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# CHAPTER THREE

# EXPERIMENTAL

## 3.1 Source and Authentication of Plant Materials

The plant materials were collected from various locations in Malaysia and were identified by Dr. K. M. Wong (Formerly Institute of Science Biology, University of Malaya, and Forest Research Centre, Sabah) and L. E. Teo (Herbarium, Chemistry Department, University of Malaya). All plant materials were screened for their alkaloidal constituents before any chemical analysis was carried out. Voucher specimens are deposited at Forest Research Centre (FRC), Sepilok, Sandakan and the Herbarium, Chemistry Department, University of Malaya (UM).

Table 3.1 : Source and authentication of plant materials

| Herbarium Specimen No. | Locality | Species | Date of Collection | Herbarium |
|---|---|---|---|---|
| SAN 138327 | Sabah | *K. pauciflora* | Feb 1994 | FRC (Sandakan) |
| KL 3632 | Johor | *K. grandifolia* | Feb 1990 | Chem. Dept. (UM) |
| GK 401, 541 | | (*K. lapidilecta*) | May 1989/90 | |

## 3.2 General

Melting points were determined with a Mel-Temp melting point apparatus and were uncorrected. Optical rotations were determined on a Jasco P-1020 automatic digital polarimeter. UV spectra were obtained on a Shimadzu UV-3101 PC spectrophotometer in absolute ethanol. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR or a Perkin-Elmer RX1 FT-IR spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra data were recorded in $CDCl_3$ using tetramethylsilane (TMS) as internal standard on a JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. Coupling constants (*J*) are reported in Hz and $\delta$ in ppm. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo K$\alpha$ fine-focus sealed tube ($\lambda$ = 0.71073 Å), at 100K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on $F^2$ (SHELXL-97). ESIMS and HRESIMS were obtained using Agilent 6530 Q-TOF mass spectrometer. HREIMS was obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. All solvents were distilled prior to use with the exception of diethyl ether, which was passed through activated neutral alumina before use.

## 3.3 Chromatographic Methods

### 3.3.1 Column Chromatography

Flash chromatography was performed using Merck silica gel 9385 (230-400 Mesh ASTM). The ratio of silica gel to the sample was approximately 30:1 for crude samples and 100:1 for semi-pure fractions. The gel was made into slurry with chloroform before

it was packed onto the column and was allowed to equilibrate for at least an hour before use. The solvent systems normally used to elute the column were chloroform with increasing methanol gradient or diethyl ether with increasing ethyl acetate gradient. When diethyl ether-hexanes was used as an eluting solvent, the column was packed by the dry packing method. Fractions were monitored by thin layer chromatography (TLC) and appropriate fractions were combined and where necessary subjected to further separation by re-chromatography or centrifugal preparative TLC.

### 3.3.2 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was routinely used to detect and separate the various alkaloids. It was also used extensively for testing and selecting the right solvent systems for separating the alkaloids. The crude alkaloidal extracts, fractions from chromatography, and isolated pure alkaloids, were examined by TLC using pre-coated 5 × 10 cm aluminium plates, 0.25 mm thickness, silica gel 60 $F_{254}$ (Merck, Darmstadt, G. F.R). The TLC plates were spotted with a piece of fine glass capillary tube and then developed in saturated chromatographic tanks with various solvent systems at room temperature. The alkaloidal spots of the developed TLC plates were visualized under UV light (254 nm), followed by spraying reagents, which formed orange spots. The $hR_f$ values of the alkaloids are tabulated in Table 3.2.

Table 3.2 : The $hR_f$ values of alkaloids isolated from *Kopsia pauciflora* and *Kopsia grandifolia*

| Alkaloids | Solvent Systems | | | | | |
|---|---|---|---|---|---|---|
| | a | b | c | d | e | f |
| Compound **1** | 51 | 20 | 38 | 69 | 79 | 31 |
| Compound **2** | 38 | 11 | 40 | 63 | 78 | 14 |
| Compound **3** | 4 | 0 | 6 | 28 | 65 | 8 |
| Compound **4** | 19 | 3 | 17 | 42 | 69 | 4 |
| Compound **5** | 54 | 26 | 44 | 62 | 75 | 37 |

Table 3.2, continued

| Alkaloids | Solvent Systems | | | | | |
|---|---|---|---|---|---|---|
| | a | b | c | d | e | f |
| Compound **6** | 29 | 5 | 22 | 40 | 65 | 4 |
| Compound **7** | 35 | 7 | 33 | 58 | 75 | 8 |
| Compound **8** | 58 | 41 | 55 | 73 | 77 | 68 |
| Compound **9** | 29 | 14 | 23 | 56 | 70 | 18 |
| Compound **10** | 17 | 5 | 10 | 18 | 51 | 9 |
| Compound **11** | 10 | 4 | 5 | 19 | 42 | 8 |
| Compound **12** | 10 | 5 | 8 | 22 | 45 | 8 |
| Tetrahydroalstonine (**13**) | 84 | 66 | 78 | 69 | 90 | 88 |
| Leuconoxine (**14**) | 34 | 10 | 36 | 60 | 77 | 15 |
| *N*(1)-Carbomethoxy-5,22-dioxokopsane (**15**) | 41 | 11 | 38 | 50 | 74 | 17 |
| Kopsanone (**16**) | 43 | 23 | 32 | 58 | 73 | 43 |
| Kopsifine (**17**) | 36 | 9 | 34 | 47 | 63 | 10 |
| Decarbomethoxykopsifine (**18**) | 28 | 5 | 16 | 27 | 41 | 6 |
| Paucidactine B (**19**) | 39 | 4 | 30 | 43 | 61 | 17 |
| Kopsamine (**20**) | 53 | 24 | 41 | 67 | 75 | 48 |
| Kopsamine *N*-oxide (**21**) | 0 | 0 | 0 | 6 | 31 | 0 |
| Kopsinine (**22**) | 35 | 12 | 18 | 38 | 52 | 45 |
| *N*(1)-Methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**) | 25 | 6 | 12 | 42 | 65 | 20 |
| *N*(1)-Methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**) | 29 | 13 | 22 | 60 | 73 | 25 |
| Kopsinine *N*-oxide (**25**) | 0 | 0 | 0 | 5 | 24 | 0 |
| *N*(1)-Methoxycarbonyl-11,12-dimethoxykopsinaline (**26**) | 46 | 12 | 24 | 50 | 70 | 41 |
| Kopsilongine (**27**) | 45 | 18 | 20 | 46 | 73 | 49 |
| Pleiocarpine (**28**) | 47 | 23 | 36 | 60 | 70 | 44 |
| 12-Methoxypleiocarpine (**29**) | 33 | 9 | 17 | 38 | 65 | 26 |
| Pleiocarpine *N*-oxide (**30**) | 0 | 0 | 0 | 7 | 26 | 0 |
| (+)-Eburnamenine (**31**) | 30 | 24 | 25 | 53 | 68 | 40 |
| (+)-Eburnamonine (**32**) | 37 | 19 | 19 | 66 | 82 | 33 |
| (−)-Eburnamine (**33**) | 39 | 11 | 11 | 24 | 39 | 34 |
| (+)-Isoeburnamine (**34**) | 26 | 9 | 22 | 33 | 51 | 21 |
| (+)-19-oxoeburnamine (**35**) | 30 | 11 | 10 | 22 | 40 | 21 |
| (−)-19(*R*)-Hydroxyisoeburnamine (**36**) | 5 | 0 | 0 | 11 | 36 | 0 |
| (+)-19(*R*)-Hydroxyeburnamine (**37**) | 5 | 0 | 0 | 8 | 37 | 0 |
| (−)-Norpleiomutine (**38**) | 10 | 0 | 3 | 24 | 44 | 7 |
| (−)-Demethylnorpleiomutine (**39**) | 0 | 0 | 0 | 3 | 4 | 0 |
| (+)-Kopsoffinol (**40**) | 3 | 0 | 3 | 16 | 40 | 0 |
| Grandilodine A (**41**) | 24 | 10 | 14 | 32 | 57 | 21 |
| Grandilodine B (**42**) | 29 | 5 | 22 | 56 | 68 | 9 |
| Grandilodine C (**43**) | 17 | 4 | 19 | 53 | 71 | 3 |
| Lapidilectine A (**44**) | 37 | 16 | 25 | 58 | 75 | 38 |
| Isolapidilectine A (**45**) | 24 | 12 | 17 | 43 | 58 | 25 |
| Lapidilectam (**46**) | 26 | 8 | 25 | 58 | 65 | 8 |
| Lapidilectine B (**47**) | 50 | 25 | 48 | 74 | 77 | 42 |

a. Ethyl acetate: Diethyl ether (1:1)
b. Ethyl acetate: Hexanes (1:1)
c. Ethyl acetate: Chloroform (1:1)
d. Methanol: Chloroform (1:25)
e. Methanol: Chloroform (1:10)
f. Diethyl ether

