Oxidative Burst Inhibitory and Cytotoxic Indoloquinazoline and Furoquinoline Alkaloids from *Oricia suaveolens*

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Two new β-indoloquinazoline alkaloids, orisuaveoline A (**1**) and orisuaveoline B (**2**), two new furoquinoline alkaloids, quinosuaveoline A (**5**) and quinosuaveoline B (**6**), and 12 known compounds were isolated from *Oricia suaveolens*. The structures of the new compounds were deduced by spectroscopic studies. The absolute configuration of nkolbisine (**4**) was also determined. Compounds **2**, **3**, **6**−**8**, **10**, and **14** were evaluated for oxidative burst inhibitory activity in a chemoluminescence assay and for cytotoxicity against A549 lung carcinoma cells.

Immune suppression and cytotoxic activity affecting the function of the immune system have been reported for many synthetic and natural agents.[1−3] Various disease conditions such as infections, organ transplantation, cancer, rheumatoid arthritis, and systemic lupus erythromatosus are currently treated with novel immunomodulating agents.[4]

In a continuing search for bioactive molecules from Cameroonian rainforest medicinal plants, *Oricia suaveolens* (Engl.) Verd. (Rutaceae), a monotypic genus, was studied.[5] Previous phytochemical investigations on the stem bark of this plant have yielded oricine,[6,7] tecleanone, evoxanthine, kokusaginine, halfordinine, 1,3-dimethoxy-*N*-methylacridone, and lupeol.[8] In this report, we describe the isolation and structural elucidation of two new β-indoloquinazoline alkaloids (**1** and **2**), two new furoquinoline alkaloids (**5** and **6**), and the absolute configuration of nkolbisine (**4**), together with the immunomodulatory and cytotoxic activity of these isolated compounds.

The stems and the leaves of *Oricia suaveolens* were extracted separately with MeOH. Each MeOH extract was subjected to bioassay-guided fractionation based on its inhibitory activity against the oxidative burst of whole blood (98%) (Figure S1, Supporting Information). Initial solvent−solvent extraction of the MeOH crude extracts indicated that the bioactivity was concentrated in *n*-hexane- and CH_2Cl_2-soluble portions. These extracts were combined on the basis of similar TLC profiles and immunomodulatory inhibitions and were subjected to column chromatography (silica gel) and preparative TLC. This led to the isolation of four new (**1**, **2**, **5**, and **6**) and 12 known compounds. By comparison with the reported data,[9−12] the known compounds were identified as 2-methoxyruteacaprine (**3**), (*S*)-nkolbisine (**4**), tecleaverdoornine (**7**), skimmianine (**8**), 7-hydroxy-8-methoxydictamine (**9**), kokusaginine (**10**), maculine (**11**), 5-methoxymaculine (**12**), flindersiamine (**13**), limonin (**14**), lupeol, and oleanolic acid. Compounds **3** and **14** were isolated for the first time from this genus.[10,13]

Orisuaveoline A (**1**) was obtained as a yellow, amorphous solid, showing a positive reaction with $FeCl_3$, indicating its phenolic nature. The molecular composition was found to be $C_{18}H_{13}N_3O_2$ by HREIMS ($[M]^+$ at *m/z* 303.1001, calcd 303.1008). The UV spectrum showed a highly conjugated system with absorption bands characteristic of indolopyridoquinazoline-type alkaloids.[14,15] The presence of a NH and a carbonyl group in compound **1** was inferred by the IR bands at 3384 and 1656 cm^{-1}, respectively. The ^1H NMR spectrum (Table 1) of compound **1** exhibited a deshielded proton at δ 13.10 (brs, H−N), a free hydroxyl function at δ 8.90 (brs, 1H), which was exchangeable with D_2O, and two symmetrical triplets at δ 4.57 ($J = 6.9$ Hz) and 3.23 ($J = 6.9$ Hz), characteristic of the C-7 and C-8 methylene protons of an indoloquinazoline alkaloid, respectively.[16] This inference was supported by the ^{13}C NMR data (Table 1), which showed characteristic signals of C-7 and C-8 at δ 41.2 and 19.7, respectively. Furthermore, the ^1H NMR spectrum of compound **1** showed four aromatic protons corresponding to an *ortho*-disubstituted ring A at δ 7.21 (t, $J = 7.4$ Hz, H-10), 7.36 (t, $J = 7.6$ Hz, H-11), 7.48 (d, $J = 7.8$ Hz, H-12), and 7.64 (d, $J = 7.9$ Hz, H-9), together with three aromatic protons for an ABX system of ring E at δ 7.24 (d, $J = 1.2$ Hz, H-1), 7.48 (dd,

