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Neoflavonoids and Related Constituents from Nepalese Propolis and Their Nitric Oxide Production Inhibitory Activity

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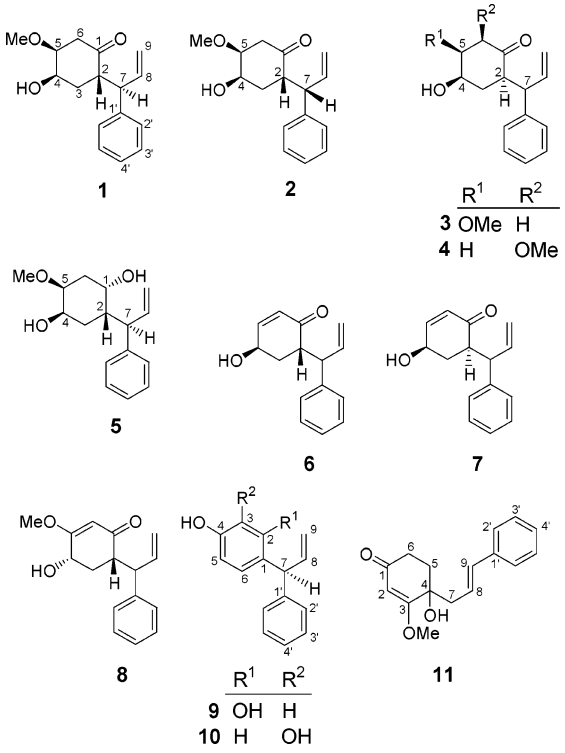
A methanolic extract of Nepalese propolis yielded 10 new open-chain neoflavonoids (**1**−**10**), a new chalcone (**11**), and eight previously reported compounds (**12**−**19**). Their structures were determined on the basis of spectroscopic data and chemical conversion. The isolated compounds other than **5**, **8**, and **16** showed dose-dependent inhibitory activities on nitric oxide production in lipopolysaccharide-activated macrophage-like J774.1 cells and were more active than a positive control, N^G-monomethyl-L-arginine (IC_{50}, 27.1 μM). The most potent activities, of **6** and **7** (IC_{50}, 0.5 μM), were greater than another positive control, caffeic acid phenethyl ester (IC_{50}, 4.8 μM).

Propolis, a resinous substance collected by bees from various plants, has a pleasant aromatic odor and yellow-green to dark brown color depending on source and age. It is used by bees to seal holes in their hive, smooth out the internal walls, and protect the entrance against intruders.[1] The plant source of propolis depends on the specific flora at the site of collection. Thus, the constituents of propolis of different places may be different.[2] Propolis is a traditional remedy in Eastern Europe and is well known for its valuable biological activities such as antibacterial, antifungal, immunostimulating, antitumor, antiinflammatory, and antioxidant.[1,3,4] Because of the broad spectrum of biological activities, propolis has become a subject of increasing interest concerning its constituents, and more than 300 compounds have been reported so far from propolis.[2,3] In our continued work on propolis of various origin[5−11] we recently found that methanol and water extracts of propolis collected from Nepal possessed potent nitric oxide (NO) production inhibitory activity (IC_{50} < 2 μg/mL) in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells. Since no previous research has been reported on Nepalese propolis, we carried out a detailed chemical investigation. The work led to the isolation of 10 new open-chain neoflavonoids (**1**−**10**) and a new chalcone (**11**), together with eight known compounds. In this paper, we report the structure elucidation of the new compounds and their NO production inhibitory activity.



Results and Discussion

The MeOH extract was subjected to a series of chromatographic separations resulting in the isolation of 10 new open-chain neoflavonoids (**1**−**10**) and a new chalcone (**11**) together with eight known compounds: (*S*)-4-methoxydalbergione (**12**),[12] cearoin (**13**),[13] 9-hydroxy-6,7-dimethoxydalbergiquinol (**14**),[14] obtusaquinol (**15**),[15] 2′,4,4′-trihydroxychalcone (**16**),[16] medicarpin (**17**),[17] (+)-vesticarpan (**18**),[18] and 4-hydroxymedicarpin (**19**).[19] The structures of compounds **12**−**19** are included in the Supporting Information.

Compound **1** was obtained as a yellow oily substance with $[\alpha]_D^{24}$ −178° ($CHCl_3$). It showed the molecular ion at *m/z* 260.1406 (M^+) in HREIMS, which corresponds to the molecular formula $C_{16}H_{20}O_3$. The IR spectrum of **1** showed absorptions of hydroxyl (3730 cm^{-1}), carbonyl (1720 cm^{-1}), and phenyl (1605, 1455 cm^{-1}) groups. The ^1H NMR spectrum of **1** exhibited signals due to a phenyl ring (δ_H 7.32−7.14, 5H), a vinyl (δ_H 6.07, 5.06, 5.00), two oxygen-substituted methines (δ_H 4.30, 3.42), two aliphatic methines (δ_H 3.70, 3.25), two methylenes (δ_H 2.77, 2.62; δ_H 1.94, 1.23), and a methoxyl group (δ_H 3.35). On the other hand, its ^{13}C NMR spectrum showed the signals of 16 carbons, including those for a ketone carbonyl (δ_C 208.2), two oxygen-substituted sp^3 carbons, and two olefinic carbons (Table 1). The partial connectivities $C_2-C_3-C_4-C_5-C_6$ and $C_2-C_7-C_8-C_9$ were deduced by the analysis of the COSY and HMQC spectra. In the HMBC spectrum, the correlations of the ketone carbonyl carbon (δ_C 208.2) with H-2, H_2-3, H-5, and H_2-6 suggested the connectivity of C-2 and C-6 via the ketone carbonyl carbon (C-1), while the correlations between H-2′,6′ and C-7 indicated the phenyl

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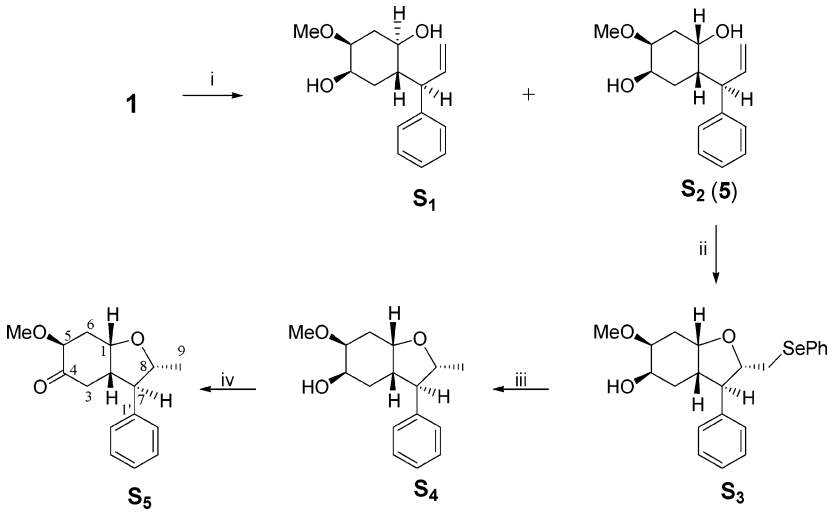

Figure 1. Chemical conversion of **1** to $\mathbf{S_5}$. Reagents and conditions: (i) $NaBH_4$, THF, CH_3OH, 0 °C, 6 h; (ii) PhSeCl, K_2CO_3, CH_2Cl_2, 4 h; (iii) Bu_3SnH, AIBN, toluene, reflux, 5 h; (iv) Jones reagent, acetone, 0 °C.