### 3.3.3 Centrifugal Preparative TLC

Centrifugal preparative TLC was carried out using a round chromatographic plate measuring 24 cm in diameter with the action of a centrifugal force to accelerate mobile phase flow across the circular plate. To prepare the chromatographic plate, the edge of the plate was secured with cellophane tape to form a mould. Silica gel (Merck 7749, 50g) was added to about 110 ml of cold distilled water. This slurry was shaken and was then quickly poured onto the circular glass plate before setting commenced. 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 ℃ for about 12 hours. The sample was dissolved in a minimum volume of a suitable solvent and loaded at the centre of the plate while the plate was spinning to form a thin band. Elution was then carried with the appropriate solvent system. The fractions were collected, concentrated by rotary evaporation, examined by TLC and combined where appropriate.

### 3.4 Spray Reagent (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 in 20 ml of distilled waer.

A stock solution was prepared by mixing equal volumes of solutions A and B.

Dragendorff's reagent was made by mixing 1ml of stock solution with 2 ml of glacial acetic acid and 10 ml of distilled water. Orange spots on the developed TLC plates indicate the presence of alkaloids.

### 3.5 Extraction of Alkaloids

The plant materials were dried and ground before extracting with 95% ethanol for 2-3 days at room temperature. The ethanol extract was filtered and the residue was then re-extracted with a fresh portion of distilled ethanol. This procedure was repeated 5 or 6 times. The combined extract was then concentrated by distillation under reduced pressure using a rotary-evaporator to about a tenth of the original volume. The concentrated extract was then added slowly into 5% hydrochloric acid with constant stirring. The acidic solution was then filtered through Kieselguhr to remove the non-alkaloidal substances. The filtrate was then basified with concentrated ammonia solution to about pH 10 with cooling and the liberated alkaloids were extracted exhaustively with chloroform. The chloroform extract was then washed with distilled water and dried over anhydrous sodium sulfate. Finally, the solvent was removed by evaporation under reduced pressure to furnish the crude alkaloidal mixture.

### 3.6 Isolation of Alkaloids

#### 3.6.1 General Procedure

The basic crude mixture obtained from the extraction procedure described above was initially fractionated by vacuum chromatography over silica gel. The column was eluted with chloroform, followed by a stepwise increase of methanol gradient. Based on TLC, the many fractions collected were pooled into several major fractions, which were then subjected to further fractionation by flash chromatography, vacuum chromatography, or centrifugal preparative TLC until pure compounds were obtained.

### 3.6.2 Isolation of Alkaloids from *Kopsia pauciflora*

Extraction of 8.5 kg of the stem-bark of *K. pauciflora* yielded *ca.* 27.7 g of crude alkaloidal mixture. This mixture was then subjected to repeated fractionation by various chromatographic methods. 40 alkaloids were obtained from this crude mixture as summarized in the flow diagram shown in Figure 3.1.

### 3.6.3 Isolation of Alkaloids from *Kopsia grandifolia*

Extraction of 9.0 kg of the stem-bark of *K. grandifolia* gave *ca.* 25.5 g of crude alkaloidal mixture. Six alkaloids were obtained from this crude mixture as summarized in the flow diagram shown in Figure 3.2. The leaves of the same plant gave *ca.* 2.5 g of crude material from the extraction of 0.5 kg of plant material. From this mixture, two alkaloids were isolated. The flow diagram depicting the isolation of the alkaloids from the leaves of *K. grandifolia* is shown in Figure 3.3.

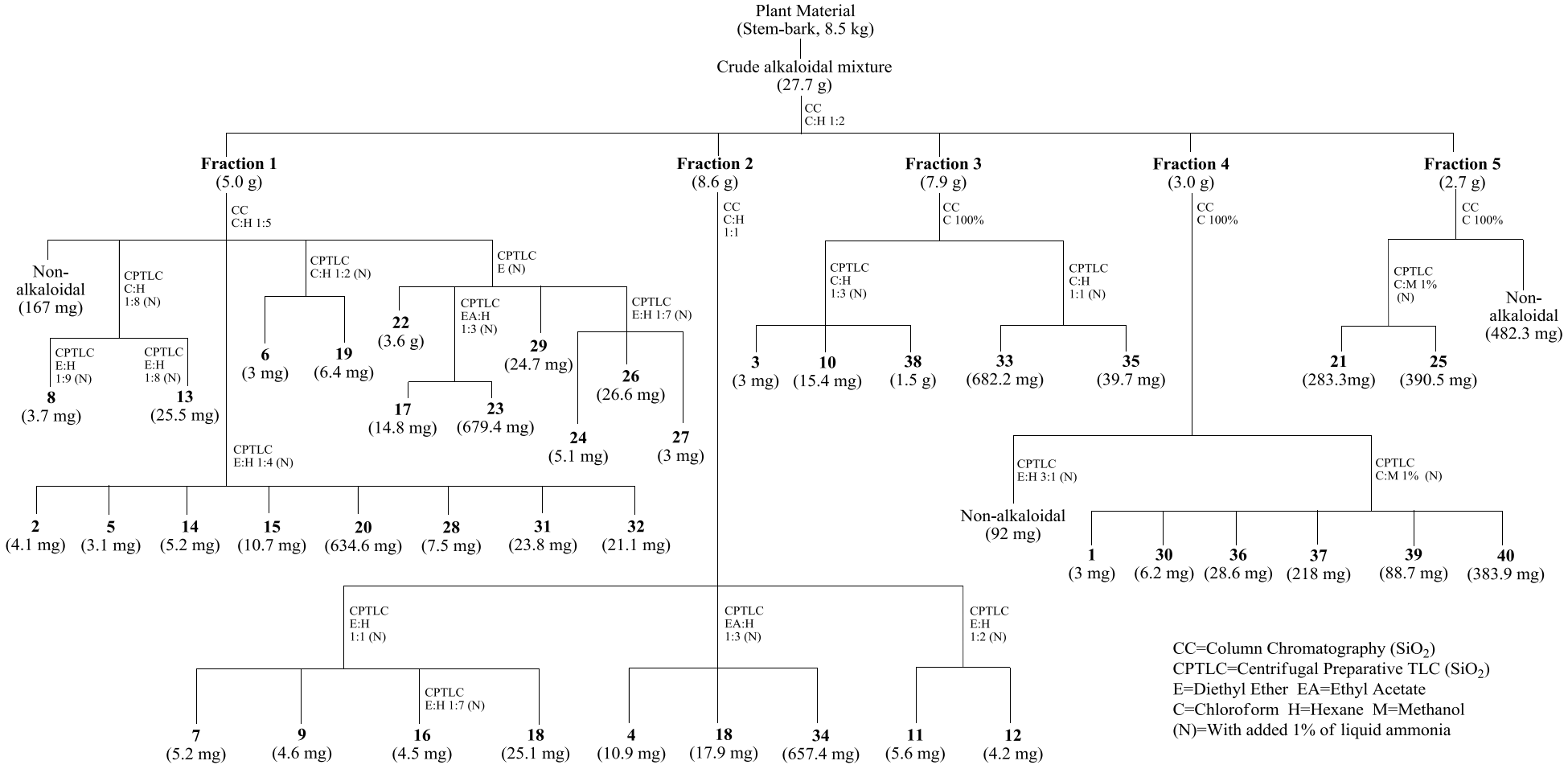


Figure 3.1 : Isolation of alkaloids from the stem-bark extract of *Kopsia pauciflora*

