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Stilbenoids of *Kobresia nepalensis* (Cyperaceae) exhibiting DNA topoisomerase II inhibition

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Abstract

Resveratrol oligomers, nepalensinol A, B and C, were isolated from the stem of *Kobresia nepalensis* (Cyperaceae). The structures were established on the basis of chemical properties and spectroscopic evidence including 2D NMR spectroscopic analysis. Nepalensinol A, B and C showed a potent inhibitory effect on topoisomerase II – stronger than etoposide (VP-16), a topoisomerase II inhibitor used as an anti-cancer drug. Nepalensinol B, in particular, exhibited the most potent activity with an IC_{50} of 0.02 μg/ml.

Keywords: *Kobresia nepalensis*; Cyperaceae; Nepalensinol A, B and C; Stilbene; Topoisomerase; Inhibitor; Anti-cancer

1. Introduction

Topoisomerases (topo I and II) play a critical cellular role by altering the topological state of DNA in events such as overwinding, underwinding and catenation. Topoisomerase II can relax supercoiled DNA and resolve knotted or catenated DNA rings (D'Arpa and Liu, 1989). Therefore, the enzyme seems to be involved in proliferative processes such as DNA replication, chromosome condensation, and chromosome segregation, it could provide a critical target for the action of a wide variety of anti-cancer drugs. In the course of a continuous search for a new class of inhibitors of topoisomerase II (Tosa et al., 1997, 1998), the methanol extract of the stem of the hitherto uninvestigated *Kobresia nepalensis* was found to show inhibitory activity against topoisomerase II (Nozaki et al., 1997). *K. nepalensis* belongs to the family Cyperaceae, which consists of a genus of about 50 species, is distributed in the northern hemisphere, especially at high altitudes in the Himalayas, China and central Asia. *K. nepalensis* is an important species in the alpine flora of the Nepal Himalayas and is economically important as pasturage (Rajbhandari and Ohba, 1991). Although this species may not have been studied before, stilbenoids have been identified from several species of the Cyperaceae (D'Abrscoa et al., 2005; Lee et al., 1998; Kawabata et al., 1989). We herein report the isolation and structures of three new active principles, and their significant inhibitory activity against topoisomerase II.

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2. Results and discussion

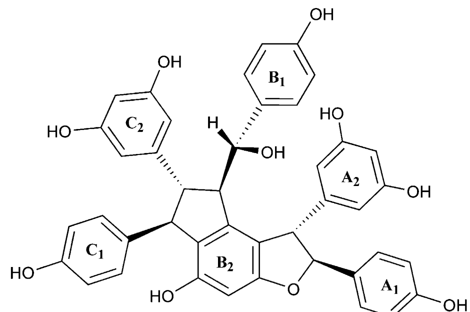
The methanol extract of air-dried stems of *K. nepalensis* was extracted successively with *n*-hexane and ethyl acetate. Bioactivity guided chromatographic separations of the active ethyl acetate extract led to the isolations of the new active principles, nepalensinol A, B and C.

Nepalensinol A (**1**), obtained as a reddish brown powder, was determined to have a molecular formula of $C_{42}H_{34}H_{10}$ from its HR-FABMS (*m/z* 737.1766 $[M+K]^+$, calcd. for $C_{42}H_{34}H_{10}K$, 737.1789), and its IR spectra exhibited an absorption band corresponding to an hydroxyl group. Acetylation and methylation of **1** gave the nona-acetate derivative (FABMS: *m/z* 1099 $[M+Na]^+$) and the octa-methylether derivative (FABMS: *m/z* 811 $[M+H]^+$), respectively, suggesting that **1** has eight phenolic hydroxy groups and one secondary hydroxy group. The ^{1}H NMR and ^{1}H–^{1}H COSY spectra showed the presence of three sets of *ortho*-coupled aromatic protons derived from three 4-hydroxyphenyl groups, two sets of aromatic protons coupled in an AX_2 system due to 3,5-dihydroxyphenyl groups, a singlet aromatic proton attributed to a penta-substituted benzene, a sequence of three aliphatic protons (H-7″, H-8″ and H-8′) connected successively, and a set of mutually coupled aliphatic proton (H-7 and H-8), as well as one hydroxymethine proton (H-7′), which coupled with an aliphatic proton (H-8′) at δ3.05 (1H, *d*, $J = 9.6$) and a hydroxy group (7′-OH) at δ3.87 (1H, *d*, $J = 3.6$). This evidence, together with the limitation imposed by twenty-six unsaturations, indicated the existence of one ring system and one cyclic ether system and six benzene rings in the structure. The 2D NMR spectra, including analysis of ^{1}H–^{1}H, ^{13}C–^{1}H COSY and HMBC spectra, allowed assignment of all proton and carbon signals as shown in Table 1, whose structure was deduced mainly from the HMBC spectrum (Fig. 2). Connections were observed between the aromatic carbons on the penta-substituted benzene ring and methine protons as follows: H-7″/C-9′, C-13′, C-14′, H-8″/C-9′, C-14′ and H-8′/C-9′, C-10′, C-14′. This indicated that the resveratrol unit C formed a indane moiety with the unit B. The correlation between H-2′(6′) and C-7′ indicated that the 4-hydroxyphenyl group (ring B_1) was substituted at C-7′. Finally, a dihydrobenzofuran ring consisting of resveratrol unit A and aromatic ring B_2 was elucidated by HMBC correlations of H-7/C-2(6), C-10′, C-11′ and H-8/C-10(14), C-10′, C-11′. Thus, structure **1** was determined as shown

