Roritoxin B (5): mp 262–265 °C dec; $[\alpha]^{25}_D$ +2.6° (*c* 0.60, $CHCl_3$); UV λ_{max} (MeOH) 254 nm (log ϵ = 4.99); MS, *m/e* 558.2106 (M^- calcd 558.2139). See Tables I and II for NMR data.

Roritoxin C (6): mp 288–290 °C dec; $[\alpha]^{25}_D$ +8.9° (*c* 0.40, $CHCl_3$); UV λ_{max} (MeOH) 254 nm (log ϵ = 4.97); MS, *m/e* 572.1955 (M^- calcd 572.1974). See Table II for NMR data.

Roritoxin D (7): mp 294–297 °C dec; $[\alpha]^{25}_D$ +30.0° (*c* 0.10, $CHCl_3$); UV λ_{max} (MeOH) 253 nm (log ϵ = 4.98); MS, *m/e* 556.1940 (M^- calcd 556.1980). See Table II for NMR data.

Acetylation of Roritoxins A and B. Roritoxin B (0.050 g) was dissolved in dichloromethane (2.5 mL). Triethylamine (1.0 mL) was added to this solution dropwise, followed by acetic anhydride (0.90 mL). A small crystal of 4-(dimethylamino)-pyridine (DMAP) was added to the mixture. At the end of 2 h the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 5% HCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on the chromatotron (1-mm silica gel plate, eluting solvent 10–30% ethyl acetate in hexane) to obtain 20 mg of roritoxin B diacetate and 18 mg of roritoxin B monoacetate. In the same manner, roritoxin A (0.010 g) gave 8 mg of roritoxin A diacetate (see Table II for ^1H NMR data).

Acetylation of Roritoxins C and D. A 100-mg portion of the 300-mg crystalline mixture of roritoxins C and D was subjected to acetylation in the same manner as described for roritoxin B. At the end of 1 h the reaction was stopped, and the reaction mixture was purified on the chromatotron (1-mm silica gel plate, 5–15% ethyl acetate in hexane as eluting solvent) to obtain 55 mg of roritoxin D acetate and 25 mg of roritoxin C acetate (see Tables I and II for NMR data).

Conversion of Roritoxin B (5) to Roritoxin D (7). A suspension of silver carbonate–Celite (70 mg) (Alfa) in 30 mL of a toluene solution of roritoxin B (30 mg) was heated under reflux, with stirring, for 2 h. The reaction mixture was filtered and the insoluble material was washed with warm CH_2Cl_2. The filtrate and wash were combined and concentrated in vacuo, and the residue was purified on the chromatotron (1-mm silica gel plate, 0–5% methanol in dichloromethane as eluting solvent). The major product obtained was roritoxin D (7) (22 mg) as shown by proton NMR and IR spectroscopy.

Conversion of Roritoxin D (7) to Roritoxin C (6). To a solution of 10 mg of **6** in 5 mL of chloroform was added 5 mg of *m*-chloroperoxybenzoic acid. After 30 h, the mixture was diluted to 10 mL with dichloromethane, washed once with 10 mL of saturated sodium bicarbonate, dried (Na_2SO_4), and concentrated in vacuo. Preparative TLC on the chromatotron (1-mm silica gel plate, 0–50% ethyl acetate in dichloromethane as eluent) yielded 5 mg of **6**.

