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Structure Elucidation and Chiral-Total Synthesis of a New Indole Alkaloid, (−)-9-Methoxymitralactonine, Isolated from *Mitragyna speciosa* in Malaysia

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Abstract—A new Corynanthe-type indole alkaloid, (−)-9-methoxymitralactonine (**1**), having a highly conjugated system was isolated from the young leaves of *Mitragyna speciosa* in Malaysia, and its structure was first deduced by spectroscopic analysis and then confirmed by chiral-total synthesis starting from optically pure epoxy-ketone and 5-methoxy-3,4-dihydro-β-carboline. The chiral HPLC analysis demonstrated that the natural 9-methoxymitralactonine contained predominantly the (−)-enantiomer over the (+)-enantiomer in the ratio of 62:38.

Introduction

Recently, we have reported the structure elucidation and total synthesis of a new indole alkaloid, (−)-mitralactonine (**2**),[1] obtained from the young leaves of Malaysian *Mitragyna speciosa* Korth. (Rubiaceae), which is an original plant for traditional folk medicine in Malay Peninsula, used for a stimulant like coca or as a substitute for opium.[2] By continuous effort for further investigation of the minor constituents of this plant,[3] we were able to find a new mitralactonine-related alkaloid. In this paper, we describe the isolation, structure elucidation, chiral-total synthesis, and analysis of the enantiomeric excess of this new Corynanthe-type indole alkaloid, (−)-9-methoxymitralactonine (**1**).

Results and Discussion

From the ethyl acetate extract of the young leaves of *M. speciosa* in Malaysia, 10 Corynanthe-type indole alkaloids, i.e. mitragynine, speciogynine, speciociliatine, paynantheine, 7α-hydroxy-7*H*-mitragynine,[3a] mitragynaline,[2b] corynantheidaline, corynantheidine, isocorynoxeine, and mitralactonine (**2**) were isolated. In addition, a porphine derivative, phaeophorbide a,[4] was obtained. Furthermore, a new indole alkaloid (**1**) could be isolated as a minor component and its structure was elucidated as follows (Fig. 1).

The new compound **1** was obtained as an orange amorphous powder, exhibiting $[\alpha]_D^{25}=-123$ (*c* 0.19, $CHCl_3$). The ^1H and ^{13}C NMR spectra of **1** showed the presence of a 9-methoxyindole nucleus, an ethane-bridge at C5–C6, an ethyl group at C-20, and a methoxycarbonyl group, which are the fundamental structural units in the common Corynanthe-type monoterpenoid indole alkaloids. The UV spectrum exhibited a long wavelength absorption at 462 nm, indicating a long conjugation in the molecule. The ^{13}C NMR and HMBC spectra disclosed the presence of six conjugated sp^2 carbons including an ester and a lactone carbonyl carbons, besides the aromatic carbons due to the indole nucleus. The quite characteristic proton signal observed at δ 6.39 (1H, singlet) was unambiguously assigned to be the proton at C14 by the HMQC spectrum, and this signal has the HMBC connectivities between the C2, C3, C15, C16 and C20 carbons. The molecular formula ($C_{22}H_{22}O_5N_2$) obtained from high-resolution mass spectrum, as well as the fact that the carbon signal at C20 resonated at δ

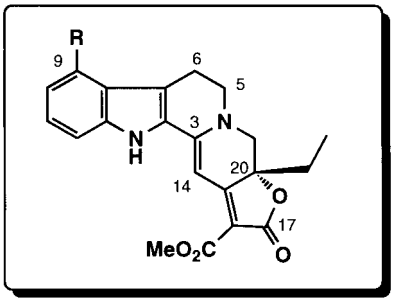

Figure 1. R=OMe: 9-methoxymitralactonine (**1**); R=H: mitralactonine (**2**).

Keywords: alkaloids; indoles; asymmetric synthesis.
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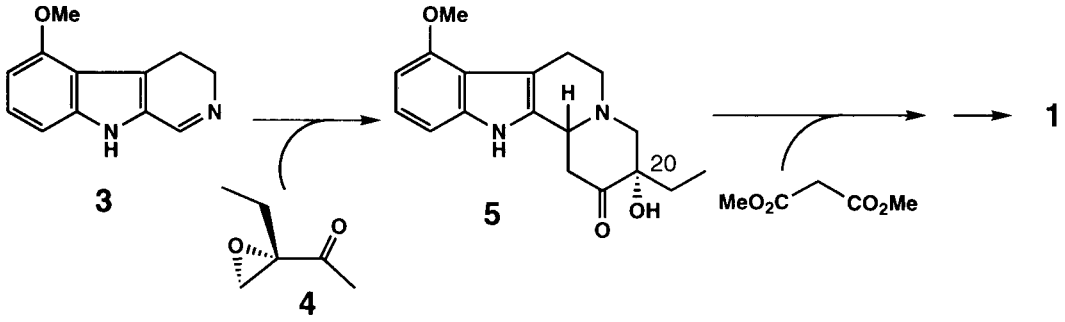


