# Komodoquinone A, a Novel Neuritogenic Anthracycline, from Marine *Streptomyces* sp. KS3

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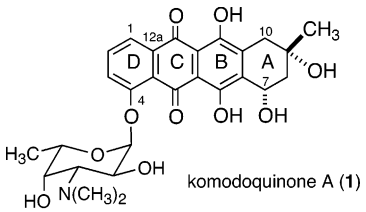
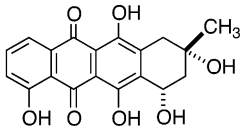
A novel anthracycline, komodoquinone A (**1**), and its aglycone, komodoquinone B (**2**), were isolated from the solid-state fermentation of the marine *Streptomyces* sp. KS3, which was isolated from marine sediment. The absolute stereostructures of **1** and **2**, except for the sugar portion, were elucidated on the basis of chemical and physicochemical evidence. Komodoquinone A (**1**) is a unique anthracycline, in which a new amino sugar is connected to the D-ring of the anthracyclinone skeleton, and was found to induce neuronal cell differentiation in the neuroblastoma cell line, Neuro 2A.

In the 1970s, natural product chemists embarked on the discovery of bioactive compounds from marine invertebrates by use of antimicrobial and cytotoxic assays. Later, several pharmaceutical companies joined this race using more sophisticated assay systems. Today, as the result of extensive research, marine natural products are recognized as a significant resource for drug discovery. Hitherto, several promising candidates for new pharmaceuticals have been discovered from marine invertebrates.[1−3] On the other hand, a growing number of biologically active metabolites have been reported from marine microorganisms during the past two decades. Now, marine microorganisms are receiving much attention as an important source in drug discovery.[4−8]

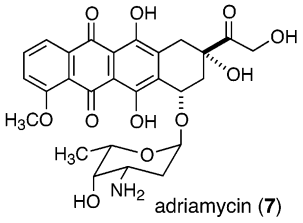
In the course of our study of bioactive substances from marine organisms, we started a search for novel bioactive secondary metabolites from marine actinomyces and fungi obtained from marine sediment.[9] Each extract of the cultured marine microorganisms was tested for neuritogenic activity against neuroblastoma Neuro 2A cells.[10,11] From the bioassay-guided separation of the solid-state fermentation of the marine *Streptomyces* sp. KS3, a novel anthracycline named komodoquinone A (**1**) and its aglycone, komodoquinone B (**2**), were found. This paper describes the elucidation of the stereostructure of komodoquinones A (**1**) and B (**2**).




komodoquinone A (**1**)




komodoquinone B (**2**)




adriamycin (**7**)

## Results and Discussion

The *Streptomyces* sp. KS3 strain was separated from marine sediment, which was collected from a depth at 50 m off Komodo Island, Indonesia. The KS3 strain was cultured at 30 °C for 2 weeks in the production medium (25 g of rice and 50 mL of artificial seawater containing 0.5% glucose and 0.3% yeast extract). The fermentation medium was extracted with acetone and a mixed solvent (MeOH−$CHCl_3$−acetone). The crude extract was partitioned into an EtOAc−water mixture to furnish an EtOAc-soluble portion and a water-soluble portion. To extract organic compounds from the water-soluble portion, the water-soluble portion was further treated with DIAION HP20. The filtered resin was washed with water and then eluted with MeOH to give a MeOH extract. This MeOH extract exhibited neuritogenic activity against neuroblastoma Neuro 2A cells at a 100 $\mu$g/mL concentaration. Then, the MeOH extract was subjected to bioassay-guided separation. The MeOH extract was fractionated by silica gel column chromatography and purified by reversed-phase HPLC to afford a new anthracycline named komodoquinone A (**1**) together with staurosporine.[12] Compound **1** exhibited neuritogenic activity[13] against (>50%) Neuro 2A cells at a concentration of 1 $\mu$g/mL in a dose-dependent manner. The EtOAc extract was also separated by silica gel column chromatography to furnish komodoquinone B (**2**).

