CHAPTER FOUR

EXPERIMENTAL

4.1 General

Melting points were determined on a Leitz Wetzler melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrophotometer. Optical rotations were measured on a Jasco P-1020 digital polarimeter. MS measurements were carried out at OIC Organic Mass Spectrometry, University of Tasmania, Tasmania, Australia. ESIMS were obtained on a Perkin Elmer API 100 instrument using ion spray method (direct sample injection) and with methanol as solvent. All air/moisture-sensitive reactions were carried out under N_2 in oven-dried glassware. THF was freshly distilled from Na/benzophenone under nitrogen while MeCN, CH_2Cl_2 and pyridine were distilled from CaH_2 under nitrogen. All other reagents were used without further purification.

4.1.1 NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA400 at 400 and 100 MHz, respectively. The deuterium signals from chloroform-d were used for the field frequency lock. All experiments were performed at ca 21 ^{o}C unless otherwise stated. The chemical shifts are expressed in δ (ppm) downfield from TMS and all *J* values are given in Hz. The multiplicity of each signal is denoted as follows: s - singlet, d - doublet, t - triplet, q - quartet, m - multiplet, br - broad.

4.1.2 Electrochemical Experiments

All electrochemical experiments were performed on a BAS 100B electrochemical analysis system using a 100 mL cylindrical glass cell (BAS MR-1195) fitted with a Teflon cell top. The electrodes used for cyclic voltammetry were Pt wire electrode (1.6 mm diameter), with Pt as the counter-electrode and Ag/AgCl/NaCl (3 M) as the reference electrode. Preparative electrolyses were performed with a Pt gauze electrode (diameter 4 cm, height 5 cm). The progress of electrolysis was monitored by TLC as well as cyclic voltammetry.

4.1.3 Chromatographic Methods

Thin layer chromatography was carried out using precoated 5 x 10 cm aluminium plates, 0.25 mm thickness, silica gel 60 F_{254} (Merck 5554). Column chromatography was performed using silica gel (Merck 9385, 230-400 Mesh ASTM). The ratio of silica gel to sample was approximately 30:1 for crude samples, and 100:1 for semi-pure fractions. The gel was made into a slurry with chloroform before it was packed onto a column and allowed to equilibrate for at least an hour before use. Preparative centrifugal TLC was carried out using a chromatotron (Harrison Research) with 1 mm thick plates 24 cm diameter of silica gel PF 254 (Merck 7749). The plate was prepared as follows. A long piece of cellophane tape was secured around the edge of the plate to form a mould. Silica gel (50 g) was added to about 100 mL of cold water and the slurry formed was poured onto the circular glass plate. The circular glass plate was rotated while the gel was being poured to obtain an even setting. The plate was then left to air dry for about an hour before being dried in an oven at 80 ^{o}C for about 12 hours. The sample was dissolved in a minimum volume of suitable solvent and loaded at the center of the plate

while the plate was rotating to form a thin band. Elution was then carried out with the appropriate solvent system.

Some of the solvent systems used were:

1. Diethyl ether
2. Diethyl ether: Hexane
3. Diethyl ether: Methanol
4. Dichloromethane
5. Dichloromethane: Hexane
6. Dichloromethane: Methanol
7. Chloroform
8. Chloroform: Hexane
9. Chloroform: Methanol
10. Ethyl acetate: Hexane

4.1.4 Dragendorff's Reagent

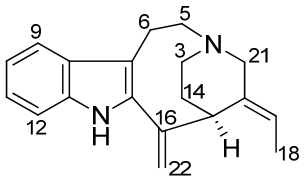
Solution A: 0.85 g of bismuth nitrate was dissolved in a mixture of 10 mL glacial acetic acid and 40 mL of distilled water.

Solution B: 8 g of potassium iodide was dissolved into 20 mL of distilled water.

A stock solution was prepared by mixing equal volumes of solutions A and B. Dragendorff's reagent was prepared by mixing 1 mL of stock solution with 2 mL of glacial acetic acid and 10 mL of distilled water. Orange spots on the developed TLC plates indicated the presence of alkaloids.

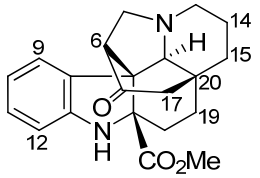
4.1.5 Starting Materials for Partial Syntheses

Pericine (**31**)[26] and methyl *N*(1)-decarbomethoxychanofruticosinate (**25**)[50] were obtained from the stem-bark and leaf extract of *K. arborea*, respectively.



31 Pericine

Pericine (31): light yellowish oil, $[\alpha]_D$ +45 (*c* 0.20, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 226 (4.05), 301 (3.85) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (m, 1H) (H-14), 1.69 (dt, *J* = 7 and 1 Hz, 3H) (H-18), 2.10 (dddd, *J* = 15, 11, 8 and 7 Hz, 1H) (H-14), 2.78 (m, 1H) (H-6), 2.81 (m, 1H) (H-3), 3.12 (m, 1H) (H-5), 3.13 (m, 1H) (H-3), 3.26 (m, 1H) (H-5), 3.26 (m, 1H) (H-21), 3.50 (ddd, *J* = 15, 11 and 3 Hz, 1H) (H-6), 3.88 (d, *J* = 16.3, 1H) (H-21), 3.98 (br d, *J* = 6.4 Hz, 1H) (H-15), 5.35 (d, *J* = 1.5 Hz, 1H) (H-22), 5.36 (d, *J* = 1.5 Hz, 1H) (H-22), 5.62 (q, *J* = 7 Hz, 1H) (H-19), 7.10 (ddd, *J* = 8, 7 and 1 Hz, 1H) (H-10), 7.18 (ddd, *J* = 8, 7 and 1 Hz, 1H) (H-11), 7.31 (m, 1H) (H-12), 7.49 (d, *J* = 8 Hz, 1H) (H-9), 8.02 (br s, 1H) (NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.4 (C-18), 24.3 (C-6), 27.7 (C-14), 42.8 (C-15), 45.3 (C-3), 53.8 (C-21), 57.8 (C-5), 110.4 (C-12), 117.6 (C-17), 118.5 (C-9), 119.3 (C-10), 120.8 (C-19), 122.2 (C-11), 128.5 (C-8), 135.2 (C-2), 135.3, (C-13), 138.8 (C-20), 145.4 (C-16); ESIMS *m/z* 279 (MH^+, $C_{19}H_{22}N_2$ + H).