Table 1. ^{13}C NMR (100 MHz) Data (δ) for Compounds **1**−**10** in $CDCl_3$

position	1	2	3	4	5	6	7	8	9	10
1	208.2	208.9	207.3	207.4	67.1	199.9	197.9	199.1	130.5	130.4
2	47.7	41.9	51.7	51.6	32.7	49.9	50.7	47.5	147.2	116.4
3	32.2	83.1	32.8	32.8	76.5	34.9	34.2	31.9	116.4	149.5
4	65.6	67.6	69.9	69.9	65.5	63.7	67.9	65.0	149.4	147.4
5	81.2	32.5	81.1	42.2	28.6	149.6	152.2	174.6	114.4	114.4
6	42.8	51.3	42.2	81.1	38.2	128.9	129.4	101.8	117.3	117.3
7	47.7	49.6	48.8	48.9	52.7	49.5	46.9	48.6	48.9	49.1
8	139.9	139.6	138.3	138.2	141.2	139.6	135.9	139.7	139.2	139.1
9	115.2	115.3	116.9	116.9	144.9	115.6	118.5	115.2	117.0	117.1
1′	115.2	141.0	143.1	143.1	142.9	141.2	142.5	141.3	141.4	141.2
2′,6′	141.3	128.7	128.5	128.4	128.6	128.9	128.5	128.5	128.6	128.6
3′,5′	128.2	128.2	127.9	127.8	127.4	127.9	128.1	128.4	128.6	128.6
4′	126.5	126.7	126.3	126.3	126.3	126.9	126.5	126.6	126.7	126.8
OMe	56.4	56.7	56.8	56.7	55.9			56.0		

Table 2. Inhibitory Effects of Constituents of Nepalese Propolis on NO Production in LPS-Activated J774.1 Cells

compound	IC_{50} (μM)[a]
1	13.2
2	9.6
3	9.6
4	2.3
5	100
6	0.5
7	0.5
8	48.5
9	5.5
10	8.4
11	19.2
12	10.2
13	15.4
14	12.8
15	6.3
16	29.3
17	10.1
18	3.4
19	5.4
CAPE	4.8
L-NMMA	27.1

[a] IC_{50} values were calculated from the mean of data of four determinations.

group to be at C-7. Likewise, a correlation was observed between the methoxyl protons and C-5, suggesting the methoxyl group to be at C-5. Thus, **1** was determined to be an open-chain neoflavanoid with a cyclohexanone ring. The relative configuration of **1** was determined from the ROESY analysis and coupling constant data. The *trans*-diaxial coupling constant for H-2 and H-3ax (J = 12.5 Hz) and for H-5 and H-6ax (J = 10.6 Hz) and the ROESY

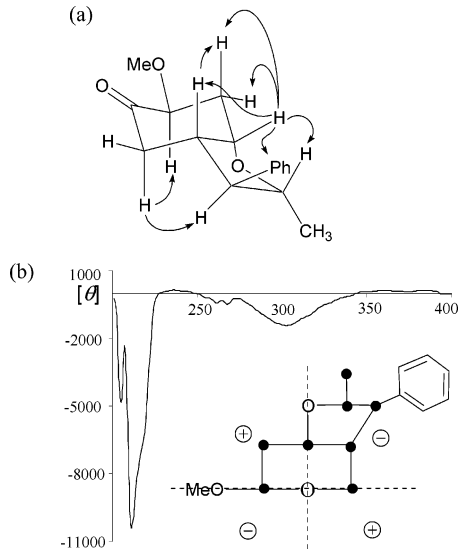

Figure 2. (a) Key NOE (arrow) observed in the difference NOE spectra and (b) CD spectrum and octant projection of $\mathbf{S_5}$.

correlations H-2/H-6ax and H-3ax/H-5 suggested the chair conformation of the cyclohexanone ring and equatorial orientation of the methoxyl group. Similarly, the ROESY correlations of H-4 with H_2-3 and H-5 indicated the equatorial orientation of H-4, i.e., the hydroxyl group to be axial. Then, to determine the absolute stereochemistry, **1** was converted to the ketone $\mathbf{S_5}$ via $NaBH_4$ reduction, selenoetherification, Bu_3SnH reduction, and Jones oxidation (Figure 1). In the difference NOE experiment on $\mathbf{S_5}$, NOE enhancements from H-1 to H-2, H_2-6, H-8, and H-2′,6′, from H-2 to H-6ax, and from H-3ax to H-5 and H-7 indicated the conformation depicted in Figure 2a. Finally, the absolute stereochemistry of $\mathbf{S_5}$ was deduced to be 1*S*,2*S*,5*S*,7*R*,8*R* by applying the octant rule[20] to the negative minimum ($[\theta]_{300}$ −1426) in its CD spectrum (Figure 2b). Thus, the absolute configuration of **1** was concluded to be 2*S*,4*R*,5*S*,7*R*.

HREIMS of compounds **2** and **3** showed the same molecular formula ($C_{16}H_{20}O_3$) as that of **1**. Their ^1H (see Experimental Section) and ^{13}C NMR (Table 1) spectra were also similar. Analysis of the COSY, HMQC, and HMBC spectra indicated **2** and **3** to be stereoisomers of **1**. The coupling constants and the ROESY correlations indicated that **2** had the same stereochemistry in the cyclohexanone

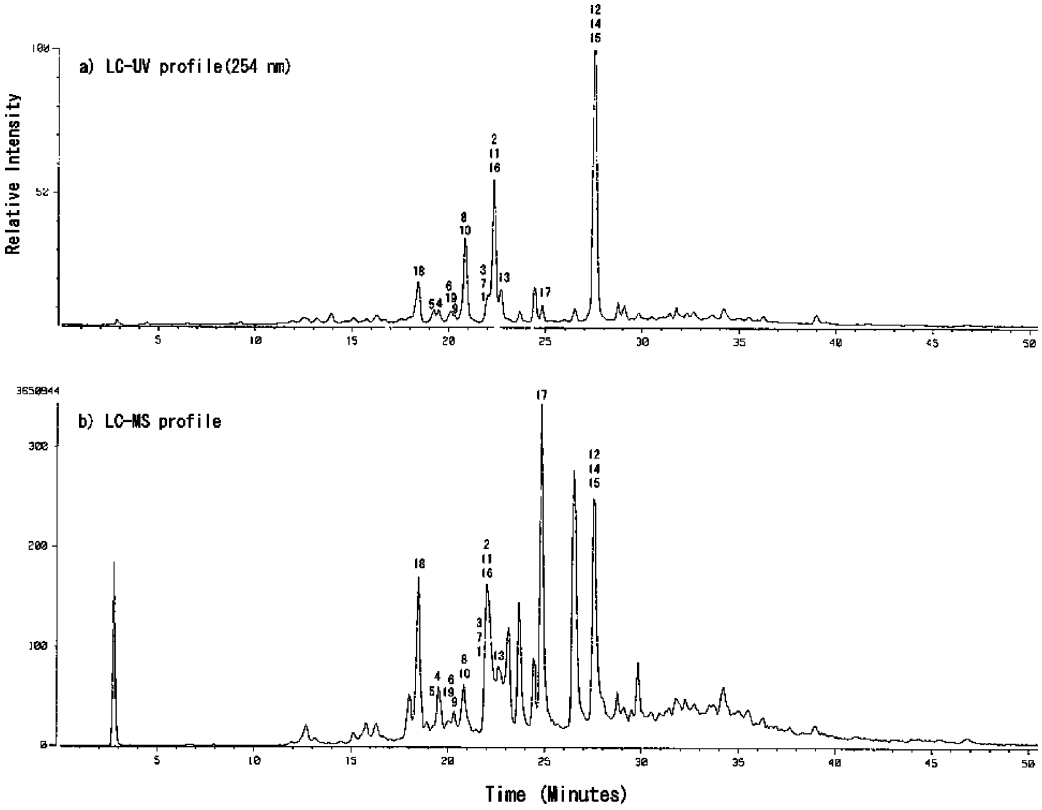

Figure 3. LC-UV profile chromatogram (a) and LC-MS profile chromatogram (b) of the MeOH extract of Nepalese propolis.

ring as **1**, and the CD spectrum revealed the absolute stereochemistry of the cyclohexanone ring to be the same as **1**. Thus, **2** should be a stereoisomer of **1** at C-7. As for compound **3**, the ROESY correlations H-2/H-4, H-2/H-6ax, H-2/H-3eq, and H-3eq/H-4 and the *trans*-diaxial coupling constant (J = 12.9 Hz) between H-2 and H-3ax indicated a chair conformation of the cyclohexanone ring, β-equatorial orientation of the 4-OH group, and β-axial orientation of the 5-OMe group. These observations and the positive maximum ($[\theta]_{291}$ +4600) in the CD spectrum indicated the 2*R*,4*R*,5*S* absolute configuration, but the absolute configuration at C-7 could not be determined due to the meager amount of compound isolated.