The FABMS of the red pigment **1** showed a quasi-molecular ion peak at *m*/*z* 529 $[M]^-$, and the molecular formula was determined as $C_{27}H_{31}NO_{10}$ by HRFABMS in conjunction with NMR analysis. The IR spectrum of **1** showed strong absorption bands due to the conjugated carbonyl (1653 $cm^{-1}$) and hydroxyl groups (3337 $cm^{-1}$). The $^1H$ NMR spectrum of **1** indicated the presence of two methyl protons [$\delta$ 1.29 (s), 1.02 (d)], *N*-methyl protons [$\delta$ 2.89 (6H, s)], five protons directly connected to heteroatoms ($\delta$ 4.95, 4.32, 4.12, 3.97, 3.82), three aromatic protons ($\delta$ 7.93, 7.83, 7.77), and an anomeric proton ($\delta$ 5.95) together with two methylene signals. The presence of these signals was confirmed by HMQC ($^1H$-detected multiple quantum coherence) spectrum. The $^{13}C$ NMR spectrum of **1** also showed the presence of two carbonyl carbons ($\delta_C$ 187.1, 185.9), three oxygenated aromatic quaternary carbons

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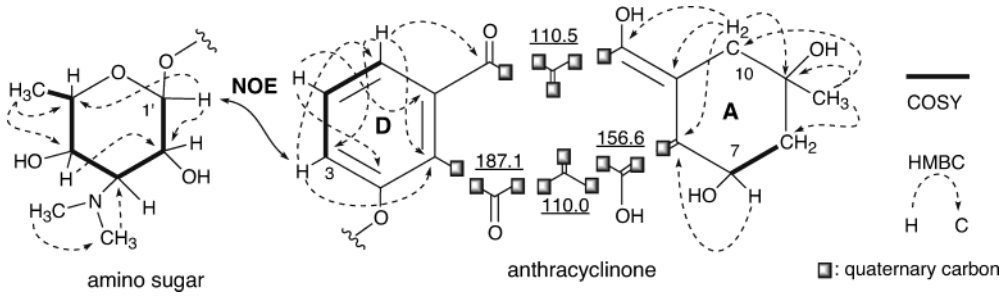


**Figure 1.** Partial structures of komodoquinone A (**1**) constructed by COSY and HMBC correlations.

**Table 1.** $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR Data for Komodoquinone A (**1**) in DMSO-$d_6$

| no. | $\delta_C$ | $\delta_H$ (mult., $J$ (Hz)) | HMBC[a] |
|---|---|---|---|
| 1 | 121.4 | 7.93 (d, 7.1) | C-3, C-4a, C-12 |
| 2 | 136.2 | 7.83 (dd, 8.2, 7.1) | C-4, C-12a |
| 3 | 123.1 | 7.77 (d, 8.2) | C-1, C-4a |
| 4 | 158.3 | | |
| 4a | 121.8 | | |
| 5 | 187.1 | | |
| 5a | 110.5[b] | | |
| 6 | 156.6 | | |
| 6a | 137.2 | | |
| 7 | 62.2 | 4.95 (br s) | C-6a |
| 8 | 41.2 | 2.02 (d, 13.9) | |
| | | 1.82 (br d, 13.9) | |
| 9 | 67.5 | | |
| 10 | 38.0 | 2.99 (d, 18.9) | C-6, C-9, C-10a, C11 |
| | | 2.52 (d, 18.9) | |
| 10a | 135.8 | | |
| 11 | 155.8 | | |
| 11a | 110.0[b] | | |
| 12 | 185.9 | | |
| 12a | 135.0 | | |
| 13 | 29.1 | 1.29 (3H, s) | |
| 1′ | 99.8 | 5.95 (br s) | C-2′, C-5′ |
| 2′ | 65.4 | 4.32 (d, 10.1) | |
| 3′ | 36.2 | 3.82 (d, 10.1) | |
| 4′ | 68.2 | 4.12 (br s) | C-2′ |
| 5′ | 69.1 | 3.97 (q, 6.3) | |
| 6′ | 15.1 | 1.02 (3H, d, 6.3) | C-4′, C-5′ |
| N-$CH_3$ | 40.9 | 2.89 (6H, s) | C-3′, N-$CH_3$ |

[a] C couples with H. [b] Assignment may be interchanged.