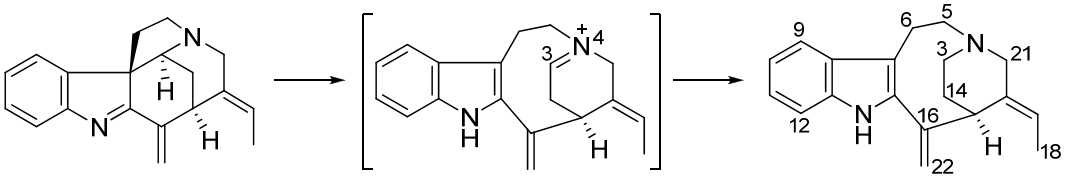


25 Methyl *N*(1)-decarbomethoxy-chanofruticosinate

Methyl *N*(1)-decarbomethoxychanofruticosinate (25): yellowish crystals (MeOH), mp 201–203 ^{o}C; $[\alpha]_D$ +266 (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 202 (4.02), 226 (3.60), 278 (2.94) nm; IR (dry film) ν_{max} 3370, 2930, 2850, 1710, 1610, 1480, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (m, 1H) (H-14α), 1.28 (m, 1H) (H-15α), 1.28 (m, 1H) (H-19α), 1.54 (br d, *J* = 13 Hz, 1H) (H-19β), 1.70 (m, 1H) (H-18α), 1.85 (m, 1H) (H-14β), 1.85 (m, 1H) (H-15β), 1.90 (m, 1H) (H-18β), 2.04 (d, *J* = 19 Hz, 1H) (H-17α), 2.50 (s, 1H) (H-21), 2.84 (d, *J* = 19 Hz, 1H) (H-17), 2.95 (m, 2H) (H-3), 2.95 (m, 1H) (H-5), 3.31 (d, *J* = 6 Hz, 1H) (H-6), 3.61 (s, 3H) (CO_2*Me*), 3.76 (dd, *J* = 11 and 6 Hz, 1H) (H-5α), 4.48 (br s, 1H) (NH), 6.76 (d, *J* = 7 Hz, 1H) (H-12), 6.79 (t, *J* = 7 Hz, 1H) (H-10), 7.09 (t, *J* = 7 Hz, 1H) (H-11), 7.11 (d, *J* = 7 Hz, 1H) (H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.4 (C-14), 27.4 (C-18), 34.6 (C-19), 34.9 (C-15), 36.1 (C-20), 42.5 (C-17), 46.5 (C-3), 52.2 (CO_2*Me*), 52.6 (C-5), 55.1 (C-6), 57.6 (C-7), 63.8 (C-21), 73.8 (C-2), 110.1 (C-12), 119.8 (C-10), 123.9 (C-9), 128.0 (C-11), 133.1 (C-8), 147.7 (C-13), 175.0 (*C*O_2Me), 209.3 (C-16); EIMS *m/z* 352 [M^+, $C_{21}H_{24}N_2O_3$] (42), 335 (10), 294 (77), 293 (100), 291 (8), 271 (3), 249 (3), 222 (10), 206 (6), 180 (11), 156 (8).

4.2 Biomimetic Partial Synthesis of Valparicine (28) and Apparicine (29)

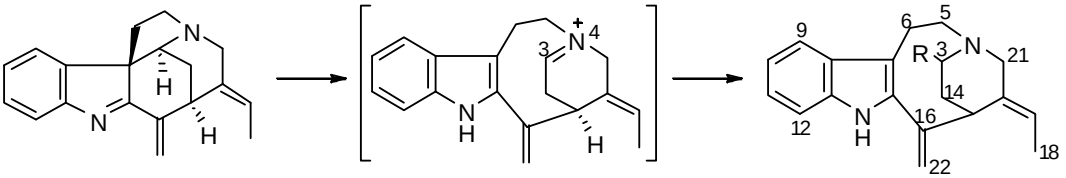
4.2.1 Trapping of the Valparicine C(3)-N(4) Iminium Ion (41) with $NaBH_4$



28 Valparicine **41** C(3)-N(4) iminium ion **31** Pericine

To a solution of valparicine (**28**) (10 mg, 0.018 mmol) in MeOH (5 mL) was added $NaBH_4$ (1.5 mg, 0.04 mmol) and the mixture was stirred for 3 h at rt. The excess solvent was removed in vacuo. Saturated NH_4Cl solution (10 ml) was then added and the mixture extracted with CH_2Cl_2 (3 x 20 mL). The combined CH_2Cl_2 extracts were then washed with water, dried (Na_2SO_4), the solvent evaporated, and the residue purified by preparative centrifugal TLC (silica gel, diethyl ether/MeOH 20:1) to afford 4.5 mg (90 %) of pericine (**31**).