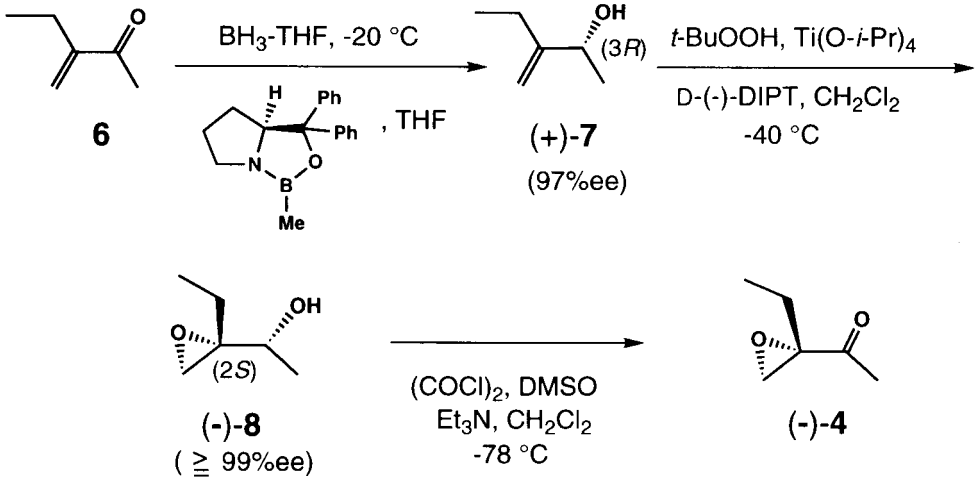
Scheme 1.

77.4 ppm, showed the presence of a lactone function constructed between the oxygen atom on C20 and the carbonyl group at the C17 position. All the above findings, as well as biogenetic consideration enabled us to compose the molecular structure of the new alkaloid to be the formula **1** having a highly conjugated pentacyclic 9-methoxy-Corynanthe-skeleton. In the ^{13}C NMR spectrum, the sp^2 carbons at C14 and C16 resonate at unusually high positions at δ 87.08 and 94.91 ppm, respectively. This abnormal phenomenon, as well as the novel chemical structure of **1**, prompted us to synthesize the new alkaloid in a chiral manner in order to confirm the structure including the absolute configuration due to one chiral center at the C20 position.

Our basic approach to **1**, which features the assembly of three fragments, i.e. 5-methoxy-3,4-dihydro-β-carboline (**3**), a chiral epoxy-ketone (**4**), and dimethyl malonate, is outlined in Scheme 1.

First, we started with the preparation of the chiral epoxy-ketone (**4**), which is an essential synthon for the construction of the functionalized tetracyclic compound (**5**). Synthesis of optically pure **4** was achieved by a combination of Corey asymmetric reduction and Sharpless asymmetric epoxidation as follows. By reduction of the enone (**6**)[5] using a chiral oxazaborolidine catalyst (0.7 equiv. of BH_3, 0.2 equiv. of (*S*)-5,5-diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine, −20°C),[6] an optically active alcohol (+)-(**7**) {$[\alpha]_D^{23}$=+9.5 (*c* 0.35, $CHCl_3$)} was obtained in 97% ee. The enantiomeric excess of **7** was determined by chiral HPLC analysis of the *p*-nitrobenzoate derivatives and the absolute configuration was deduced to be (*R*) by the proposed reaction mechanism.[6b] Next, the allylic alcohol (**7**) (97% ee) was subjected to Sharpless asymmetric epoxidation under kinetic resolution conditions[7] (1.0 equiv. of Ti(O-*i*-Pr)$_4$, 1.2 equiv. of diisopropyl D-tartrate, *t*-BuOOH, −40°C, 40.5 h) to give the (−)-epoxide (**8**) {$[\alpha]_D^{23}$=−12.4 (*c* 0.56, $CHCl_3$)} with >99% ee. The enantiomeric excess of **8** was also determined by chiral HPLC analysis of the *p*-bromobenzoate derivatives and the absolute configuration of the quaternary center was assigned to be (*S*) by using the well-established enantioselectivity principle.[7b] The secondary carbinol in **8** was then converted to a ketone by Swern oxidation to give the (−)-epoxy-ketone (**4**) {$[\alpha]_D^{24}$=−58 (*c* 0.54, $CHCl_3$)} (Scheme 2).

The thus-obtained epoxide (−)-(**4**) and 5-methoxy-3,4-dihydro-β-carboline (**11**), which was prepared from 4-methoxytryptamine (**9**)[8] through *N*-formylation and subsequent Bischler–Napieralski reaction, were condensed[9] in heated MeOH to afford two diastereomeric tetracyclic compounds (**5a** and **5b**) in 33 and 17% yields, respectively. The C3 configuration of the major and minor product was deduced by comparison of their CD spectra, as follows. In general, the Cotton effect at the long-wave region (around 300 nm) of the tetracyclic Corynante-type indole alkaloids having the C3(*S*) configuration exhibits a positive Cotton



Scheme 2.