**Table 2.** $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR Data for Komodoquinone B (**2**)

| | in $CDCl_3$ | | in DMSO-$d_6$ | |
|---|---|---|---|---|
| no. | $\delta_C$ | $\delta_H$ (mult., $J$ (Hz)) | $\delta_C$ | $\delta_H$ (mult., $J$ (Hz)) |
| 1 | 119.8 | 7.99 (d, 6.8) | 119.0 | 7.73 (d, 7.4) |
| 2 | 137.6 | 7.22 (dd, 8.0, 6.8) | 137.7[a] | 7.80 (dd, 7.7, 7.4) |
| 3 | 124.2 | 7.32 (d, 8.0) | 125.0 | 7.36 (d, 7.7) |
| 4 | 162.3 | | 162.2 | |
| 4a | 116.0 | | 116.8 | |
| 5 | 188.9 | | 188.9 | |
| 5a | 110.2 | | 111.9[b] | |
| 6 | 156.0 | | 157.8[c] | |
| 6a | 136.7 | | 139.0 | |
| 7 | 62.0 | 5.27 (d, 5.5) | 62.3 | 4.93 (br s) |
| 8 | 40.3 | 2.36 (d, 14.9) | 41.2 | 2.04 (d, 13.8) |
| | | 1.93 (dd, 14.9, 5.5) | | 1.86 (br d, 13.8) |
| 9 | 68.2 | | 68.2 | |
| 10 | 38.4 | 3.28 (d, 18.9) | 38.2 | 3.01 (d, 18.7) |
| | | 2.61 (d, 18.9) | | 2.53 (d, 18.7) |
| 10a | 137.5 | | 137.6[a] | |
| 11 | 157.2 | | 157.0[c] | |
| 11a | 110.0 | | 110.3[b] | |
| 12 | 186.1 | | 186.1 | |
| 12a | 133.2 | | 133.7 | |
| 13 | 29.8 | 1.51 (3H, s) | 30.5 | 1.32 (3H, s) |
| 4-OH | | 12.19 (s) | | |
| 6-OH | | 13.00 (s) | | |
| 11-OH | | 13.50 (s) | | |

[a–c] Assignment may be interchanged.

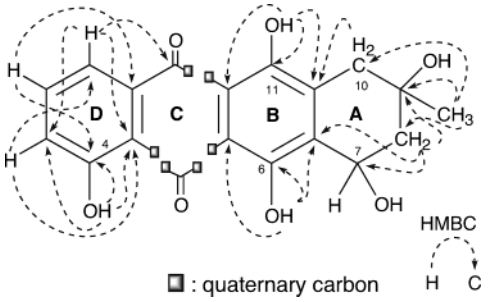


**Figure 2.** Partial structures of komodoquinone B (**2**) constructed by HMBC correlations.

($\delta_C$ 158.3, 156.6, 155.8), six aromatic quaternary carbons ($\delta_C$ 137.2, 135.8, 135.0, 121.8, 110.5, 110.0), and an oxygenated quaternary carbon ($\delta_C$ 67.5). The detailed analysis of the $^1$H−$^1$H COSY, HMQC, and HMBC ($^1$H-detected heteronuclear multiple bond connectivity) spectra of **1** measured in DMSO-$d_6$ led us to construct the partial structures assignable to an amino sugar and the A-ring and D-ring parts of anthracycline (Figure 1). The UV−vis spectrum of **1** also showed absorption maxima ($\lambda_{max}$) at 492 and 528 nm ascribable to a hydroxyl quinone chromophore. The functionality of the amino sugar moiety in **1** was found to be rare. Furthermore, the NOE correlation between H-3 ($\delta$ 7.77) in the D-ring and H-1′ ($\delta$ 5.95) in the amino sugar moiety indicated that the amino sugar combines with the hydroxyl group in the D-ring. In general, sugar moieties in most anthracyclines are known to connect through the hydroxyl groups in the A-ring. However, komodoquinone A (**1**) appeared to be a novel type of anthracycline in which the amino sugar moiety is linked to the aglycone through the hydroxyl group in the D-ring. Unfortunately, the connectivity between these partial structures, as shown in Figure 1, was not fully clarified on the basis of interpretation of the HMBC correlations. Isolation of komodoquinone B (**2**), as described below, confirmed the D-ring attachment of the sugar.

The FABMS of komodoquinone B (**2**) showed a quasi-molecular ion peak at $m/z$ 379 $[M + Na]^+$, and the molecular formula was determined as $C_{19}H_{16}NO_7Na$ by HRFABMS. The $^1$H and $^{13}$C NMR spectra of **2** (Table 2) measured in DMSO-$d_6$ were closely similar to those of komodoquinone A (**1**), except for the signals assignable to the amino sugar moiety, and komodoquinone B (**2**) was presumed to be the aglycone of **1**. Furthermore, the methanolysis of **1** by 2 N HCl/MeOH gave **2**, which was identical with komodoquinone B. On the basis of the detailed analysis of the 2D NMR spectra of **2** measured in $CDCl_3$, the AB- and D-ring structures were constructed (Figure 2). Thus, the HMBC correlations between OH-6 and C-6a, and OH-11 and C-10a, which were further correlated with the functional groups in the A-ring, were observed. The glycosidation shifts[14] observed for C-4, C-4a, and C-5 also supported the connectivity between the amino