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Orisuaveolines A (**1**) and B (**2**) in $CDCl_3$

	1		**2**	
position	δ_C	δ_H [mult, *J* (Hz)]	δ_C	δ_H [mult, *J* (Hz)]
1	116.9	7.24 (d, 1.2)	108.8	7.15 (d, 2.0)
2	162.5		163.1	
3	117.8	7.48 (dd, 8.2, 1.2)	115.6	7.50 (dd, 7.9, 2.0)
4	127.0	7.78 (d, 8.2)	127.2	8.43 (d, 7.9)
4a	118.1		118.3	
5	161.2		161.1	
7	41.2	4.57 (t, 6.9)	41.1	4.50 (t, 7.0)
8	19.7	3.23 (t, 6.9)	19.6	3.01(t, 7.0)
8a	118.7		118.4	
9	120.9	7.64 (d, 7.9)	98.4	7.26 (s)
9a	125.2		126.1	
10	120.2	7.21 (t, 7.4)	143.9	
11	125.9	7.36 (t, 7.6)	142.6	
12	112.0	7.48 (d, 7.8)	104.4	7.74 (s)
12a	138.3		134.3	
13		13.10 (brs)		13.09 (brs)
13a	126.7		126.5	
13b	144.2		146.1	
14a	151.0		150.7	
−OCH_2O−			101.6	6.04 (s)
OH		8.90 (brs)		
OMe			58.9	4.36 (s)

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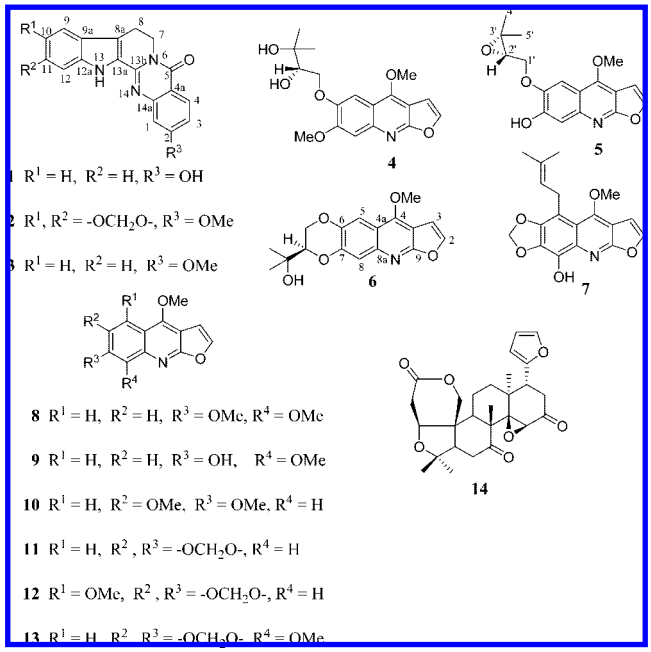
Chart 1



J = 8.2, 1.2 Hz, H-3), and 7.78 (d, J = 8.2 Hz, H-4). Together with δ_H, the δ_C values of ring A at δ 120.9 (C-9), 120.2 (C-10), 125.9 (C-11), and 112.0 (C-12), as well as for rings C and D at δ 118.7 (C-8a), 126.7 (C-13a), 144.2 (C-13b), 118.1 (C-4a), and 161.2 (C-5), were in agreement with those reported for compound **3**,[17] except for the absence of the methoxy signal in the ^{1}H NMR and ^{13}C NMR spectra of compound **1**. The complete assignments of the indoloquinazoline skeleton and its substitution pattern were based on COSY, HMQC, and HMBC experiments. In the HMBC spectrum, correlations between the aromatic low-field signal of H-4 (δ 7.78) with C-5 (δ 161.2) and C-2 (δ 162.5) and between the free hydroxyl function at δ 8.90 (OH-2) and both C-1 (δ 116.9) and C-3 (δ 117.8) indicated the position of a hydroxyl group at C-2. From the above spectroscopic data, the structure of compound **1** was determined as 8,13-dihydro-2-hydroxyindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one and named orisuaveoline A.

