Nepalensinols D—G, New Resveratrol Oligomers from *Kobresia nepalensis* (Cyperaceae) as Potent Inhibitors of DNA Topoisomerase II

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Four new resveratrol oligomers, nepalensinols D—G, were isolated from the stem of *Kobresia nepalensis* (Cyperaceae). The structures were determined by detailed NMR spectral analysis. The compounds were assessed for their inhibitory activity against human topoisomerase II, a potential target of anti-tumor agents. These stilbenoids showed potent inhibitory activity against human topoisomerase II with IC_{50} values of 5—15 μM.

Key words *Kobresia nepalensis*; nepalensinol; stilbene; topoisomerase; inhibitor

Topoisomerase II (topo II) catalyzes the conversion of topological states of DNA, such as overwinding, underwinding, and catenation, and can relax supercoiled DNA and resolve knotted or catenated DNA rings.[1] Therefore, topo II plays a crucial role in proliferative processes such as DNA replication, chromosome condensation, and chromosome segregation, which indicate that topo II is a critical target for anti-cancer drugs. In the search for new classes of inhibitors of human topo II in plant extracts, we previously isolated nepalensinols A, B and C, oligomers of resveratrol, from the stem of *Kobresia nepalensis* and showed that they are potent topo II inhibitors.[2,3] This plant belongs to the family Cyperaceae, consisting of a genus comprising about 50 species. It is distributed in the northern hemisphere, particularly at high altitudes in the Himalayas, China and Central Asia. *K. nepalensis* is an important species in the alpine flora of the Nepalese Himalayas and is an economically important pasture crop.[4] As part of our ongoing project to screen natural resources for topo II inhibitors,[5,6] further investigation of extracts of *K. nepalensis* has led to the isolation of four new stilbenoids, three of them active and one inactive. We describe here the isolation and structure determination of nepalensinols D (**1**), E (**2**), F (**3**) and nepalensinol G (**4**), as well as their inhibitory activity against human topoisomerase II.

Results and Discussion

Nepalensinol D (**1**) was obtained as a brown powder. The molecular formula was determined to be $C_{42}H_{34}O_{10}$ by HR-FAB-MS. The IR spectrum showed an absorption band at 3215 cm^{-1} attributable to a hydroxyl group. ^{1}H- and ^{13}C-NMR and ^{1}H–^{1}H COSY spectroscopic data indicate that **1** has a sequence of four aliphatic methines (H-7a, H-8a, H-8b and H-7b), two mutually coupled aliphatic methines (H-7c and H-8c), a 1,2,3,5-tetra-substituted benzene, two sets of 3,5-dihydroxyphenyl groups, and three sets of 4-hydroxyphenyl groups. These NMR signals are characteristic of a stilbene oligomer and 26 unsaturations, suggesting that **1** is a resveratrol trimer containing two rings and six benzene rings in its structure. The ^{1}H–^{1}H COSY, HMQC, and HMBC spectra allowed the assignment of all proton and carbon signals (Table 1). HMBC correlations (H-2b/C-7b, H-7b/C-9b, H-8b/C-9b, and H-10b/C-8b) among the aliphatic methine protons (H-7b and H-8b), 4-hydroxyphenyl group (ring B_1) and tetra-substituted benzene ring (ring B_2) could be attributed to a resveratrol unit B comprising these three moieties. Resveratrol units A and C were elucidated by HMBC correlations [unit A: H-2a(6a)/C-7a, H-7a/C-2a(6a), H-8a/C-9a, and H-10a(14a)/C-8a; unit C: H-2c(6c)/C-7c, H-7c/C-2c(6c), C-9c, H-8c/C-9c, C-10c(14c), and H-10c(14c)/C-8c]. Taken together with 26 unsaturations of **1** and the presence of three resveratrol units above mentioned, HMBC correlations from H-8c to C-13b, C-14b and the chemical shift of C-7c (δ_C 93.8) indicated the formation of a dihydrobenzofuran ring at C-7c and C-8c of the C unit, with a tetra-substituted benzene ring (ring B_2). Finally, connection from H-7a to H-7b in the ^{1}H–^{1}H COSY spectrum, in addition to the chemical shifts of C-7a and C-7b (C-7a: δ_C 81.6 and C-7b: δ_C 84.8), suggest that the resveratrol unit A is coupled to the unit B at C-8a and C-8b to form a tetrahydrofuran ring. Thus, the planar structure of **1** was determined as shown in Fig. 1 and was found to be the same planar structure as for nepalensinol C.[2,3] The distinct spectroscopic data and specific rotation for **1** and nepalensinol C indicate that the two compounds are diastereometric. The relative stereochemistry was established by NOESY experiments (Fig. 2). NOE interactions were observed between H-2c(6c)/H-8c, and H-7c/H-10c(14c), which indicate that the configuration of two phenyls at C-7c and C-8c were *trans*. NOEs observed in H-7b/H-8a and H-2b(6b)/H-10b suggest that H-8a, H-7b, and H-8b are *cis*-oriented to each other. A strong NOE was observed between H-7b and H-8a, which can be observed only when these protons are situated on the same side of a reference plane (β-configuration) and C-8b forms the flap of the envelope conformation. An NOE observed for H-7a/H-10b suggests that H-7a and the ring B_2 are *cis*-oriented. The small value ($J_{H\text{-}7b,8b}$=4.6 Hz)