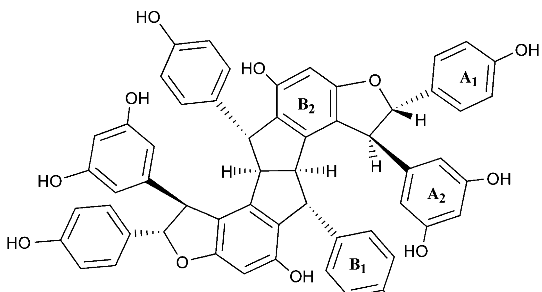
Table 1
^{1}H and ^{13}C NMR spectroscopic data of nepalensinol A (**1**), B (**2**) and C (**3**)

No.	**1**		**2**[a]		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		133.4		134.0		131.1
2(6)	7.31 (2H, *d*, 8.6)	127.7	7.11 (2H, *d*, 8.5)	126.6	7.16 (2H, *d*, 8.5)	128.4
3(5)	6.87 (2H, *d*, 8.6)	115.4	6.75 (2H, *d*, 8.5)[b]	115.4	6.76 (2H, *d*, 8.5)	115.0
4		157.5		157.3		157.2
7	5.48 (1H, *d*, 7.2)	93.6	5.31 (1H, *d*, 1.7)	93.2	4.50 (1H, *d* , 9.5)	87.2
8	4.95 (1H, *d*, 7.2)	57.7	4.31 (1H, *d*, 1.7)	56.4	3.54 (1H, *dd*, 11.3, 9.5)	55.6
9		146.7		148.0		140.6
10 (14)	6.40 (2H, brs)	108.0	6.29 (2H, brs)	106.0	5.86 (2H, *d*, 2.1)	107.2
11(13)		158.4		159.5		158.3
12	6.30 (1H, *t*, 2.2)	101.6	6.31 (lH, *t*, 2.0)	101.6	6.08 (1H, *t*, 2.1)	101.5
1′		136.0		137.9		132.2
2′(6′)	6.58 (2H, *d*, 8.7)	128.4	6.75 (2H, *d*, 8.5)[b]	128.6	7.04 (2H, *d*, 8.5)	129.0
3′(5′)	6.54 (2H, *d*, 8.7)	114.7	6.56 (2H, *d*, 8.5)	114.8	6.64 (2H, *d*, 8.5)	114.5
4′		156.6		155.3		156.6
7′	4.30 (1H, *dd*, 3.5, 9.6)	77.8	4.29 (1H, *s*)	49.6	5.33 (1H, *d* , 9.4)	81.9
8′	3.05 (1H, *d*, 9.6)	57.5	3.97 (1H, *s*)	59.9	3.98 (1H, *dd*, 11.3, 9.4)	51.6
9′		150.5		143.9		137.5
10′		119.9		125.9	6.12 (1H, *d*, 1.9)	107.8
11′		161.4		154.9		158.4
12′	6.40 (1H, *s*)	96.0	6.20 (1H, *s*)	96.1	6.02 (1H, *d*, 1.9)	95.0
13′		154.2		162.6		161.1
14′		122.7		115.6		119.5
1″		145.0				133.4
2″(6″)	6.93 (2H, *d*, 8.5)	128.8			7.06 (2H, *d*, 8.5)	127.6
3″(5″)	6.77 (2H, *d*, 8.5)	115.0			6.88 (2H, *d*, 8.5)	115.5
4″		155.7				157.6
7″	4.16 (1H, *s*)	55.8			5.28 (1H, *d*, 6.0)	93.3
8″	2.89 (1H, *s*)	58.2			4.32 (1H, *d*, 6.0)	57.7
9″		145.0				145.6
10″(14″)	5.90 (2H, *d*, 2.1)	105.5			6.29 (2H, *d*, 2.1)	107.0
11″(13″)		158.2				158.8
12″	6.06 (1H, *t*, 2.1)	100.5			6.41 (1H, *t*, 2.1)	102.3
7′-OH	3.87 (1H, *d*, 3.6)					
OH	8.70, 8.50, 8.39 8.13, 7.94					