The ^{1}H and ^{13}C NMR spectra of **4** closely resembled those of **3**, but analysis of the COSY and HMQC spectra revealed C-5 to be an aliphatic methylene and C-6 to be an oxygen-substituted methine. The HMBC analysis indicated the methoxyl group to be at C-6 instead at C-5 as in **3**. ROESY correlations H-2/H-4, H-2/H-5ax, H-5ax/H-6, and H-5eq/H-6 and NOE enhancements from H-4 to both H-5ax and H-5eq indicated a boat conformation of the cyclohexanone ring and β-equatorial orientation of the 4-OH group. The small coupling constants between H-5α and H-6 (J = 3.2 Hz) and between H-5β and H-6 (J = 4.4 Hz) indicated β-axial orientation of the methoxyl group. A positive maximum ($[\theta]_{286}$ +1700) due to n→π^* transition in the CD spectrum indicated *R* configuration at C-2, i.e., **4** to have a 2*R*,4*R*,6*R* configuration. But, due to the meager amount of the compound isolated, the absolute configuration at C-7 could not be determined.

The HREIMS of **5** showed the molecular ion at *m/z* 262.1572 (M^+), consistent with the molecular formula $C_{16}H_{22}O_3$. The ^{1}H and ^{13}C NMR spectra and the $[\alpha]_D$ value (+26.4°, $CHCl_3$) were the same as those of synthesized compound **S$_2$** (Figure 1). Thus, **5** was determined to be the alcohol **S$_2$**.

The molecular formulas of compounds **6** and **7** ($C_{15}H_{16}O_2$) were determined by HREIMS. Their IR spectra showed the presence of hydroxyl and α,β-unsaturated ketone functionalities. Their ^{1}H NMR spectra displayed signals due to a *cis*-olefin, a vinyl group, an oxygen-substituted methine, a methylene, two methines, and a phenyl group. Their ^{13}C NMR spectra displayed 15 signals corresponding to the above groups and an α,β-unsaturated ketone (Table 1). The partial structures C(6)H=C(5)H−C(4)H(O)−C(3)H$_2$−C(2)H−C(7)H−C(8)H=C(9)H$_2$ were deduced by the COSY and HMQC spectra, while the HMBC correlations of the ketone carbonyl carbon with H-2, H-3, H-5, H-6, and H-7 suggested the carbonyl carbon to be C-1, and the correlation between C-7 and H-1′,6′ and between C-1′ and H-7 indicated the location of the phenyl group at C-7. In **6**, on the other hand, small coupling constants for H-2 and H$_2$-3 (J = 4.9 Hz) and ROESY correlations H-2/H-3α and H-2/H-3β suggested the 1-phenyl-2-propenyl group to be α-axial, and the large coupling constant between H-3β and H-4 (J = 10.7 Hz) suggested the β-equatorial orientation of the 4-OH group. In addition, by applying an inverse octant rule,[21,22] **6** was determined to have an *S* configuration at C-2 ($[\theta]_{322}$ +748), i.e., 2*S*,4*R*. Similarly, the configuration of **7** was determined to be 2*R*,4*R*, on the basis of the coupling constants of H-3ax with H-2 (J = 12.4 Hz) and H-4 (J = 10.7 Hz), the ROESY correlation between H-2 and H-4, and the negative minimum ($[\theta]_{339}$ −3664) in the CD spectrum. The absolute configuration at C-7 of both **6** and **7** could not be determined due to meager amounts obtained.

The ^{1}H and ^{13}C NMR spectra of **8** closely resembled those of **6**, but were characterized by the absence of a signal due to an olefinic proton and the presence of a methoxyl signal. The methoxyl group was deduced to be at C-5 on the basis of HMBC correlations between the methoxyl protons and C-5. ROESY correlations H-2/H-3α, H-2/H-3β, H-4/H-3α, and H-4/H-3β and their coupling constants indicated an

α-axial orientation of both the 4-hydroxyl and 1-phenyl-2-propenyl groups. The CD data ($[\theta]_{307}$ +30704) indicated C-2 to have an *S* configuration, i.e., 2*S*,4*S*, but the absolute configuration at C-7 could not be determined due to the meager amount of available sample.

Compounds **9** and **10** both had the molecular formula $C_{15}H_{14}O_2$. Their ^{1}H and ^{13}C NMR spectra resembled those of **14** and **15**, but they indicated the presence of a 1,2,4-trisubstituted benzene ring instead of the 1,2,4,5-tetrasubstituted benzene ring in **14** and **15**. HMBC correlations between H-7 and the carbons at δ_C 130.5 (C-1), 147.2 (C-2), and 117.3 (C-6) and the coupling patterns of H-3 (d, J = 2.6 Hz), H-5 (dd, J = 8.6, 2.6 Hz), and H-6 (d, J = 8.6 Hz) in **9** suggested the two hydroxyl groups to be located at C-2 and C-4. Similarly, HMBC correlations between H-7 and the carbons at δ_C 130.4 (C-1), 116.4 (C-2), and 117.3 (C-6) and the coupling patterns of H-2 (d, J = 2.9 Hz), H-5 (d, J = 8.5 Hz), and H-6 (dd, J = 8.5, 2.9 Hz) in **10** suggested the hydroxyl groups to be located at C-3 and C-4. Their absolute stereochemistry was determined to be 7*S* by comparing their $[\alpha]_D$ values and CD data with those of related compounds.[23,24] In addition, the absolute stereochemistry of the known compound **14**, which has not been determined in the literature,[14] was determined to be 7*R* by the same method.

Compound **11** had the molecular formula $C_{16}H_{18}O_3$. Its IR spectrum showed the presence of hydroxyl and α,β-unsaturated ketone groups. The ^{1}H NMR spectrum showed signals due to a monosubstituted benzene ring, three methylenes, three olefinic protons, and a methoxyl group. The ^{13}C NMR spectrum displayed signals due to 16 carbons including those of an α,β-unsaturated carbonyl group (δ_C 198.3), an oxygen-substituted sp^2 carbon (δ_C 177.6), and an oxygen-substituted quaternary carbon (δ_C 72.0). The partial structures C(7)H_2−C(8)H=C(9)H and C(5)H_2−C(6)-H_2 were obtained from the COSY and HMQC spectra. The planar structure was deduced from the HMBC correlations of H-2, H-6, and H-5 with C-1 and C-4, of the methoxyl protons with C-3, of the methylene protons at δ_H 2.74 and 2.62 (H_2-7) with C-4, and of the olefinic proton at δ_H 6.50 (H-9) with the carbons of the phenyl ring.

Thus, we report 11 new flavonoids (**1**−**11**) together with eight known compounds (**12**−**19**, structures in the Supporting Information). Among the isolated compounds, **1**, **12**, and **15** were the major constituents of Nepalese propolis. Compounds **12** and **15** were earlier reported as constituents of *Dalbergia* and *Machaerium* woods,[15,24−28] while neoflavonoids **13** and **14** were reported only from *Dalbergia* species.[13,26−30] Moreover, "Chitwan", where the propolis used in this study was collected, has many plants of various *Dalbergia* species such as *D. sissoo.* Thus, the source of Nepalese propolis might be plants belonging to the genus *Dalbergia*. Neoflavonoids such as obtusaquinone, *R*-3,4-dimethoxydalbergione, *R*-4-methoxydalbergione, and other quinones isolated from *Dalbergia* species have been reported as allergens.[31−33] They are also reported to inhibit the growth of microorganisms, such as the marine borer *Teredo navalis*, and are reported to be useful in protective coatings on ships.[31] As bees prepare propolis to protect their hive from foreign intruders, including microorganisms, these neoflavanoids may have a defensive role in protecting the hive. However, the use of propolis as a health food that contains such allergens should be a matter of further research. To the best of our knowledge, this is the first report on the isolation of open-chain neoflavonoids from propolis.