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Table 1. ^{13}C-NMR Data for Nepalensinol D (**1**), E (**2**), F (**3**) and G (**4**)

Carbon No.	**1**	**2**	**3**[a)]	**4**
1a	135.7	132.8	134.2	127.9
2a (6a)	128.2	128.6	130.4	130.2
3a (5a)	115.3	114.6	116.5	116.5
4a	157.7	156.3	158.5[b)]	158.5
7a	81.6	81.5	93.7	89.0
8a	56.8	54.6	52.7	49.3
9a	141.4	135.9	141.9	143.4
10a	107.6[b)]	106.6[b)]	117.9	120.3
11a	158.0[b)]	157.6[b)]	157.7	159.4
12a	100.5	101.5	102.0	102.0
13a	158.0[b)]	157.6[b)]	156.8	157.7
14a	107.6[b)]	106.6[b)]	106.7	107.0
1b	129.9	129.4	134.7	134.0
2b (6b)	128.9	128.8	130.9	128.3
3b (5b)	114.4	115.2	114.4	115.8
4b	157.4	157.1	155.6	156.3
7b	84.8	84.4	44.9	40.0
8b	52.1	52.5	42.0	42.0
9b	136.3	138.1	141.5	138.2
10b	110.3	104.9	123.0	142.0
11b	157.0	159.0	158.5[b)]	171.2
12b	95.0	95.5	94.7	100.2
13b	161.1	161.0	159.7	178.4
14b	121.1	122.1	107.9	178.3
1c	131.8	132.8		134.8
2c (6c)	128.9	127.9		129.1
3c (5c)	115.3	115.3		115.3
4c	156.6	157.3		155.7
7c	93.8	94.1		40.1
8c	56.9	58.4		48.0
9c	145.9	147.5		139.4
10c	107.9[b)]	109.8		119.8
11c	158.2[b)]	159.2		160.5
12c	102.0	96.0		95.7
13c	158.2[b)]	159.5		157.8
14c	107.9[b)]	122.7		112.8
1d		132.9		130.8
2d (6d)		128.4		130.4
3d (5d)		115.5		116.0
4d		157.7		158.6
7d		93.0		88.6
8d		55.1		49.4
9d		147.9		142.9
10d		106.6[b)]		119.6
11d		159.3[b)]		159.5
12d		101.4		100.9
13d		159.3[b)]		157.3
14d		106.6[b)]		106.6

a) **3** has symmetry in the structure (C-1a to C-14b). *b*) Overlapping.

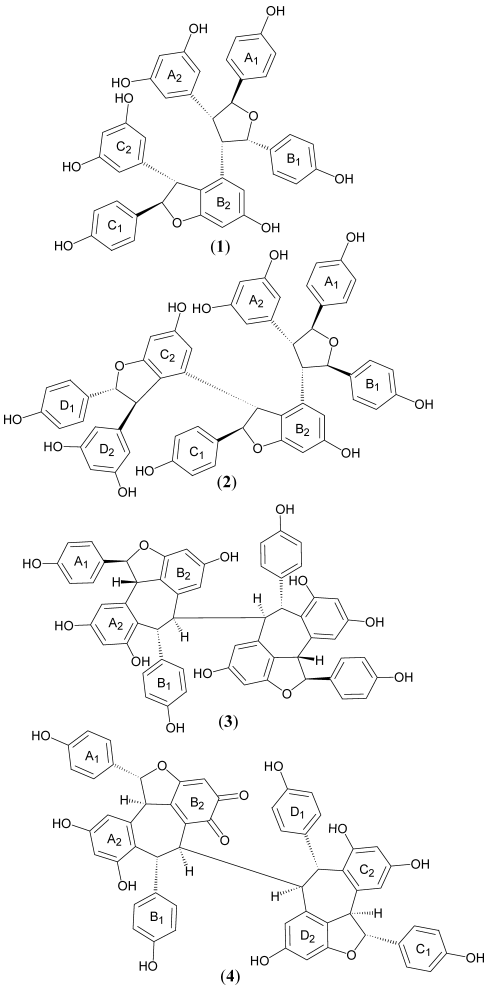

Fig. 1. Structures of Nepalensinols D (**1**), E (**2**), F (**3**) and G (**4**)