Isolation of Isoepiepoformin (3) from *M. roridum* CL-514 (ATCC 20605). Spores of CL-514 grown on Sabouraud-dextrose agar were transferred to 300 mL of a sterile medium which contained 20.0 g of glucose, 2.0 g each of malt extract, yeast extract, and peptone, 1.0 g each of KH_2PO_4 and NH_4Cl, and 0.5 g of $MgSO_4 \cdot 7H_2O$ per 1 L of distilled water. The 2-L Erlenmeyer flask containing 300 mL of inoculated medium was placed on a gyratory shaker (150 rpm) and incubated at 28 °C for 60 h. At the end of this time, the inoculum was transferred into 3 L of another sterile medium (3 × 1 L per 4-L Erlenmeyer flask) which contained 40.0 g of sucrose, 10.0 g of glycerol, 5.0 g of NaCl, 3.0 g of K_2HPO_4, 1.0 g of $NH_4H_2PO_4$, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per 1 L of distilled water. The culture was again incubated at 28 °C on a gyratory shaker (150 rpm). At the end of 4 days, the mycelium was removed by filtration through cheese cloth. The filtrate was extracted with 3 × 3 L of ethyl acetate, and the mycelium was extracted with 3 × 1 L of methanol in a sonicator. The methanol extracts were combined and washed with hexane and concentrated and the aqueous solution was extracted with ethyl acetate (3 × 1 L). The ethyl acetate extracts were all combined and concentrated in vacuo to give 5 g of a red oil. The crude extract obtained was subjected to filtration chromatography (25 g of flash grade silica gel) with dichloromethane (1 L), 5% methanol in dichloromethane (2 L), and methanol (500 mL) as eluents. The fraction eluted with 5% methanol in dichloromethane upon TLC analysis gave a spot which turned deep blue in color when sprayed with NBP reagent.[8] This fraction (2.4 g) which was in the form of a red oil was loaded onto a 4-mm thick silica gel plate in two portions and subjected to purification on the chromatotron. At the start, dichloromethane was used as eluting solvent. This was followed by 1%, 3%, and 5% methanol in dichloromethane and 100% methanol. Upon TLC analysis, the 1–3% methanol in dichloromethane fractions were found to contain the epoformin-type compound. This pale red oily fraction (≃1 g) was further purified on the chromatotron (4-mm silica gel plate, eluting solvent 1–3% methanol in dichloromethane) to afford 750 mg of isoepiepoformin (**3**) as a pale yellow oil: $[\alpha]^{25}_D$ +36.4° (*c* 0.50 chloroform); UV λ_{max} (MeOH) 230 nm (log ϵ = 3.94); CD [θ] +3240 (*c* 0.10, methanol); MS, *m/e* 140.0459 (M^- calcd 140.0472); ^1H NMR δ 2.03 (3 H, d, J = 1.3 Hz, methyl), 3.38 (1 H, ddd, J = 0.7, 3.0, and 3.3 Hz, H-1), 3.75 (1 H, dd, J = 1.3 and 3.3 Hz, H-6), 4.43 (after H–D exchange) (1 H, dd, J = 0.7 and 1.3 Hz, H-5), and 5.75 (1 H, m, H-3); ^{13}C NMR δ 21.4 (Me), 52.2 (C-1), 56.9 (C-6), 66.6 (C-5), 124.1 (C-3), 156.0 (C-4), and 193.3 (C-2).

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β-Methyleneglutamic Acid and β-Methyleneglutamine

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The novel β,γ-unsaturated amino acids β-methyleneglutamic acid (I) and β-methyleneglutamine (II) are readily prepared by addition of the protected aminomalonates III and VIII to the allenes ethyl buta-2,3-dienoate (IV) and cyanoallene (VII) followed by acid hydrolysis. Byproducts of the reaction are 4-amino-3-methylbut-2-enoic acid hydrochloride (VI) and 4-amino-3-methylbut-2-enamide hydrochloride (X).

β,γ-Unsaturated amino acids[1,2] constitute a useful class of enzyme inhibitors. In this paper, we describe highly efficient syntheses of two new candidate inhibitors: β-methylene-D,L-glutamic acid (I) and β-methylene-D,L-

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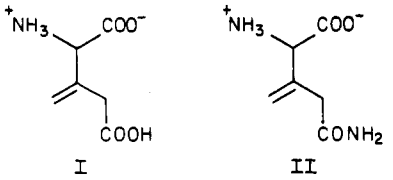
glutamine (II).

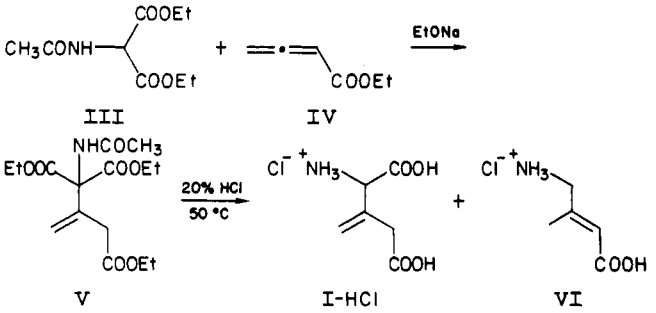


I II

There is substantial incentive to explore potential inhibitors in the glutamate and glutamine series.[3] Glutamic acid is the biological precursor to the important neurotransmitter γ-aminobutyric acid (GABA) which is formed in a pyridoxal phosphate mediated decarboxylation reaction. Glutamate and glutamine play critical roles in the etiology of tumor development.[4] Both amino acids are important as substrates in transamination reactions. The goal of the present research was to develop glutamine and glutamate antagonists.