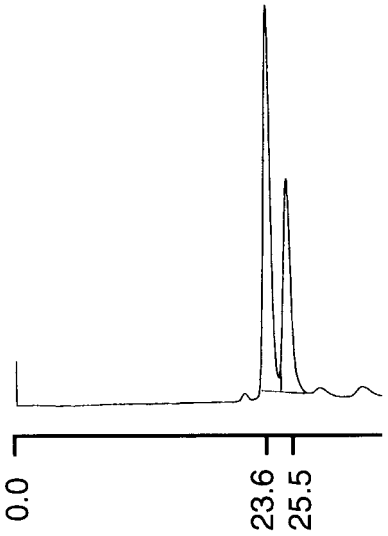


Figure 2. Chiral HPLC analysis of natural (−)-9-methoxymitralactonine (**1**) (conditions: Chiralcel OD; 20% EtOH/*n*-hexane; flow rate 0.5 mL/min; column temperature 30°C).

curve; on the contrary, that of C3(*R*) configuration shows a negative curve.[10] In fact, the major and minor products, respectively, show the positive and negative Cotton curves in the long-wave region, indicating that **5a** has *S* and **5b** has *R* configuration, respectively, at the C3 position. The major isomer (**5a**) was subjected to Knoevenagel condensation with dimethyl malonate in refluxing toluene in the presence of $AcONH_4$ and AcOH to give directly the pentacyclic product (**12**) having a lactone residue in 51% yield. Interestingly, the same product (**12**) was obtained starting from the minor isomer (**5b**), through isomerization at C3 during condensation under acidic conditions. Finally, the double bond was introduced to the C3-14 position in **12** by a two-step operation: (i) *t*-BuOCl, Et_3N, (ii) ethanolic HCl then $NaHCO_3$ (Scheme 3). The synthetic compound (**1**) was identified with the natural product by comparison of their chromatographic behaviors, UV, ^1H and ^{13}C NMR, and mass spectra. The observed optical rotation of the synthetic compound showed levorotation similar to the natural product; however, the specific rotation was very different $\{[\alpha]_D^{24}=-838$ (*c* 0.10, $CHCl_3)\}$ from that of natural (**1**) $\{[\alpha]_D^{18}=-123$ (*c* 0.19, $CHCl_3)\}$. Then, we synthesized racemic 9-methoxymitralactonine (**1**) starting from the achiral epoxy-ketone (**4**) and analyzed the enantiomeric purity of both the synthetic (±)-(**1**) and (−)-(**1**) and the natural product using chiral column chromatography. As a result, it was found that the natural 9-methoxymitralactonine contained predominantly the (−)-enantiomer over the (+)-enantiomer in the ratio of 62:38 (Fig. 2).

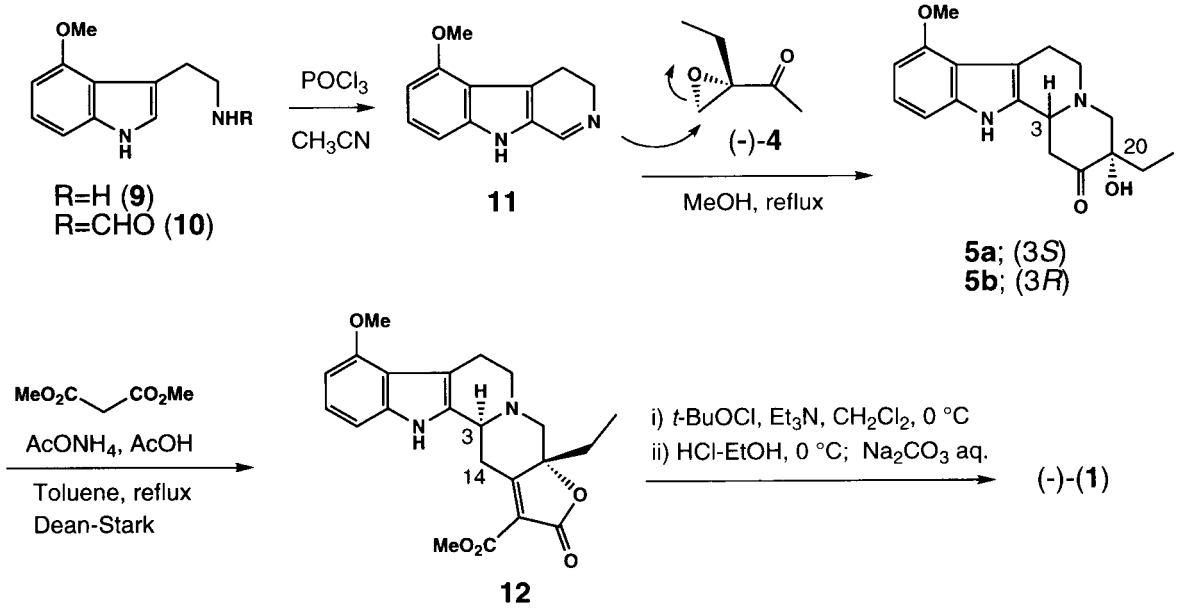
Experimental

General

UV: recorded in MeOH. Hitachi U 3400. ^1H and ^{13}C NMR spectra: recorded at 500 and 125.65 MHz, respectively (ppm, *J* in Hz with TMS as int. standard). JEOL JNM A-500. EI-MS: direct probe insertion at 70 eV. JEOL JMS-AM20. FAB-MS: JEOL JMS-HX110. CD: JASCO J-720WI. Optical Rotation: JASCO DIP-140. TLC: precoated Kieselgel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Kieselgel 60 [Merck, 70–230 (for open chromatography) and 230–400 mesh (for flash chromatography)], Sephadex LH-20 [Pharmacia Biotech]. MPLC: silica gel prepacked column Kusano CPS-HS-221-05. Prep. TLC: silica gel 60 GF_{254} (Merck 7730, 0.5 mm thick).