and an additional NOE [H-2b(6b)/H-10b] suggests the *cis* relationship of H-7b and H-8b. This was also supported by small *J*-value (4.2 Hz) between *cis*-oriented protons at H-7b and H-8b in kobophenol A.[7] In addition, NOEs for H-2a(6a)/H-8a and H-7a/H-10a(14a) revealed that H-7a is *trans*-oriented to H-8a. Finally, the relative configuration of C-8b and C-8c was determined to be *rel*-(8*b**S***,8*c**R***) by NOE enhancements (H-7a/H-10b and H-8c/H-2b). Thus, the relative stereochemistry of **1** was determined as shown in Fig. 2 and was found to be a diastereomer of nepalensinol C at C-7a and C-8a.[2,3]

Nepalensinol E (**2**), a brown powder, gave an $[M+H]^+$ ion peak at *m*/*z* 925.2857 in HR-FAB-MS, corresponding to a molecular formula of $C_{56}H_{44}O_{13}$. The ^{1}H-NMR spectrum displayed characteristic features of a resveratrol oligomer. The ^{13}C-NMR spectrum (Table 1) and the molecular formula indicate that **2** is a tetramer. ^{1}H-NMR and ^{1}H–^{1}H COSY spectra showed the presence of two sets of mutually coupled aliphatic methine protons (H-7c and H-8c, H-7d and H-8d), a sequence of four aliphatic methine protons (H-7a, H-8a, H-7b, and H-8b) attributable to a tetra-substituted tetrahydrofuran, two sets of *meta*-coupled aromatic protons on a tetra-substituted benzene, two sets of 3,5-dihydroxyphenyl protons, and four sets of 4-hydroxyphenyl protons. The HMBC spectra of **2** displayed the same correlations as for **1** among resveratrol units A, B, and C. The tetrahydrofuran moiety composed of units A and B, and the dihydrobenzofuran moiety composed of units B and C were established by HMBC correlations, as depicted in Fig. 3. Additional HMBC correlations [H-10c/C14c, H-12c/C-14c, H-8d/C-9c, C-14c, H-2d(6d)/H-7d and H-10d(14d)/C-8d] revealed that resveratrol unit C was coupled with unit D at C-7d and C-8d to form another dihydrobenzofuran ring. Finally, the planar structure of **2** was determined as shown in Fig. 1 and was found to be the same planar structure as that of kobophenol A isolated from *Carex kobomugi*.[7] The relative stereochemistry was elucidated from the NOESY spectrum. NOESY correlations of H-2a(6a)/H-8b and H-7b/H10b suggest that H-7a and H-8b are *trans*-oriented to each other and the configuration of H-7b and H-8b was *trans* (Fig. 3, left). NOE enhancements [H-2c(6c)/H-8c, H-7d/H-10d(14d), and H-8d/H-2d(6d)] indi-

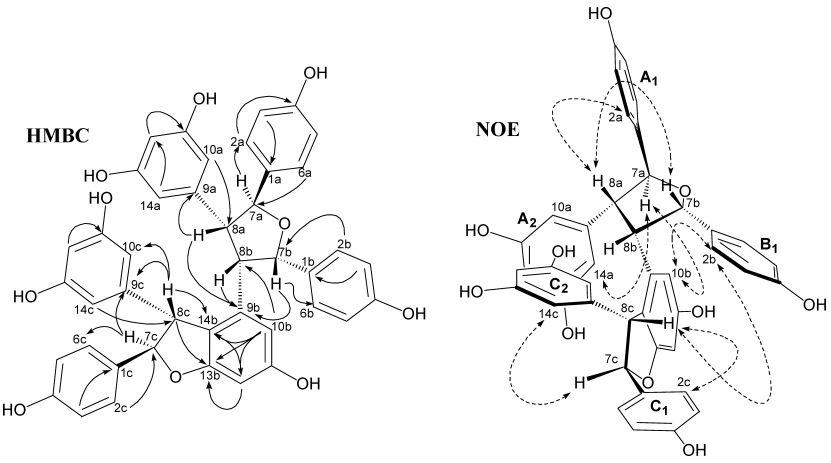

Fig. 2. HMBC and NOESY Correlations in Nepalensinol D (**1**)