Results and Discussion

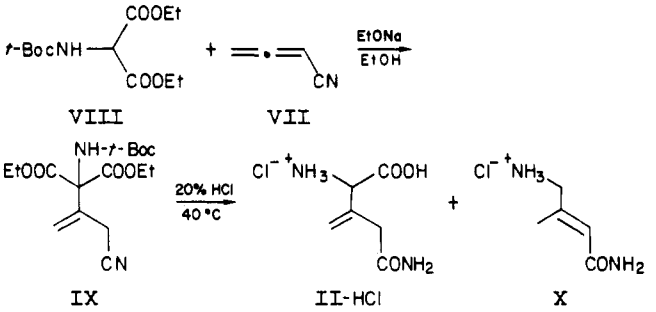
Synthesis of β-methylene-D,L-glutamic acid (I) was carried out in two steps. Diethyl acetamidomalonate (III) was condensed with ethyl allenecarboxylate[5] (IV) in the



III IV V I-HCl VI

presence of a catalytic amount of sodium ethoxide in ethanol. The adduct V was obtained in 94% yield, and the integrity of the unconjugated double bond was completely maintained. The adduct V was heated at 50 °C with 20% hydrochloric acid, resulting in sequential ester hydrolysis and decarboxylation to the desired β-methyleneglutamic acid hydrochloride (I-HCl). In the course of the reaction, further decarboxylation of I-HCl occurred, leading to the amino acid VI. The desired product I-HCl was separated from this and other impurities by ion exchange chromatography and isolated in 67% yield. The structure of I-HCl was established by spectroscopic means. Neutralization of I-HCl then yielded the desired β-methylene-D,L-glutamic acid (I). The amount of the byproduct VI can be controlled by closely monitoring the hydrolysis reaction. It was demonstrated that heating I yielded VI by decarboxylation. The amino acid VI was also cleanly separated in the course of the ion exchange chromatography and was isolated in 13% yield.

Condensation of the cyanoallene (VII) with diethyl *N*-*t*-Boc-aminomalonate (VIII) yielded (89%) the adduct IX. Acid hydrolysis of IX then led to the desired β-



VIII VII IX II-HCl X

methylene-D,L-glutamine hydrochloride (II-HCl) in 23% yield. The decarboxylation product X was a byproduct of this reaction. The hydrochlorides II-HCl and X were separated by repeated trituration with 50:1 acetone–EtOH. The most important part of the structural assignment required establishing whether the product II-HCl was the nitrile or the amide. The ^{13}C NMR spectrum provided clear evidence demonstrating that the product must be the amide II-HCl. The proton-decoupled ^{13}C NMR spectrum (D_2O) showed six lines at 35.2, 64.7, 113.0, 134.9, 170.6, and 172.4 ppm. Had the nitrile group survived the acidic hydrolysis conditions, the carbonyl region would not show two peaks, instead a nitrile band at ca. 120 ppm[6] would have been observed. The proton-coupled ^{13}C NMR spectrum confirmed the structural assignment showing a carbonyl singlet at δ 172.4, a carbonyl singlet at 170.6, a quaternary vinyl singlet at 134.9, a vinyl triplet at 113.0 (J = 163.3 Hz), a methine doublet at 64.7 (J = 161.1 Hz), and a methine triplet at 35.2 (J = 139.2 Hz).

Experimental Section

Ethyl Buta-2,3-dienoate (IV).[5] In a 500-mL three-necked, round-bottomed flask, 17.4 g (0.05 mol) of ethyl (triphenylphosphoranylidene)acetate and 150 mL of dichloromethane were combined and flushed with nitrogen. The solution was stirred at room temperature as 5.05 g (0.05 mol) of triethylamine in 50 mL of dichloromethane was added dropwise over a period of 5 min. After 10 min, 3.93 g (0.05 mol) of acetyl chloride in 50 mL of dichloromethane was added dropwise to the vigorously stirred solution over a period of 15 min. Stirring was continued for an additional 0.5 h after which the clear yellow mixture was distilled bulb-to-bulb under vacuum (10 mtorr) at 25 °C.