As a part of our ongoing research on propolis, we previously established an LC-MS technique for the analysis of propolis samples from locations in Brazil, Peru, China, and The Netherlands.[8] To compare Nepalese propolis with those of other origin, further work on LC-MS was carried out. Both LC-UV and LC-MS fingerprint profiles were obtained using the LC-UV handling mode in a JEOL JMS-700T four-sector mass spectrometer, in the negative atmospheric pressure chemical ionization (APCI) mode (Figure 3). Peak identification in the profile was carried out on the basis of the retention time and pseudomolecular ion $(M - H)^-$ of all the standard compounds.

The isolated compounds were assayed against NO production by LPS-activated macrophage-like J774.1 cells. All of the compounds displayed significant concentration-dependent inhibition of NO production in macrophage-like J774.1 cells. The activities of **1**−**3**, **9**−**15**, **17**, and **19** were greater than a positive control, N^G-monomethyl-L-arginine (L-NMMA; IC_{50}, 27.1 μM), a nonselective nitric oxide synthase (NOS) inhibitor,[34] while the activities of **4**, **6**, **7**, and **18** were more potent than another positive control, caffeic acid phenethyl ester (CAPE; IC_{50}, 4.8 μM), an inhibitor of nuclear factor κB activation[35] (Table 2). CAPE is an active principle of Europian propolis, reported to inhibit inducible nitric oxide synthase of Raw 264.7 cells[36] and to show an antiinflammatory effect in a rat model of carrageenin-induced subcutaneous inflammation by increasing leukocyte apoptosis and decreasing leukocyte concentration in exudate.[37]

In the present study, structure−activity relationships were deduced as follows. Among the open-chained neoflavanoids, those having a ketone carbonyl at C-1 in ring A exhibited stronger activity than those without (**1**, **2**, **3**, **4**, **6**, **7**, **8**, **12** > **5**). Compounds without a substituent at C-5 showed stronger activity than those having a methoxyl substituent (**4** > **1**, **2**, **3**; **6**, **7** ≫ **8**). Among the compounds possessing the phenyl ring A system, an increase in the number of hydroxyl groups improved activity (**9**, **10**, **15** > **14**). These phenomena were also observed in pterocarpans (**17**, **18** > **16**). Conversely, an increase in the number of methoxyl groups in ring A decreased the activity (**6**, **7** > **8**, and **15** > **14**). The absence of a vinyl group at C-7 also showed a decrease in activity (**15** > **13**).

Cell viability in the present experiment was determined by the MTT method to find out whether inhibition of NO production was due to the cytotoxicity of tested compounds (data not shown). Compounds **6** and **7** exhibited cytotoxicity above 50 μM concentration. However, the IC_{50} values of all the compounds lie within the nontoxic concentration range, suggesting that the inhibition of nitrite accumulation was not due to its cytytoxic properties but was due to its inhibitory activity on NO production. NO is produced by inducible NOS (iNOS) and acts as a host defense by damaging pathogenic DNA and as a regulatory molecule with homeostatic activities.[38] However, excessive production has detrimental effects on many organ systems of the body, leading to tissue damage and even leading to a fatal development (septic shock). Therefore, the effective inhibition of NO accumulation by inflammatory stimuli may be beneficial for thereapy.[39,40]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solution. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in

δ values. EIMS and HREIMS measurements were performed on a JEOL JMS-700T spectrometer using a direct inlet system at the ionization voltage of 70 eV. CD spectra were measured in a JASCO J-805 spectropolarimeter. Column chromatography was performed with BW-820MH silica gel (Fuji Silysia, Aichi, Japan) and LiChroprep RP-18 (Merck, Darmstadt, Germany). Analytical and preparative TLC was carried out on precoated silica gel $60F_{254}$ or RP-$18F_{254S}$ plates (Merck, 0.25 or 0.50 mm thickness).

Biological Material. Nepalese propolis was collected at Chitwan, Nepal, in 2001. A voucher specimen (TMPW24045) is preserved in the Museum of Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. Nepalese propolis (500 g) was successively extracted with H_2O and MeOH under reflux to give H_2O (45 g) and MeOH (55 g) extracts, respectively. Part of the MeOH extract (20 g) was chromatographed on silica gel (5 × 46 cm) with a MeOH−$CHCl_3$ solvent system to give five fractions [1: MeOH−$CHCl_3$ (1:99) eluate, 2.64 g; 2: MeOH−$CHCl_3$ (2:98) eluate, 2.06 g; 3: MeOH−$CHCl_3$ (3:97) eluate, 1.38 g; 4: MeOH−$CHCl_3$ (5:95) eluate, 5.97 g; 5: MeOH−$CHCl_3$ (7:93) eluate, 1.88 g].

Fraction 2 (2.06 g) was rechromatographed on silica gel (3.5 × 36 cm) with MeOH−$CHCl_3$ (1% → 5% MeOH) to afford four subfractions (2-1, 504 mg; 2-2, 374 mg; 2-3, 201 mg; 2-4, 326 mg). Subfraction 2-1 gave **12** (24.0 mg). Subfractions 2-2 to 2-4 were rechromatographed on silica gel (2 × 34 cm) with 2% MeOH−$CHCl_3$, followed by normal-phase preparative TLC with CH_3CN−C_6H_6 (1:10), to give **13** (70.1 mg), **14** (25.2 mg), and **16** (60.4 mg), respectively.

Fraction 3 (1.38 g) was rechromatographed on silica gel (3 × 35 cm) with 2.5% MeOH−$CHCl_3$ to give **15** (357 mg).

Fraction 4 (5.97 g) was rechromatographed on ODS with CH_3CN−acetone−H_2O (1:1:5 → 1:1:1) to afford four subfractions (4-1, 534 mg; 4-2, 1.45 g; 4-3, 930 mg; 4-4, 811 mg). Subfraction 4-1 (534 mg) was rechromatographed on silica gel (2 × 37 cm) with 10% CH_3CN−C_6H_6, followed by normal-phase preparative TLC with CH_3CN−C_6H_6 (1:10), to give **2** (80.6 mg), **3** (12.2 mg), **4** (8.1 mg), and **8** (10.8 mg). Subfraction 4-2 (1.45 g) was rechromatographed on ODS with CH_3CN−acetone−H_2O (1:1:5 → 1:1:1), followed by normal-phase preparative TLC with 2% MeOH−$CHCl_3$ or reversed-phase preparative TLC with CH_3CN−acetone−H_2O (1:1:3), to give **5** (182 mg), **6** (9.3 mg), **7** (6.7 mg), **9** (60.6 mg), and **10** (35.0 mg). Subfraction 4-3 (930 mg) gave **1**. Subfraction 4-4 (811 mg) was rechromatographed on ODS with CH_3CN−acetone−H_2O (1:1:5 → 1:1:1), followed by normal-phase preparative TLC with 10% CH_3CN−C_6H_6, to give **11** (22.2 mg), **17** (20.0 mg), **18** (33.9 mg), and **19** (57.3 mg). Structures of **12**−**19** are shown in the Supporting Information.