[a] Compound **2** has symmetry in the structure (C1–C14).
[b] Overlapping.



(1)



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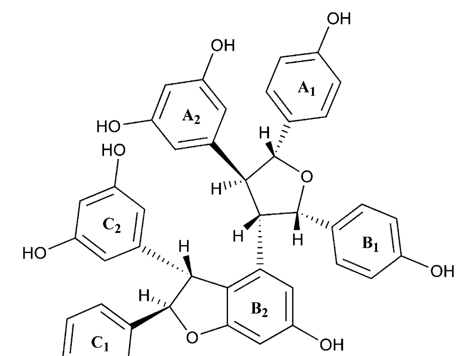
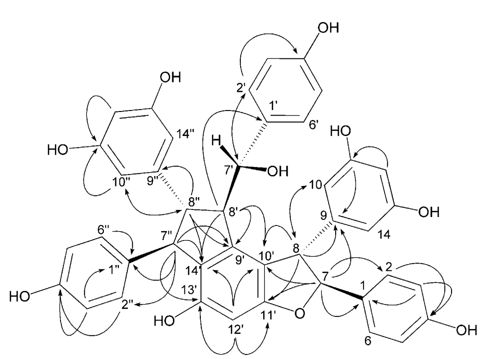


(3)

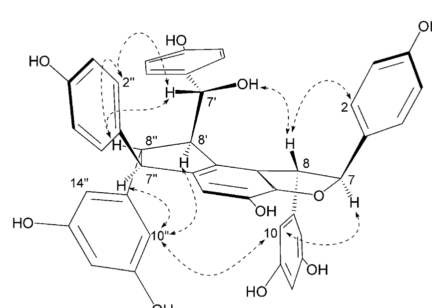
Fig. 1. Structures of nepalensinol A (**1**), B (**2**) and C (**3**).

in Fig. 1. The relative stereostructure was assigned from results of analysis of NOESY experiments (Fig. 2). NOE interactions were observed between H-2/H-8 and H-7/H-10, which showed that the phenyls at C-7 and at C-8 were situated in a *trans*-orientation to each other. NOEs also appeared between H-2″/H-8″, H-7″/H-10″, and H-8′/H-10″, suggesting that H-8′, H-7″ and phenyl at C-8″ were *cis*-orientated to each other. NOEs observed between H-10/H-10″ indicated that H-8 was *cis*-orientated to H-8″. Finally, the relative configuration at C-7′ was determined to be *rel*-7′*S* by the NOE enhancements (H-7′/H-2″, H-8″, and OH-7′/H-8), supported by the fact that the proton signal of H-8″ in the ^{1}H NMR spectrum appeared at relatively higher field with overlapping of the aryl proton at C-7′ (Ohyama et al., 1995). Thus, relative stereostructure of **1** was determined.