4.2.2 Trapping of the Valparicine C(3)-N(4) Iminium Ion (41) with Acetone

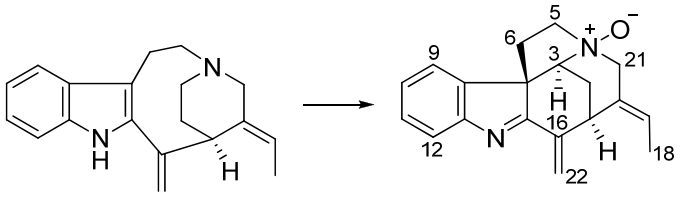


R = CH_2COCH_3

28 Valparicine **41** C(3)-N(4) iminium ion **42** 3-Acetonylpericine

To a mixture of valparicine (**28**) (5 mg, 0.018 mmol), silica gel (0.1 g) and acetone (5 mL) was added a trace amount of concentrated NH_3, and the mixture was stirred for 2 h at rt. The mixture was filtered through a patch of silica gel, the solvent evaporated in vacuo, and the residue purified by preparative centrifugal TLC (silica gel, diethyl ether) to afford 5.1 mg (88%) of 3-acetonylpericine (**42**). **3-Acetonylpericine (42):** colorless prisms (acetone); mp 193–194 °C; $[\alpha]_D$ +51 (*c* 0.20, $CHCl_3$); IR (dry film) ν_{max} 3399 (NH), 1700 (C=O, ester) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.68 (br d, *J* = 6.5 Hz, 3H) (H-18), 1.68 (m, 1H) (H-14), 1.79 (ddd, *J* = 14.6, 6.5 and 1.5 Hz, 1H) (H-14), 1.93 (s, 3H) ($CH_2CO\mathit{CH_3}$), 2.10 (dd, *J* = 14.9 and 6.5 Hz, 1H) ($\mathit{CH_2}COCH_3$), 2.35 (dd, *J* = 14.9 and 6.5 Hz, 1H) ($\mathit{CH_2}COCH_3$), 2.68 (ddd, *J* = 14.7, 3.7 and 2.0 Hz, 1H) (H-6), 3.84 (d, *J* = 16.2 Hz, 1H) (H-21), 3.95 (br d, *J* = 4.5 Hz, 1H) (H-15), 5.33 (d, *J* = 1.2 Hz, 1H) (H-22), 5.35 (d, *J* = 1.2 Hz, 1H) (H-22), 5.65 (qt, *J* = 6.5 and 2.0 Hz, 1H) (H-19), 7.08 (ddd, *J* = 7.9, 7.1 and 1.1 Hz, 1H) (H-10), 7.16 (ddd, *J* = 8.0, 7.1 and 1.1 Hz, 1H) (H-11), 7.29 (d, *J* = 8.0 Hz, 1H) (H-12), 7.47 (d, *J* = 7.9 Hz, 1H) (H-9); 8.19 (br s, 1H) (NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.5 (C-18), 24.1 (C-6), 30.8 ($CH_2CO\mathit{CH_3}$), 33.5 (C-14), 43.6 (C-15), 51.5 (C-3), 51.9 ($\mathit{CH_2}COCH_3$), 52.5 (C-21), 110.4 (C-12), 112.5 (C-7), 118.0 (C-17), 118.4 (C-9), 119.2 (C-10), 120.5 (C-19), 122.2 (C-11), 128.5 (C-8), 134.9 (C-2), 135.3 (C-13), 138.5 (C-20), 144.7 (C-16), 208.6 ($CH_2\mathit{C}OCH_3$); EIMS *m/z* 334 $[M]^+$ (23), 319 $[M - CH_3]^+$ (10), 305 (4), 291 $[M - COCH_3]^+$ (22), 277 $[M - CH_2COCH_3]^+$ (95), 276 (100), 261 (38), 246 (36), 232 (52), 218 (18), 206 (15), 194 (34), 180 (10), 170 (40), 144 (9), 121 (11), 106 (5), 93 (12), 58 (15), 43 (25). HREIMS *m/z* 334.2035 (calcd for $C_{22}H_{26}N_2O$, 334.2045).

4.2.3 Oxidation of Pericine (31) to Pericine *N*-oxide (43)

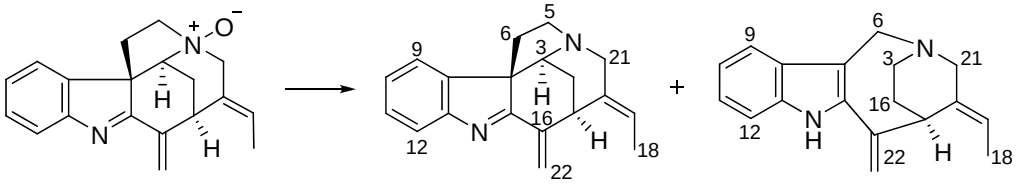


31 Pericine **43** Pericine *N*-oxide

To a stirred solution of pericine (**31**) (100 mg, 0.36 mmol) in CH_2Cl_2 (10 mL) was added *m*-CPBA (74 mg, 0.43 mmol), and the mixture was stirred for 30 min at 0 ^oC. The mixture was quenched with 1 M Na_2SO_3 (10 mL), extracted with CH_2Cl_2 (3 x 20 mL) and the combined CH_2Cl_2 extracts were then washed with water, dried (Na_2SO_4), the solvent evaporated, and the residue purified by preparative centrifugal TLC (silica gel, $CHCl_3$/MeOH 4:1) to give 88 mg (83%) of pericine *N*-oxide (**43**). **Pericine *N*-oxide (43)**: light yellowish prisms (chloroform); mp 205–208 °C; $[\alpha]_D$ +78 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 227 (4.41), 301 (4.23) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.71 (m, 1H) (H-14), 1.75 (ddd, *J* = 7.1, 2.4 and 1.7 Hz, 1H) (H-18), 2.62 (tt, *J* = 14.1 and 7.1 Hz, 1H) (H-14), 3.10 (br d, *J* = 14 Hz, 1H)(H-6), 3.65 (dd, *J* = 14.1 and 7 Hz, 1H) (H-3), 3.72 (m, 1H) (H-5), 3.84 (m, 1H) (H-6), 3.86 (m, 1H) (H-5), 3.90 (m, 1H) (H-3), 3.94 (m, 1H) (H-15), 4.16 (d, *J* = 15 Hz, 1H) (H-21), 4.43 (dt, *J* = 15 and 2.0 Hz, 1H) (H-21), 5.43 (s, 1H) (H-22), 5.53 (s, 1H) (H-22), 5.95 (br q, *J* = 7.1 Hz, 1H) (H-19), 7.13 (ddd, *J* = 8, 7.1 and 1.0 Hz, 1H) (H-10), 7.22 (ddd, *J* = 8, 7.1 and 1.0 Hz, 1H) (H-11), 7.37 (d, *J* = 8 Hz, 1H) (H-12), 7.42 (d, *J* = 8 Hz, 1H) (H-9), 8.62 (br s, 1H) (NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.7 (C-18), 22.6 (C-6), 26.6 (C-14), 40.4 (C-15), 66.2 (C-3), 71.9 (C-21), 74.9 (C-5), 108.3 (C-7), 110.8 (C-12), 118.1 (C-9), 119.1 (C-17), 120.2 (C-10), 123.2 (C-11), 127.1 (C-19), 128.1 (C-20), 132.3 (C-8), 135.5 (C-2), 135.5 (C-13), 142.6 (C-16); EIMS *m/z* 278 $[M - O]^+$ (100), 263 $[M - O - Me]^+$ (78),