Compound 1: yellow oil; $[\alpha]_D^{24}$ −178° (*c* 0.075, $CHCl_3$); IR ν_{max} 3650, 1720, 1605, 1510, 1470, 1455, 1370, 1340, 1120 cm^{-1}; CD (*c* 3.841 × 10^{-4} M, EtOH) $[\theta]_{291}$ −5263; ^1H NMR (CD_3OD) δ 7.32−7.14 (5H, m, Ph), 6.07 (1H, ddd, *J* = 17.3, 10.3, 7.8 Hz, H-8), 5.06 (1H, dd, *J* = 10.3. 1.2 Hz, H-9), 5.00 (1H, dd, *J* = 17.3, 1.2 Hz, H-9), 4.30 (1H, br s, H-4), 3.70 (1H, dd, *J* = 8.4, 7.8 Hz,H-7), 3.42 (1H, ddd, *J* = 10.6, 5.1, 2.9 Hz, H-5), 3.35 (1H, s, OMe), 3.25 (1H, ddd, *J* = 12.5, 8.4, 5.5 Hz, H-2), 2.77 (1H, dd, *J* = 12.6, 5.1 Hz, H-6), 2.62 (1H, dd, *J* = 12.6, 10.6 Hz, H-6), 1.94 (1H, ddd, *J* = 14.3, 5.5, 2.6 Hz, H-3), 1.23 (1H, ddd, *J* = 14.3, 12.5, 2.6 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 260.1406 [calcd for $C_{16}H_{20}O_3$, 260.1412].

Compound 2: yellow waxy substance; $[\alpha]_D^{24}$ −57.0° (*c* 0.05, $CHCl_3$); IR ν_{max} 3350, 1690, 1490, 1455, 1370, 1340, 1220, 1000 cm^{-1}; CD (*c* 3.841 × 10^{-4} M, EtOH) $[\theta]_{290}$ −3466; ^1H NMR (CD_3OD) δ 7.35−7.15 (5H, m, Ph), 6.00 (1H, ddd, *J* = 17.1, 10.0, 8.0, H-8), 5.02 (1H, d, *J* = 8.0 Hz, H-9), 5.00 (1H, d, *J* = 17.1 Hz, H-9), 4.03 (1H, ddd, *J* = 7.6, 5.4, 3.6 Hz, H-4), 3.63 (1H, dd, *J* = 10.0, 8.8 Hz, H-7), 3.50 (1H, dt, *J* = 7.4, 3.6 Hz, H-5), 3.36 (3H, s, OMe), 3.02 (1H, ddd, *J* = 8.8, 5.8, 5.4 Hz, H-2), 2.87 (1H, dd, *J* = 13.4, 3.6 Hz, H-6), 2.50 (1H, dd, *J* = 13.4, 7.4 Hz, H-6), 1.76 (1H, ddd, *J* = 13.4, 5.8, 3.6 Hz, H-3), 1.55 (1H, ddd, *J* = 13.4, 7.6, 5.4 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 260.1382 [calcd for $C_{16}H_{20}O_3$, 260.1412].

Compound 3: white waxy substance; $[\alpha]_D^{24}$ +180.1° (*c* 0.045, $CHCl_3$); IR ν_{max} 3570, 1715, 1490, 1455, 1330, 1220, 995 cm^{-1}; CD (*c* 3.841 × 10^{-4} M, EtOH) $[\theta]_{292}$ +4600; ^1H NMR (CD_3OD) δ 7.30 − 7.15 (5H, m, Ph), 5.89 (1H, ddd, *J* = 17.3, 10.4, 7.8 Hz, H-8), 5.11 (1H, dd, *J* = 17.3, 1.0 Hz, H-9), 5.10 (1H, dd, *J* = 10.4, 1.0 Hz, H-9), 4.10 (1H, ddd, *J* = 10.7, 4.4, 3.2 Hz, H-4), 3.91 (1H, t, *J* = 7.8 Hz, H-7), 3.81 (3H, s, OMe), 3.76 (1H, dt, *J* = 4.4, 3.2 Hz, H-5), 2.83 (1H, ddd, *J* = 12.9, 7.8 Hz, 5.1, H-2), 2.74 (1H, dd, *J* = 14.4, 4.4 Hz, H-6), 2.39 (1H, dd, *J* = 14.4, 3.2, H-6), 2.16 (1H, ddd, *J* = 11.7, 4.4, 5.1 Hz, H-3), 1.98 (1H, ddd, *J* = 12.9, 11.7 Hz, 10.7, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 260.1445 [calcd for $C_{16}H_{20}O_3$, 260.1412].

Compound 4: white waxy substance; $[\alpha]_D^{24}$ +210.5° (*c* 0.06, $CHCl_3$); IR ν_{max} 3560, 1715, 1495, 1455, 995 cm^{-1}; CD (*c* 3.841 × 10^{-4} M, EtOH) $[\theta]_{286}$ +1700; ^1H NMR (CD_3OD) δ 7.30−7.15 (5H, m, Ph), 5.86 (1H, ddd, *J* = 16.8, 10.0, 8.2 Hz, H-8), 5.11 (1H, dd, *J* = 16.8, 0.72 Hz, H-9), 5.09 (1H,dd, *J* = 10.0, 0.72 Hz, H-9), 4.08 (1H, br s, H-4), 3.90 (1H, t, *J* = 8.2 Hz, H-7), 3.75 (1H, dd, *J* = 4.4, 3.2 Hz, H-6), 3.38 (1H, d, *J* = 2.6 Hz, OMe), 2.76 (1H,ddd, *J* = 12.2, 8.2, 5.4 Hz, H-2), 2.70 (1H, dd, *J* = 14.4, 4.4 Hz, H-5), 2.38 (1H, dd, *J* = 14.4, 3.2 Hz, H-5), 2.15 (1H, ddd, *J* = 12.9, 5.4, 4.4 Hz, H-3), 1.95 (1H, ddd, *J* = 12.9, 12.2, 10.5 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 260.1400 [calcd for $C_{16}H_{20}O_3$, 260.1412].

Compound 5: colorless oil; $[\alpha]_D^{22}$ +26.4° (*c* 0.055, $CHCl_3$); IR ν_{max} 3590, 3350, 1490, 1455, 1350, 1025 cm^{-1}; ^1H NMR (CD_3OD) δ 7.30−7.15 (5H, m, Ph), 5.97 (1H, dt, *J* = 16.9, 10.0 Hz, H-8), 5.12 (1H, dd, *J* = 16.9, 1.2 Hz, H-9), 5.01 (1H, dd, *J* = 10.0, 1.2 Hz, H-9), 4.23 (1H, br d, *J* = 2.4 Hz, H-1), 4.02 (1H, br d, *J* = 2.4 Hz, H-4), 3.53 (1H, ddd, *J* = 11.7, 4.6, 2.4 Hz, H-5), 3.28 (1H, s, OMe), 3.19 (1H, t, *J* = 10.0 Hz, H-7), 2.18 (1H, m, H-2), 2.18 (1H, m, H-6), 1.81 (1H, ddd, *J* = 13.9, 11.7, 2.2 Hz, H-6), 1.45 (2H, m, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 262.1572 [calcd for $C_{16}H_{22}O_3$, 262.1568].

Compound 6: brown oil; $[\alpha]_D^{22}$ +173.7° (*c* 0.05, $CHCl_3$); IR ν_{max} 3600, 1675, 1600, 1450 cm^{-1}; CD (*c* 4.380 × 10^{-4} M, EtOH) $[\theta]_{322}$ +748; ^1H NMR (CD_3OD) δ 7.36−7.20 (5H, m, Ph), 6.83 (1H, dd, *J* = 10.4, 3.4 Hz, H-5), 6.02 (1H, ddd, *J* = 16.8, 10.2, 8.7 Hz, H-8), 5.94 (1H, d, *J* = 10.4 Hz, H-5), 5.02 (1H, dd, *J* = 10.2, 1.0 Hz, H-9), 4.93 (1H, dd, *J* = 16.8, 1.0 Hz, H-9), 4.57 (1H, br d, *J* = 8.7 Hz, H-4), 3.64 (1H, t, *J* = 8.7 Hz, H-7), 2.97 (1H, dt, *J* = 8.7, 4.9 Hz, H-2), 1.97 (1H, ddd, *J* = 13.1, 8.7, 4.9 Hz, H-3), 1.95 (1H, ddd, *J* = 13.1, 4.9, 5.2 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 228.1108 [calcd for $C_{15}H_{16}O_2$, 228.1150].