Nepalensinol B (**2**), a brown powder, gave an $[M + H]^+$ ion peak at m/z 907.2757 (calcd. for $C_{56}H_{43}O_{12}$, 907.2754) in its HR FABMS, corresponding to the molecular formula of $C_{56}H_{42}O_{12}$. The symmetrical nature of the molecule was revealed by the number of ^{13}C NMR spectroscopic signals in comparison to its molecular formula. The ^{1}H NMR and ^{1}H–^{1}H COSY spectra showed the presence of two sets of *ortho*-coupled aromatic protons assignable to a 4-hydroxyphenyl group, a set of 3,5-dihydroxyphenyl group, an aromatic proton on a penta-substituted benzene ring as a singlet, and a set of mutually coupled aliphatic protons (H-7 and H-8) from a dihydrofuran ring, in addition to two aliphatic methine protons (H-7′ and H-8′) characteristic of chemical shifts and coupling patterns observed in pallidol (Khan et al., 1986). These spectroscopic data were similar to those of ampelopsin H, a resveratrol oxidation tetramer (Oshima and Ueno, 1993), isolated from *Ampelopsis brevipodunculata*, with the exception of the chemical shifts and coupling constants of two aliphatic protons (**2**: H-7 δH 5.31, $J = 1.7$, H-8 δH 4.31, $J = 1.7$, ampelopsin H: H-7 δH 5.29 , $J = 8.0$, H-8 δH 4.88, $J = 8.0$) on the dihydrofuran ring. ^{1}H–^{1}H COSY and HMBC spectra revealed that **2** has the same structure (Fig. 2) as ampelopsin H, and the NMR signals were assigned as shown in Table 1. The relative stereochemistry was deduced by the NOESY experiments (Fig. 3): NOEs of H-7/H-10 and H-2/H-8 showed that the configuration of two methine protons (H-7 and H-8) on the dihydrofuran ring was *trans*. NOEs of H-8′/H-2′, H-8 indicated that two methine protons (H-8 and H-8′) and 4-hydroxyphenyl at C-7′ was *cis*-orientated to each other. Compound **2** had a symmetrical resveratrol dimer in its molecule. Thus, the



HMBC



NOE

Fig. 2. HMBC and NOESY correlations in nepalensinol A (**1**).

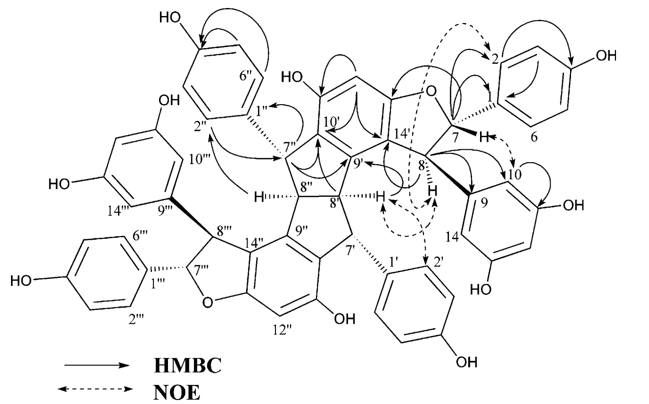


Fig. 3. HMBC and NOESY correlations in nepalensinol B (**2**).

relative stereostructure of **2** was determined as shown in Fig. 3. indicating that nepalensinol B (**2**) is a stereoisomer of ampelopsin H, although the stereochemistry of the latter has still not been exactly determined (Oshima and Ueno, 1993). Additionally, nepalensinol B and stenophyllol C are found to be same compound. Stenophyllol C has been isolated form *Sophora stenophylla* (Ohyama et al., 1998). These compounds have been isolated independently from different species at almost same time (Nozaki et al., 1997).