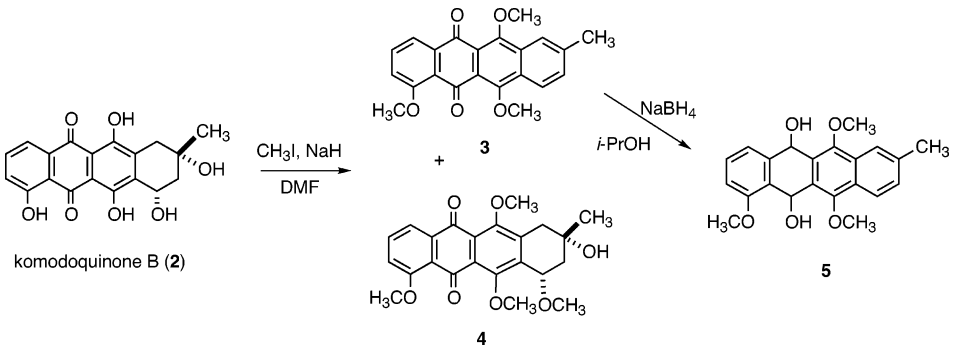


**Figure 3.** Chemical conversions of komodoquinone B (**2**).

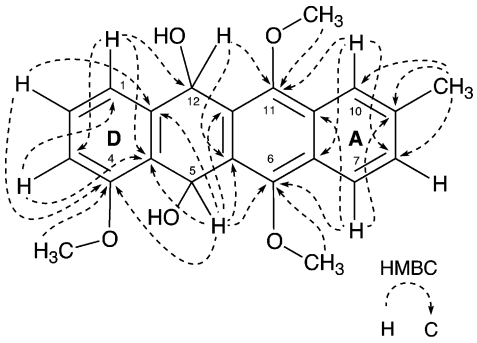


**Figure 4.** HMBC correlations of **5**.

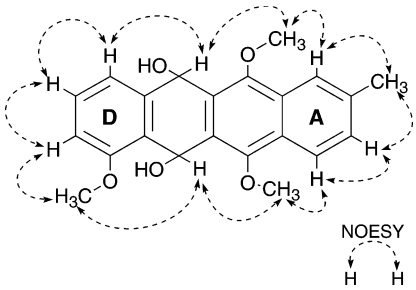


**Figure 5.** NOESY correlations of **5**.

sugar and the hydroxyl group in the D-ring. However, the chemical shifts of the carbons at both C-5a and C-11a were very similar to each other, and no HMBC correlation, which connects the B- and D-rings, was observed.

To confirm the anthracyclinone skeleton of **2** and the position of the hydroxyl group in the D-ring, the following chemical conversion of **2** was executed. Komodoquinone B (**2**) was treated with NaH and $CH_3I$ to furnish a tetramethylated derivative **4** and a trimethylated derivative **3**, in which both hydroxyl groups in the A-ring were dehydrated. Compound **3** was further reduced by $NaBH_4$ to furnish **5** (Figure 3).

The structure of compound **5** was revealed by a detailed 2D NMR analysis. Thus, in the HMBC spectrum of **5**, many correlations (e.g., between H-1 and C-12; H-5 and C-4, 4a, 5a, 6; H-7 and C-6; H-10 and C-11; H-12 and C-11), which connected the ring parts with each other, were observed (Figure 4). The structure of **5** was further confirmed by the NOESY correlations (e.g., between H-5 and $OCH_3$-4, $OCH_3$-6; H-10 and $OCH_3$-11; H-12 and H-1, $OCH_3$-11) shown in Figure 5. On the basis of this evidence, the structure of **5** and the planar structures of komodoquinones A (**1**) and B (**2**) were determined as shown.

The relative stereostructure of the A-ring in komodoquinone A (**1**) and komodoquinone B (**2**) was elucidated on the basis of the detailed analysis of the $^1H$ NMR and NOESY spectra of **2** and the tetramethylated derivative

