupling experiments at the proton resonances corresponding to the δ-protons of arginine, the γ-protons of Gla, and the α-protons of all three residues were performed (Bruker Model WM250). One equivalent (0.0041 g, 0.012) mmol) of cesium carbonate was then added to the NMR tube and the solution placed under a vacuum to remove the carbon dioxide formed. The normal proton spectrum and the corresponding homonuclear decoupled spectra were recorded. These same spectra were also recorded at concentrations of **16** of 25 mM, 10 mM, 5 mM, and 1 mM.

Acknowledgment. This research was supported by Grant HL-20161 from the National Heart, Lung, Blood Institute, U.S. Public Health Service. We also acknowledge the contributions of Dr. David L. Harris and Ms. M. W. Pendergraft.

Registry No. 1, 60686-50-2; 2, 64153-37-3; 3, 87136-42-3; 4, 76804-70-1; 5, 76822-52-1; 6, 87173-10-2; 7·H_3CCO_2H, 87136-44-5; 8, 10342-52-6; 9, 14317-83-0; 10, 47689-13-4; 11, 29359-35-1; 12, 87136-45-6; 13·Li, 87136-46-7; 14, 87136-47-8; 15·Me-p-$C_6H_4SO_3H$, 87136-49-0; 16·F_3CCO_2H, 87136-51-4; 17·F_3CCO_2H·Me-p-$C_6H_4SO_3H$, 87136-53-6; H-Arg-OH, 74-79-3; Z-Lys(BOC)-OH·DCHA, 2212-76-2; H-Gly-OMe·HCl, 5680-79-5; N-(benzyloxycarbonyl)-L-aspargine, 2304-96-3; L-leucine methyl ester hydrochloride, 7517-19-3; vitamin K, 12001-79-5.

Novel Biogenetic Pathways from (+)-Reticuline. Three Dimeric Alkaloids: (+)-Vanuatine, (+)-Vateamine, and (+)-Malekulatine

Jean Bruneton

CEPM, U.E.R. des Sciences Médicales et Pharmaceutiques, 49000 Angers, France

Maurice Shamma,* Robert D. Minard, and Alan J. Freyer

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

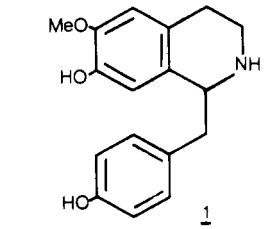
Hélène Guinaudeau

Faculté de Médecine et de Pharmacie, 87032 Limoges Cedex, France

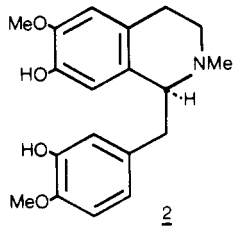
Received April 13, 1983

The bark of *Hernandia peltata* Meissner (Hernandiaceae), gathered in the Republic of Vanuatu (New Hebrides), has yielded the bis(benzylisoquinolines) (+)-vanuatine (**5**), (+)-vateamine (**6**), and (+)-malekulatine (**7**). These are the first-known dimers of (+)-reticuline (**2**). Compounds **5** and **6** are products of tail-to-tail oxidative coupling, whereas **7** involves head-to-tail coupling.

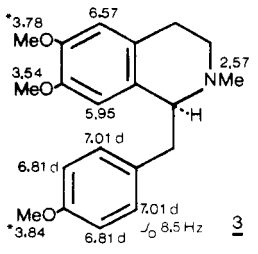
The isoquinoline skeleton is incorporated into well over 1000 alkaloids, and most of these compounds may be broken down into two broad categories depending upon whether they are derived biogenetically from the tetrahydrobenzylisoquinolines (±)-coclaurine (**1**) or (+)-reticuline (**2**). (±)-Coclaurine-derived alkaloids tend to dimerize to provide more than 250 different bis(benzylisoquinolines).[1] Alternatively, they may undero intramolecular oxidative coupling to proaporphines, which can rearrange to aporphines.



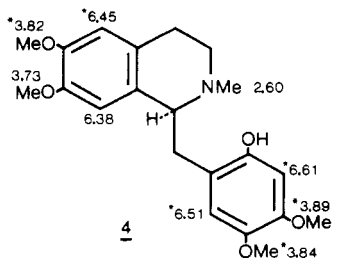
1



2



3



4

The in vivo chemistry of (+)-reticuline (**2**), which possesses an additional oxygen in the bottom ring, differs in some important respects from that of (±)-coclaurine (**1**). There is a proclivity for internal cyclization, due to the added activation of the bottom ring, to generate a pavine, an isopavine, a dibenzopyrrocoline, a berbine, or an aporphine. The tendency to dimerize is minimal, and in fact only nine bis(benzylisoquinolines) are known that incorporate a reticuline moiety bonded to a coclaurine unit, while none were known that originate from bonding between two reticulines.[1,2]

We now report the first three bis(benzylisoquinoline) alkaloids derived from the condensation of two (+)-reticuline units. Of added significance is the fact that each of these three dimers is formed by a different mode of

(1) Guha, K. P.; Mukherjee, B.; Mukherjee, R. *J. Natural Products* **1979**, *42*, 1. Schiff, P. L., Jr. *Ibid.* **1983**, *46*, 1.

(2) Some 30 aporphine–benzylisoquinoline alkaloids of the thalicarpine type are known. These alkaloids are formed biogenetically through coupling of a fully formed aporphine unit with (+)-reticuline (**2**). See: Sidjimov, A. K.; Marekov, N. L. *Phytochemistry* **1982**, *21*, 871. Bhakuni, D. S.; Jain, S. *Tetrahedron* **1982**, *38*, 729.

0022-3263/83/1948-3957$01.50/0