Nepalensinol C (**3**), a brown powder, had a molecular formula of $C_{42}H_{34}O_{10}$ was determined by HR FABMS (*m*/*z* 731.1786 $[M+K]^+$, calcd. for $C_{42}H_{34}O_{10}K$, 731.1789). The ^1H, ^{13}C and ^1H–^1H COSY spectra indicated the presence of three sets of *ortho*-coupled aromatic protons assignable to three 4-hydroxy phenyl groups, two sets of *meta*-coupled aromatic protons on a 3,5-dihydroxyphenyl group, and a *meta*-coupled aromatic proton on a 1,2,3,5-tetrasubstituted benzene ring. Further, the ^1H NMR spectra showed the presence of two mutually coupled aliphatic protons (H-7″ and H-8″), one of which was bonded to an oxygen bearing carbon, in addition to a sequence of four aliphatic methine protons (H-7, H-7′, H-8 and H-8′) connected, two of which were attached to carbons adjacent to oxygen. Acetylation of **3** with Ac_2O/pyridine yielded an octa-acetate derivative, with the spectra showing that there were no longer signals assignable to hydroxyl functions. These data indicated that **3** had eight phenolic hydroxy groups, and consequently, that there were two ether rings, one attributable to 2,3-diaryl-2,3-dihydrobenzofuran ring and the other as a tetrahydrofuran ring, which accounted for four successive aliphatic protons in the ^1H–^1H COSY spectrum. Analysis of the ^1H–^1H COSY, HMQC and HMBC spectra enabled the complete assignment of all protons and carbons as shown in Table 1. In the HMBC spectrum (Fig. 4, left), distinct cross-peaks observed between H-7/C-2(6), H-8/C-10(14), H-7′/C-2′(6′), H-8′/C-9′, H-7″/C-2″ (6″), and H-8″/C-10″ (14″) revealed the connections in each resveratrol unit A, B and C. The tetrahydrofuran ring comprised of resveratrol units A and B was elucidated by ^1H–^1H COSY correlations (H-7 to H-7′). HMBC correlations of H-8″/C-14′ and H-8″/C-13′ indicated that the resveratrol unit C formed a dihydrofuran ring with the aromatic ring B_2. As the results, structure **3** was determined as that in Fig. 1. To confirm the relative stereochemistry, NOESY experiments were performed (Fig. 4, right), and NOE correlations of H-7″/H-10″ and H-8″/H-2″ suggested that the configuration of two phenyls (rings C_1 and C_2) was *trans.* On the tetrahydrofuran ring, NOEs of H-7/H-8′ H-10 and H-2/H-8 indicated H-7, H-8′, and phenyl (ring A_2) was *cis*-oriented to each other. The configuration of H-8 and H-7′ was confirmed to be *trans* by NOEs of H-8/H-6′ and H-7′/H-8′. The NOE of H-8/H-10′ suggested H-10′ on ring B_2 was oriented to H-8. Therefore, the configuration of H-8′ and H-8″ was determined to be *rel*-(8′***S*** and 8″***R***) on the basis of NOEs of H-2′/H-8″ and H-8′/H-14″. Thus, the relative stereostructure of **3** was determined as in Fig. 1.

The inhibitory activity of nepalensinol A (**1**), B (**2**) and C (**3**) against topoisomerase II (topo II) was evaluated by the inhibitory effect against the decatenation activity of topo II on kinetoplast DNA. The compounds **1**–**3** were subjected to topo II assay at various concentrations. The IC_{50} values were determined from at least three individual experiments with three replicates for each concentration. Compounds **1**–**3** showed inhibitory activities (IC_{50} values: 0.30 μg/ml

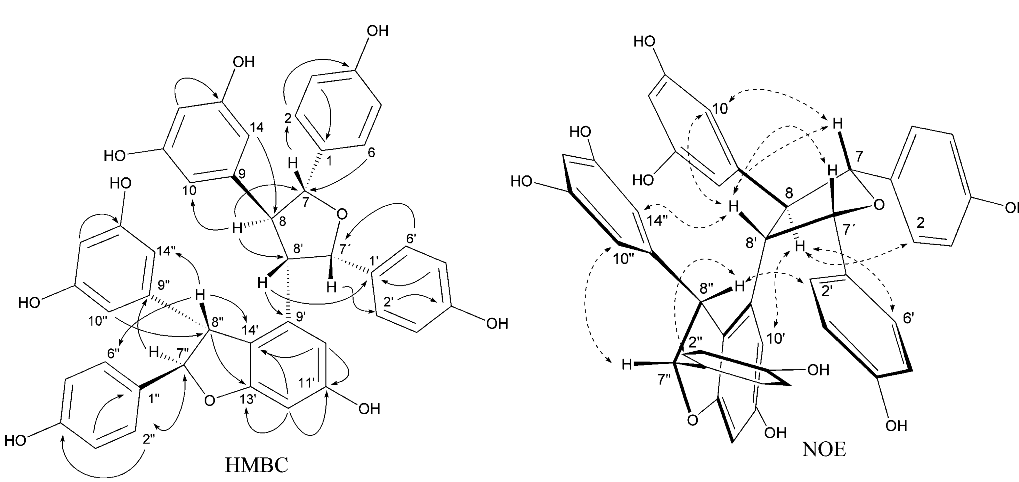


Fig. 4. HMBC and NOESY correlations in nepalensinol C (**3**).