249 (34), 235 (51), 220 (67), 209 (62), 194 (43), 180 (30), 167 (31), 122 (46), 108 (15), 43 (23); ESIMS *m*/*z* 295 (MH^+, $C_{19}H_{22}N_2O$ + H).

4.2.4 Synthesis of Valparicine (28) and Apparicine (29) via the Potier-Polonovski Reaction



43 Pericine *N*-oxide **28** Valparicine **29** Apparicine

General Procedure

To a stirred solution of pericine *N*-oxide (**43**) in CH_2Cl_2, was added TFAA. The mixture was quenched with 10% NaOH and extracted with CH_2Cl_2. The combined organic phase was dried (Na_2SO_4), the solvent evaporated, and the residue purified by preparative centrifugal TLC (silica gel, ether) to give valparicine (**28**) and apparicine (**29**) (See Table 2.5).

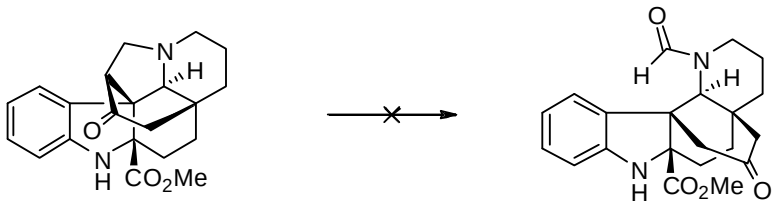
Valparicine (28): colorless oil, $[\alpha]_D$ –40 (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (3.87), 297 (3.60) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (dt, *J* = 14.2 and 2.7 Hz, 1H) (H-14), 1.78 (d, *J* = 7 Hz, 3H) (H-18), 1.97 (ddd, *J* = 12.9, 6.1 and 4.1 Hz, 1H) (H-6), 2.01 (dt, *J* = 14.2 and 3.2 Hz, 1H) (H-14), 2.42 (ddd, *J* = 12.9, 9.2 and 6.5 Hz, 1H) (H-6), 3.23 (ddd, *J* = 11, 6.5 and 4.1 Hz, 1H) (H-5), 3.29 (d, *J* = 15.0 Hz, 1H) (H-21), 3.36 (ddd, *J* = 11, 9.2 and 6.1 Hz, 1H) (H-5), 3.77 (dt, *J* = 15.0 and 1.6 Hz, 1H) (H-21), 3.85 (s, 1H) (H-15), 4.10 (s, 1H) (H-3), 5.39 (s, 1H) (H-22), 5.52 (qd, *J* = 7 and 1.3 Hz, 1H) (H-19), 6.02 (s, 1H) (H-22), 7.22 (td, *J* = 7.5 and 1 Hz, 1H) (H-10), 7.34 (td, *J* = 7.5 and

1 Hz, 1H) (H-11), 7.37 (d, *J* = 7.5 Hz, 1H) (H-9), 7.61 (d, *J* = 7.5 Hz, 1H) (H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.7 (C-18), 27.0 (C-14), 36.7 (C-6), 37.3 (C-15), 54.5 (C-21), 56.5 (C-5), 65.1 (C-3), 65.1 (C-7), 116.4 (C-22), 119.8 (C-19), 120.7 (C-12), 121.0 (C-9), 125.8 (C-10), 127.9 (C-11), 139.2 (C-20), 144.5 (C-8), 144.6 (C-16), 154.2 (C-13), 186.4 (C-2); EIMS *m/z* 276 $[M]^+$ (100), 261 $[M - CH_3]^+$ (49), 246 (53), 232 (87), 218 (43), 194 (59), 170 (50), 143 (20), 130 (17), 115 (56), 93 (28), 84 (32), 57 (22), 49 (32), 40 (86); HREIMS *m/z* 276.1624 (calcd for $C_{19}H_{20}N_2$, 276.1626).