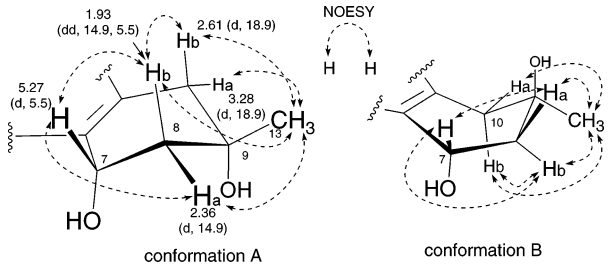


**Figure 6.** NOESY correlations of the A-ring in komodoquinone B (**2**).

**4**. As shown in Figure 6, H-7 observed at $\delta$ 5.27 was coupled with H-8b ($\delta$ 1.93) by 5.5 Hz and was not coupled with H-8a ($\delta$ 2.36). In addition, the NOE correlations between H-7 and H-8a, 8b; H-13 and H-8a, 8b, 10a, 10b; and H-8b and H-10b were observed in the NOESY spectrum of **2**. On the basis of the evidence, two conformations for the A-ring [a chair form having 7,9-*syn*-dihydroxyl groups (conformation A) and a boat form having 7,9-*anti*-dihydroxyl groups (conformation B)] were proposed. In the case of the NOESY spectrum of the tetramethylated derivative **4**, correlations similar to those for **2** were observed except for an additional important correlation between $OCH_3$-7 and H-8a. On this basis, the relative stereostructure of komodoquinone B (**2**) was confirmed to be an anthracyclinone with 7,9-*syn*-dihydroxyl groups. Komodoquinone B (**2**) appears to be identical with a synthetic compound,[15] and this is the first case of its isolation from a natural source. Komodoquinones A (**1**) and B (**2**) are rare anthracyclines having a 9-methyl substituent (e.g., nogalamycin,[16] steffinomycin,[17] and mutactimycin[18]).

Next, the absolute configuration of the C-7 hydroxy group in komodoquinone B (**2**) was examined by application of the modified Mosher's method.[19] Thus, **2** was treated with (−)- or (+)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA), 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl), and *N*,*N*-(dimethylamino)pyridine (DMAP) in $CH_2Cl_2$ at room temperature to obtain the 4,7-di-*O*-(−)-MTPA ester **6a** and 4,7-di-*O*-(+)-MTPA ester **6b**, respectively. All the protons of **6a** and **6b** were assigned, and the absolute configuration at C-7 was determined as *S* by the analysis of $\Delta\delta$ values (Figure 8). Additionally, a stronger anisotropic effect for the H-8a was observed in comparison with that for the 8-axial proton (H-8b). These data indicated that the 8-equatorial proton (H-8a) was located near the benzene ring in the MTPA group. This result also suggested that the defined chair conformation of the A-ring in **1** and **2** was correct.

The amino sugar portion of komodoquinone A (**1**) shares the same planar structure as mycaminose,[20] which has been found in macrolide antibiotics. The relative stereostructure of the amino sugar portion in **1** was established according to the following. The coupling constant of 10.1

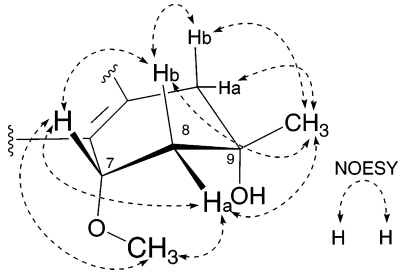


**Figure 7.** NOESY correlations of tetramethylated derivative **4**.

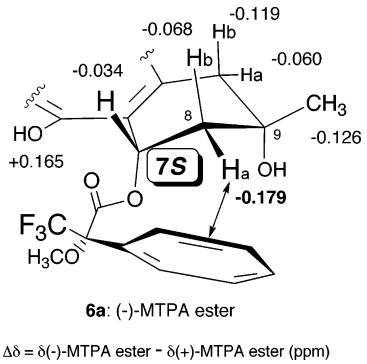


**Figure 8.** Application of modified Mosher's method to komodoquinone B (**2**).

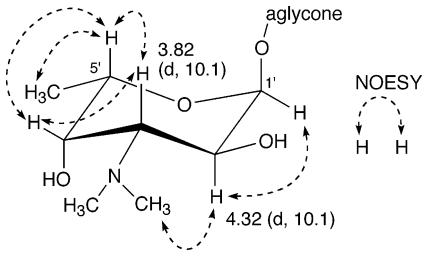


**Figure 9.** NOESY correlations of the amino sugar moiety in komodoquinone A (**1**).

Hz between H-2′ and H-3′ indicated a diaxial relationship between these two protons. In the NOESY spectrum of the amino sugar portion in **1**, the correlations between H-1′ and H-2′; H-3′ and H-4′, 5′; H-5′ and H-4′, 6′; and N-$CH_3$ and H-2′ were observed. Therefore, the relative stereostructure of the amino sugar moiety was clarified as shown in Figure 9. It is noteworthy that this amino sugar is the C-4 stereoisomer of mycaminose and a novel type of amino sugar. The absolute stereostructures of the sugar moiety in komodoquinones A (**1**) and B (**2**) are under continuous investigation.