for **1**, 0.02 μg/ml for **2** and 7.0 μg/ml for **3**), stronger than the positive control of etoposide (VP-16, $IC_{50} = 70$ μg/ml). Compound **2** exhibited the most potent activity, which is 3×10^3 times stronger than etoposide, VP16 (Hande, 1998). To evaluate the specificity of topo II inhibition, the effect of those compounds on the activity of a *Hin*dIII restriction enzyme was examined. *Hin*dIII enzyme recognizes DNA as a substrate and catalyzes the cleavage of DNA at a specific sequence. The IC_{50} values of **1** and **3** against *Hin*dIII activity were higher than 100 μg/ml. The specific inhibitory effects of **1** and **3** on topo II indicate that these might be suitable candidates as specific inhibitors of topo II, although the specificity of **2** could not be determined experimentally, because of its strong fluorescence in agarose gel. This is the first report that stilbenoids show a potent inhibitory activity on topoisomerase II. These findings thus provide new lead compounds for anti-cancer drug. The mechanism of inhibition and structure-activity relationships of these stilbenoids are now under investigation, and will be the subject of future efforts.

3. Experimental

3.1. General

All melting points were measured on a melting-point apparatus (Gallenkamp, UK) and are uncorrected. Optical rotations were measured with a SEPT-200 polarimeter (Horiba, Japan). UV spectra were recorded on a U-3210 spectrophotometer (Hitachi, Japan), and IR spectra on a MODEL 1720 spectrometer (Perkin–Elmer, Japan). ^{1}H and ^{13}C NMR spectra were recorded on an ARX400 spectrometer (Brüker, Germany), with chemical shifts shown in δ-values from TMS as the internal reference, with peak multiplicities quoted in Hz. Mass spectra were measured on a JMS-700 spectrometer (JEOL, Japan). Column chromatography was carried out on silica gel 60 (70–230 and 230–400 mesh, Merck, Japan), and Sephadex LH-20 (Amersham Biosciences, Japan) columns. Medium pressure liquid chromatography (prepacked ODS column, 15 × 300 mm, Kusano Kagakukikai, Japan), and HPLC (prep Nova-Pack HR C18, 25 × 100 mm, Waters, Japan) were also utilized.

3.2. Plant material

Stems of *K. nepalensis* were collected in the Himalaya mountains, Nepal, in August 1995. A voucher specimen of (No. OUS-1104) has been deposited in the herbarium of Okayama University of Science (OKAY).

3.3. Extraction and isolation

Dried stems (1.5 kg) of *K. nepalensis* were extracted with MeOH (8 l) at room temperature for two weeks, with the combined extracts concentrated under reduced pressure. The resultant residue was suspended in water and partitioned with *n*-hexane, EtOAc, and *n*-BuOH, respectively. In the first inhibitory assay, the EtOAc extracts showed a significant inhibitory activity on topoisomerase II at 100 μg/ml. The EtOAc extracts (50 g) was subjected to silica gel column chromatography (CC, 90 × 12 cm) eluting with a gradient mixture of $CHCl_3$–MeOH to give ten fractions. Fraction 7 (2.8 g) showing the inhibitory activity was purified by silica gel CC (50 × 3 cm) with gradient elution ($CHCl_3$:MeOH:H_2O = 10:2:0.1–9:3:0.2) to give two active subfractions (7A: 320 mg and 7B: 440 mg). Each active fractions 7A and 7B were separated by Sephadex LH-20 CC (50 × 2.5 cm, $CHCl_3$:MeOH = 1:1) to give active fractions $7A_1$, and $7B_1$, respectively. The active fraction $7A_1$ (95 mg) was separated by ODS medium pressure liquid chromatography (MeOH:H_2O = 1:2) and active fractions were combined. Further purification of the active fraction by ODS-HPLC (CH_3CN:H_2O = 23:77) gave nepalensinol B (**2**: 5.1 mg), and C (**3**: 14 mg). The active fraction $7B_1$ (220 mg) was purified by ODS medium pressure liquid chromatography (MeOH:H_2O = 1:2) and ODS-HPLC (CH_3CN:H_2O = 23:77) to give nepalensinol A (**1**: 80 mg).