Apparicine (29): colorless oil, $[\alpha]_D$ −177 (*c* 0.03, $CHCl_3$); IR (dry film) ν_{max} 3262 (NH), 1620 (C=C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (dd, *J* = 6.8 and 2 Hz, 1H) (H-18), 1.90 (ddt, *J* = 13, 7 and 2.5 Hz, 1H) (H-14), 2.17 (dddd, *J* = 13, 9, 8 and 5 Hz, 1H) (H-14), 3.07 (dddd, *J* = 13, 9, 7 and 2 Hz, 1H) (H-3), 3.21 (br d, *J* = 15 Hz, 1H) (H-21), 3.43 (ddd, *J* = 13, 8 and 2.5 Hz, 1H) (H-3), 3.82 (dt, *J* = 15 and 2 Hz, 1H) (H-21), 3.92 (br s, 1H) (H-15), 4.26 (d, *J* = 17.6 Hz, 1H) (H-6), 4.52 (d, *J* = 17.6 Hz, 1H) (H-6), 5.26 (s, 1H) (H-22), 5.27 (q, *J* = 6.8 Hz, 1H) (H-19), 5.40 (s, 1H) (H-22), 7.06 (ddd, *J* = 8, 7 and 1 Hz, 1H) (H-10), 7.18 (ddd, *J* = 8, 7 and 1 Hz, 1H) (H-11), 7.29 (dt, *J* = 8 and 1 Hz, 1H) (H-12), 7.41 (br d, *J* = 8 Hz, 1H) (H-9), 7.92 (br s, 1H) (NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.5 (C-18), 29.5 (C-14), 41.2 (C-15), 45.3 (C-3), 54.1 (C-6), 54.3 (C-21), 110.2 (C-12), 110.9 (C-7), 112.3 (C-22), 118.6 (C-9), 119.3 (C-10), 120.3 (C-19), 123.0 (C-11), 129.0 (C-8), 131.9 (C-20), 135.6 (C-13), 137.6 (C-2), 145.1 (C-16); ESIMS *m/z* 265 (MH^+, $C_{18}H_{20}N_2$ + H).

4.3 Electrochemically-mediated Partial Synthesis of Danuphylline B (30)

4.3.1 Direct Electrochemical Oxidation of Methyl *N*(1)-decarbomethoxychano-fruticosinate (25)

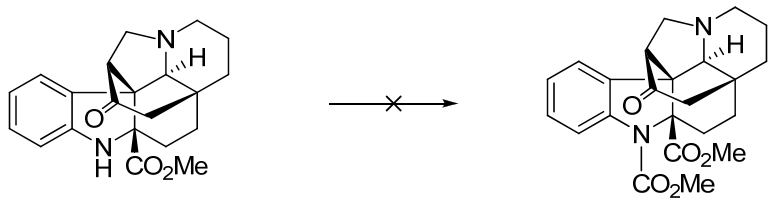


25 Methyl *N*(1)-decarbomethoxy-chanofruticosinate

30 Danuphylline B

A solution of **25** (12.4 mg, 0.02 mmol) in 50 mL of a mixed solvent (30% CH_2Cl_2-MeCN) containing Et_4NClO_4 (0.1 M) and 2,6-lutidine (0.2 mmol) was placed in a divided cell under nitrogen. The anodic potential (Pt gauze) was maintained at 1.1 V vs Ag/AgCl and the electrolysis continued until 2 $Fmol^{-1}$ of charge had been transferred. The progress of electrolysis was also monitored by cyclic voltammetry. In the course of the electrooxidation, the originally colorless solution changed to a deep orange-brown coloration. The solution was then evaporated to dryness and CH_2Cl_2 (12 mL) was added. The CH_2Cl_2 extract was then filtered through a patch of silica gel and the solvent evaporated in vacuo yielded a dark brown mixture. TLC of the crude product mixture did not show formation of any significant product.

4.3.2 Attempted Protection of the Indolic NH of Methyl *N*(1)-decarbomethoxy-chanofruticosinate (25) with Methyl Chloroformate



25 Methyl *N*(1)-decarbomethoxy-chanofruticosinate

To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and TEA (20 μL, 0.15 mmol) was added dropwise methyl chloroformate (14 μL, 0.15 mmol), and the mixture was stirred at rt. After stirring for 24 hours, TLC analysis showed only the presence of starting material.

To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and dimethylaminopyridine (DMAP, 0.04 mg, 0.033 mmol) was added dropwise methyl chloroformate (14 μL, 0.15 mmol), and the mixture was stirred at rt. After stirring for 17 hours, TLC analysis showed only the presence of starting material.

4.3.3 Attempted Protection of the Indolic NH of Methyl *N*(1)-decarbomethoxy-chanofruticosinate (25) with Boc Anhydride under Mild Conditions

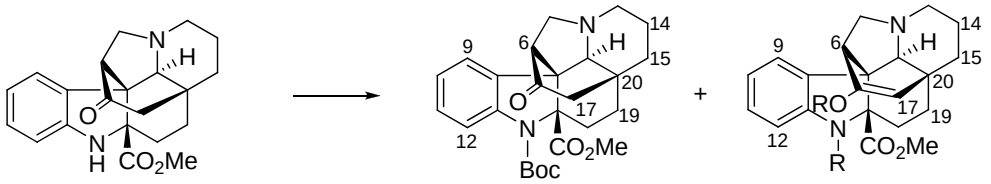
To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and Boc anhydride (14.6 μL, 0.059 mmol) was added TEA (4 μL, 0.059 mmol), and the mixture was stirred at rt. After stirring for 16 hours, TLC analysis showed only the presence of starting material.

To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and Boc anhydride (14.6 μL, 0.059 mmol) was added *N*,*N*-diisopropylethylamine (10 μL, 0.059 mmol), and the mixture was stirred at rt. After stirring for 17 hours, TLC analysis showed only the presence of starting material.

To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and Boc anhydride (14.6 μL, 0.059 mmol) was added pyridine (5 μL, 0.059 mmol), and the mixture was stirred at rt. After stirring for 17 hours, TLC analysis showed only the presence of starting material.

To a stirred solution of **25** (10 mg, 0.03 mmol), CH_2Cl_2 (5 mL) and Boc anhydride (14.6 μL, 0.059 mmol) was added TEA (4 μL, 0.059 mmol) and DMAP (0.7 mg, 0.006 mmol), and the mixture was stirred at rt. After stirring for 17 hours, TLC analysis showed only the presence of starting material.