Extraction and isolation of 9-methoxymitralactonine

Fresh young leaves (315 g) of *Mitragyna speciosa* Korth., collected in Malaysia were moistened with 10% ammonia water and allowed to stand overnight. They were then macerated with ethyl acetate three times and filtered. The



Scheme 3.

combined filtrate was concentrated under reduced pressure to give a crude extract (3.25 g). The extract was roughly separated by silica gel flash column chromatography. The column was eluted with *n*-hexane/AcOEt–AcOEt/MeOH gradient to give the ten fractions. The 60% – 80% AcOEt/*n*-hexane eluent from the first SiO_2 column chromatography was subjected to Sephadex (LH-20) chromatography (MeOH) to give a fraction containing 9-methoxymitralactonine, which was further purified by SiO_2 medium-pressure liquid chromatography (50% AcOEt/*n*-hexane) and then by ODS column chromatography (25% H_2O/MeOH) to afford 1.9 mg of 9-methoxymitralactonine (**1**).

9-Methoxymitralactonine (1). A dark orange amorphous powder. R_f value; 0.3 [SiO_2, solvent system: AcOEt/*n*-hexane 2:1]. $[\alpha]_D^{25}=-123$ (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ): 462 (4.51), 435 (sh), 340 (3.72), 267 (sh), 220 (4.21) nm. EIMS *m/z* (%): 394 (M^+, 20), 365 (14), 350 (74), 319 (17), 292 (35), 291 (39), 275 (14), 97 (100). HR-EIMS: Calcd for $C_{22}H_{22}O_5N_2$: 394.1529, found: 394.1531. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.64 (1H, s, *N*a-H), 7.32 (1H, dd, *J*=8.1, 8.1 Hz, H-11), 6.97 (1H, d, *J*=8.1 Hz, H-12), 6.49 (1H, d, *J*=8.1 Hz, H-10), 6.39 (1H, s, H-14), 3.93 (3H, s, 9-OCH_3), 3.88 (3H, s, 22-OCH_3), 3.66 (1H, d, *J*=12.2 Hz, H-21), 3.56 (1H, d, *J*=12.2 Hz, H-21), 3.50–3.68 and 3.28–3.41 (4H, m, H_2-5 and H_2-6), 1.80–1.94 (2H, m, H_2-19), 0.95 (3H, dd, *J*=7.4, 7.4 Hz, H_3-18). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 169.74 (C-15), 168.61 (C-17), 163.14 (C-22), 154.91 (C-9), 149.24 (C-3), 140.07 (C-13), 126.46 (C-11), 125.52 (C-2), 116.80 (C-7), 115.74 (C-8), 105.17 (C-12), 99.74 (C-10), 94.91 (C-16), 87.08 (C-14), 77.36 (C-20), 55.23 (9-OCH_3), 54.86 (C-21), 50.49 (22-OCH_3), 50.41 (C-5), 29.60 (C-19), 21.75 (C-6), 7.12 (C-18).

Preparation of (*R*)-(+)-alcohol (7) by asymmetric reduction of enone (6). A solution of borane–THF complex (28 mL, 28 mmol) was added dropwise at room temperature to a solution of (*S*)-5,5-diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine (2.14 g, 7.72 mmol) in dry THF (40 mL) under argon atmosphere. After stirring at the same temperature for 40 min, the mixture was cooled to −20°C. To this solution, a cold mixture of **6** (4.16 g, 42.5 mmol) in dry THF (40 mL) was added dropwise for 30 min, then the reaction mixture was stirred for 17.5 h at −20°C. MeOH (14 mL) was added to the reaction mixture and the resulting solution was gradually warmed to room temperature. The mixture was then poured into saturated NH_4Cl solution (400 mL) and the whole mixture was extracted with Et_2O four times. The organic layer was washed with water, dried ($MgSO_4$) and concentrated to ca. 100 mL under 180 mmHg at 20°C. The residue was passed through a short column of silica gel and again concentrated to ca. 20 mL under 180 mmHg at 30°C. The residue was distilled under reduced pressure (45–87°C, 70 mmHg) to afford 2.77 g (65%) of alcohol **7** as a colorless oil. $[\alpha]_D^{23}=+9.5$ (*c* 0.35, $CHCl_3$). IR ν_{max} (neat): 3426, 1642 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.04 (1H, dd, *J*=1.1, 1.1 Hz, =C*H*), 4.81 (1H, d, *J*=1.5 Hz, =C*H*), 4.28 (1H, ddd, *J*=6.5, 6.5, 6.5 Hz, –C*H*(OH)CH_3), 2.02–2.15 (2H, m, –C$H_2$$CH_3$), 1.30 (3H, d, *J*=6.5 Hz, –CH(OH)CH_3), 1.08 (3H, dd, *J*=7.4, 7.4 Hz, –CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 154.91 (–*C*=CH_2), 107.09 (–C=*C*H_2), 71.09 (–*C*H(OH)CH_3), 24.24 and 22.19 (–*C*$H_2$$CH_3$ and –CH(OH)*C*H_3), 12.26 (–CH_2*C*H_3). Owing to the high volatility of **7**, mass spectral data including high resolution MS could not be obtained. Therefore, the mass spectral data was obtained for the *p*-nitrobenzoyl derivative as described below.