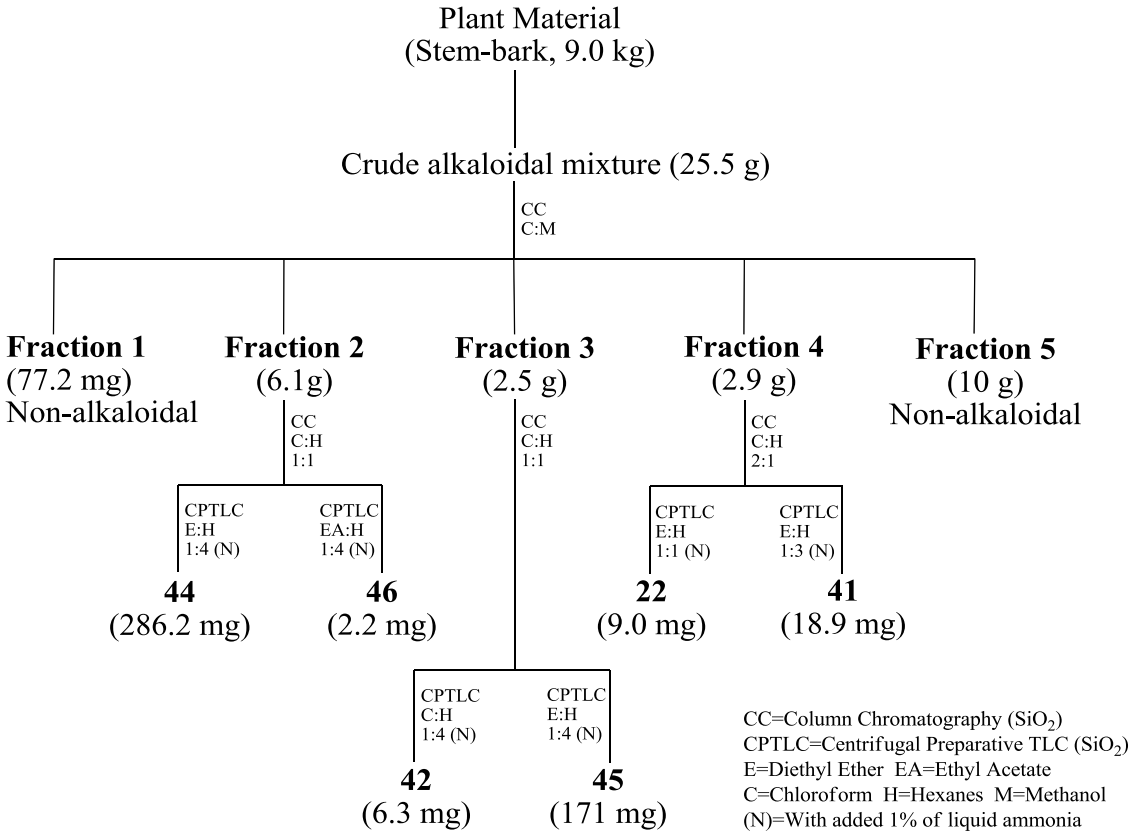


Figure 3.2 : Isolation of alkaloids from the stem-bark extract of *Kopsia grandifolia*

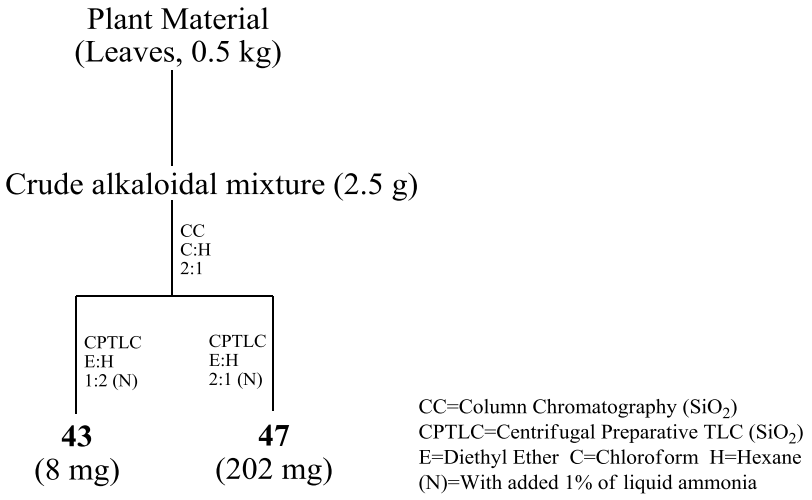


Figure 3.3 : Isolation of alkaloids from the leaf extract of *Kopsia grandifolia*

### 3.7 Compound Data

**Alkaloids from *K. pauciflora***

**Compound 1 :** Light yellowish amorphous solid and subsequently colorless block crystals ($CH_2Cl_2$–hexanes); mp 206–208 $^oC$; $[\alpha]^{25}_D$ +30 (*c* 0.15, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 210 (4.01), 224 (4.15), 250 (3.69), and 289 (3.00) nm; IR (dry film) $\upsilon_{max}$ 3240, 1734, 1685 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.2; ESIMS *m/z* 441 ($MH^+$); HRESIMS *m/z* 441.1665 (calcd for $C_{23}H_{24}N_2O_7$ + H, 441.1656). HMBC: $^2J$ H(5) to C(6); H(6) to C(5), C(22); H(9) to C(10); H(10) to C(11); H(15) to C(20); H(17) to C(16), C(20); H(18) to C(2); H(19) to C(20); 16-OH to C(16). $^3J$ H(5) to C(3), C(7), C(21), C(22); H(6) to C(8); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(15) to C(21); H(17) to C(2), C(15), C(21); H(18) to C(7), C(16), C(20); H(19) to C(2), C(17); H(21) to C(5), C(6), C(8), C(15), C(17); $NCO_2Me$ to $NCO_2Me$; 16-OH to C(2), C(17); $OCH_2O$ to C(11), C(12).

**Crystallographic data of 1 :** Colorless block crystals, $C_{23}H_{24}N_2O_7$, *M*r = 440.44, monoclinic, space group $P2_1$, $a$ = 9.9492(5) Å, $b$ = 8.2037(4) Å, $c$ = 13.0494(7) Å; $\alpha = \gamma = 90^o$, $\beta = 109.845(3)^o$, $V$ = 1002(9) $Å^3$, $T$ = 100 K, $Z$ = 2, $D_{calcd}$ = 1.460 $gcm^{-3}$, crystal size 0.06 x 0.15 x 0.58 $mm^3$, $F(000)$ = 464. The final $R_1$ value is 0.0504 (w$R_2$ = 0.1220) for 2175 reflections [$I>2\sigma(I)$].