3.3.1. Nepalensinol A (*1*)

Reddish brown powder; m.p. 310 °C (decomp.); $[\alpha]_D - 161.7°$ (MeOH, *c* 0.2); UV λ_{max} (MeOH) nm (log ε): 224 (4.53), 278 (4.17); IR(KBr) ν_{max} 3364 (*brd*), 1620, 1523, 1463, 1353, 1001, 831 cm^{-1}; For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; Positive FABMS *m*/*z* 721 $[M + Na]^+$, Negative FABMS *m*/*z* 697 [M − H], HR FABMS (positive) *m*/*z* 737.1766 $[M + K]^+$ for $C_{42}H_{34}O_{10}K$ (Δmmu 2.3).

3.3.2. Nepalensinol A nona-acetate

Acetylation of **1** (5.5 mg) with pyridine-Ac_2O (1:1) by the usual procedure afforded the nona-acetate (7.7 mg) as a pale yellow powder; m.p. 133 °C; $[\alpha]_D + 16.0°$ ($CHCl_3$, *c* 0.4); UV λ_{max} (MeOH) nm (log ε): 293 (3.77); IR ($CHCl_3$) ν_{max} 1767, 1615, 1509, 1455, 1371, 1228, 1201, 1126, 779 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.63 (3H, *s*), 1.69 (3H, *s*), 2.24 (6H, *s*), 2.26 (6H, *s*), 2.27 (6H, *s*), 2.31 (3H, *s*), 3.09 (1H, *t*, *J* = 5.4), 3.42 (1H, *t*, *J* = 6.0), 4.16 (1H, *d*, *J* = 5.5), 4.70 (1H, *d*, *J* = 6.0), 5.73 (1H, *d*, *J* = 6.0), 5.91 (1H, *d*, *J* = 6.9), 6.37 (2H, *d*, *J* = 2.0), 6.65 (2H, *d*, *J* = 8.5), 6.70–6.75 (4H, *m*), 6.84 (2H, *d*, *J* = 8.6), 6.88 (3H, *brs*), 6.90 (2H, *d*, *J* = 8.6), 7.16 (2H, *d*, *J* = 8.6), 7.41 (2H, *d*, *J* = 8.6); Positive FABMS *m*/*z* 1099 $[M + Na]^+$.

3.3.3. Nepalensinol A octa-methyl ether

1 (7.8 mg) was refluxed with K_2CO_3 (50 mg) and dimethyl sulfate (300 μl) in dry acetone (3 ml) for 6 h. The methyl ether (5.5 mg) was isolated by silica gel column chromatography ($CHCl_3$:EtOAc = 8:1) to give a pale yellow powder; m.p. 85 °C; $[\alpha]_D - 20.8°$ ($CHCl_3$, *c* 0.3); UV λ_{max} (MeOH) nm (log ε): 284 (3.85); IR ($CHCl_3$) ν_{max} 1608, 1511, 1466, 1249, 1158, 833 cm^{-1}; ^{1}H NMR ($CDCl_3$)δ: 2.85 (1H, *s*), 2.93 (1H, *d*, *J* = 8.7), 3.59 (6H, *s*), 3.73 (6H, *s*), 3.81 (3H, *s*), 3.82 (3H, *s*), 4.12 (1H, *dd*, $J = 7.15$, 14.3), 4.31 (1H, *d*, $J = 9.7$), 4.40 (1H, *s*), 4.78 (1H, *d*, $J = 8.4$), 5.50 (1H, *d*, $J = 8.4$), 5.77 (2H, *d*, $J = 2.2$), 6.17 (1H, *t*, $J = 2.2$), 6.30 (3H, *brs*), 6.56 (3H, *m*), 6.65 (2H, *d*, $J = 8.6$), 6.79 (2H, *d*, $J = 8.7$), 6.90 (4H, *m*), 7.34 (2H, *d*, $J = 8.7$); Positive FABMS *m*/*z* 811 $[M + H]^+$.

3.3.4. Nepalensinol B (2)

Brown powder. m.p. 260 °C (decomp.); $[\alpha]_D - 24.4°$ (MeOH, *c* 0.2); UV λ_{max} (MeOH) nm (log ε): 212 (3.95), 285(4.13); IR (KBr) ν_{max} 3296 (brd), 1620, 1518, 1455, 1353, 1238, 1149, 1094, 1005, 835, 695 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see in Table 1; Positive FABMS *m*/*z* 907 $[M + H]^+$, HR FABMS (positive) *m*/*z* 907.2757 $[M + H]^+$ for $C_{56}H_{43}O_{12}$ (Δmmu 0.2).