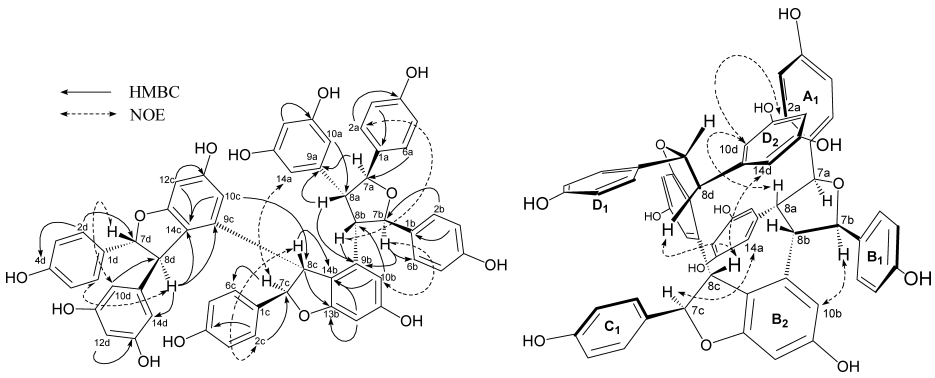

Fig. 3. HMBC and NOESY Correlations in Nepalensinol E (**2**)

cated that the configuration of the two phenyls at C-7c/C-8c and at C-7d/C-8d in the dihydrobenzofurans is *trans* (Fig. 3, left). Taken together with the orientation of the B_2 ring based on the NOEs for H-7b/H-10b, the NOE of H-7c/H-14a suggests that the configuration of H-8a and H-8b is *cis* and that of H-8b and H-7c is *rel*-(8*b**S***, 7*c**R***), as shown in Fig. 3 (right). The NOEs of H-8c/H-8d and H-14d indicate that H-8d and ring D_2 are oriented to the same direction as H-8c. Ring D_2 is located near H-8a and ring A_1 on the basis of NOEs for H-10d/H-2a and H-8a. Thus, the relative configuration of the three furans was elucidated to be *rel*-(8*b**S***, 8*c**R***, and 8*d**R***) (Fig. 3, right). Thus, the stereochemistry of **2** was determined as in Fig. 3 and this confirms that **2** is a stereoisomer of kobophenol A at H-7b, H-7c and H-8c.[7)]

Nepalensinol F (**3**) was isolated as a brown powder and a molecular formula of $C_{56}H_{42}O_{12}$ was elucidated by HR-FAB-MS (*m*/*z* 929.2553 $[M+Na]^+$). The symmetrical nature of **3** was revealed by the number of ^{13}C-NMR signals (Table 1) for its molecular formula. This indicated that **3** is a resveratrol tetramer composed of two symmetrical dimers. ^{1}H-NMR and ^{1}H–^{1}H COSY spectra showed two sets of 4-hydroxyphenyl protons and two sets of *meta*-coupled aromatic protons on a 1,3,4,5-tetra-substituted benzene, together with two sets of mutually coupled aliphatic methine protons [H-7a: δ_H 5.83 (d, J=8.6 Hz), H-8a: δ_H 5.33 (d, J=8.6 Hz); and H-7b: δ_H 3.99 (d, J=0.8 Hz), H-8b: δ_H 5.98 (d, J=0.8 Hz)]. In the HMBC spectrum, unit A was deduced from correlations for H-7a/C-2a(6a), C-9a, H-8a/C-1a, C-2a(6a), C-9a, C-10a, and H-14a/C-10a. Connections in the unit B were deduced from the HMBC correlations for H-7b/C-2b(6b), H-8b/C-10b, C-14b, H-12b/C-11b, and H-14b/C-10b. Taken together with the presence of resveratrol A unit and 1,3,4,5-tetrasubstituted benzene ring in B unit, the methines at C-7a and C-8a of unit A were connected with C-11b in an ether linkage and with C-10b in the B_2 ring, respectively, to form a dihydrobenzofuran, based on HMBC correlations (H-8a/C-11b, H-14a/C10b) and the chemical shift of C-7a (δ_C 93.7). Furthermore, the connection between C-7b and C-10a was confirmed by HMBC correlations (H-8b/C-11a and H-7b/C-11a). Thus, the planar structure of **3** was determined as shown in Fig. 1, indicating that **3** is a stereoisomer of hopeaphenol, which has been isolated from the heartwood of *Hopea odorata* or *Balanocarpus heimii*.[8)] Three stereoisomers have been reported for hopeaphenol: isohopeaphenol,[9)] pauciflorol C,[10)] and vateriaphenol B.[11)] Among these, hopeaphenol and isohopeaphenol possess a symmetrical plane, as does compound **3**. However, the NMR data for **3** did not agree with those for hopeaphenol and isohopeaphenol. Although the stereochemistry of H-7c and H-8c in vateriaphenol B (corresponding to H-7b and H-8b at dimer unit in **3**) was drawn as α and β in Fig. 1 in a previous report,[10)] the correct structure of vateriaphenol B is β (H-7c) and α (H-8c), as described in the text (not symmetrical, see Table 2). Thus, the relative structural stereochemistry of nepalensinol F (**3**) is different from that of vateriaphenol B.