The solution collected in a liquid nitrogen trap was redistilled at 50 °C to remove dichloromethane and that residue was distilled under vacuum, yielding 3.166 g (57%) of colorless allene IV, bp 80 °C/60 torr. The 300-MHz proton NMR spectrum ($CDCl_3$) showed a one-proton triplet (J = 6.5 Hz) at δ 5.64, a two-proton doublet (J = 6.5 Hz) at 5.23, a two-proton quartet (J = 7.1 Hz) at 4.21, and a three-proton triplet (J = 7.1 Hz) at 1.29. The IR spectrum (neat) showed bands at 2950 (w, C–H), 1960 (m, C=C=C), and 1700 cm^{-1} (s, CO).

Diethyl 2-Acetamido-2-carbethoxy-3-methylenepentane-1,5-dioate (V). In a 50 mL, three-necked, round-bottomed flask, 6 mg (0.26 mmol) of sodium metal was reacted with 12 mL of dry ethanol under a nitrogen atmosphere. To this solution was added 5.25 g (0.024 mol) of diethyl acetamidomalonate (III), and the mixture was cooled to 0 °C. A solution of 3.12 g (0.027 mol) of ethyl buta-2,3-dienoate[4] (IV) in 1 mL of dry ethanol was added

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dropwise by using a syringe. After 1 h the reaction mixture became a homogeneous yellow solution. At this point, 16 mg (0.26 mmol) of acetic acid and 100 mL of H_2O were added and the mixture was extracted with five 100-mL portions of ether. The organic extract was dried with $MgSO_4$. Evaporation of the solvent gave 11.12 g of colorless oil. Chromatography on 500 g of silica gel, eluting with 1:1 EtOAc–hexanes, yielded 7.473 g (94%) of white solid V, mp 56–57 °C.

The 300-MHz proton NMR spectrum ($CDCl_3$) showed a one-proton singlet at δ 7.00, two one-proton vinyl singlets at δ 5.50 and 5.39, a four-proton multiplet at 4.25, a two-proton quartet (J = 7.1 Hz) at 4.12, a two-proton singlet at 3.44, a three-proton singlet at 2.08, and a nine-proton multiplet at 1.25. The proton-decoupled ^{13}C NMR spectrum ($CDCl_3$) showed 12 lines at 171.7, 169.2, 166.6, 136.1, 120.6, 69.4, 62.7, 60.7, 39.1, 22.7, 14.1, and 13.8 ppm. The coupled ^{13}C NMR spectrum showed three carbonyl singlets at δ 171.7, 169.2, 166.6, a vinyl quaternary singlet at 136.1, a vinyl triplet at 120.6 (J = 161.1 Hz), a quaternary carbon singlet at 69.4, a methylene triplet at 62.7 (J = 148.9 Hz), a methylene triplet at 60.7 (J = 151.3 Hz), a methylene triplet at 39.1 (J = 131.8 Hz), a methyl quartet at 22.7 (J = 126.9 Hz), a methyl quartet at 14.1 (J = 126.9 Hz), and a methyl quartet at 13.8 (J = 129.4 Hz). The IR spectrum ($CHCl_3$) showed bands at 3400 (w, NH), 2940 (w, CH), 1715 (s, CO), 1650 (m, C=C), 1460 (m, C=C), and 1190 cm^{-1} (s). The mass spectrum (15 eV) showed peaks at m/z (relative intensity) 329 (1, M^+), 284 (5, M – OEt), 270 (4), 257 (21), 256 (100, $M^+ - CO_2Et$), 242 (14), 214 (40), 196 (20), 168 (10), and 113 (5); exact mass calcd for $C_{13}H_{18}NO_6$ (M – OEt)$^+$ 284.1134, found 284.1141.

Anal. Calcd for $C_{15}H_{23}NO_7$: C, 54.70; H, 7.04; N, 4.25. Found: C, 54.77; H, 7.17; N, 4.11.

β-Methylene-D,L-glutamic Acid Hydrochloride (I-HCl) and 4-Amino-3-methylbut-2-enoic Acid Hydrochloride (VI). In a 250-mL, round-bottomed flask, 3.970 g (0.012 mol) of adduct V was placed in 50 mL of 20% HCl, and the mixture was stirred in a 50 °C oil bath under a nitrogen-filled balloon. After 48 h, the evaporation of solvent gave 2.101 g (89%) of slightly yellow foam. The NMR spectrum showed that the solid was a 4:1 mixture of β-methylene-D,L-glutamic acid hydrochloride (I-HCl) and 4-amino-3-methylbut-2-enoic acid hydrochloride (VI) and some small impurities. The crude product was chromatographed on 70 mL of Dowex-50X8-400 resin. The column was eluted with 900 mL of water followed by 300 mL of 2% HCl; 75-mL fractions were taken. In fractions 6–9, 0.235 g (13%) of VI was isolated as a white solid (mp 110–111 °C).