To determine the optical purity of **7**, two *p*-nitrobenzoate derivatives {FAB-MS (NBA) *m/z*: 250 ([M+1]$^+$). HR-FABMS (NBA): Calcd for $C_{13}H_{16}O_4N$: 250.1079, found: 250.1072} were, respectively, prepared by conventional method starting from the racemic and chiral alcohols obtained by the above reaction. They were analyzed by HPLC using chiral column chromatography (Chiral Cel OB, Daicel Chemical Industries, Ltd., solvent; 1% *iso*-propanol in *n*-hexane, flow rate; 0.5 mL/min, column temperature; 30°C). The benzoate derived from optically active **3** exhibited two peaks at 16.4 and 19.0 min in a ratio of 98.6:1.4 (97% ee).

Preparation of (−)-epoxide (8) by asymmetric epoxidation of (+)-allylic alcohol (7). To a stirred solution of freshly distilled diisopropyl D-tartrate (7.77 g, 33.2 mmol) and dry CH_2Cl_2 (120 mL), freshly distilled Ti(O-*i*-Pr)$_4$ (8.0 mL, 27.1 mmol) was added under argon atmosphere and the mixture was cooled to −40°C. To this solution a mixture of (+)-**7** (97% ee, 2.66 g, 26.6 mmol) and dry CH_2Cl_2 was added and the resulting mixture was stirred at −40°C for 45 min. A cold solution of *t*-BuOOH in toluene (3.5 M, 7.7 mL) diluted with dry CH_2Cl_2 (10 mL) was added dropwise to the above reaction mixture for 20 min and the mixture was stirred at −40°C for 40.5 h under argon atmosphere. The reaction was quenched by addition of 10% L-tartaric acid solution (110 mL) at −40°C and the whole mixture was introduced into a separatory funnel. After separation of the organic layer, the remaining aqueous layer was extracted with Et_2O three times. The combined organic layer was washed with water, dried over $MgSO_4$ and concentrated to ca. 50 mL under 180 mmHg at 23°C. After dilution with Et_2O (50 mL) a 1 N NaOH solution (50 mL) was added and the whole mixture was stirred at 0°C for 45 min. The solution was introduced into the separatory funnel. After separation of the organic layer, the remaining aqueous layer was extracted with Et_2O three times. The combined organic layer was washed with water, dried over $MgSO_4$ and concentrated to ca. 150 mL under 200 mmHg at 21°C. The residue was passed through a short column of silica gel and again concentrated to ca. 20 mL under 180 mmHg at 22°C. The residue was distilled under reduced pressure (60–96°C, 65 mmHg) to afford 1.71 g (56%) of epoxide **8** as a colorless oil. $[\alpha]_D^{23}=-12.4$ (*c* 0.56, $CHCl_3$). IR ν_{max} (neat): 3500, 2975 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.94 (1H, dddd, *J*=6.3, 6.3, 6.3 Hz, 1.7, –C*H*(OH)CH_3), 2.88 (1H, d, *J*=4.6 Hz, methylene proton of oxiran ring), 2.65 (1H, d, *J*=4.6 Hz, methylene proton of oxiran ring), 2.08 (1H, br.s, –CH(O*H*)CH_3), 1.62–1.83 (2H, m, –C$H_2$$CH_3$), 1.24 (3H, dd, *J*=6.3, 0.8 Hz, –CH(OH)CH_3), 0.93 (3H, dd, *J*=7.6, 7.6 Hz, –CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 65.86, 63.04, 47.84, 23.71, 18.50, 7.97. Owing to the high volatility of **8**, mass spectral data including high resolution MS could not be obtained. Therefore, the mass spectral data was obtained for the *p*-bromobenzoyl derivative as described below.

The ^{1}H NMR spectrum of distilled **8** indicated the presence of a single isomer. The presence of the diastereomer of **8**, i.e. (2*R*, 3*R*)-**8** and/or (2*S*, 3*S*)-**8**, could not be observed by comparison with that of the diastereomeric mixture prepared by *m*-CPBA oxidation of the racemic alcohol **7**. To determine the optical purity of **8**, *p*-bromobenzoyl derivative {FABMS (NBA) *m/z*: 301 ([M+3]$^+$), 299 ([M+1]$^+$). HR-FABMS (NBA): Calcd for $C_{13}H_{16}O_3{}^{79}Br$: 299.0283; found: 299.0288, calcd for $C_{13}H_{16}O_3{}^{81}Br$: 301.0262; found: 301.0250} were prepared by conventional method. They were analyzed by HPLC using a chiral column chromatography (Chiral Cel OB, Daicel Chemical Industries Ltd, solvent; 1% *iso*-propanol in *n*-hexane, flow rate; 0.5 mL/min, column temperature; 30°C). The benzoate derived from optically active **8** obtained by above reaction exhibited one peak at 26.0 min; on the other hand, the benzoate derivative containing (2*S*, 3*R*)-**8** and (2*R*, 3*S*)-**8** gave two peaks at 20.9 min and 26.0 min, revealing that the enantiomeric excess of **8** obtained by the Sharpless epoxidation was >99%.