Compound 7: brown oil; $[\alpha]_D^{22}$ +214.0° (*c* 0.09, $CHCl_3$); IR ν_{max} 3595, 1680, 1600, 1450, 1380 cm^{-1}; CD (*c* 4.380 × 10^{-4} M, EtOH) $[\theta]_{339}$ −3664; ^1H NMR (CD_3OD) δ 7.32−7.10 (5H, m, Ph), 6.86 (1H, dd, *J* = 8.2, 1.9 Hz, H-5), 6.02 (1H, ddd, *J* = 17.6, 9.7, 8.6 Hz, H-8), 5.98 (1H, d, *J* = 8.2 Hz, H-5), 5.18 (1H, dd, *J* = 9.7, 1.2 Hz, H-9), 5.17 (1H, dd, *J* = 17.6, 1.2 Hz, H-9), 4.50 (1H, br s, H-4), 4.32 (1H, dd, *J* = 8.6, 4.1 Hz, H-7), 2.64 (1H, dt, *J* = 12.4, 4.1 Hz, H-2), 2.34 (1H, ddd, *J* = 13.9, 4.8, 4.1 Hz, H-3), 1.90 (1H, ddd, *J* = 13.9, 12.4, 10.7 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 228.1135 [calcd for $C_{15}H_{16}O_2$, 228.1150].

Compound 8: dark brown oil; $[\alpha]_D^{22}$ +26.7° (*c* 0.11, $CHCl_3$); IR ν_{max} 3400 br, 1655, 1615 cm^{-1}; CD (*c* 3.871 × 10^{-4} M, EtOH) $[\theta]_{307}$ +30704; ^1H NMR (CD_3OD) δ 7.32−7.18 (5H, m, Ph), 6.10 (1H, dd, *J* = 17.6, 10.4, 7.6 Hz, H-8), 5.29 (1H, s, H-5), 5.07 (1H, dd, *J* = 10.4, 1.2 Hz, H-9), 5.02 (1H, dd, *J* = 17.6, 1.2 Hz, H-9), 4.42 (1H, dd, *J* = 5.4, 4.8 Hz, H-4), 3.85 (1H, t, *J* = 7.6 Hz, H-7), 3.71 (1H, s, OMe), 3.02 (1H, ddd, *J* = 7.6, 4.9, 4.3 Hz, H-2), 1.99 (1H, ddd, *J* = 13.7, 5.4, 4.9 Hz, H-3), 1.84 (1H, ddd, *J* = 13.7, 4.8, 4.3 Hz, H-3); ^{13}C NMR, see Table 1; HREIMS *m/z* 258.1243 [calcd for $C_{16}H_{18}O_3$, 258.1256].

Compound 9: dark brown greasy substance; $[\alpha]_D^{22}$ +34.7° (*c* 0.06, $CHCl_3$); IR ν_{max} 3600, 1675, 1600, 1450 cm^{-1}; CD (*c* 4.419 × 10^{-4} M, EtOH) $[\theta]_{286}$ +1700; ^1H NMR (CD_3OD) δ 7.31−7.15 (5H, m, Ph), 6.64 (1H, d, *J* = 8.6 Hz, H-5), 6.56 (1H, dd, *J* = 8.6, 2.6 Hz, H-5), 6.53 (1H, d, *J* = 2.6 Hz, H-3), 6.25 (1H, ddd, *J* = 16.5, 10.5, 6.6 Hz, H-8), 5.98 (1H, d, *J* = 16.5 Hz, H-9), 5.22 (1H, d, *J* = 10.5 Hz, H-9), 4.90 (1H, d, *J* = 6.6 Hz,

H-7); ^{13}C NMR, see Table 1; HREIMS *m/z* 226.0989 [calcd for $C_{15}H_{14}O_2$, 226.0994].

Compound 10: dark brown greasy substance; $[\alpha]_D^{22}$ +36.5° (*c* 0.05, $CHCl_3$); IR ν_{max} 3600, 1600, 1490 cm^{-1}; CD (*c* 4.419 × 10^{-4} M, EtOH) $[\theta]_{304}$ −2807; ^{1}H NMR (CD_3OD) δ 7.35 − 7.2 (5H, m, Ph), 6.71 (1H, d, *J* = 8.5 Hz, H-5), 6.64 (1H, d, *J* = 8.5, 2.9 Hz, H-5), 6.54 (1H, d, *J* = 2.9 Hz, H-2), 5.86 (1H, m, H-8), 5.30 (1H, d, *J* = 10.3 Hz, H-9), 5.01 (1H, d, *J* = 17.1 Hz, H-9), 4.90 (1H, d, *J* = 6.6 Hz, H-7); ^{13}C NMR, see Table 1; HREIMS *m/z* 226.0980 [calcd for $C_{15}H_{14}O_2$, 226.0994].

Compound 11: brown oil; $[\alpha]_D^{22}$ −14.2° (*c* 0.055, $CHCl_3$); IR ν_{max} 3530, 1720, 1620, 1600, 1500 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.40−7.21 (5H, m, Ph), 6.50 (1H, d, *J* = 15.9 Hz, H-9), 6.22 (1H, ddd, *J* = 15.9, 8.3, 6.8 Hz, H-8), 5.35 (1H, s, H-2), 3.76 (3H, s, OMe), 2.74 (1H, dd, *J* = 14.2, 6.8 Hz, H-7), 2.62 (1H, dd, *J* = 14.2, 8.3 Hz, H-7), 2.56 (1H, dt, *J* = 17.3, 5.3 Hz, H-6), 2.42 (1H, ddd, *J* = 17.3, 11.0, 5.1 Hz, H-6), 2.22 (1H, dt, *J* = 13.6, 5.1 Hz, H-5), 2.10 (1H, ddd, *J* = 13.6, 11.0, 5.3 Hz, H-5); ^{13}C NMR (CD_3OD) δ 198.3 (C-1), 177.6 (C-3), 136.9 (C-1′), 134.5 (C-9), 128.6 (C-2′,6′), 128.3 (C-4′), 127.6 (C-3′,5′), 123.4 (C-8), 102.0 (C-2, C-7), 72.0 (C-4), 56.2 (OMe), 33.9 (C-6), 33.0 (C-5); HREIMS: *m/z* 258.1249 [calcd for $C_{16}H_{18}O_3$, 258.1256].