Orisuaveoline B (**2**) was also obtained as a yellow, amorphous powder. The molecular composition was found to be $C_{20}H_{15}N_3O_4$ by HREIMS ([M]$^+$ at *m*/*z* 361.1062, calcd 361.1063). This value was 58 mass units higher than that of compound **1**, suggesting the presence of additional units such as CO_2 and CH_2 in compound **2**. The UV absorption bands (249, 329, 342, 392 nm) and the IR spectrum (1217, 1299, 1442, 1602, 1656, 2924, 3384 cm^{-1}) also suggested a β-indoloquinazoline skeleton for compound **2**.[14,15] The ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, and HMBC spectra of compound **2** showed the presence of the same aromatic spin systems as in compound **1** (Table 1). The ^{1}H NMR spectrum revealed the presence of one methoxy group (δ 4.36) and the absence of the free hydroxyl group observed in **1**. This indicated that the free hydroxyl group in **1** was replaced by a methoxy group in **2**. Furthermore, the ^{1}H NMR spectrum of **2** showed a singlet at δ 6.04, corresponding to a methylenedioxy group, and two aromatic protons, resonating as singlets at δ 7.26 and 7.74.[18] The presence of the methoxy and the methylenedioxy moieties was confirmed by the ^{13}C NMR and DEPT spectra, which showed two signals corresponding to oxygenated aromatic carbons at δ 142.6 (C-11) and 143.9 (C-10), one signal of a methylenedioxy group at δ 101.6, two aromatic methine carbons at δ 98.4 (C-9) and 104.4 (C-12), and a methoxy carbon at δ 58.9. The position of the methylenedioxy group was deduced by the HMBC correlations from H-12 (δ 7.74) to C-10 (δ 143.9) and C-9a (δ 126.1), H-9 (δ 7.26) to C-11 (δ 142.6), C-8a (δ 118.4), and C-12a (δ 134.3), and H-N (δ 13.09) to C-12 (δ 104.4) and C-9a (δ 126.1). These observations supported the positions C-10 and C-11 for the presence of the methylenedioxy group. From the above spectroscopic data, the structure of compound **2** was deduced as 8,13-dihydro-2-methoxy-10,11-methylenedioxyindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one and named orisuaveoline B.

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Quinosuaveolines A (**5**) and B (**6**) in $CDCl_3$

	5		**6**	
position	δ_C	δ_H [mult, J (Hz)]	δ_C	δ_H [mult, J (Hz)]
2	143.0	7.90(d, 2.7)	142.7	7.53(d, 2.0)
3	105.2	7.38(d, 2.7)	105.2	6.99(d, 2.0)
3a	101.0		102.3	
4	155.0		155.8	
4a	112.1		114.2	
5	100.9	7.54(s)	106.9	7.65(s)
6	148.6		142.2	
7	155.6		147.3	
8	106.6	7.31(s)	112.4	7.49(s)
8a	141.8		141.9	
9	162.5		163.5	
1′	68.8	4.30(dd, 11.1; 5.4) 4.10(dd, 11.1; 6.0)	64.9	4.50(dd, 11.5; 2.0) 4.14(dd, 11.5; 9.0)
2′	62.6	3.28(dd, 6.0; 5.4)	79.4	4.08(dd, 9.0; 2.0)
3′	58.7		70.7	
4′	24.5	1.35(s)	26.1	1.36(s)
5′	19.3	1.35(s)	25.3	1.43(s)
OH		9.80(brs)		3.80(brs)
OMe	58.9	4.32(s)		4.40(s)

Compound **4** was obtained as a yellow powder. The molecular composition was found to be $C_{18}H_{21}NO_6$ by HREIMS ([M]$^+$, *m*/*z* 347.1377, calcd 347.1369). Its UV absorptions at 229, 252, 307, and 321 nm suggested a furoquinoline skeleton.[9] Compound **4** was identified by comparison of its spectroscopic data (MS, UV, IR, and 1D and 2D NMR) with literature data as nkolbisine, previously reported from *Teclea ouabanguiensis*.[9] The *S*-configuration of compound **4** at C-2′ was determined for the first time in the present study from the strongly positive Cotton effect [248 ($\Delta\varepsilon$ +3.59), 220 ($\Delta\varepsilon$ +0.10) nm] and by comparison of the $[\alpha]^{25}_D$ value of **4** with data for (*S*)-1-[(6,7-dimethoxyfuro[2,3-*b*]quinolin-4-oxy]-3-methylbutane-2,3-diol, (*S*)-porritoxinol, and (*S*)-peucedanol, which have similar 2,3-dihydroxy-3-methylbutyl moieties.[19,20] These data supported the configuration of nkolbisine (**4**) as *S*.