Preparation of (−)-ketone (4) by Swern oxidation of carbinol (8). To a stirred solution of oxalyl chloride (0.75 mL, 8.60 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise DMSO (1.2 mL, 16.91 mmol) in dry CH_2Cl_2 (7 mL) at −78°C under argon atmosphere. After stirring the reaction mixture for 30 min, a solution of (−)-**8** (800 mg, 6.90 mmol) in dry CH_2Cl_2 (12 mL) was added dropwise, and the reaction mixture was stirred for 1.5 h at −78°C. Then Et_3N (4.8 mL, 34.44 mmol) was added, and the reaction mixture was allowed to warm to room temperature gradually. Water (25 mL) was added to the reaction mixture. The organic layer was separated, and the aqueous layer was extracted with Et_2O three times. The combined organic layer was washed with water, dried over $MgSO_4$ and concentrated to ca. 20 mL under 200 mmHg at 15–21°C. The residue was distilled under reduced pressure (44–81°C, 82–96 mmHg) to afford 327 mg (41%) of the ketone **4** as a colorless oil. $[\alpha]_D^{24}$=−58 (*c* 0.54, $CHCl_3$). IR ν_{max} (neat): 3750, 1704, 1642 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.93 (1H, d, *J*=4.9 Hz, H-1), 2.89 (1H, d, *J*=4.9 Hz, H-1), 2.04 (3H, s, –$COCH_3$), 2.14, 1.64 (2H, each m, H_2-3), 0.94 (3H, ddd, *J*=7.5, 7.5 Hz, 0.8, H_3-4). ^{13}C NMR (125 MHz, $CHCl_3$) δ: 207.7, 63.29, 50.44, 23.87, 23.00, 8.63.

Preparation of 5-methoxy-3,4-dihydro-β-carboline (11). To a solution of *N*b-formyl-4-methoxytryptamine (**10**) (869 mg, 3.99 mmol) (which was prepared by condensation of 4-methoxytryptamine (**9**) and formic acid, in CH_3CN (10 mL)) $POCl_3$ (500 μL, 5.36 mmol) was added at 0°C and the reaction mixture was heated under reflux for 1 h under argon atmosphere. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in aqueous 1 N HCl solution. The insoluble material was filtered off and the filtrate was alkalified with aqueous conc. NH_3 solution. By suction filtration of the resulting precipitate, **11** (518 mg, 65%) was obtained as yellow amorphous powder. UV (MeOH) λ_{max}: 349, 317, 249, 220, 208, 205 nm. IR ν_{max} (KBr): 3185, 1623, 1580, 1544, 1519, 1425, 1373, 1262, 1174, 1107 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (1H, br.s, *N*a-H), 8.33 (1H, br.s, H-1), 7.17 (1H, dd, *J*=8.0, 8.0 Hz, H-7), 6.97 (1H, d, *J*=8.0 Hz, H-8), 6.48 (1H, d, *J*=8.0 Hz, H-6), 3.92 (3H, s, –OCH_3), 3.88 (2H, dd, *J*=7.6, 7.6 Hz, H_2-3), 3.09 (2H, dd, *J*=7.6, 7.6 Hz, H_2-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.51, 151.29, 138.21, 126.97, 125.57, 116.64, 116.20, 104.99, 99.94, 55.23, 48.56, 20.67. EIMS *m/z* (%): 200 (M$^+$, 100), 199 (92), 184 (24), 149 (26), 105 (48). HR-EIMS: Calcd for $C_{12}H_{12}ON_2$: 200.0950, found: 200.0959.