For confirmation of the relative stereochemistry, a difference NOE spectrum was measured (Fig. 4). NOE enhancement of H-2b(6b) was observed when H-8b was irradiated,

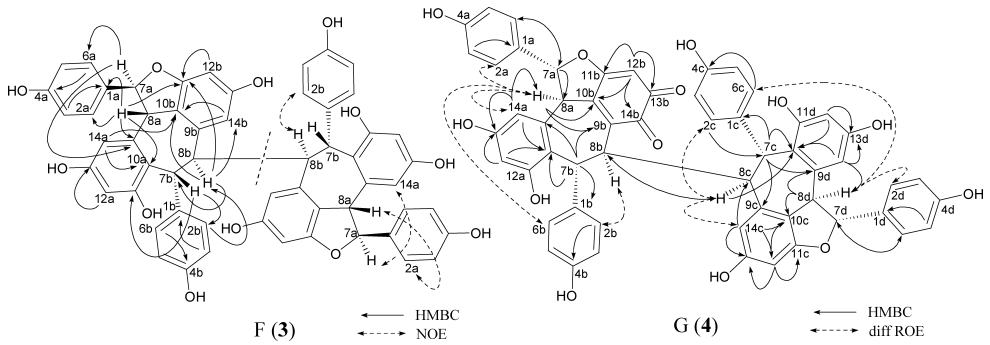

Fig. 4. 2D NMR Correlations in Nepalensinols F (**3**) and G (**4**)

Table 2. Differences in Stereochemistry among Five Isomers

		H-7a	H-8a	H-7b	H-8b	H-7c	H-8c	H-7d	H-8d
Nepalensinol F (**3**)	Symmetrical	α	β	β	α				
Hopeaphenol	Symmetrical	α	β	α	β				
Isohopeaphenol	Symmetrical	α	β	β	β				
Pauciflorol C	—	α	β	β	β	β	α	α	β
Vateriaphenol B	—	α	β	β	α	β	α	β	α

indicating that H-7b is *trans*-oriented to H-8b. NOEs for H-2a(6a)/H-8a and H-7a/H-14a suggest that the configuration of H-7a and H-8a is *trans*. The stereochemistry of hopeaphenol was determined previously by X-ray crystallography.[8,12] The relative configurations of H-7a/H-8a and H-7b/H-8b in hopeaphenol are both *trans*, which are the same configurations as in **3** at H-7a/H-8a (*trans*) and H-7b/H-8b (*trans*). This evidence suggests that **3** is a diastereomer of hopeaphenol at H-7a(7b) and H-8a(8b). The configurations of five stereoisomers are listed in Table 2. Thus, the relative stereochemistry of **3** was determined as shown in Fig. 4.