Reduction of Compound 1. Compound **1** (96.2 mg) in 5 mL of THF was treated at 0 °C with $NaBH_4$ (55.9 mg) in MeOH (5 mL). After 3 h, an excess of acetone was added to the reaction mixture, which was then partitioned with water and EtOAc. The EtOAc layer was evaporated to dryness and then subjected to normal-phase preparative TLC with 30% CH_3CN−C_6H_6 to give $\mathbf{S}_1$ (17.9 mg) and $\mathbf{S}_2$ (22.6 mg). $\mathbf{S}_1$: ^{1}H NMR (CD_3-OD) δ 7.35−7.20 (5H, m, Ph), 6.17 (1H, ddd, *J* = 17.1, 10.2, 8.6 Hz, H-8), 5.90 (1H, br d, *J* = 10.2 Hz, H-9), 5.17 (1H, br d, *J* = 17.1 Hz, H-9), 3.93 (1H, br d, *J* = 2.7 Hz, H-4), 3.57 (3H, s, OMe), 2.27 (1H, m, H-7), 3.45 (1H, dt, *J* = 9.3, 3.9 Hz, H-1), 3.19 (1H, ddd, *J* = 9.3, 3.9, 2.7 Hz, H-5), 2.27 (1H, m, H-2), 1.97 (1H, dt, *J* = 12.7, 3.9 Hz, H-6), 1.85 (1H, dt, *J* = 12.7, 9.3 Hz, H-6), 1.83 (1H, dt, *J* = 13.6, 2.7 Hz, H-3), 0.97 (1H, ddd, *J* = 13.6, 11.2, 2.7 Hz, H-3). $\mathbf{S}_2$: $[\alpha]_D^{22}$ +26.4° (*c* 0.055, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 7.30−7.15(5H, m, Ph), 5.97 (1H, dt, *J* = 16.9, 10.0 Hz, H-8), 5.12 (1H, dd, *J* = 16.9, 1.2 Hz, H-9), 5.01 (1H, dd, *J* = 10.0, 1.2 Hz, H-9), 4.23 (1H, br d, *J* = 2.2 Hz, H-1), 4.02 (1H, br d, *J* = 2.4 Hz, H-4), 3.53 (1H, ddd, *J* = 11.7, 4.6, 2.4 Hz, H-5), 3.28 (3H, s, OMe), 3.19 (1H, t, *J* = 10 Hz, H-1), 2.18 (1H, m, H-6), 2.18 (1H, m, H-2), 1.81 (1H, ddd, *J* = 13.9, 11.7, 2.2 Hz, H-6), 1.45 (2H, m, H-3).

Selenoetherification of $\mathbf{S}_2$. To a magnetically stirred solution of $\mathbf{S}_2$ (13.5 mg) in dry CH_2Cl_2 (1 mL) were added PhSeCl (10.5 mg) and K_2CO_3 (7.6 mg), and the mixture was stirred at room temperature until the red-orange solid of PhSeCl was dissolved and TLC indicated completion of the reaction.[41] The product mixture was partitioned with H_2O and CH_2Cl_2, and the organic layer was separated, dried, and subjected to normal-phase preparative TLC with 30% CH_3CN−C_6H_6 to afford selenoether $\mathbf{S}_3$ (17.2 mg): ^{1}H NMR (CD_3OD) δ 6.82−6.58 (5H, m, Ph), 4.60 (1H, dt, *J* = 9.3, 4.3 Hz, H-1), 4.40 (1H, dt, *J* = 12.4, 5.6 Hz, H-8), 3.60 (1H, br d, *J* = 2.6 Hz, H-4), 3.4 (3H, s, OMe), 3.31 (1H, ddd, *J* = 11.4, 4.4, 2.6 Hz, H-5), 3.15 (2H, d, *J* = 2.6 Hz, H-9), 3.12 (1H, dd, *J* = 12.4, 5.6 Hz, H-7), 2.84 (1H, ddd, *J* = 12.9, 8.5, 2.6 Hz, H-3), 2.40 (1H, ddd, *J* = 12.9, 4.6, 3.6 Hz, H-3), 2.34 (1H, dt, *J* = 11.2, 4.3 Hz, H-6), 2.28 (1H, dddd, *J* = 12.4, 8.5, 9.3, 4.6 Hz, H-2), 1.81 (1H, ddd, *J* = 11.4, 11.2, 4.3 Hz, H-6).

Reduction of Selenoether $\mathbf{S}_3$. To a solution of selenoether $\mathbf{S}_3$ (15.1 mg) in freshly distilled toluene (1 mL) were added *n*-Bu_3SnH (15.9 mg) and azobisisobutyronitrile (AIBN, 0.02 M toluene solution, 1 mL). The mixture was degassed with a stream of argon for 15 min and heated to 110 °C for 6 h under argon.[41] The mixture was partitioned with H_2O and $CHCl_3$, and the organic layer was dried and subjected to normal-phase preparative TLC with 30% CH_3CN−C_6H_6 to afforded $\mathbf{S}_4$ (7.5 mg): ^{1}H NMR (CD_3OD) δ 7.32−7.18 (5H, m, Ph), 4.44 (1H, ddd, *J* = 9.8, 7.2, 3.4 Hz, H-1), 4.35 (1H, dq, *J* = 8.7, 6.3 Hz, H-8), 3.50 (1H, dt, *J* = 13.9, 3.4 Hz, H-5), 3.40 (1H, br d, *J* = 3.4 Hz, H-4), 3.28 (3H, s, OMe), 2.51 (1H, dd, *J* = 11.4, 8.7 Hz, H-7), 2.30 (1H, m, H-2), 2.14 (1H, dt, *J* = 10.7, 3.4 Hz, H-3), 1.70 (1H, m, H-3), 1.64 (1H, dt, *J* = 13.9, 3.4 Hz, H-6), 1.26 (1H, dt, *J* = 13.9, 7.2, Hz, H-6), 1.56 (1H, d, *J* = 6.3 Hz, H-9).

Oxidation of $\mathbf{S}_4$. To the stirred solution of $\mathbf{S}_4$ (6.1 mg) in CH_2Cl_2 (1.5 mL) was Jones reagent drop by drop at 0 °C. After the reaction was completed, the solution was extracted with CH_2Cl_2. The organic layer was dried and chromatographed on silica gel with 3% MeOH−$CHCl_3$ to give ketone $\mathbf{S}_5$ (4.0 mg): ^{1}H NMR (CD_3OD) δ 7.32 (2H, m, H-2′,6′), 7.27 (1H, m, H-4′), 7.18 (2H, m, H-3′,5′), 4.57 (1H, dt, *J* = 9.3, 4.2 Hz, H-1), 4.00 (1H, dq, *J* = 9.0, 5.8 Hz, H-8), 3.90 (1H, dd, *J* = 10.5, 4.8 Hz, H-5), 3.50 (3H, s, OMe), 2.81 (1H, dddd, *J* = 9.3, 6.8, 6.8, 4.2 Hz, H-2), 2.63 (1H, dd, *J* = 15.5, 6.8 Hz, H-3), 2.46 (1H, dt, *J* = 14.4, 4.2 Hz, H-6), 2.40 (1H, dd, *J* = 9.0, 6.8 Hz, H-7), 2.35 (1H, dd, *J* = 15.6, 4.2 Hz, H-3), 2.10 (1H, ddd, *J* = 14.4, 10.5, 4.2 Hz, H-6), 1.27 (3H, d, *J* = 5.8 Hz, H-9); ^{13}C NMR (CD_3OD) δ 209.5 (C-4), 139.6 (C-1′), 128.9 (C-2′,6′), 127.7 (C-3′,5′), 127.2 (C-4′), 81.6 (C-8), 79.1 (C-5), 74.8 (C-1), 60.7 (C-7), 58.3 (OMe), 47.2 (C-2), 40.5 (C-3), 34.1 (C-6), 19.1 (C-9); CD (*c* 3.844 × 10^{-4}M, EtOH) $[\theta]_{300}$ −1426; HREIMS *m/z* 260.1389 [calcd for $C_{15}H_{14}O_2$, 260.1412].

LC-MS Analysis. The LC-MS analyses were performed using a JEOL JMS-700T four-sector mass spectrometer, coupled to a Hewlett-Packard (Waldbronn, Germany) model HP1100 HPLC system, operated in the negative atmospheric pressure chemical ionization (APCI) mode [capillary temperature, 400 °C; ion injection time, 0.1 s; column, Shim-pack (Shimadzu, Kyoto, Japan) CLC-ODS (150 × 6.0 mm i.d.); column temperature, 40 °C; mobile phase, linear gradient of 0.5% acetic acid:MeOH from 70:30 (v/v) to 20:80 in 30 min; flow-rate, 1 mL/min]. Samples of the standards **1**−**19** were dissolved in MeOH (5 mg/mL), and 10 μL aliquots were injected for LC-MS analysis. The Nepalese propolis extract was dissolved in HPLC grade MeOH (5 mg/mL), filtered using a Millipore (Millipore, Bedford, MA) SJLG 250 filter, and aliquots of the filtrate (10 μL) were injected for analysis.