So far, many anthracyclines are well known to exhibit antibiotic and/or antitumor activities. However, komodoquinone A (**1**) is the first example that has neuritogenic activity against the neuroblastoma cell line (Neuro 2A). Compound **1** induced a morphological change[21,22] with multipolar processes emanating from the cell body at the concentration of 1 $\mu$g/mL. On the contrary, adriamycin (**7**), the representative anthracycline, showed no neuritogenic activity at a 3 $\mu$g/mL concentration, and komodoquinone B (**2**), the aglycone of **1**, showed only weak activity at a concentration of 30 $\mu$g/mL. These data suggested that the amino sugar moiety, which was connected to the D-ring, might be important for neuritogenic activity.

Komodoquinones A (**1**) and B (**2**) together with staurosporine were produced when the KS3 strain was cultured in the solid-state medium based on rice.[23] No production of **1** and **2** was observed in the case of the culture of the KS3 strain in liquid medium (ISP1 medium prepared from artificial seawater), and only komodoquinone B (**2**) was produced by cultures using liquid medium containing rice as an additive.

## Experimental Section

**General Experimental Procedures.** The following instruments were used to obtain physical data: a JASCO DIP-370 digital polarimeter for specific rotations; a JASCO FT/IR-5300 infrared spectrometer for IR spectra; a JEOL JMS SX-102 mass spectrometer for FABMS and HRFABMS; a JEOL JNM LA-500 (500 MHz) for $^1$H NMR spectra. In the $^1$H NMR spectra, chemical shifts are recorded as ppm ($\delta$) relative to TMS as an internal standard. Silica gel (Merck 60-230 mesh) and precoated thin-layer chromatography (TLC) plates (Merck, Kiesel gel, 60$F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying acidic *p*-anisaldehyde solution (*p*-anisaldehyde 25 mL, *c*-$H_2SO_4$ 25 mL, AcOH 5 mL, EtOH 425 mL) with subsequent heating.

**Culture Conditions and Extraction.** The *Streptomyces* sp. KS3 strain was isolated from marine sediment collected at a depth of 50 m off Komodo Island, Indonesia, in 2001 and deposited in our laboratory. From the cultural characteristics, carbon utilization, and 16SrDNA sequence, the KS3 strain was classified as *Streptomyces* sp. The KS3 strain was cultured under static conditions in 500 mL flasks (× 50) at 30 °C for 2 weeks using the production medium (25 g of rice and 50 mL of artificial seawater containing 0.5% glucose and 2% yeast extract). The culture was extracted with acetone (3 L) and a mixed solvent ($CHCl_3$−MeOH−acetone 1:2:4, 3 L). The organic solvent was evaporated under reduced pressure to give an extract, which was further partitioned into an EtOAc−water mixture. The water layer was treated with DIAION HP20 and stirred gently for 15 min at room temperature. After filtration, the recovered resin was washed with water and eluted with MeOH. The MeOH eluate was evaporated under reduced pressure to give a MeOH extract (4.2 g). The EtOAc layer was evaporated under reduced pressure to give an EtOAc extract (4.2 g).

**Isolation of Komodoquinones A (1) and B (2).** The MeOH extract (4.2 g) was fractionated by $SiO_2$ column chromatography [$CHCl_3$−MeOH−water (lower phase)] to give three fractions (A−C). The active fraction B (200 mg) was further separated by reversed-phase HPLC (Cosmosil 5Ph, 10 × 250 mm, MeOH−$H_2O$−$CHCl_3$, 7:3:0.5) to furnish komodoquinone A (**1**, 20 mg) as a red neuritogenic-active substance. The EtOAc extract (4.2 g) was separated by $SiO_2$ column chromatography (*n*-hexane−EtOAc) to give three fractions (EA−EC). Fraction EB (320 mg) was washed with MeOH to give an insoluble red pigment, komodoquinone B (**2**, 110 mg).

**Komodoquinone A (1):** $[\alpha]^{25}_D$ +30° (*c* 0.05, MeOH); IR (KBr) $\nu_{max}$ 3337, 2935, 1653, 1614, 1585 cm$^{-1}$; UV−vis (MeOH) $\lambda_{max}$ ($\epsilon$) 233 (19200), 252 (18800), 289 (9600), 492 (5300), 525 (3200) nm; $^1$H and $^{13}$C NMR data, see Table 1; FAB MS *m/z* 529 [M]$^-$; HRFAB MS *m/z* 529.1948 [M]$^-$ (calcd for $C_{27}H_{31}NO_{10}$, 529.1967).