The 300-MHz proton NMR spectrum (D_2O) of VI showed a one-proton singlet at δ 5.85, a two-proton singlet at 4.03, and a three-proton singlet at 2.10. The proton-decoupled ^{13}C NMR spectrum (D_2O) showed five lines at 14.4, 52.2, 120.2, 163.2, and 177.7 ppm. The coupled ^{13}C NMR spectrum (D_2O) showed a methyl quartet at δ 14.4 (J = 128.3 Hz), a methylene triplet at 52.2 (J = 145.8 Hz), a vinyl methine doublet at 120.2 (J = 175.5 Hz), a quaternary vinyl singlet at 163.2, and a carbonyl singlet at 177.7. The IR spectrum (KBr) showed bands at 3400–2600 (br, OH, NH), 1650 (s, C=O), and 1210 cm^{-1} (w).

In fraction 14, 1.580 g (67%) of white amorphous amino acid hydrochloride (I-HCl) was obtained. The 300-MHz proton NMR spectrum (D_2O) of I-HCl showed a two-proton singlet at δ 5.57, a one-proton singlet at 4.77, and a two-proton AB quartet at 3.42 and 3.41 (J = 17.2). The proton-decoupled ^{13}C NMR spectrum (D_2O) showed six lines at 39.3, 56.7, 123.2, 133.2, 169.9, and 174.7 ppm. The coupled ^{13}C NMR spectrum (D_2O) showed a methylene triplet at δ 39.3 (J = 125.7 Hz), a methine doublet at 56.7 (J = 148.9 Hz), a vinyl methylene triplet at 123.2 (J = 161.1 Hz), a quaternary vinyl singlet at 133.3, and two carbonyl singlets at 169.9 and 174.7. The IR spectrum (KBr) showed bands at 3600–2700 (br, OH, NH), 1600 (S, C=O), 1320, and 1160 cm^{-1}. The negative laser desorption (LD) mass spectrum showed m/z (relative intensity) 159 (8, M^- – HCl), 158 (32), 127 (23), 112 (82), 37, 35 (68 and 100, Cl^-). The positive LD mass spectrum showed m/z (relative intensity) 160 (5, M^+ – Cl), 145 (18), 56 (75), and 39 (100).

The β-methylene-D,L-glutamic acid hydrochloride (I-HCl) does not have a sharp melting point: it decomposes at 125 °C.

β-Methylene-D,L-glutamic Acid (I). In a 250-mL, round-bottomed flask, 7.88 g (0.024 mol) of diethyl 2-acetamido-2-carbethoxy-3-methylenepenta-1,5-dioate (V) was placed in 100 mL of 20% HCl, and the mixture was stirred at 50 °C under nitrogen. After 24 h, the evaporation of solvent gave 4.079 g of slightly yellow foam. The product was dissolved in 3 mL of deionized water and treated with small aliquots of 50% sodium hydroxide at 0 °C until the pH of the solution was 3.2. At this point 2.438 g (64%) of a white solid precipitated from the solution. Recrystallization from 30 mL of hot water yielded 1.127 g (30%) of white crystalline amino acid I, mp 187–193 °C. The 300-MHz proton NMR spectrum (D_2O, DCl) was identical with that of β-methylene-D,L-glutamic acid hydrochloride (I-HCl). The IR spectrum (KBr) showed bands at 3350 (w), 3200–2700 (br, s), 1600 (s, C=O), 1400 (s), 1280 (m) and 1170 cm^{-1} (s). The negative LD mass spectrum showed m/z (relative intensity) 158 (34, M^- – H), 115 (100, M^- – CO_2), 97 (53), 80 (35), and 26 (35). The positive LD mass spectrum showed m/z (relative intensity) 160 (18, M^+ H), 142 (10, $M^+ - NH_3$), 114 (20), 97 (35), 71 (40), 69 (40), 39 (100), and 23 (99).