Synthesis of the tetracyclic ketone (5a and 5b). A solution of (−)-epoxide (**4**) (194 mg, 1.70 mmol) and 5-methoxy-3,4-dihydro-β-carboline (**11**) (167 mg, 0.84 mmol) in dry MeOH (3 mL) was heated under reflux for 5 h under argon atmosphere. The reaction mixture was cooled and then poured onto the saturated aqueous $NaHCO_3$. The whole was extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was separated by SiO_2 column chromatography (*n*-hexane/AcOEt 3:1) to give **5a** (85.3 mg, 33%) and **5b** (43.4 mg, 17%). Major isomer **5a** (more polar compound); colorless amorphous powder. UV (MeOH) λ_{max} (log ϵ): 293 (3.82), 286 (sh, 3.84), 268 (3.96), 225 (4.58) nm. IR ν_{max} (KBr): 3316, 2943, 2700–2840, 1721, 1621, 1569, 1510, 1462, 1348, 1257, 1104 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.74 (1H, br.s, *N*a-H), 7.06 (1H, dd, *J*=7.9, 7.9 Hz, H-11), 6.93 (1H, d, *J*=7.9 Hz, H-12), 6.49 (1H, d, *J*=7.9 Hz, H-10), 4.05 (1H, s, –OH), 3.89 (3H, s, –OCH_3), 3.82 (1H, m, H-3), 3.11–3.20 (2H, each m, H-5 and H-6), 3.07 (1H, d, *J*=11.9 Hz, H-21), 3.04–3.09 (1H, m, H-6), 3.03 (1H, dd, *J*=13.7 Hz, 10.4, H-14), 2.84–2.91 (1H, m, H-5), 2.77 (1H, dd, *J*=13.7 Hz, 4.1, H-14), 2.65 (1H, d, *J*=11.9 Hz, H-21), 1.89–1.97 (1H, m, H-19), 1.62–1.70 (1H, m, H-19), 0.96 (3H, dd, *J*=7.5, 7.5 Hz, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 207.92 (C-15), 154.52 (C-9), 137.49 (C-13), 130.24 (C-2), 122.89 (C-11), 117.03 (C-8), 108.47 (C-7), 104.38 (C-12), 100.02 (C-10), 79.38 (C-20), 63.33 (C-21), 58.56 (C-3), 55.24 (-OCH_3), 52.05 (C-5), 41.96 (C-14), 25.47 (C-19), 23.16 (C-6), 7.08 (C-18). EIMS *m/z* (%): 314 (M$^+$, 100), 313 (63), 214 (95), 200 (70), 186 (46). HR-FABMS: Calcd for $C_{18}H_{23}O_3N_2$: 315.1709, found: 315.1700. $[\alpha]_D^{24}$=+87.6 (*c* 0.27, $CHCl_3$). CD (0.41 mM, MeOH, 26°C), λ_{nm} ($\Delta\epsilon$): 337 (0), 310 (+1.3), 296 (0), 291 (sh, −1.2), 284 (sh, −1.4), 267 (−2.5), 246 (−1.4), 234 (−2.6), 230 (0), 219 (+12.3), 203 (0). Minor isomer **5b** (less polar compound); pale yellow amorphous powder. UV (MeOH) λ_{max} (log ϵ): 293 (3.90), 282 (sh, 3.91), 266 (4.02), 225 (4.59) nm. IR ν_{max} (KBr): 3316, 2943, 2700–2830, 1721, 1621, 1510, 1462, 1348, 1104 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.66 (1H, br.s, *N*a-H), 7.06 (1H, dd, *J*=8.1, 8.1 Hz, H-11), 6.93 (1H, d, *J*=8.1 Hz, H-12), 6.49 (1H, d, *J*=8.1 Hz, H-10), 3.89 (3H, s, –OCH_3), 3.79 (1H, s, OH), 3.53–3.57 (1H, m, H-3), 3.22 (1H, d, *J*=11.3 Hz, H-21), 3.13–3.18 (1H, m, H-6), 3.08–3.12 (1H, m, H-5), 3.03–3.07 (1H, m, H-6), 2.82 (1H, dd, *J*=13.3, 3.2 Hz, H-14), 2.75 (1H, dd, *J*=13.3, 11.9 Hz, H-14), 2.64–2.69 (1H, m, H-5), 2.53 (1H, d, *J*=11.3 Hz, H-21), 2.21–2.30 (1H, m, H-19), 1.81–1.89 (1H, m, H-19), 0.82 (3H, dd, *J*=7.5, 7.5 Hz, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 211.22 (C-15), 154.54 (C-9), 137.51 (C-13), 130.63 (C-2), 122.82 (C-11), 117.08 (C-8), 108.99 (C-7), 104.29 (C-12), 100.01 (C-10), 79.35 (C-20), 65.97 (C-21), 59.74 (C-3), 55.25 (-OCH_3), 52.15 (C-5), 42.97 (C-14), 30.67 (C-19), 23.94 (C-6), 6.94 (C-18). EIMS *m/z* (%): 314 (M$^+$, 96), 313 (52), 214 (100), 200

(48), 186 (36). HR-FABMS: Calcd for $C_{18}H_{23}O_3N_2$: 315.1709, found: 315.1690. $[\alpha]_D^{24}$ −145 (*c* 0.35, $CHCl_3$). CD (0.43 mM, MeOH, 26.0°C), λ_{nm} ($\Delta\epsilon$): 319 (0), 296 (−3.7), 287 (sh, −2.7), 279 (sh, −2.0), 270 (0), 258 (+2.3), 245 (+1.7), 233 (+3.5), 229 (0), 220 (−11.3), 204 (0).