4.3.4 Protection of the Indolic NH of Methyl *N*(1)-decarbomethoxychanofruticosinate (25) with Boc Anhydride in the Presence of a Strong Base



25 Methyl *N*(1)-decarbomethoxychanofruticosinate

51

52 R = Boc

General Procedure

To a stirred solution of **25**, Boc_2O and THF was added dropwise NaHMDS (1 M), and the mixture was stirred for 45 min at rt. The mixture was quenched with saturated

NH_4Cl (15 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried (Na_2SO_4), the solvent evaporated in vacuo, and the residue purified by preparative centrifugal TLC (SiO_2, Hexanes/AcOEt 5:3) to give N-Boc protected derivative **51** and doubly-acylated enol carbonate **52**.

Table 4.1: Reaction of Compound **25** with Boc Anhydride in the Presence of NaHMDS or NaH under Different Conditions

Entry	Temperature (^oC)	Type of base	Amount of Boc_2O (equiv)	Amount of base (equiv)	Yield (%)	
					51	**52**
1	rt	NaHMDS	2.2	2.2	26	62
2	rt	NaHMDS	0.55	0.55	10	32
3	–78	NaHMDS	2.2	2.2	5	18
4	40	NaHMDS	2.2	2.2	15	43
5	rt	NaHMDS	0.95	1	no significant product	
6	0	NaH	2.2	2.2	no significant product	

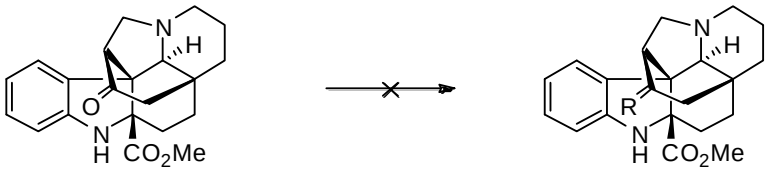
Note: Entry 1, optimized condition; Entry 5, the order of addition is **25**, NaHMDS, followed by Boc_2O; Due to paucity of **25**, further optimization was not carried out.

Compound 51: colorless oil; $[\alpha]_D$ +106 (*c* 0.65, $CHCl_3$); IR (dry film) ν_{max} 1744, 1711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (m, 1H) (H-19), 1.33 (m, 1H) (H-15), 1.44 (m, 1H) (H-14), 1.50 (s, 9H) ($NCO_2C\mathit{Me}_3$), 1.61 (m, 1H) (H-14), 1.65 (m, 1H) (H-15), 1.83 (m, 1H) (H-19), 2.15 (d, *J* = 19.5 Hz, 1H) (H-17), 2.41 (m, 1H) (H-18), 2.50 (s, 1H) (H-21), 2.58 (m, 1H) (H-18), 2.71 (d, *J* =19.5 Hz, 1H) (H-17), 2.78 (d, *J* = 11.5 Hz, 1H) (H-5), 2.82 (d, *J* = 6 Hz, 1H) (H-6), 2.97 (d, *J* = 6 Hz, 1H) (H-3), 2.99 (m, 1H) (H-3), 3.57 (s, 3H) ($CO_2\mathit{Me}$), 3.65 (dd, *J* = 11.5 and 6.5 Hz, 1H) (H-5), 6.96 (td, *J* = 8 and 1 Hz, 1H) (H-10), 7.21 (td, *J* = 8 and 1 Hz, 1H) (H-11), 7.32 (dd, *J* = 8 and 1 Hz, 1H) (H-9), 7.78 (br d, *J* = 8 Hz, 1H) (H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.3 (C-14), 25.1 (C-18), 28.2 ($NCO_2C\mathit{Me}_3$), 34.4 (C-19), 35.6 (C-15), 36.3 (C-20), 43.7 (C-17), 46.4 (C-

3), 52.0 (CO_2*Me*), 52.1 (C-5), 56.9 (C-6), 57.6 (C-7), 67.6 (C-21), 73.2 (C-2), 82.4 (NCO_2*C*Me_3), 114.1 (C-12), 122.6 (C-10), 124.9 (C-9), 128.4 (C-11), 133.4 (C-8), 141.6 (C-13), 152.4 (N*C*O_2CMe_3), 171.1 (*C*O_2Me), 208.2 (C-16); EIMS *m/z* 452 $[M]^+$ (3), 293 (100), 249 (2), 222 (5), 180 (3). HREIMS *m/z* 452.2316 (calcd for $C_{26}H_{32}N_2O_5$, 452.2311).

Compound 52: colorless oil; $[\alpha]_D$ +99 (*c* 0.71, $CHCl_3$); IR (dry film) ν_{max} 1811, 1752, 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.25 (m, 1H) (H-14), 1.32 (m, 1H) (H-15); 1.39 (m, 1H) (H-19), 1.47 (s, 9H) (OCO_2C*Me*$_3$), 1.52 (s, 9H) (NCO_2C*Me*$_3$), 1.71 (m, 1H) (H-14), 1.71 (m, 1H) (H-18), 1.82 (m, 1H) (H-15), 1.86 (m, 2H) (H-19), 2.56 (d, *J* = 5 Hz, 1H) (H-6), 2.73 (s, 1H) (H-21), 2.77 (dd, *J* = 13.5 and 8 Hz, 1H) (H-18), 2.92 (t, *J* = 14 Hz, 1H) (H-3), 2.99 (t, *J* = 14 Hz, 1H) (H-3), 3.21 (d, *J* = 10 Hz, 1H) (H-5), 3.66 (dd, *J* = 10 and 5 Hz, 1H) (H-5), 3.74 (s, 3H) (CO_2*Me*), 4.82 (s, 1H) (H-17), 7.01 (t, *J* = 8 Hz, 1H) (H-10), 7.24 (t, *J* = 8 Hz, 1H) (H-11), 7.67 (d, *J* = 8 Hz, 1H) (H-9), 7.88 (br s, 1H) (H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.2 (C-14), 26.5 (C-18), 27.7 (OCO_2C*Me*$_3$), 28.1 (NCO_2C*Me*$_3$), 31.6 (C-19), 36.7 (C-20), 37.5(C-15), 47.2 (C-6), 48.0 (C-3), 51.5 (CO_2*Me*), 57.9 (C-5), 60.9 (C-7), 64.5 (C-21), 73.0 (C-2), 82.0 (OCO_2*C*Me_3), 82.6 (NCO_2*C*Me_3), 114.5 (C-12), 120.7 (C-17), 122.9 (C-10), 126.0 (C-9), 128.4 (C-11), 132.8 (C-8), 140.9 (C-13), 150.1 (O*C*O_2CMe_3), 150.1 (N*C*O_2CMe_3), 151.2 (C-16), 171.4 (*C*O_2Me); EIMS *m/z* 552 $[M]^+$ (5), 435 (7), 393 (15), 337 (21), 263 (4), 222 (8), 180 (5), 144 (3). HREIMS *m/z* 552.2837 (calcd for $C_{31}H_{40}N_2O_7$, 552.2836).