**Komodoquinone B (2):** $[\alpha]^{25}_D$ +6° (*c* 0.05, $CHCl_3$); IR (KBr) $\nu_{max}$ 3362, 2924, 1736, 1599 cm$^{-1}$; UV−vis (MeOH) $\lambda_{max}$ ($\epsilon$) 234 (15600), 245 (1200), 292 (3700), 492 (6200), 526 (4500) nm; $^1$H and $^{13}$C NMR data, see Table 2; FABMS *m/z* 379 [M + Na]$^+$; HRFABMS *m/z* 379.0794 [M + Na]$^+$ (calcd for $C_{19}H_{16}O_7Na$, 379.0793).

**Methanolysis of Komodoquinone A (1).** A solution of **1** (4.5 mg) in 2 N HCl−MeOH (0.5 mL) was refluxed for 1 h. The reaction mixture was evaporated under reduced pressure, and the resulting residue was washed with MeOH to furnish komodoquinone B (**2**, 0.7 mg).

**Methylation of Komodoquinone B (2).** A solution of **2** (35 mg) in DMF (6 mL) was treated with NaH (60 mg) and $CH_3I$ (150 $\mu$L). The reaction mixture was stirred at room temperature for 3 h under an Ar atmosphere. The solution was partitioned with an EtOAc−water mixture, and the EtOAc layer was dried over $MgSO_4$ and evaporated under reduced

pressure. The residue was purified by reversed-phase HPLC (Cosmosil 5C18-AR, MeOH−$H_2O$, 7:3) to obtain **3** (21 mg) and **4** (3.5 mg).

**Compound 3:** $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.26 (1H, d, $J$ = 8.5 Hz, H-7), 8.13 (1H, s, H-10), 7.82 (1H, d, $J$ = 7.9 Hz, H-1), 7.64 (1H, dd, $J$ = 7.9, 7.9 Hz, H-2), 7.55 (1H, d, $J$ = 8.5 Hz, H-8), 7.2 (1H, overlap, H-3), 4.16 (3H, s, −OC$H_3$), 4.13 (3H, s, −OC$H_3$), 4.04 (3H, s, −OC$H_3$), 2.60 (3H, s, H-13); FABMS $m/z$ 363 $[M + H]^+$; HRFABMS $m/z$ 363.1230 $[M + H]^+$ (calcd for $C_{22}H_{19}O_5$, 363.1232).

**Compound 4:** $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 7.73 (1H, d, $J$ = 7.7 Hz, H-1), 7.62 (1H, dd, $J$ = 8.3, 7.7 Hz, H-2), 7.2 (1H, overlap, H-3), 4.85 (1H, d, $J$ = 5.1 Hz, H-7), 4.00 (3H, s, OC$H_3$-6), 3.98 (3H, s, OC$H_3$-4), 3.90 (3H, s, OC$H_3$-11), 3.56 (3H, s, OC$H_3$-7), 3.28 (1H, d, $J$ = 17.9 Hz, H-10), 2.62 (1H, d, $J$ = 17.9 Hz, H-10), 2.44 (1H, d, $J$ = 14.5 Hz, H-8), 1.62 (1H, dd, $J$ = 14.5, 5.1 Hz, H-8), 1.43 (3H, s, H-13); FABMS $m/z$ 413 $[M + H]^+$; HRFABMS $m/z$ 413.1607 $[M + H]^+$ (calcd for $C_{23}H_{25}O_7$, 413.1601).

**Reduction of 3.** A solution of **3** (12 mg) in *i*-PrOH (6 mL) was treated with $NaBH_4$ (60 mg) and stirred at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure and partitioned into an EtOAc−water mixture. The EtOAc layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by reversed-phase HPLC (Cosmosil 5C18-AR, MeOH−water, 7:3) to give **5** (3.8 mg).

**Compound 5:** IR (KBr) $\nu_{max}$ 3472, 2935, 2361, 1597 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ ($\epsilon$) 232 (4800) nm; $^1$H NMR (600 MHz, $C_6D_6$) $\delta$ 8.09 (1H, d, $J$ = 8.5 Hz, H-7), 8.02 (1H, d, $J$ = 7.5 Hz, H-1), 7.82 (1H, s, H-10), 7.29 (1H, dd, $J$ = 7.5, 7.5 Hz, H-2), 7.2 (1H, overlap, H-8), 7.00 (1H, s, H-5), 6.51 (1H, d, $J$ = 7.5 Hz, H-3), 6.45 (1H, s, H-12), 5.68 (1H, s, OH-12), 3.91 (3H, s, OC$H_3$-6), 3.46 (3H, s, OC$H_3$-11), 3.28 (3H, s, OC$H_3$-4), 2.29 (3H, s, H-13); $^{13}$C NMR (150 MHz, $C_6D_6$) $\delta_C$ 156.8 (C-4), 151.9 (C-6), 149.6 (C-11), 141.5 (C-12a), 136.3 (C-9), 129.7 (5a-C), 129.0 (C-10a), 128.5 (C-8), 128.3 (C-2), 127.1 (C-6a), 125.9, (C-11a), 124.8 (C-4a), 123.2 (C-7), 119.5 (C-1), 109.2 (C-3), 66.6 (C-12), 63.2 (4-OC$H_3$), 61.3 (6-OC$H_3$), 58.9 (C-5), 58.6 (11-OC$H_3$), 21.7 (C-9); FABMS $m/z$ 365 $[M + H]^+$; HRFABMS $m/z$ 365.1384 $[M + H]^+$ (calcd for $C_{22}H_{21}O_5$, 365.1389).