Diethyl *N*-*tert*-Butoxycarbonylaminomalonate (VIII). In a 500-mL, round-bottomed flask fitted with a reflux condenser, 21.1 g (0.1 mol) of diethyl aminomalonate was suspended in 200 mL of chloroform. A solution of 8.4 g (0.1 mol) of $NaHCO_3$ in 150 mL of H_2O, 20 g (0.34 mol) of NaCl, and 24.00 g (0.11 mol) of di-*tert*-butyl dicarbonate was added and the mixture was refluxed under a nitrogen-filled balloon overnight. After 12 h, the reaction was cooled and the layers were separated. The aqueous layer was extracted with three 200-mL portions of $CHCl_3$. The $CHCl_3$ solutions were combined and dried with anhydrous $MgSO_4$. Evaporation of the solvent gave 36.8 g of slightly yellow liquid. The crude product was placed on 300 g of silica gel and eluted with 1:9 EtOAc–hexanes, yielding 26.8 g (98%) of colorless oil.

The 300-MHz proton NMR spectrum ($CDCl_3$) showed a broad one-proton doublet at δ 5.57 (J = 7.97 Hz), a one-proton doublet at 4.95 (J = 7.97 H), a four-proton multiplet at 4.2–4.3, a nine-proton singlet at 1.45, and a six-proton triplet at 1.30 (J = 7.15 Hz). The IR spectrum ($CHCl_3$) showed bands at 3400 (w, NH), 2975 (m, CH), 1725 (s, CO), 1480 (m), 1360 (m), and 1255 cm^{-1} (s). The mass spectrum (15 eV) showed peaks at m/z (relative intensity) 275 (0.4, M^+), 220 (10), 202 (23), 176 (8), 175 (5), 174 (11), 158 (7), 147 (18), 129 (7), and 102 (100).

Ethyl 2-(*N*-*tert*-Butoxycarbonylamino)-2-carbethoxy-3-methylene-4-cyanobutanoate (IX). In a 25-mL, three-necked, round-bottomed flask, 0.5 mg (0.02 mmol) of sodium metal was reacted with 1 mL of dry ethanol. To this solution was added 0.550 g (2 mmol) of diethyl *N*-*tert*-butoxycarbonylaminomalonate (VIII), and the mixture was cooled to 0 °C. A solution of 0.150 g (2.3 mmol) of cyanoallene (VII) in 0.5 mL of dry ethanol was added dropwise using a syringe. The reaction was stirred under a nitrogen-filled balloon in an ice bath. After 1 h, 1.2 mg (0.02 mmol) of acetic acid and 50 mL of H_2O were added, and the mixture was extracted with three 50-mL portions of ether. The combined organic extracts were dried with $MgSO_4$. Evaporation of solvent gave 0.602 g (89%) of white solid (mp 44.5–45.5 °C). The 300-MHz proton NMR spectrum ($CDCl_3$) showed a one-proton singlet at δ 5.54, a four-proton multiplet at 4.33–4.22, a two-proton singlet at 3.63, a nine-proton singlet at 1.44, and a six-proton triplet at 1.29 (J = 7.3 Hz). The proton-decoupled ^{13}C NMR spectrum (D_2O) showed 11 lines at 166.3, 154.0, 135.2, 119.5, 117.7, 81.1, 68.9, 63.1, 28.2, 21.9, and 13.9 ppm. The coupled ^{13}C NMR spectrum ($CDCl_3$) showed carbonyl singlets at δ 166.3 and 154.0, a quaternary vinyl singlet at 135.2, a vinyl methylene triplet at 119.5 (J = 161.1 Hz), a nitrile singlet at 117.7, a quaternary carbon singlet at 81.1, a quaternary carbon singlet at 68.9, a methylene triplet at 63.1 (J = 148.9 Hz), a methyl quartet at 28.2 (J = 126.9 Hz), a methylene triplet at 21.9 (J 141.6 Hz), and a methyl quartet at 13.9 (J = 126.9 Hz). The IR spectrum ($CHCl_3$) showed bands at 3400 (w, NH), 2960 (m, CH), 1730 (s, CO), 1720 (s, CO), 1450 (s, C=C), 1360 (m), and 1260 cm^{-1} (s). The mass spectrum (15 eV) showed peaks at m/z (relative intensity) 340 (0.5, M^+), 284 (25, $M^+ - C_4H_8$), 267 (6, $M^+ - CO_2Et$), 239 (6), 212 (12), 211 (100, $M^+ - C_4H_8 - CO_2Et$), 167 (56), 102 (22), and 57 (32); exact mass calcd for $C_{12}H_{16}N_2O_6$ 284.1008, found 284.1008.