3.3.5. Nepalensinol C (3)

Brown powder; m.p. 290 °C (decomp.); $[\alpha]_D - 56.3°$ (MeOH, *c* 0.6,); UV λ_{max} (MeOH) nm (log ε): 218 (3.84), 278 (4.15); IR (KBr) ν_{max} 3210 (*brd*), 1612, 1523, 1459, 1344, 1225, 1153, 1094, 1009, 839, 686 cm^{-1}, For ^1H and ^{13}C NMR spectroscopic data, see Table 1; Positive FABMS *m*/*z* 737 $[M + H]^+$, HR FABMS (Positive) *m*/*z* 737.1786 $[M + K]^+$ for $C_{42}H_{34}O_{10}K$ (Δmmu 0.3).

3.3.6. Nepalensinol C octa-acetate

Acetylation of **3** (1.7 mg) with pyridine-Ac_2O (1:1) by the usual procedure afforded the octa-acetate (2.7 mg) as pale yellow powder; m.p. 137 °C; $[\alpha]_D - 17.1°$ ($CHCl_3$, *c* 0.1); UV λ_{max} (MeOH) nm (log ε): 277 (4.15), 238 (4.14); IR ($CHCl_3$) ν_{max} 1767, 1615, 1596, 1509, 1455, 1371, 1285, 1201, 1126, 779 cm^{-1}; ^1H NMR ($CDCl_3$)δ: 2.24 (3H, *s*), 2.25 (9H, *s*), 2.27 (3H, *s*), 2.29 (6H, *s*), 2.32 (3H, *s*), 3.36 (1H, *t*, $J = 7.8$), 3.52 (1H, *t*, $J = 7.3$), 4.00 (1H, *d*, $J = 6.0$), 4.93 (1H, *d*, $J = 8.9$), 5.36 (2H, *m*), 6.41 (1H, *d*, $J = 1.9$), 6.44 (1H, *d*, $J = 1.9$), 6.49 (2H, *d*, $J = 2.0$), 6.59 (2H, *brs*), 6.77 (1H, *t*, $J = 2.0$), 6.85 (3H, *m*), 7.10 ~ 7.02 (8H, *m*), 7.28 (2H, *d*, $J = 8.7$); Positive FABMS *m*/*z* 1073 $[M + K]^+$.

3.4. Biological assays

3.4.1. Topoisomerase II assay

Purified human topoisomerase II was purchased from TopoGen, Inc (USA) and kinetoplast DNA was purified from *Crithidia fasciculata* with cesium chloride using step gradient centrifugation as described in a previous report (Englund, 1979). Topoisomerase II activity was assessed by a decatenation reaction of kinetoplast DNA (Miller et al., 1981). The assay was performed in a reaction mixture (20 µl) containing 50 mM Tris–HCl (pH 7.9), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA (pH 8.0), 0.5 mM ATP, 30 µg/ml bovine serum albumin, and 0.25 µg/ml of kinetoplast DNA, as described previously (Tsutsui et al., 1986). A DMSO solution of the stilbenoids was diluted with Tris–HCl buffer (pH 7.9) and then added in the reaction mixture to be 0.05% final concentration (V/V) of DMSO. The reaction was initiated by adding 1 µl of topoisomerase II (0.75 U). After incubation at 30 °C for 30 min, the reaction was terminated by addition of 4 µl of the solution (0.66% SDS and 0.33 mg/ml proteinase K) prior to the analysis of DNA products by 0.8% agarose gel electrophoresis. Gels were stained with ethidium bromide and photographed under UV light. Monomer minicircles released from the kinetoplast DNA were quantified by NIH image software (NIH, USA).

3.4.2. HindIII assay

*Hin*dIII activity was assessed by conversion of supercoiled plasmid DNA to the linear form. The assay was performed in a reaction mixture (20 µl) containing 10 mM Tris–HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, and 200 ng of pBluescript SK(-) DNA with or without test compounds. The reaction was started by adding 1 µl of *Hin*dIII (1.25 U; New England Biolab., USA). After incubation at 37 °C for 60 min, the plasmids were separated by agarose gel electrophoresis in the presence of ethidium bromide. The linear form DNAs generated by *Hin*dIII reaction were quantified by densitometry.

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