Nitric Oxide Inhibitory Assay. The J774.1 cell line was propagated in 75 cm^2 plastic culture flasks (Falcone, Becton Dickinson, NJ), containing RPMI 1640 medium supplemented with penicillin G (100 U/mL), streptomycin (100 U/mL), and 10% FCS. The cells were harvested with trypsin and diluted to a suspension in fresh medium. The cells were seeded in 96-well plastic plates with 1 × 10^5 cells/well and allowed to adhere for 2 h at 37 °C in a humidified atmosphere containing 5% CO_2. Then, the medium was replaced with fresh medium, containing LPS (10 μg/mL) and test compounds at indicated concentrations, and the cells were incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent.[42] Briefly, 50 μL of the supernatant from incubates was mixed with equal volume of Griess reagent (0.05% sulfanilamide and 0.05% naphthylenediamide dihydrochloride in 2.5% H_3PO_4) and was allowed to stand for 10 min at room temperature. Absorbance at 550 nm was measured using an HTS 7000 microplate reader (Perkin-Elmer, CT). The blank correction was carried out by subtracting the absorbance due to medium only from the absorbance reading of each well. The percentage inhibition was calculated as follows: % inhibition = $[(A_c - A_s)/A_c] \times 100$, where A_c and A_s are absorbance of control group treated with LPS alone and absorbance of the sample, respectively.

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Supporting Information Available: Figure S1, showing the structures of the known compounds (**12**−**19**) isolated form Nepalese propolis, and Figure S2, showing the key HMBC and selected ROESY correlations observed for **1**, **4**, **5**, and **6**, are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Burdock, G. A. *Food Chem. Toxicol.* **1998**, *36*, 347−363.
(2) Bankova, S. B.; De Castro, S. L.; Marcucci, M. C. *Apidologie* **2000**, *31*, 3−15.
(3) Marcucci, M. C. *Apidologie* **1995**, *26*, 83−99.
(4) Banskota A. H.; Tezuka Y.; Kadota, S. *Phytother. Res.* **2001**, *15*, 561−571.
(5) Banskota, A. H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Nat. Prod.* **2000**, *63*, 1277−1279.
(6) Banskota, A. H.; Tezuka, Y.; Prasain, J. K.; Matsushige, K.; Saiki, I.; Kadota, S. *J. Nat. Prod.* **1998**, *61*, 896−900.
(7) Banskota, A. H.; Tezuka, Y.; Adnyana, I. K.; Midorikawa, K.; Matsushige, K.; Message, D.; Huertas, A. A. G.; Kadota, S. *J. Ethnopharmacol.* **2000**, *72*, 239−246.
(8) Midorikawa, K.; Banskota, A. H.; Tezuka, Y.; Nagaoka, T.; Matsushige, K.; Message, D.; Huertas, A. A. G.; Kadota, S. *Phytochem. Anal.* **2001**, *12*, 366−373.
(9) Banskota, A. H.; Nagaoka, T.; Sumioka, L. Y.; Tezuka, Y.; Awale, S.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Ethnopharmacol.* **2002**, *80*, 67−73.
(10) Usia, T.; Banskota, A. H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Nat. Prod.* **2002**, *65*, 673−676.
(11) Than, M. M.; Banskota A. H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Trad. Med.* **2003**, *20*, 20−29.
(12) Donnelly, D. M. X.; Thompson, J. C.; Whalley, W. B.; Ahmad, S. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1737−1745.
(13) Muangnoicharoen, N.; Frahm, A. W. *Phytochemistry* **1982**, *21*, 767−772.
(14) Pathak, V.; Shirota, O.; Sekita, S.; Hirayama, Y.; Hakamata, Y.; Hayashi, T.; Yanagawa, T.; Satake, M. *Phytochemistry* **1997**, *46*, 1219−1223.
(15) Seshadri, T. R. *Phytochemistry* **1972**, *11*, 881−898.
(16) Bate-Smith, E. C.; Swain, T. *J. Chem. Soc.* **1953**, 2185−2187.
(17) Smith, D. G.; McInnes, A. G.; Higgins, V. J.; Millar, R. L. *Physiol. Plant Pathol.* **1971**, *1*, 41−44.
(18) Kurosawa, K.; Ollis, D. W.; Redman, B. T.; Sutherland, I. O.; Gottlieb, O. R. *Phytochemistry* **1978**, *17*, 1413−1415.
(19) Ingham, J. L. *Phytochemistry* **1976**, *15*, 1489−11495.
(20) Moffitt, W.; Woodward, R. B.; Moscowitz, A.; Klyne, W.; Djerassi, C. *J. Am. Chem. Soc.* **1961**, *83*, 4013−4018.
(21) Snatzke, G. *Tetrahedron* **1965**, *21*, 413−419.
(22) Zaitsev, V. G.; Sachava, D. G.; Yankovskaya, G. S.; Garbuz, N. I. *Chirality* **2000**, *12*, 287−290.
(23) Eyton, W. B.; Ollis, W. D.; Sutherland, O. I.; Gottlieb, O. R.; Magalhaes, M. T.; Jackman, L. M. *Tetrahedron* **1966**, *21*, 2683−2696.
(24) Donnelly, D. M. X.; O'Reilly, J.; Thompson, J. *Phytochemistry* **1972**, *11*, 823−826.
(25) Letcher, R. M.; Shirley, M. I. *Phytochemistry* **1976**, *15*, 353−354.
(26) Donnelly, D. M. X.; Criodain, T. O.; O'Sullivan, M. *Proc. R. Ir. Acad., Sect. B* **1983**, 39−48.
(27) Ramesh, P.; Yuvarajan, C. R. *Indian J. Heterocycl. Chem.* **1995**, *4*, 315−316.
(28) Chan, S. C.; Chang, Y. S.; Kuo, S. C. *Phytochemistry* **1997**, *46*, 947−949.
(29) Chan, S. C.; Chang, Y. S.; Wang, J. P.; Chen, S. C.; Kuo, S. C. *Planta Med.* **1998**, *64*, 153−158.
(30) Rother, A.; Edwards, J. M. *Phytochemistry* **1994**, *36*, 911−916.
(31) Hausen, B. M. *Derm. Beruf. Umwelt* **1982**, *30*, 189−192.
(32) Hausen, B. M.; Munster, G. *Derm. Beruf. Umwelt* **1983**, *31*, 110−117.
(33) Guanche, A. D.; Prawer, S. *Am. J. Contact Dermat.* **2003**, *14*, 90−92.
(34) Rees, D. D.; Palmer, R. M.; Schulz, R.; Hodson, H. F.; Moncada, S. *Br. J. Pharmacol.* **1990**, *101*, 746−752.
(35) Natrajan, K.; Shingh, S.; Burke, T. R., Jr.; Grungerger, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9090−9095.
(36) Song, Y. S.; Park, E. H.; Hur, G. M.; Ryu, Y. S.; Lee, Y. S.; Lee, J. Y. *Cancer Lett.* **2002**, *175*, 53−61.
(37) Orban, Z. N.; Mitsiades, T. R.; Burke, M.; Tsokos; Chrousos, G. P. *Neuroimmunomodulation* **2000**, *7*, 99−105.
(38) Kuo, P. C.; Schroeder, R. T. *Ann. Surg.* **1995**, *211*, 220−235.
(39) Vincent, J.-L.; Zhang, H.; Szabo, C.; Preiser J.-C. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, 1781−1785.
(40) Feihl, F.; Waeber, B.; Liaudet, L. *Pharmacol., Ther.* **2001**, *91*, 179−213.
(41) Nicolaou, K. C.; Magolda, R. L.; Sipio, W. J.; Barnette, W. E.; Lysenko, Z.; Joullie, M. M. *J. Am. Chem.* Soc. **1980**, *102*, 3784−3793.
(42) Dirsch, V. M.; Stuppner, H.; Vollmar, A. M. *Planta Med.* **1998**, *64*, 423−426.

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