Quinosuaveoline A (**5**), $[\alpha]^{25}_D$ −25.5, was obtained as an amorphous, yellow powder. The molecular composition was found to be $C_{17}H_{17}NO_5$ by HREIMS ([M]$^+$ at *m*/*z* 315.1015, calcd 315.1107). Its UV absorptions at 245, 251, 308, and 321 nm suggested a furoquinoline skeleton.[9] The ^{1}H NMR spectrum of compound **5** (Table 2) revealed the presence of a free hydroxyl function at δ 9.80 (1H, brs), exchangeable with D_2O, and two aromatic proton resonances as singlets at δ 7.31 (H-8) and 7.54 (H-5). The absence of correlations between these protons in the COSY spectrum indicated that they are in the *para* position of the tetrasubstituted aromatic ring A. Furthermore, the ^{1}H NMR spectrum showed two protons corresponding to a pair of AB doublets centered at δ 7.38 and 7.90 (J = 2.7 Hz), characteristic H-2 and H-3 of a furan ring, respectively, and a singlet at δ 4.32 assigned to the C-4 methoxy group in furoquinoline alkaloids. In addition to the resonances assigned above and those required for the furoquinoline nucleus, the ^{13}C NMR spectrum (Table 2) displayed five carbon signals for two methyls at δ 24.5 (C-4′) and 19.3 (C-5′), one oxymethylene at δ 68.8 (C-1′), one oxymethine δ 62.6 (C-2′), and one quaternary carbon at δ 58.7 (C-3′). These data were in agreement with the presence of a 2,3-epoxy-3-methylbutyl ether in **5**.[21,22] This was further supported by the ^{1}H NMR spectrum, which showed signals at δ 4.30 (H-1′a), 4.10 (H-1′b), 3.28 (H-2′), and 1.35 (CH_3-4′ and CH_3-5′). The EIMS indicated a (2,3-epoxy-3-methylbutyl)oxy moiety from the base peak at *m*/*z* 230, consistent with the loss of a C_5H_9O fragment (*m*/*z* 85), via a β-H transfer mechanism common in aromatic ethers,[21] from the molecular ion

at *m/z* 315. The positions of the free hydroxyl function and the (2,3-epoxy-3-methylbutyl)oxy moiety were determined by the NOESY cross-peaks between H-5 (δ 7.54) and CH_3O-4 (δ 4.32), as well as between H-1′ (δ 4.30; 4.10) and H-5 (δ 7.54), which indicated clearly that the 2,3-epoxy-3-methylbutyl ether was located at the C-6 position. The NOESY spectrum also showed correlation between the free hydroxyl group at δ 9.80 and H-8 (δ 7.31), indicating the position of the hydroxyl group at C-7. The absolute configuration of **5** was determined on the basis of circular dichroism (CD) spectroscopic analysis. Thus, the CD spectrum of **5** showed the same positive Cotton effect [252 ($\Delta\varepsilon$ +4.90), 215 ($\Delta\varepsilon$ +0.60) nm] in the same region as (*S*)-nkolbisine (**4**), which indicated the absolute configuration of C-2′ to be *S*. From the above spectroscopic studies, the structure of compound **5** was determined as (*S*)-(−)-6-[(2,3-epoxy-3-methylbutyl)oxy]-4-methoxy-7-hydroxylfuran[2,3-*b*]quinoline and named quinosuaveoline A.

Quinosuaveoline B (**6**) was obtained as a colorless, amorphous powder. Its specific rotation was found to be $[\alpha]^{25}_D$ +42.5. The molecular composition was found to be $C_{17}H_{17}NO_5$ by HREIMS ($[M]^+$ at *m/z* 315.1015, calcd 315.1107). The IR and UV spectra also suggested a furoquinoline alkaloid skeleton in compound **6**.[9] The ^1H and ^{13}C NMR data of **6** (Table 2) were very similar to those of **5**, suggesting that they are indeed positional isomers. The differences observed in their ^{13}C NMR spectra (δ 25.3, C-5′; 26.1, C-4′; 70.7, C-3′; 79.4, C-2′; and 64.9, C-1′) indicated that the 2,3-epoxy-3-methylbutyl ether group of **5** is replaced by a fused (2-hydroxyl-2-propyl)dioxane ring in **6**.[23,24] The presence of this group was further supported by the ^1H NMR spectrum, from the signals at δ 4.50 (H-1′a), 4.14 (H-1′b), 4.08 (H-2′), 1.36 (H-4′), and 1.43 (H-5′) and by the fragmentation pattern of the EIMS, which exhibited significant peaks at *m/z* 297 (loss of H_2O, *m/z* 18) and 228 (loss of $C_5H_{11}O$, *m/z* 87). The position of the fused (2-hydroxyl-2-propyl)dioxane ring was determined from the HMBC and NOESY. In the HMBC spectrum, correlations observed from H-5 (δ 7.65) to C-4 (δ 155.8), C-7 (δ 147.3), C-8a (δ 141.9), C-6 (δ 142.2), and C-4a (δ 114.2); H-8 (δ 7.49) to C-6 (δ 142.2), C-4a (δ 114.2), C-7 (δ 147.3), and C-8a (δ 141.9); and H-1′a (δ 4.50) and H-1′b (δ 4.14) to C-6 (δ 142.2), C-3′ (δ 70.7), and C-2′ (δ 79.4) suggested that the substituted dioxane ring was fused to the C-6/C-7 positions on the furoquinoline skeleton. A singlet at δ 7.65 was assigned to H-5, based on its cross-peak in the NOESY spectrum with the C-4 methoxy group at δ 4.40. Since both aromatic proton singlets were in the *para* position, it was deduced that the remaining singlet at δ 7.49 was at H-8. The absolute configuration of **6** was determined as *R* on the basis of circular dichroism (CD) spectroscopic analysis, which showed a strong negative Cotton effect [248 ($\Delta\varepsilon$ −22.19), 225 ($\Delta\varepsilon$ +7.45) nm]. From the above spectroscopic studies, the structure of **6** was assigned as (*R*)-(+)-6,7-[(3-hydroxyl-3-methylbutane-1,2-dioxy)]-4-methoxyfuran[2,3-*b*]quinoline and named quinosuaveoline B.