**Compound 2 :** Light yellowish oil and subsequently colorless block crystals ($CH_2Cl_2$–MeOH); mp 180–182 $^oC$; $[\alpha]^{25}_D$ –22 (*c* 0.13, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (4.29), 255 (3.72), and 291 (3.38) nm; IR (dry film) $\upsilon_{max}$ 3344, 1729, 1694 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.3; ESIMS *m/z* 339 ($MH^+$); HRESIMS *m/z*

339.1704 (calcd for $C_{20}H_{22}N_2O_3$ + H, 339.1703). HMBC: $^2J$ H(6) to C(5), C(7); H(10) to C(11); H(15) to C(20); H(16) to C(2), C(17), *C*$O_2$Me; H(17) to C(16), C(20); H(18) to C(2), C(19); H(19) to C(18), C(20); H(21) to C(7); NH to C(13). $^3J$ H(5) to C(7), C(14), C(21); H(6) to C(2), C(8), C(21); H(9) to C(7), C(11); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(17), C(19); H(16) to C(7), C(18); H(17) to C(2), C(15), C(19), C(21), *C*$O_2$Me; H(18) to C(7), C(16), C(20); H(19) to C(17), C(21); H(21) to C(5), C(6), C(8), C(14), C(15), C(17), C(19); $CO_2$*Me* to *C*$O_2$Me; NH to C(7), C(8).

**Crystallographic data of 2 :** Colorless block crystals, $C_{20}H_{22}N_2O_3.CH_3OH$, *M*r = 370.44, orthorhombic, space group $P2_12_12_1$, $a$ = 6.7581(2) Å, $b$ = 11.4548(3) Å, $c$ = 23.3157(6) Å; $V$ = 1804.93(9) Å$^3$, $T$ = 100 K, $Z$ = 4, $D_{calcd}$ = 1.363 gcm$^{-3}$, crystal size 0.58 x 0.25 x 0.13 mm$^3$, $F$(000) = 792. The final $R_1$ value is 0.0332 (w$R_2$ = 0.993) for 2163 reflections [$I>2\sigma(I)$].

**Compound 3 :** Light yellowish oil and subsequently colorless needles ($CH_2Cl_2$–hexanes); mp 268–270 $^o$C; $[\alpha]^{25}_D$ +135 (*c* 0.11, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 205 (3.19), 211 (3.13), 229 (2.89), and 279 (2.99) nm; IR (dry film) $\upsilon_{max}$ 3349, 1720 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.4; ESIMS *m/z* 391 ($MH^+$); HRESIMS *m/z* 391.2019 (calcd for $C_{24}H_{26}N_2O_3$ + H, 391.2016). HMBC: $^2J$ H(6) to C(5), C(7); H(10) to C(9), C(11); H(15) to C(14), C(20); H(16) to C(2), *C*$O_2$Me; H(17) to C(16), C(20); H(18) to C(2), C(19); H(19) to C(18), C(20); H(21) to C(7); H(22) to C(3), C(23); H(24) to C(14), C(23). $^3J$ H(5) to C(7), C(21); H(6) to C(2), C(8), C(21); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(17), C(19), C(21), C(24); H(16) to C(7), C(18); H(17) to C(15), C(19), C(21), *C*$O_2$Me; H(18) to C(7), C(16), C(20); H(19) to C(2), C(17), C(21); H(21) to

C(3), C(6), C(8), C(17); $CO_2$*Me* to *C*$O_2$Me; H(22) to C(14), C(24); H(24) to C(3). NOESY: H(5α)/H(5β), H(6α), H(9); H(5β)/H(22); H(6α)/H(5α), H(6β); H(6β)/H(6α), H(17β); H(9)/H(5α), H(10), H(21); H(10)/H(11); H(11)/H(12); H(12)/H(11); H(14)/H(15α), H(21), H(24α); H(15β)/H(15α), H(17α); H(15α)/H(14), H(15β); H(16)/H(17α), H(18β), H(19β); H(17α)/H(15β), H(16), H(17β), H(19β); H(17β)/H(6β), H(17α); H(18β)/H(16), H(18α); H(18α)/H(18β), H(19α), H(21); H(19α)/H(18α), H(21); H(19α)/H(18α), H(21); H(19β)/H(16), H(18β), H(19α); H(21)/H(14), H(15α), H(18α), H(19α); H(22)/H(5β); H(24β)/H(15β), H(24α); H(24α)/H(14), H(24β).

**Crystallographic data of 3 :** Colorless needles, $C_{24}H_{26}N_2O_3$, $CH_2Cl_2$, *M*r = 475.39, orthorhombic, space group $P2_12_12_1$, *a* = 6.6620(4) Å, *b* = 9.8110(5) Å, *c* = 34.4440(18) Å; *V* = 2251.3(2) Å$^3$, *T* = 100 K, *Z* = 4, $D_{calcd}$ = 1.403 gcm$^{-3}$, crystal size 0.42 x 0.04 x 0.02 mm$^3$, *F*(000) = 1000. The final $R_1$ value is 0.0600 (w$R_2$ = 0.1664) for 2773 reflections [$I>2\sigma(I)$]. The absolute configuration was determined on the basis of Flack parameter[161] of –0.02(12), refined using 1930 Friedel pairs.

**Compound 4 :** Light yellowish amorphous solid; mp 158–160 $^oC$; $[\alpha]^{25}_D$ +156 (*c* 0.72, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (3.75), 245 (3.63), and 303 (4.24) nm; IR (dry film) $\upsilon_{max}$ 3349, 1727 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.5; ESIMS *m/z* 365 (MH$^+$); HRESIMS *m/z* 365.1858 (calcd for $C_{22}H_{24}N_2O_3$ + H, 365.1860). HMBC: $^2J$ H(3) to C(14); H(5) to C(6); H(6) to C(5), C(7); H(10) to C(11); H(12) to C(11), C(13); H(15) to C(14), C(20); H(16) to C(2), C(17), *C*$O_2$Me; H(17) to C(16), C(20); H(18) to C(2), C(19); H(19) to C(20); H(21) to C(7), C(20); CHO to C(14). $^3J$ H(3) to *C*HO, C(5), C(15), C(21); H(5) to C(21); H(6) to C(2), C(8), C(21); H(9) to C(7), C(11),

C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(3), C(17), C(21), *C*HO; H(16) to C(7), C(18); H(17) to C(2), C(15), C(19), C(21), *C*$O_2$Me; H(18) to C(7), C(16), C(20); H(19) to C(2), C(15), C(17), C(21); H(21) to C(3), C(5), C(6), C(8), C(15), C(17), C(19); $CO_2$*Me* to *C*$O_2$Me; CHO to C(3), C(15).

**Compound 5 :** Light yellowish oil; $[\alpha]^{25}_D$ +123 (*c* 0.16, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 210 (3.92), 242 (3.67), 257 (3.41), and 293 (3.27) nm; IR (dry film) $\upsilon_{max}$ 3336, 1736 $cm^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.6; ESIMS *m/z* 323 ($MH^+$); HRESIMS *m/z* 323.1758 (calcd for $C_{20}H_{22}N_2O_2$ + H, 323.1754). HMBC: $^2J$ H(3) to C(14); H(5) to C(6); H(10) to C(9), C(11); H(11) to C(12); H(12) to C(11); H(14) to C(15); H(15) to C(14); H(16) to C(2), C(22); H(17) to C(16), C(20); H(18) to C(2), C(19); H(19) to C(18); H(21) to C(7). $^3J$ H(3) to C(5), C(15), C(21); H(5) to C(21); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(3), C(21); H(16) to C(7); H(17) to C(19), C(21), C(22); H(18) to C(7), C(16), C(20); H(19) to C(21); H(21) to C(3), C(5), C(6), C(8); NH to C(7), C(8).