Nepalensinol G (**4**), a reddish brown powder, has a molecular formula of $C_{56}H_{40}O_{13}$, as determined by HR-FAB-MS, indicating that **4** is a resveratrol tetramer, confirmed by the number of ^{13}C-NMR signals (Table 1). The ^{1}H- and ^{13}C-NMR spectra closely resemble those of hopeaphenol; however, **4** has an asymmetrical nature, in contrast to hopeaphenol. A resveratrol dimer of an asymmetrical unit displayed almost identical NMR signals as hopeaphenol,[13] but another resveratrol dimer showed two additional carbonyl signals (δ_C 178.3 and 178.4) and the disappearance of *meta*-coupled aromatic protons on the 3,5-dioxygenated phenyl group (ring B_2) in the NMR spectra of **4**. This evidence strongly implies that **4** is an oxidation product of hopeaphenol. The ^{1}H-NMR and ^{1}H–^{1}H COSY spectra showed signals attributable to four sets of 4-hydroxyphenyl protons, three sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetra-substituted benzene, together with an olefinic proton (H-12b) and four sets of two mutually coupled aliphatic methine protons. The HMBC correlations between olefinic protons (H-12b) and two carbonyl carbons (C-13b, C-14b), in addition to the correlations of H-12b/C-11b and C-10b, reveal that **4** has a *ortho*-quinone moiety in the B_2 ring. The connections of the other moieties were established by HMBC correlations (Fig. 4) and the planar structure determined is shown in Fig. 1. Difference ROE experiments were conducted to confirm the relative stereochemistry (Fig. 4). ROE enhancements for H-2a(6a) and H-2b(6b) were observed when H-8a was irradiated, which suggests that H-8a is *trans*-oriented to H-7a and H-7b. ROEs observed for H-8d/H-2d(6d) and H-8d/H-2c(6c) reveal that H-8d is *trans*-oriented to H-7c and H-7d. The configurations of H-7b/H-8b and H-7c/H-8c were determined to be both *trans* by additional ROEs for H-2b(6b)/H-8b and H-2c(6c)/H-8c. Although the configurations of H-8b and H-8c could not be revealed by the spectroscopic methods used, the relative stereochemistry was elucidated as shown in Fig. 4, except for the configuration of H-8b and H-8c.

The inhibitory activity of nepalensinols D—G (**1**—**4**) against topo II was evaluated in terms of the inhibitory effect against the decatenation activity of topo II on kinetoplast DNA. Compounds **1**—**4** 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. Daunorubicin, an anthracycline-type antibiotic, is a potent inhibitor of topo II and is clinically valuable as an anticancer drug.[14] Daunorubicin was used as a positive control and showed an IC_{50} value of 9.1 μM in this assay. Nepalensinol F (**3**) showed more potent inhibitory activity (IC_{50}=5.5 μM) than daunorubicin. Nepalensinol D (**1**) and E (**2**) exhibited almost the same activity (**1**: IC_{50}=14.8 μM, **2**: IC_{50}=11.7 μM) as daunorubicin. In contrast, **4** showed no inhibitory activity (IC_{50}>50 μM). The mechanism of inhibition and the structure–activity relationships of these resveratrol oligomers are now under investigation and will be published elsewhere in the near future.

Experimental

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). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL lambda 500 NMR spectrometer (JEOL, Japan). Chemical shifts are shown as δ values from TMS as the internal reference. Peak multiplicities are quoted in Hz. Mass spectra were measured on a JMS-700 spectrometer (JEOL, Japan). Column chromatography was carried out on columns of sil-

ica gel 60 (70—230 and 230—400 mesh, Merck, Japan), Sephadex LH-20 (Amersham Biosciences, Japan), a medium-pressure liquid chromatography prepacked ODS column (15×300 mm, Kusano Kagakukikai, Japan), and HPLC prep Nova-Pack HR C18 and Nova-Pack Phenyl (Waters, Japan).

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

Extraction and Isolation Dried stem (1.5 kg) of *Kobresia nepalensis* was extracted with MeOH. The MeOH solution was concentrated under reduced pressure and the residue was poured into water. The suspended solution was partitioned successively with *n*-hexane, EtOAc, and *n*-BuOH. EtOAc extracts (50 g) that showed inhibitory activity against topoisomerase II at 100 μg/ml were applied to silica gel column chromatography (CC) (90×12 cm) and eluted with a gradient of $CHCl_3$–MeOH to yield 10 fractions. Fraction 7 (2.8 g) showed the strongest inhibitory activity and 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 fractions (7A, 320 mg; 7B, 440 mg). Nepalensinol A was isolated from active fraction 7B as previously described. Active fractions 7A were combined and separated by Sephadex LH-20 CC (50×2.5 cm, $CHCl_3$/MeOH 1 : 1) to give two active subfractions ($7A_1$ and $7A_2$). Potent active fraction $7A_1$ (95 mg) contained nepalensinols B, and C as previously described.[3] Moderately active fraction $7A_2$ (85 mg) was further purified by HPLC (Nova-Pack phenyl, 5 mmϕ×100 mm, CH_3CN/H_2O 3 : 7) to afford nepalensinol E (**2**, 10 mg). Active fraction 8 (1.2 g) was fractionated by silica gel CC ($CHCl_3$/MeOH/H_2O 9 : 4 : 0.2). Active fractions were combined and separated by Sephadex LH-20 CC (30×2 cm, $CHCl_3$/MeOH 1 : 1) to give active fraction 8A. Fraction 8A (25 mg) was purified by HPLC (Nova-Pack ODS, 20 mmϕ×100 mm, CH_3CN/H_2O 23 : 77) to give nepalensinol D (**1**, 4.2 mg). Active fraction 9 (1.65 g) was purified by silica gel CC ($CHCl_3$/MeOH/H_2O 9 : 4 : 0.2). Combined active fractions (0.25 g) were fractionated by Sephadex LH-20 CC (30×2 cm, $CHCl_3$/MeOH 1 : 1) to give active fraction 9A. Fraction 9A (92 mg) was further separated by ODS medium-pressure liquid chromatography (MeOH/H_2O, 1 : 2→1 : 1) to give nepalensinols F (**3**, 7.3 mg) and G (**4**, 11.3 mg).