4.3.5 Attempted Protection of the C-16 Carbonyl of Methyl *N*(1)-decarbomethoxychanofruticosinate (25) as a Ketal Group



25 Methyl *N*(1)-decarbomethoxy-chanofruticosinate

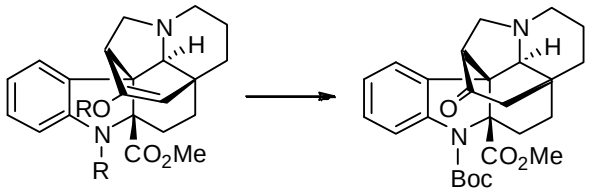
To a stirred solution containing toluene (5 ml), *p*-toluenesulfonic acid (10 mg, 0.059 mmol), and molecular sieves (3 Å, 0.15 g) were added ethylene glycol (10 μL, 0.18 mmol) followed by **25** (10 mg, 0.03 mmol). The mixture was refluxed for 6 h, filtered through a patch of silica gel, and the solvent removed in vacuo. TLC analysis of the crude product mixture showed only the presence of starting material.

To a stirred solution containing methanol (5 mL), **25** (11.2 mg, 0.032 mmol), and ethylene glycol (11 μL, 0.19 mmol), was added trimethylsilyl chloride (8 μL, 0.064 mmol). The mixture was stirred at rt for 16 h, and the solvent removed in vacuo. No definitive products were obtained although starting material had been consumed.

To a stirred solution containing ethylene glycol (1 mL) and copper (II) chloride (0.5 mg, 0.003 mmol) was added **25** (11.5 mg, 0.033 mmol). The mixture was stirred at 80 ^{o}C for 1 h, and then partitioned between water (20 mL) and CH_2Cl_2 (20 mL). The phases were then separated and the organic phase was washed with water (3 x 20 mL), dried, and the solvent remove in vacuo. TLC analysis of the crude product mixture showed only the presence of starting material.

To a stirred CH_2Cl_2 (5 mL) containing TMSOTf (0.54 μL, 0.003 mmol) were successively added 1,2-bis(trimethylsiloxy)ethane (25 mL, 0.1 mmol) and **25** (11.5 mg, 0.033 mmol) at −78 °C. The mixture was stirred at −78 °C for 6 h, quenched by addition of dry pyridine (1 mL), poured into a saturated $NaHCO_3$ solution (10 mL), and extracted with CH_2Cl_2 (3 x 20 mL). The combined extracts were dried (Na_2SO_4), and the solvent removed in vacuo. TLC analysis of the crude product mixture showed only the presence of starting material.

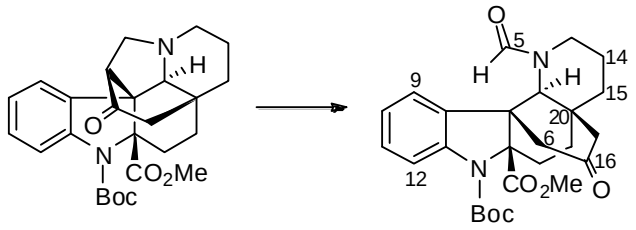
4.3.6 Krapcho Decarboxylation of the Doubly-acylated Enol Carbonate 52



52 R = Boc **51**

A mixture of **52** (13.5 mg, 0.024mmol), LiCl (2.0 mg, 0.048 mmol), H_2O (0.1 g) and DMSO (4 mL) was stirred for 6 h at 100−110 °C. The mixture was then cooled in ice, and H_2O (40 mL) was added, followed by brine (3 mL). The mixture was extracted with CH_2Cl_2 (3 x 30 mL), and the combined CH_2Cl_2 extracts were then washed with saturated NaCl, dried (Na_2SO_4), the solvent evaporated in vacuo, and the residue purified by preparative centrifugal TLC (SiO_2, Hexanes/AcOEt 5:3) to give 7.0 mg (61%) of **51**.