Anal. Calcd for $C_{16}H_{24}N_2O_6$: C, 56.46; H, 7.11; N, 8.23. Found: C, 56.54; H, 7.27; N, 8.29.

β-Methylene-D,L-glutamine Hydrochloride (II-HCl) and 4-Amino-3-methylbut-2-enamide Hydrochloride (X). In a 1-L, round-bottomed flask, 6.0 g (0.0176 mol) of ethyl 2-*N*-*t*-Boc-

amino-2-carbethoxy-3-methylene-4-cyanobutanoate was placed in 600 mL of 20% HCl, and the mixture was stirred in a 40 °C oil bath under a nitrogen-filled balloon. After 22 h, the solvent was removed under vacuum, yielding 3.208 g (94%) of yellow foam. Trituration with 150 mL of 50:1 acetone–EtOH solution gave 2.030 g (60%) of brown solid. The NMR showed that the solid was a 1.6:1 mixture of β-methylene-D,L-glutamine hydrochloride and 4-amino-3-methylbut-2-enamide hydrochloride. When the mixture was triturated with five 150-mL portions of 50:1 acetone–EtOH, 0.790 g (23%) of pure β-methylene-D,L-glutamine hydrochloride (II-HCl) was obtained as a white solid, mp 123–131 °C dec.

The 300-MHz proton NMR spectrum (D_2O) showed a one-proton doublet at δ 5.58 (J = 2.2 Hz), a one-proton singlet at 5.17, and a two-proton singlet at 3.75. The proton-decoupled ^{13}C NMR spectrum (D_2O) showed six lines at 35.2, 64.7, 113.0, 134.9, 170.6, and 172.4 ppm. The coupled ^{13}C NMR spectrum (D_2O) showed a carbonyl singlet at δ 172.4, a carbonyl singlet at 170.6, a quaternary vinyl singlet at 134.9, a vinyl triplet at 113.0 (J = 163.6 Hz), a methine doublet at 64.7 (J = 161.1 Hz), and a methylene triplet at 35.2 (J = 139.2 Hz). The IR spectrum (KBr) showed bands at 3340 (m, NH), 3200–2900 (s, OH), 1680 (s, CO), 1430 (m), and 1390 cm^{-1} (m). The positive LD mass spectrum showed m/z (relative intensity) 194 (27, M^+), 141 (19), 97 (84, $M^+ - HCl - NH_2 - CO_2H$), 80 (35), 38 (55), and 23 (100). The negative LD mass spectrum showed m/z (relative intensity) 174 (40), 172 (85), 170 (100), 168 (61), 163 (28), 161 (38), 159 (37, $M^- - Cl$), 35 (36), and 26 (69).

The combined acetone–EtOH solution was evaporated and triturated once again with 150 mL of 50:1 acetone–EtOH, yielding 0.230 g (7%) of pure 4-amino-3-methylbut-2-enamide hydrochloride as a brown solid. The 300-MHz proton NMR spectrum (D_2O) showed a one-proton singlet at δ 6.21, a two-proton singlet at 4.37, and a three-proton singlet at 2.18. The proton-decoupled ^{13}C NMR spectrum (D_2O) showed a carbonyl singlet at δ 167.8, a quaternary vinyl singlet at 166.3, a vinyl doublet at 116.7 (J = 178.2 Hz), a methylene triplet at 57.2 (J = 144.0 Hz), and a methyl quartet at 14.0 (J = 126.9 Hz). The IR spectrum (KBr) showed bands at 3200–3000 (m, NH, OH), 2310 (w), and 1655 cm^{-1} (s, C=O). The amide does not have a sharp melting point, it begins to decompose at 185 °C, becoming progressively darker at higher temperatures.

β-Methylene-D,L-glutamine (II). In a 10-mL, round-bottomed flask, 0.400 g (2.06 mmol) of β-methylene-D,L-glutamine hydrochloride (II-HCl) was dissolved in 1 mL of deionized water, and the solution was stirred at 0 °C in an ice bath. An excess of propylene oxide (0.360 g, 6.18 mmol) was added, and the mixture was stirred at 0 °C under a nitrogen-filled balloon. After 1 h, the pH of the mixture became 2.77 and a white solid had precipitated. An additional 0.360 g (6.18 mmol) of propylene oxide was added. After 3.5 h, the pH of the mixture became 5.93. At this point the white precipitate was centrifuged and washed three times each with 1 mL of cold EtOH yielding 0.216 g (66%) of white solid, mp 130–144 °C dec.