Nepalensinol D (**1**): A reddish brown powder; mp 230 °C (decomp.); $[\alpha]_D$=−82.0° (c=0.3, MeOH); UV λ_{max} (MeOH) nm (log ε): 280 (4.12); IR (KBr) ν_{max}: 3215 (br d), 1612, 1525, 1453, 1340 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.38 (1H, d, J=8.8 Hz, H-8c), 3.77 (1H, dd, J=6.8, 4.6 Hz, H-8b), 4.07 (1H, dd, J=9.5, 6.8 Hz, H-8a), 4.92 (1H, d, J=8.8 Hz, H-7c), 5.36 (1H, d, J=4.6 Hz, H-7b), 5.81 (1H, d, J=9.5 Hz, H-7a), 6.01 (1H, d, J=2.0 Hz, H-12b), 6.07 (1H, t, J=1.8 Hz, H-12a), 6.09 (2H, d, J=2.0 Hz, H-10c, H-14c), 6.19 (2H, d, J=1.8 Hz, H-10a, H-14a), 6.40 (1H, d, J=2.0 Hz, H-10b), 6.41 (1H, d, J=2.0 Hz, H-12c), 6.63 (2H, d, J=8.5 Hz, H-3b, H-5b), 6.74 (2H, d, J=8.5 Hz, H-2c, H-6c), 6.79 (2H, d, J=8.6 Hz, H-3a, H-5a), 6.81 (2H, d, J=8.5 Hz, H-3c, H-5c), 6.90 (2H, d, J=8.5 Hz, H-2b, H-6b), 7.41 (2H, d, J=8.6, H-2a, H-6a); HR-FAB-MS (positive) m/z 721.2039 [M+Na]$^+$ for $C_{42}H_{34}O_{10}Na$ (Δ mmu 1.1).

Nepalensinol E (**2**): A brown powder; mp 250 °C (decomp.); $[\alpha]_D$=−307.8° (c=0.5, MeOH); UV λ_{max} (MeOH) nm (log ε): 282 (4.15); IR (KBr) ν_{max}: 3292, 1612, 1513, 1452, 1349, 1235, 1145 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.61 (1H, dd, J=12.0, 10.3 Hz, H-8b), 3.94 (1H, dd, J=12.0, 9.3 Hz, H-8a), 3.95 (1H, d, J=4.8 Hz, H-8c), 4.09 (1H, d, J=5.0 Hz, H-8d), 4.70 (1H, d, J=9.3 Hz, H-7a), 5.01 (1H, d, J=10.3 Hz, H-7b), 5.09 (1H, d, J=4.8 Hz, H-7c), 5.23 (1H, d, J=5.0 Hz, H-7d), 5.68 (1H, d, J=2.0 Hz, H-10c), 5.93 (1H, d, J=2.0 Hz, H-12c), 6.04 (2H, br s, H-10a, H-14a), 6.17 (1H, d, J=2.0 Hz, H-12b), 6.24 (2H, d, J=2.1 Hz, H-10d, H-14d), 6.29 (1H, t, J=2.1 Hz, H-12a), 6.33 (1H, t, J=2.1 Hz, H-12d), 6.54 (2H, d, J=8.6 Hz, H-3c, H-5c), 6.58 (2H, d, J=8.6 Hz, H-3b, H-5b), 6.65 (2H, d, J=8.6 Hz, H-3a, H-5a), 6.66 (1H, d, J=2.0 Hz, H-10b), 6.68 (4H, d, J=8.6 Hz, H-2c, H-6c, H-3d, H-5d), 6.76 (2H, d, J=8.6 Hz, H-2b, H-6b), 6.79 (2H, d, J=8.6 Hz, H-2a, H-6a), 6.88 (2H, d, J=8.6 Hz, H-2d, H-6d); HR-FAB-MS (positive) m/z 925.2857 [M+H]$^+$ for $C_{56}H_{45}O_{13}$ (Δ mmu 0.3).