4.3.7 Anodic Oxidation of Compound 51

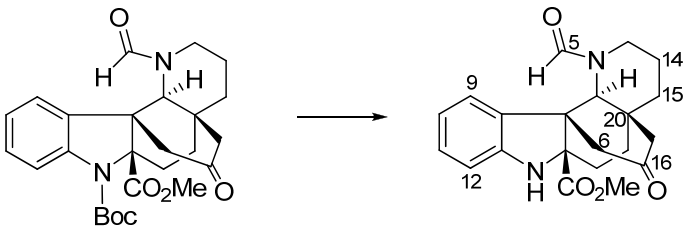


51 **53**

A solution of **51** (12.4 mg, 0.02 mmol) in 50 mL of a mixed solvent (30% CH_2Cl_2-MeCN) containing Et_4NClO_4 (0.1 M) and 2,6-lutidine (0.2 mmol) was placed in a divided cell under nitrogen. The anodic potential (Pt gauze) was maintained at 1.2 V vs Ag/AgCl and the electrolysis continued until 2 $Fmol^{-1}$ of charge had been transferred. The progress of electrolysis was also monitored by cyclic voltammetry. The solution was then evaporated to dryness and CH_2Cl_2 (12 mL) was added. The precipitated electrolyte was then filtered off and the residue washed with CH_2Cl_2. The CH_2Cl_2 extract was then chromatographed over silica gel (CH_2Cl_2) via preparative centrifugal TLC to afford the Boc-protected danuphylline B **53** (3.7 mg, 30 %). **Compound 53:** colorless oil; $[\alpha]_D$ +28 (*c* 0.39, $CHCl_3$). IR (dry film) ν_{max} 1738, 1712, 1673 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (m, 1H) (H-15), 1.58 (s, 9H) (s, $NCO_2C\mathit{Me}_3$,), 1.70 (m, 2H) (H-19), 1.71 (m, 1H) (H-15), 1.76 (m, 1H) (H-14), 1.89 (m, 1H) (H-14), 2.37 (d, *J* = 17 Hz, 1H) (H-6), 2.50 (d, *J* = 20 Hz, 1H) (H-17), 2.54 (m, 1H) (H-18), 2.71 (d, *J* = 20 Hz, 1H) (H-17), 2.72 (m, 1H) (H-3), 2.83 (d, *J* = 17 Hz, 1H) (H-6), 3.20 (br d, *J* = 16.5 Hz, 1H) (H-18), 3.34 (s, 1H) (H-21), 3.57 (s, 1H) ($CO_2\mathit{Me}$), 4.52 (dd, *J* = 14 and 9 Hz, 1H) (H-3), 6.38 (s, 1H) (H-5), 6.88 (d, *J* = 7.5 Hz, 1H) (H-10), 7.04 (t, *J* = 7.5 Hz, 1H) (H-11), 7.31 (t, *J* = 7.5 Hz, 1H) (H-9), 7.81 (br d, *J* = 7.5 Hz, 1H) (H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.4 (C-14), 23.7 (C-18), 28.4 ($NCO_2C\mathit{Me}_3$), 29.9 (C-15), 34.4 (C-

20), 35.0 (C-3), 39.4 (C-6), 39.4 (C-19), 46.1 (C-17), 52.7 (CO_2*Me*), 54.1 (C-7), 62.2 (C-21), 78.6 (C-2), 83.1 (NCO_2*C*Me$_3$), 115.5 (C-12), 122.8 (C-11), 123.8 (C-10), 129.2 (C-8), 129.8 (C-9), 143.7 (C-13), 153.6 (N*C*O$_2$CMe$_3$), 165.8 (C-5), 170.7 (*C*O$_2$Me), 207.0 (C(16)); EIMS *m/z* 468 $[M]^+$ (2), 368 (10), 309 (100), 281 (11), 252 (14), 222 (7), 180 (4), 156 (7). HREIMS *m/z* 468.2260 (calcd for $C_{26}H_{32}N_2O_6$, 468.2260).

4.3.8 Deprotection of the Boc-protected Danuphylline B 53



53 **30** Danuphylline B

Neat TMSOTf (11 μL, 0.061 mmol) was added dropwise to a solution of **53** (6 mg, 0.013 mmol) in CH_2Cl_2 (5 mL) at rt. The flask was left open to air so that adventitious H_2O would create a small amount of triflic acid. After 3 h, the solution was partitioned between saturated Na_2CO_3 solution (15 mL) and CH_2Cl_2 (15 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was purified by preparative centrifugal TLC (SiO_2, Hexanes/AcOEt 5:3) to give 4.6 mg (97%) of danuphylline B (**30**). **Danuphylline B (30):** colorless oil; $[\alpha]_D$ + 67 (*c* 0.68, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 212 (4.02), 239 (3.68), 294 (3.34) nm; IR (dry film) ν_{max} 3347, 1731, 1661 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (dt, *J* = 14 and 9 Hz, 1H) (H-15), 1.63 (m, 1H) (H-15), 1.65 (m, 1H) (H-19), 1.75 (m, 1H) (H14), 1.96 (m, 1H) (H-14), 1.96 (m, 1H) (H-19), 2.01 (m, 1H) (H-18), 2.18 (m, 1H) (H-18), 2.45 (d, *J* = 20 Hz, 1H) (H-17), 2.70 (d, *J* = 17 Hz, 1H) (H-6), 2.72 (d, *J* = 20 Hz, 1H) (H-17), 2.73 (m, 1H) (H-

3), 2.85 (d, *J* = 17 Hz, 1H) (H-6), 3.62 (s, 3H) (CO_2*Me*), 3.68 (s, 1H) (H-21), 4.58 (dd, *J* = 12 and 9 Hz, 1H) (H-3), 6.62 (s, 1H) (H-5), 6.84 (m, 1H) (H-10), 6.86 (m, 1H) (H-9), 6.86 (m, 1H) (H-12), 7.18 (ddd, *J* = 8, 7 and 2 Hz, 1H) (H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.2 (C-14), 26.7 (C-18), 29.8 (C-15), 34.0 (C-20), 34.7 (C-3), 39.0 (C-19), 39.2 (C-6), 45.9 (C-17), 52.5 (CO_2*Me*), 53.9 (C-7), 59.7 (C-21), 77.0 (C-2), 111.1 (C-12), 119.9 (C-10), 123.9 (C-9), 128.8 (C-8), 129.3 (C-11), 148.9 (C-13), 165.7 (H-5), 174.2 (*C*O_2Me), 207.9 (C-16); EIMS *m*/*z* 368 $[M]^+$ (7), 339 $[M - CHO]^+$ (7), 309 $[M - CO_2Me]^+$ (100), 281 (19), 264 (5), 252 (29), 224 (11), 188 (6), 156 (10), 124 (20), 110 (5), 96 (17), 83 (8), 57 (4), 40 (24); HREIMS *m*/*z* 368.1735 (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).