The IR spectrum (KBr) showed bands at 3300–2800 cm^{-1} (br s, NH, OH), 1720–1580 (br s, C=O), and 1350 cm^{-1} (br s). The NMR spectrum were taken as the hydrochloride and were identical with those of (II-HCl) described above.

Decarboxylation of β-Methylene-D,L-glutamic Acid (I). In a 50-mL, round-bottomed flask 0.030 g (0.194 mmol) of β-methylene-D,L-glutamic acid (I) was placed in 5 mL of 20% HCl. The mixture was stirred under a nitrogen-filled balloon in a 50 °C oil bath. After 2 days, evaporation of the solvent under vacuum gave 0.029 g of white amorphorous solid. The 300-MHz proton NMR spectrum (D_2O) showed a one-proton singlet at δ 5.85, a two-proton singlet at 4.03, a three-proton singlet at 2.10 together with starting material. The peaks resulting from decarboxylation are identical with those of the amino acid VI.

Decarboxylation of β-Methylene-D,L-glutamine Hydrochloride (II-HCl). In a 50-mL, round-bottomed flask 0.010 g (0.05 mmol) of β-methylene-D,L-glutamine hydrochloride was placed in 5 mL of deionized water, and the mixture was stirred under nitrogen in a 50 °C oil bath. After 2 days, evaporation of the solvent under vacuum gave 0.009 g of white amorphorous solid. The 300-MHz proton NMR spectrum (D_2O) showed a one-proton singlet at δ 6.21, a two-proton singlet at 4.37, and a three-proton singlet at 2.18, together with starting material. The new peaks in the spectrum correspond to the decarboxylated product (X).

Registry No. I, 97402-98-7; I-HCl, 102831-40-3; II, 97402-99-8; II-HCl, 102831-41-4; III, 1068-90-2; IV, 14369-81-4; V, 102831-42-5; VI, 102831-43-6; VII, 1001-56-5; VIII, 102831-44-7; IX, 102831-45-8; X, 102831-39-0; ethyl (triphenylphosphoranylidene)acetate, 1099-45-2; acetyl chloride, 75-36-5; diethyl aminomalonate, 6829-40-9; di-*tert*-butyl dicarbonate, 24424-99-5.

Studies in Biomimetic Alkaloid Syntheses. 14. Controlled, Selective Syntheses of Catharanthine and Tabersonine, and Related Desethyl Compounds, through Generation of 15-Oxosecodine Intermediates

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The syntheses of isolated 15-oxo-$\Delta^{20(21)}$-secodine (**13**) and desethyl-15-oxo-$\Delta^{20(21)}$-secodine (**32**) from methyl 1,2,3,4,5,6-hexahydroazepino[4,5-*b*]indole-5-carboxylate (**14**) by spiroquaternization, or alternatively by a bridged azepine pathway, are described. Thermolyses of these compounds gave 15-oxovincadifformine (**18**) and desethyl-15-oxovincadifformine (**41**). Subsequent transformations led to tabersonine (**2**) and desethylvincadifformine (**43**), respectively. On O-silylation of the 15-oxosecodines **13** and **32** 15-(silyloxy)catharanthine (**23**) and the corresponding desethyl compound (**44**) were formed. Transformation to 15-oxo- and 15-β-hydroxycoronanidines (**25**, **27**) and their desethyl analogues, **47** and **48**, and to catharanthine (**1**) and desethylcatharanthine (**50**) are discussed.

Without rival in modern alkaloid chemistry has been the sustained interest in solutions to the syntheses of the catharanthine (**1**) and the tabersonine (**2**) classes of alkaloids. The stimulus for these endeavors derives from more than an intrinsic challenge inherent in the demands of stereochemically controlled assembly of polycyclic molecules, that is piqued in the catharanthine–ibogamine class by the unique isoquinuclidine skeleton. Further attention to the problem comes from biogenetic considerations of a common origin of the structurally very dissimilar molecular frameworks of catharanthine (**1**) and tabersonine (**2**), which was proposed, with differing organic chemical rationales, to explain the formation of these alkaloids.[1-4]

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(2) Wenkert, E.; Bringi, N. V. *J. Am. Chem. Soc.* **1959**, *81*, 1474, 6535.

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