Nepalensinol F (**3**): A brown powder; mp >300 °C (decomp.); $[\alpha]_D$ +26.3° (c=0.4, MeOH); UV λ_{max} (MeOH) nm (log ε): 284 (4.16); IR (KBr) ν_{max}: 3339, 2923, 1610, 1507, 1457, 1362, 1129 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.99 (1H, d, J=0.8 Hz, H-7b), 5.33 (1H, d, J=8.6 Hz, H-8a), 5.45 (1H, d, J=2.2 Hz, H-14b), 5.83 (1H, d, J=8.6 Hz, H-7a), 5.89 (1H, d, J=2.2 Hz, H-12b), 5.98 (1H, d, J=0.8 Hz, H-8b), 6.30 (2H, d, J=8.6 Hz, H-3b, H-5b), 6.35 (1H, d, J=2.2 Hz, H-12a), 6.37 (1H, d, J=2.2 Hz, H-14a), 6.51 (2H, d, J=8.6 Hz, H-2b, H-6b), 6.95 (2H, d, J=8.5 Hz, H-3a, H-5a), 7.53 (2H, d, J=8.5 Hz, H-2a, H-6a); HR-FAB-MS (positive) m/z 929.2553 [M+Na]$^+$ for $C_{56}H_{42}O_{12}Na$ (Δ mmu 2.1).

Nepalensinol G (**4**): A reddish brown powder; mp >300 °C (decomp.); $[\alpha]_D$ +66° (c=0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 223 (4.93), 282 (4.30); IR (KBr) ν_{max}: 3306, 1694, 1596, 1511, 1442, 1367, 1228, 1173 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.65 (1H, dd, J=6.3, 4.3 Hz, H-8c), 4.09 (1H, d, J=9.5 Hz, H-8a), 4.21 (1H, d, J=12.0 Hz, H-8d), 4.22 (1H, br d, H-8b), 5.47 (1H, s, H-12b), 5.60 (1H, d, J=2.1 Hz, H-14c), 5.75 (1H, d, J=12.0 Hz, H-7d), 5.75 (1H, d, J=2.1 Hz, H-12c), 5.99 (1H, d, J=4.3 Hz, H-7c), 6.03 (1H, br d, H-7b), 6.04 (1H, d, J=9.5 Hz, H-7a), 6.31 (1H, br d, H-14d), 6.41 (1H, br d, H-14a), 6.46 (1H, d, J=2.0 Hz, H-12d), 6.57 (2H, d, J=8.5 Hz, H-3c, H-5c), 6.58 (1H, br d, H-12a), 6.67 (2H, d, J=8.5 Hz, H-3b, H-5b), 6.80 (2H, d, J=8.5 Hz, H-2b, H-6b), 6.81 (2H, d, J=8.5 Hz, H-3d, H-5d), 6.85 (2H, d, J=8.5 Hz, H-2c, H-6c), 6.86 (2H, d, J=8.5 Hz, H-3a, H-5a), 7.16 (2H, d, J=8.5 Hz, H-2a, H-6a), 7.17 (2H, d, J=8.5 Hz, H-2d, H-6d); HR-FAB-MS (positive) m/z 921.2572 [M+H]$^+$ for $C_{56}H_{41}O_{13}$ (Δ mmu 2.5).

Topoisomerase II Assay Purified human topoisomerase II was purchased from TopoGen, Inc (U.S.A.) and kinetoplast DNA was purified from *Crithidia fasciculata* by cesium chloride step-gradient centrifugation as described by Englund.[15] Topoisomerase II activity was assessed by decatenation reaction of kinetoplast DNA.[16] The assay was performed in a reaction mixture of 20 ml 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.[17] A DMSO solution of the stilbenoids was diluted with Tris–HCl buffer (pH 7.9), and then added to the reaction mixture to the desired concentrations (0.05% final concentration 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 the addition of 4 μl of a solution of 0.66% SDS and 0.33 mg/ml proteinase K. Analysis of the DNA products was then carried out 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, U.S.A.).

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