Knoevenagel reaction of 5a. A mixture of **5a** (56 mg, 0.18 mmol), dimethyl malonate (330 μL, 2.89 mmol), $AcONH_4$ (30.5 mg, 0.40 mmol) and AcOH (21 μL, 0.37 mmol) in dry toluene (2.5 mL) was refluxed under argon atmosphere with Dean–Stark apparatus for 3.5 h. The reaction mixture was cooled and then poured onto the saturated aqueous $NaHCO_3$. The whole was extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was separated by SiO_2 column chromatography (*n*-hexane/AcOEt 2:1) to give **12** (36 mg, 51%). Pale yellow amorphous powder. UV (MeOH) λ_{max} (log ϵ): 293 (3.85), 285 (sh, 3.91), 270 (4.02), 226 (4.67) nm. IR ν_{max} (KBr): 3365, 2951, 2700–2840, 1768, 1652, 1511, 1438, 1334, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): 8.02 (1H, br.s, *N*a-H), 7.06 (1H, dd, *J*=7.9, 7.9 Hz, H-11), 6.93 (1H, d, *J*=7.9 Hz, H-12), 6.49 (1H, d, *J*=7.9 Hz, H-10), 4.12 (1H, dd, *J*=12.7, 2.9 Hz, H-14), 3.94 (3H, s, 22-OCH_3), 3.89 (3H, s, 9-OCH_3), 3.43 (1H, d, *J*=10.7 Hz, H-21), 3.37 (1H, br.d, *J*=9.4 Hz, H-3), 3.03–3.09 (1H, m, H-5), 3.03–3.15 (2H, m, H_2-6), 2.72 (1H, ddd, *J*=11.4, 11.4, 4.6 Hz, H-5), 2.49 (1H, dd, *J*=12.7, 9.4 Hz, H-14), 2.42 (1H, d, *J*=10.7 Hz, H-21), 2.16–2.25 and 2.08–2.16 (2H, each m, H-19), 0.81 (3H, dd, *J*=7.5, 7.5 Hz, H_3-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 180.47 (C-15), 167.51 (C-17), 162.05 (C-22), 154.54 (C-9), 137.60 (C-13), 130.30 (C-2), 122.90 (C-11), 117.06 (C-16), 116.93 (C-8), 109.10 (C-7), 104.35 (C-12), 99.98 (C-10), 85.67 (C-20), 64.96 (C-21), 59.94 (C-3), 55.24 (9-OCH_3), 52.55 (C-5), 52.39 (22-OCH_3), 32.36 (C-14), 28.25 (C-19), 23.97 (C-6), 7.05 (C-18). EI-MS *m/z* (%): 396 (M^+, 100), 395 (94), 337 (17), 213 (92). HR-FABMS: Calcd for $C_{22}H_{24}O_5N_2$: 396.1686, found: 396.1667. $[\alpha]_D^{26}$=+63.7 (*c* 1.29, $CHCl_3$). CD (0.28 mM, MeOH, 26.0°C), λ_{nm} ($\Delta\epsilon$): 328 (0), 283 (+2.1), 264 (+1.0), 231 (+58.1), 223 (0), 216 (−35.4).

Knoevenagel reaction of 5b. Under the same reaction conditions described above, the isomer **5b** (24.8 mg) was subjected to Knoevenagel condensation to give **12** (10 mg, 32%) together with recovered starting material **5b** (10.5 mg, 42%). Compound **12** obtained by this reaction was completely identical with the product from **5a** by comparison of their physical and spectroscopic data.

Conversion of 12 to (−)-9-methoxymitralactonine (1). To a stirred solution of **12** (23.6 mg, 0.06 mmol) in dry CH_2Cl_2 (1 mL) was successively added Et_3N (12 μL, 0.084 mmol) and then *t*-BuOCl (14 μL, 0.12 mmol) at −20°C under argon atmosphere. After stirring the reaction mixture for 3 h, the volatile material was thoroughly removed under reduced pressure. The residue was dissolved in 5 N ethanolic HCl (1.5 mL) at 0°C and the mixture was stirred at the same temperature for 1.5 h. The reaction mixture was poured onto the chilled saturated aqueous $NaHCO_3$ and was extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was separated by SiO_2 column chromatography (*n*-hexane/AcOEt 1:1) to give (−)-**1** (19 mg, 81%). Orange amorphous powder, $[\alpha]_D^{24}$=−838 (*c* 0.1, $CHCl_3$). CD (0.58 mM, MeOH, 26.0°C), λ_{nm} ($\Delta\epsilon$): 507 (0), 475 (−5.1), 463 (−0.1), 427 (−5.1), 368 (0), 337 (+3.9), 294 (+0.6), 261 (+8.9), 237 (sh, 1.6), 234 (0), 222 (−5.2), 212 (0). The chromatographic behaviors and UV, ^1H and ^{13}C NMR, and mass spectral data were identical with those of the natural product.

Chiral HPLC analysis of synthetic (−)-9-methoxymitralactonine, (±)-9-methoxymitralactonine and natural 9-methoxymitralactonine. Three samples were analyzed by HPLC using a chiral column chromatography (Chiral Cel OD, Daicel Chemical Industries Ltd, solvent; 20% EtOH in *n*-hexane, flow rate; 0.5 mL/min, column temperature; 30°C). The synthetic racemate, which was prepared by the same procedure described above starting from racemic epoxy-ketone (**4**), exhibited two peaks at 23.6 and 25.5 min in a ratio of 50:50. The synthetic and natural (−)-mitralactonine exhibited corresponding peaks in a ratio of 100:0 and 62:38, respectively.

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