**Compound 6 :** Light yellowish oil; $[\alpha]^{25}_D$ +208 (*c* 0.12, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (3.76), 243 (3.35), 257 (3.08), and 295 (2.96) nm; IR (dry film) $\upsilon_{max}$ 3332, 1756, 1698 $cm^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.7; ESIMS *m/z* 337 ($MH^+$); HRESIMS *m/z* 337.1552 (calcd for $C_{20}H_{20}N_2O_3$ + H, 337.1547). HMBC: $^2J$ H(3) to C(14); H(6) to C(5), C(7); H(10) to C(9), C(11); H(11) to C(10); H(14) to C(3), C(15); H(15) to C(20); H(16) to C(2), C(17), C(22); H(17) to C(16), C(20); H(18) to C(19); H(19) to C(18), C(20); H(21) to C(7), C(20). $^3J$ H(3) to C(5), C(15), C(21); H(6) to C(8), C(21), C(22); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(14) to C(20); H(15) to C(3), C(17), C(19), C(21); H(16)

to C(7); H(17) to C(2), C(19), C(21), C(22); H(18) to C(7), C(16), C(20); H(19) to C(2), C(15), C(17), C(21); H(21) to C(5), C(6), C(8), C(19); NH to C(7).

**Compound 7 :** Light yellowish oil; $[\alpha]^{25}_{D}$ +88 (*c* 0.11, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 212 (4.00), 248 (3.46), and 282 (2.86) nm; IR (dry film) $\upsilon_{max}$ 1756, 1693 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.8; ESIMS *m/z* 409 ($MH^+$); HRESIMS *m/z* 409.1754 (calcd for $C_{23}H_{24}N_2O_5$ + H, 409.1758). HMBC: $^{2}J$ H(6) to C(5), C(22); H(9) to C(10); H(10) to C(11); H(11) to C(10), C(12); H(14) to C(15); H(15) to C(14), C(20); H(16) to C(17); H(17) to C(20); H(18) to C(2), C(19); H(19) to C(18), C(20); H(21) to C(7), C(20). $^{3}J$ H(3) to C(15), C(21); H(6) to C(8), C(21); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(14) to C(20); H(15) to C(3), C(17), C(19), C(21); H(16) to C(7), C(20); H(17) to C(15), C(19), C(21), C(22); H(18) to C(7), C(16), C(20); H(19) to C(2), C(15), C(17), C(21); H(21) to C(5), C(6), C(8), C(15), C(17); $NCO_2$*Me* to N*C*$O_2$Me; 12-OMe to C(12).

**Compound 8 :** Light yellowish oil and subsequently light orange block crystals ($CH_2Cl_2$– MeOH); mp 190−192 °C; $[\alpha]^{25}_{D}$ +414 (*c* 0.26, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 210 (4.25), 234 (4.55), 257 (3.96), 308 (3.36), and 376 (3.40) nm; IR (dry film) $\upsilon_{max}$ 2855, 2799, 1610 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.9; ESIMS *m/z* 311 ($MH^+$); HRESIMS *m/z* 311.1756 (calcd for $C_{19}H_{22}N_2O_2$ + H, 311.1754). HMBC: $^{2}J$ H(5) to C(6); H(6) to C(2), C(5); H(10) to C(11); H(14) to C(15); H(15) to C(14), C(20); H(16) to C(17); H(17) to C(16), C(20); H(18) to C(19); H(19) to C(20). $^{3}J$ H(3) to C(15), C(21); H(5) to C(2), C(3), C(21); H(6) to C(7), C(21); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9); H(12) to C(8), C(10); H(14) to C(20); H(15) to C(3), C(17), C(21); H(16) to C(2), C(19), C(20); H(17) to C(15), C(19), C(21); H(18) to C(20); H(19) to C(15), C(21); H(21) to C(3), C(7), C(17). NOE: H(3β)/H(3α),

H(14), H(21); H(3α)/H(3β), H(14); H(5β)/H(3β), H(5α), H(18); H(6)/H(5); H(9)/H(10); H(12)/H(11), H(16); H(14)/H(3α,β), H(15α); H(15β)/H(14), H(19); H(16)/H(17α,β); H(17β)/H(16), H(17α), H(19); H(17α)/H(16), H(17β); H(18)/H(15β), H(19), H(21); H(19)/H(15β), H(17β), H(18); H(21)/H(18).

**Crystallographic data of 8 :** Light orange block crystals, $C_{19}H_{22}N_2O_2$, *M*r = 310.39, monoclinic, space group $P2_1$, $a$ = 14.2796(4) Å, $b$ = 7.9986(2) Å, $c$ = 15.7780(5) Å; $\alpha = \gamma = 90^o$, $\beta = 116.781(2)^o$, $V$ = 1608(8) Å$^3$, $T$ = 100 K, $Z$ = 4, $D_{calcd}$ = 1.281 gcm$^{-3}$, crystal size 0.10 x 0.22 x 0.72 mm$^3$, $F(000)$ = 664. The final $R_1$ value is 0.0409 (w$R_2$ = 0.0916) for 3270 reflections [$I>2\sigma(I)$].

**Compound 9 :** Colorless oil; $[\alpha]^{25}_D$ −152 (*c* 0.11, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (4.12), 243 (4.37), 282 (3.91), and 299 (3.79) nm; IR (dry film) $\upsilon_{max}$ 1707, 1630 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.10; ESIMS *m/z* 309 ($MH^+$); HRESIMS *m/z* 309.1601 (calcd for $C_{19}H_{20}N_2O_2$ + H, 309.1598). HMBC: $^2J$ H(3) to C(14); H(5) to C(6); H(6) to C(5), C(7); H(10) to C(9); H(11) to C(12); H(17) to C(16), C(20); H(18) to C(19); H(21) to C(2), C(20). $^3J$ H(5) to C(3), C(7), C(21); H(6) to C(2); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(19); H(17) to C(15), C(19); H(18) to C(20); H(21) to C(3), C(7), C(15).

**Compound 10 :** Light yellowish amorphous solid; mp 178–180 $^o$C; $[\alpha]^{25}_D$ −17 (*c* 0.82, MeOH); UV (EtOH) $\lambda_{max}$ (log ε) 209 (3.91), 227 (3.99), and 277 (3.43) nm; IR (dry film) $\upsilon_{max}$ 3315, 1704 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.11; ESIMS *m/z* 311 ($MH^+$); HRESIMS *m/z* 311.1759 (calcd for $C_{19}H_{22}N_2O_2$ + H, 311.1754). HMBC: $^2J$ H(6) to C(5), C(7); H(17) to C(16), C(20); H(18) to C(19); H(21) to C(2), C(20). $^3J$ H(3) to C(15); H(5) to C(3), C(7), C(21); H(9) to C(11), C(13); H(10) to C(8); H(11) to

C(9), C(13); H(12) to C(8), C(10); H(15) to C(17); H(16) to C(20); H(17) to C(15), C(21); H(21) to C(3), C(15).

**Compound 11 :** Light yellowish oil and subsequently light orange block crystals ($CH_2Cl_2$–MeOH); mp 162–164 $^oC$; $[\alpha]^{25}_D$ –157 (*c* 0.07, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 225 (4.21), 258 (4.29), 302 (3.79), and 309 (3.79) nm; IR (dry film) $\upsilon_{max}$ 3364 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.12; ESIMS *m/z* 295 ($MH^+$); HRESIMS *m/z* 295.1806 (calcd for $C_{19}H_{22}N_2O$ + H, 295.1805). HMBC: $^2J$ H(3) to C(14); H(5) to C(6); H(6) to C(5), C(7); H(9) to C(8); H(14) to C(15); H(15) to C(14), C(20); H(16) to C(17); H(17) to C(16), C(20); H(18) to C(19); H(19) to C(20); H(21) to C(2), C(20). $^3J$ H(3) to C(15), C(21); H(5) to C(3), C(7), C(21); H(6) to C(2); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(3), C(17), C(19), C(21); H(16) to C(2), C(20); H(17) to C(19), C(21); H(18) to C(20); H(19) to C(15), C(17), C(21); H(21) to C(3), C(7), C(15), C(19).