**MTPA Esterification of Komodoquinone B (2).** A solution of **2** (1.1 mg) in $CH_2Cl_2$ was treated with (−)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) (1.2 mg), 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (2.9 mg), and *N*,*N*-(dimethylamino)pyridine (DMAP) (0.3 mg) at room temperature for 30 min under Ar atmosphere. The reaction was quenched by 5% HCl and extracted with EtOAc. The EtOAc layer was washed with saturated aqueous $NaHCO_3$ and brine. After evaporation of the organic solvent, the residue was purified by silica gel chromatography (*n*-hexane−EtOAc, 4:1) to furnish (−)-MTPA ester **6a** (1.3 mg). A solution of **2** (1.3 mg) in $CH_2Cl_2$ was similarly treated with (+)-MTPA (3.2 mg), EDCI·HCl (2.9 mg), and DMAP (0.3 mg) to afford (+)-MTPA ester **6b** (0.5 mg).

**(−)-MTPA ester 6a:** $^1$H NMR (600 MHz, $CDCl_3$,) $\delta$ 13.37 (1H, s, O$H$-6), 13.19 (1H, s, O$H$-11), 8.40 (1H, d, $J$ = 7.4 Hz, H-1), 7.86 (1H, dd, $J$ = 8.1, 7.4 Hz, H-2), 7.4−7.2 (m, 3-H and $C_6H_5$), 6.61 (d, $J$ = 3.3 Hz, H-7), 3.85 (3H, s, −OC$H_3$), 3.58 (3H, s, −OC$H_3$), 3.27 (1H, d, $J$ = 18.9 Hz, H-10a), 2.53 (1H, d, $J$ = 18.9 Hz, H-10b), 2.16 (1H, d, $J$ = 14.9 Hz, H-8a), 2.05 (1H, dd, $J$ = 14.9, 3.3 Hz, H-8b), 1.34 (3H, s, H-13); FABMS $m/z$ 788 $[M]^+$; HRFABMS $m/z$ 788.1713 $[M]^+$ (calcd for $C_{39}H_{30}O_{11}F_6$, 788.1692).

**(+)-MTPA ester 6b:** $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 13.23 (1H, s, O$H$-11), 13.20 (1H, s, O$H$-6), 8.39 (1H, d, $J$ = 7.4 Hz, H-1), 7.85 (1H, dd, $J$ = 8.1, 7.4 Hz, H-2), 7.4−7.2 (11H, m, 3-H and $C_6H_5$), 6.65 (1H, d, $J$ = 3.8 Hz, H-7), 3.82 (3H, s, −OC$H_3$), 3.53 (3H, s, −OC$H_3$), 3.33 (1H, d, $J$ = 18.8 Hz, H-10a), 2.65 (1H, d, $J$ = 18.8 Hz, H-10b), 2.34 (1H, d, $J$ = 14.9 Hz, H-8a), 2.12 (1H, dd, $J$ = 14.9, 3.8 Hz, H-8b), 1.47 (3H, s, H-13); FABMS $m/z$ 788 $[M^+]$; HRFABMS $m/z$ 788.1725 $[M]^+$ (calcd for $C_{39}H_{30}O_{11}F_6$, 788.1692).

**Assay[13] for Neuritogenic Activity in Neuro 2A Cells.** Neuro 2A cells were grown in Dulbecco's modified essential medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS). The cells were kept in an incubator at 37 °C with 5% $CO_2$. The cells were plated on 24-well plates at a density of $2 \times 10^4$ per well with 1 mL of culture medium. After 24 h cultivation, the medium was exchanged for fresh medium, and the 10 $\mu$L of EtOH test sample solution (several concentration) was added to each well. After 24 or 48 h incubation, morphological changes in the cells were observed under a microscope.

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