**Crystallographic data of 11 :** Light orange block crystals, $C_{19}H_{22}N_2O$, *M*r = 294.39, monoclinic, space group $P2_1$, *a* = 8.6391(10) Å, *b* = 7.9260(10) Å, *c* = 11.5438(2) Å; $\alpha = \gamma = 90^o$, $\beta = 98.4100(10)^o$, *V* = 781.945(19) $Å^3$, *T* = 100 K, *Z* = 2, $D_{calcd}$ =1.250 $gcm^{-3}$, crystal size 0.15 x 0.16 x 0.34 $mm^3$, *F*(000) = 316. The final $R_1$ value is 0.0373 (w$R_2$ = 0.1035) for 1705 reflections [$I>2\sigma(I)$].

**Compound 12 :** Light yellowish oil and subsequently colorless block crystals ($CH_2Cl_2$–hexanes); mp 150–152 $^oC$; $[\alpha]^{25}_D$ –47 (*c* 0.19, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 226 (4.11) and 280 (3.75) nm; IR (dry film) $\upsilon_{max}$ 3395 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.13; ESIMS *m/z* 341 ($MH^+$); HRESIMS *m/z* 341.2220 (calcd for $C_{21}H_{28}N_2O_2$ + H, 341.2224). HMBC: $^2J$ H(6) to C(5), C(7); H(9) to C(8); H(15) to

C(14), C(20); H(17) to C(16), C(20); H(18) to C(19); H(21) to C(2), C(20); H(22) to C(23); H(23) to C(22). $^{3}J$ H(3) to C(15), C(21); H(5) to C(3), C(7), C(21); H(6) to C(2); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(15) to C(19); H(16) to C(20), C(22); H(17) to C(15), C(19), C(21); H(18) to C(20); H(19) to C(15), C(17); H(21) to C(3), C(7), C(15), C(19); H(22) to C(16).

**Crystallographic data of 12 :** Colorless block crystals, $C_{21}H_{28}N_2O_2$, *M*r = 340.45, orthorhombic, space group $P2_12_12_1$, $a$ = 8.4666(3) Å, $b$ = 12.1895(4) Å, $c$ = 16.9938(6) Å; $V$ = 1753.82(10) Å$^3$, $T$ = 100 K, $Z$ = 4, $D_{calcd}$ =1.289 gcm$^{-3}$, crystal size 0.41 x 0.09 x 0.06 mm$^3$, $F$(000) = 736. The final $R_1$ value is 0.0516 (w$R_2$ = 0.1050) for 1497 reflections [$I>2\sigma(I)$].

**Tetrahydroalstonine (13) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −53 (*c* 0.10, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 227 (4.31), 247 (3.77), 282 (3.64), and 291 (3.49) nm; IR (dry film) $\nu_{max}$ 3370, 1703 cm$^{-1}$. $^{1}$H NMR and $^{13}$C NMR data, see Table 2.15; ESIMS *m/z* 353 ($MH^+$, $C_{21}H_{24}N_2O_3$ + H).

**Leuconoxine (14) :** Colorless oil; $[\alpha]^{25}_{D}$ −71 (*c* 0.08, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 205 (4.42), 244 (3.82), 244 (3.82), and 280 (3.16) nm; IR (dry film) $\nu_{max}$ 1695, 1677 cm$^{-1}$; $^{1}$H NMR and $^{13}$C NMR data, see Table 2.16; ESIMS *m/z* 325 ($MH^+$, $C_{19}H_{22}N_2O_2$+H).

***N*(1)-Carbomethoxy-5,22-dioxokopsane (15) :** Colorless oil; $[\alpha]^{25}_{D}$ +73 (*c* 0.12, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 210 (4.38), 241 (4.11), and 281 (3.46) nm; IR (dry film) $\nu_{max}$ 1757, 1713, 1687 cm$^{-1}$; $^{1}$H NMR and $^{13}$C NMR data, see Tables 2.17 and 2.18, respectively; ESIMS, *m/z* 379 ($MH^+$, $C_{22}H_{22}N_2O_4$+H).

**Kopsanone (16) :** Colorless oil; $[\alpha]^{25}_{D}$ +126 (*c* 0.20, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 207 (4.38), 244 (3.84), and 294 (3.50) nm; IR (dry film) $\nu_{max}$ 3336, 1738 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.17 and 2.18, respectively; ESIMS, *m/z* 307 ($MH^+$, $C_{20}H_{22}N_2O$ +H).

**Kopsifine (17) :** Colorless oil; $[\alpha]^{25}_{D}$ +97 (*c* 0.04, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 223 (4.41), 250 (3.94), 285 (3.16), and 295 (3.10) nm; IR (dry film) $\nu_{max}$ 3295, 1765, 1684 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.17 and 2.18, respectively; EIMS *m/z* 438 ($M^+$, $C_{23}H_{22}N_2O_7$).

**Decarbomethoxykopsifine (18) :** Colorless oil; $[\alpha]^{25}_{D}$ +52 (*c* 0.07, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 220 (4.72), 243 (4.23), and 288 (3.51) nm; IR (dry film) $\nu_{max}$ 3344, 1761, 1682 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.19; EIMS *m/z* 380 ($M^+$, $C_{21}H_{20}N_2O_5$).

**Paucidactine B (19) :** Colorless oil; $[\alpha]^{25}_{D}$ +2 (*c* 0.11, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 224 (4.71), 244 (4.30), 285 (3.60), and 295 (3.52) nm; IR (dry film)$\nu_{max}$ 3333, 1766, 1703, 1691 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.19; ESIMS *m/z* 455 ($MH^+$, $C_{23}H_{22}N_2O_8$ + H).

**Kopsamine (20) :** Colorless crystals ($CHCl_3$–$Et_2O$); mp 200–201 ℃; $[\alpha]^{25}_{D}$ –47 (*c* 0.21, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 227 (4.36), 248 (3.92), and 286 (2.92) nm; IR (dry film) $\nu_{max}$ 3315, 1736, 1684 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.20; ESIMS *m/z* 457 ($MH^+$, $C_{24}H_{28}N_2O_7$ + H).

**Kopsamine *N*-oxide (21) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −29 (*c* 0.36, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 227 (4.15), 246 (3.69), and 286 (2.15) nm; IR (dry film) $\nu_{max}$ 3292, 1732, 1684 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.20; ESIMS, *m/z* 473 ($MH^+$, $C_{24}H_{28}N_2O_8$ + H).

**Kopsinine (22) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −68 (*c* 0.24, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 205 (4.56), 246 (3.88), and 296 (3.48) nm; IR (dry film) $\nu_{max}$ 3350, 1728 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.21 and 2.22, respectively; ESIMS, *m/z* 339 ($MH^+$, $C_{21}H_{26}N_2O_2$ + H).

***N*(1)-Methoxycarbonyl-12-methoxy-Δ$^{16,17}$-kopsinine (23) :** Light yellowish needles (EtOH); mp 150−152 ℃; $[\alpha]^{25}_{D}$ −60 (*c* 0.10, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 218 (4.50), 251 (4.01), and 282 (3.45) nm; IR (dry film) $\nu_{max}$ 1717 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.21 and 2.22, respectively; ESIMS *m/z* 425 ($MH^+$, $C_{24}H_{28}N_2O_5$ + H).

***N*(1)-Methoxycarbonyl-12-hydroxy-Δ$^{16,17}$-kopsinine (24) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −121 (*c* 0.04, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 218 (3.98), 248 (3.61), and 290 (3.13) nm; IR (dry film) $\nu_{max}$ 3400, 1716, 1674 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.21 and 2.22, respectively; EIMS *m/z* 410 ($M^+$, $C_{23}H_{26}N_2O_5$).

**Kopsinine *N*-oxide (25) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −56 (*c* 0.26, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 204 (4.31), 243 (3.77), and 294 (3.39) nm; IR (dry film) $\nu_{max}$3347, 1725 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.23 and 2.24, respectively; ESIMS, *m/z* 355 ($MH^+$, $C_{21}H_{26}N_2O_3$ + H).

***N*(1)-Methoxycarbonyl-11,12-dimethoxykopsinaline (26) :** Colorless prisms ($Et_2O$); mp 167–168 ℃; $[\alpha]^{25}_D$ −21 (*c* 0.09, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 223 (4.44), 251 (3.91), 286 (3.31), and 292 (3.32) nm; IR (dry film) $\nu_{max}$ 3323, 1736, 1677 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Tables 2.23 and 2.24, respectively; ESIMS, *m/z* 473 ($MH^+$, $C_{25}H_{32}N_2O_7$ + H).

**Kopsilongine (27) :** Light yellowish oil; $[\alpha]^{25}_D$ −21 (*c* 0.09, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 217 (4.34), 254 (3.92), 282 (3.22), and 288 (3.20) nm; IR (dry film) $\nu_{max}$ 3318, 1737, 1675 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Tables 2.23 and 2.24, respectively; ESIMS *m/z* 443 ($MH^+$, $C_{24}H_{30}N_2O_6$ + H).

**Pleiocarpine (28) :** Colorless prisms ($Et_2O$); mp 149–150 ℃; $[\alpha]^{25}_D$ −169 (*c* 0.08, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 204 (4.45), 244 (3.88), 281 (3.99), and 290 (3.51) nm; IR (dry film) $\nu_{max}$ 1736, 1674 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Tables 2.25 and 2.26, respectively; ESIMS, *m/z* 397 ($MH^+$, $C_{23}H_{28}N_2O_4$ + H).

**12-Methoxypleiocarpine (29) :** Colorless oil; $[\alpha]^{25}_D$ −82 (*c* 0.15, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 217 (4.40), 253 (3.99), and 283 (3.50) nm; IR (dry film) $\nu_{max}$ 1723, 1699 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Tables 2.25 and 2.26, respectively; ESIMS *m/z* 427 ($MH^+$, $C_{24}H_{30}N_2O_5$ + H).

**Pleiocarpine *N*-oxide (30) :** Light yellowish oil; $[\alpha]^{25}_D$ −103 (*c* 0.27, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 208 (3.45), 244 (3.19), 256 (2.81), and 284 (2.31) nm; IR (dry film) $\upsilon_{max}$ 1709 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Tables 2.25 and 2.26, respectively; ESIMS *m/z* 413 ($MH^+$, $C_{23}H_{28}N_2O_5$ + H).

**(+)-Eburnamenine (31) :** Colorless oil; $[\alpha]^{25}_{D}$ +216 (*c* 0.07, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 223 (3.95), 259 (3.98), 303 (3.42), 310 (3.44), and 362 (2.48) nm; IR (dry film) $\nu_{max}$ 1638 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.27; EIMS *m/z* 278 ($M^+$, $C_{19}H_{22}N_2$).

**(+)-Eburnamonine (32) :** Light yellowish oil; $[\alpha]^{25}_{D}$ +108 (*c* 0.24, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 207 (4.40), 246 (4.46), 270 (4.18), and 302 (3.91) nm; IR (dry film) $\nu_{max}$ 1716 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.27; ESIMS *m/z* 295 ($MH^+$, $C_{19}H_{22}N_2O$ + H).

**(–)-Eburnamine (33) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −77 (*c* 0.11, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 205 (4.13), 229 (4.30), 282 (3.79), and 292 (3.67) nm; IR (dry film) $\nu_{max}$ 3325$cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.28; EIMS *m/z* 296 ($M^+$, $C_{19}H_{22}N_2O$).

**(+)-Isoeburnamine (34) :** Light yellowish oil; $[\alpha]^{25}_{D}$ +93 (*c* 0.12, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 207 (3.66), 230 (3.92), 283 (3.32), and 290 (3.23) nm; IR (dry film) $\nu_{max}$ 3315 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.28; ESIMS, *m/z* 297 ($MH^+$, $C_{19}H_{25}N_2O$ +H).

**(+)-19-Oxoeburnamine (35) :** Light yellowish oil; $[\alpha]^{25}_{D}$ +83 (*c* 0.06, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 202 (3.81), 229 (4.00), 282 (3.39), and 292 (3.25) nm; IR (dry film) $\nu_{max}$ 3324, 1702 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.29 and 2.30, respectively; EIMS *m/z* 310 ($M^+$, $C_{19}H_{22}N_2O_2$).

**(–)-19(*R*)-Hydroxyisoeburnamine (36) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −16 (*c* 0.18, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 203 (4.39), 229 (4.53), 282 (3.74), and 292 (3.83) nm; IR (dry film) $\nu_{max}$ 3296 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.29 and 2.30, respectively; EIMS, *m/z* 312 ($M^+$, $C_{19}H_{24}N_2O_2$).

**(+)-19(*R*)-Hydroxyeburnamine (37) :** Colorless crystals (EtOH); mp 246-248 $^{o}C$; $[\alpha]^{25}_{D}$ +111 (*c* 0.09, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 201 (3.86), 229 (4.02), 283 (3.42), and 291 (3.31) nm; IR (dry film) $\nu_{max}$ 3298 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.29 and 2.30, respectively; EIMS, *m/z* 312 ($M^+$, $C_{19}H_{24}N_2O_2$).

**(–)-Norpleiomutine (38) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −49 (*c* 1.21, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 208 (4.69), 229 (4.51), 255 (4.09), 287 (3.97), and 293 (3.97) nm; IR (dry film) $\nu_{max}$ 3348, 1730 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.31; ESIMS *m/z* 617 ($MH^+$, $C_{40}H_{48}N_4O_2$+ H).

**(–)-Demethylnorpleiomutine (39) :** Light yellowish oil; $[\alpha]^{25}_{D}$ −87 (*c* 1.21, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (4.48), 230 (4.34), 254 (3.99), 287 (3.89), and 292 (3.84) nm; IR (dry film) $\nu_{max}$ 3341, 1720 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.32; ESIMS *m/z* 603 ($MH^+$, $C_{39}H_{46}N_4O_2$+ H).

**(+)-Kopsoffinol (40) :** Light yellowish oil; $[\alpha]^{25}_{D}$ +22 (*c* 0.43, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 211 (4.57), 231 (4.49), 254 (4.06), 287 (3.94), and 293 (3.94) nm; IR (dry film) $\nu_{max}$ 3340, 1728 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.33; ESIMS *m/z* 633 ($MH^+$, $C_{40}H_{48}N_4O_3$+ H).

**Alkaloids from *K. grandifolia***

**Grandilodine A (41) :** Light yellowish oil and subsequently light yellowish block crystals ($CH_2Cl_2$–MeOH); mp 120–122 $^oC$; $[\alpha]^{25}_D$ –76 (*c* 1.46, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (3.04), 253 (2.71), and 289 (2.13) nm; IR (dry film) $\upsilon_{max}$ 1731, 1704 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.35; ESIMS *m/z* 443 ($MH^+$); HRESIMS *m/z* 443.21880 (calcd for $C_{24}H_{30}N_2O_6$ + H, 443.21821). HMBC: $^2J$ H(6) to C(5), C(7); H(9) to C(8); H(10) to C(11); H(11) to C(10), C(12); H(15) to C(14); H(17) to C(20); H(18) to C(2), C(19); H(19) to C(18), C(20). $^3J$ H(5) to C(7), C(20); H(6) to C(2), C(21); H(9) to C(7), C(11), C(13); H(10) to C(12); H(11) to C(13); H(17) to C(15), *C*$O_2$Me; H(19) to C(15); 21-OMe to C(21); $CO_2$*Me* to *C*$O_2$Me; $NCO_2$*Me* to N*C*$O_2$Me. NOESY: H(6α)/H(9); H(6β)/H(5); H(9)/H(6α), H(10); H(10)/H(9), H(11); H(15β)/H(15α), H(17α); H(16)/H(17α), H(19β); H(17α)/H(15β), H(16), $CO_2$*Me*; H(18α)/H(19α), $NCO_2$*Me*; H(19β)/H(19α), H(16); H(19α)/$NCO_2$*Me;* $CO_2$*Me*/H(17α); $NCO_2$*Me*/ H(18α), H(19α).

**Crystallographic data of 41 :** Light yellowish block crystals, $C_{24}H_{30}N_2O_6$, *M*r = 442.50, orthorhombic, space group $P2_12_12_1$, *a* = 8.0067(2) Å, *b* = 11.2455(3) Å, *c* = 24.1247(7) Å, *V* = 2172.17(10) $Å^3$, *T* = 100 K, *Z* = 4, $D_{calcd}$ = 1.353 $gcm^{-3}$, crystal size 0.15 x 0.20 x 0.61 $mm^3$, *F*(000) = 944. The final $R_1$ value is 0.0465 (w$R_2$ = 0.1028) for 2913 reflections [$I>2\sigma(I)$].

**Grandilodine B (42) :** White amorphous solid and subsequently colorless block crystals ($CH_2Cl_2$–hexanes); mp 204–206 $^oC$; $[\alpha]^{25}_D$ +66 (*c* 0.39, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 210 (3.80), 252 (3.48), and 286 (2.78) nm; IR (dry film) $\upsilon_{max}$ 1734, 1690 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, see Table 2.36; ESIMS *m/z* 455 ($MH^+$); HRESIMS

*m/z* 455.1836 (calcd for $C_{24}H_{26}N_2O_7$ + H, 455.1813). HMBC: $^2J$ H(5) to C(6); H(6) to C(5), C(7); H(9) to C(10); H(10) to C(11); H(11) to C(10), C(12); H(12) to C(11), C(13); H(14) to C(3), C(15); H(15) to C(14), C(20); H(16) to C(2), C(17), *C*$O_2$Me; H(17) to C(16), C(20); H(18) to C(2), C(19); H(19) to C(18), C(20). $^3J$ H(5) to C(3), C(7), C(20); H(6) to C(2), C(8); H(9) to C(7), C(11), C(13); H(10) to C(8), C(12); H(11) to C(9), C(13); H(12) to C(8), C(10); H(14) to C(20); H(15) to C(3), C(17), C(19); H(16) to C(18), C(20); H(17) to C(2), C(15), C(19), *C*$O_2$Me; H(18) to C(7), C(16), C(20); H(19) to C(2), C(15); 21-OMe to C(21); $CO_2$*Me* to *C*$O_2$Me; $NCO_2$*Me* to N*C*$O_2$Me. NOESY: H(6β)/H(16); H(9)/H(6α), H(10); H(10)/H(9); H(12)/H(11); H(16)/H(6β), H(17β); H(17β)/H(16).

**Crystallographic data of 42 :** Colorless block crystals, $C_{24}H_{26}N_2O_7$, *M*r = 454.47, monoclinic, space group $P2_1$, $a$ = 8.6505(2) Å, $b$ = 8.0985(2) Å, $c$ = 15.3926(4) Å, $\alpha = \gamma = 90^o$, $\beta$ = 90.0057(2)$^o$, $V$ = 1078.34(5) Å$^3$, $T$ = 100 K, $Z$ = 2, $D_{calcd}$ = 1.400 gcm$^{-3}$, crystal size 0.08 x 0.28 x 0.47 mm$^3$, $F$(000) = 480. The final $R_1$ value is 0.0335 (w$R_2$ = 0.0790) for 2351 reflections [$I>2\sigma(I)$].

**Grandilodine C (43) :** Light yellowish oil; $[\alpha]^{25}_D$ +61 (*c* 0.55, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (4.08), 241 (3.99), and 286 (3.25) nm; IR (dry film) $\upsilon_{max}$ 1772, 1691 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, see Table 2.37; ESIMS *m/z* 381 ($MH^+$); HRESIMS *m/z* 381.14544 (calcd for $C_{21}H_{20}N_2O_5$ + H, 381.14450). HMBC: $^2J$ H(5) to C(6); H(6) to C(5), C(7); H(10) to C(11); H(11) to C(12); H(12) to C(11); H(14) to C(3), C(15); H(15) to C(14), C(20); H(17) to C(16), C(20). $^3J$ H(5) to C(3), C(7), C(20); H(6) to C(2), C(8); H(9) to C(7), C(11), C(13); H(10) to C(12); H(11) to C(9), C(13); H(14) to C(20); H(15) to C(3), C(17), C(19); H(17) to C(2), C(19); $NCO_2$*Me* to N*C*$O_2$Me.

**Lapidilectine A (44) :** White amorphous solid; mp 79–81 ℃; $[\alpha]^{25}_{D}$ –34 (*c* 2.02, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (3.61), 225 (3.47), 253 (3.35), and 289 (2.78) nm; IR (dry film) $\nu_{max}$ 1728, 1701 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.39 and 2.40, respectively; ESIMS, *m/z* 441 ($MH^+$, $C_{24}H_{28}N_2O_6$ + H).

**Isolapidilectine A (45) :** Light yellowish amorphous solid; mp 80–82 ℃; $[\alpha]^{25}_{D}$ +89 (*c* 1.76, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 211 (2.93), 228 (2.67), 252 (2.46), and 283 (1.91) nm; IR (dry film) $\nu_{max}$ 1725, 1701 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.39 and 2.40, respectively; ESIMS, *m/z* 441 ($MH^+$, $C_{24}H_{28}N_2O_6$ + H).

**Lapidilectam (46) :** Light yellowish oil; $[\alpha]^{25}_{D}$ +128 (*c* 0.47, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (4.02), 228 (3.84), 254 (3.69), and 288 (2.99) nm; IR (dry film) $\nu_{max}$ 1728, 1694 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Tables 2.39 and 2.40, respectively; ESIMS, *m/z* 455 ($MH^+$, $C_{24}H_{26}N_2O_7$ + H).

**Lapidilectine B (47) :** Orange amorphous solid; mp 188–190 ℃; $[\alpha]^{25}_{D}$ +23 (*c* 0.45, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 209 (2.60), 242 (2.48), and 282 (1.65) nm; IR (dry film) $\nu_{max}$ 1757, 1705 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR data, see Table 2.41; ESIMS, *m/z* 367 ($MH^+$, $C_{21}H_{22}N_2O_4$ + H).

### 3.8 Catalytic Hydrogenation of lapidilectine A (44)

**General Procedure :** Lapidilectine A (**44**) (15.3 mg, 0.035 mmol) was dissolved in MeOH (5 mL) and then stirred over 10% Pd/C (3 mg) under hydrogen atmosphere at room temperature for 1 h. The catalyst was removed by filtration over celite.

Evaporation of the solvent in vacuo, followed by chromatography of the resulting residue (silica gel, $CHCl_3$–MeOH) provided grandilodine A (**41**) (10.6 mg, 69%).

### 3.9 Cytotoxicity Assays

Cytotoxicity assays were carried out on KB (human oral epidermoid carcinoma) cell line. The cells were maintained in culture flasks in Eagle's MEM, supplemented with 10% fetal calf serum and kanamycin (60 $\mu$g/mL). The KB cells ($1.5\times10^5$ /mL) were seeded in 0.2 ml of culture medium/well in 96-well plates (Corning Glass Works). The cells were treated in triplicate with graded concentrations of 5 $\mu$L test samples and were then incubated in a 5% carbon dioxide atmosphere at 37 ℃ for 72 h. The MTT assay was used to measure the cytotoxicity effect.[162] The activity was shown as the $IC_{50}$ value, which was the concentration ($\mu$g/mL) of test compound to give 50% inhibition of cell growth.