Compound **6** could result biosynthetically from nucleophilic attack of the C-7 hydroxyl group at C-2′ of the epoxide of quinosuaveoline A (**5**) with dioxane formation and inversion of the configuration at the stereogenic center.

Compounds **2**, **3**, **6**−**8**, **10**, and **14** were screened over a range of concentrations (3.1−50 μg/mL) for their oxidative burst inhibitory potential. These compounds were shown to possess inhibitory activity upon activation with serum opsonized zymosan, which was tested in vitro for oxidative burst studies of whole blood and polymorphoneutrophils (PMNs). Compounds **2**, **8**, and **14** showed significant effects on the oxidative burst of the whole blood (IC_{50} range 26.1−51.3 μM), while compounds **2**, **3**, and **6**−**8** showed inhibition of the oxidative burst of the PMNs (IC_{50} range 14.5−35.5 μM) (Table 3). PMNs were activated using two different cell activators: the serum opsonized zymosan and phorbol-12-myristate-13-acetate (PMA). The test compounds exhibited a clear suppressive effect on phagocyte oxidative burst response upon activation with serum opsonized zymosan in a dose-dependent manner (Figure S2, Supporting Information). However, a weak stimulatory effect was observed when the cells were activated with PMA (data not shown).

Table 3. Effect of Compounds **2**, **3**, **6**−**8**, **10**, and **14** on Oxidative Burst of Whole Blood, Isolated Polymorphoneutrophils, and Activated PMNs with Luminol Substrate

	IC_{50} (μM)	
compound	whole blood + luminol	PMNs + luminal
2	31.8 ± 11.6	22.4 ± 4.1
3	48.8 ± 0.5	27.4 ± 0.5
6	56.8 ± 6.7	14.5 ± 0.0
7	54.4 ± 5.8	21.2 ± 1.5
8	51.3 ± 3.9	35.5 ± 1.8
10	74.9 ± 37.0	61.8 ± 7.7
14	26.1 ± 1.1	38.2 ± 9.4
ibuprofen	54.3 ± 9.2	12.1 ± 3.0

Compounds **1**, **3**, **8**, **9**, and **11**−**13** were evaluated for their cytotoxicity against the lung adenocarcinoma A549 cell line using a sulforhodamine B (SRB) method and cisplatin as positive control. Among the tested compounds, **11**−**13** were the most potent, with IC_{50} values of 9.5, 7.9, and 8.9 μM, respectively. All other compounds exhibited IC_{50} values of >10 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured in methanol on a JASCO DIP-360 digital polarimeter using a 10 cm cell. Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. CD spectra were measured on a JASCO J-810 spectropolarimeter. Infrared spectra were recorded on a JASCO 302-A spectrophotometer. ESIMS was recorded on a Finnigan LCQ with a Rheos 4000 quaternary pump (Flux Instrument). EIMS were recorded on a Varian MAT 311A spectrometer (70 eV). HREIMS were performed on a JEOL HX 110 mass spectrometer. The ^1H and ^3C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX 500 NMR spectrometer. Chemical shifts are reported in δ (ppm) using TMS as internal standard. Column chromatography was carried out on silica gel (70−230 mesh, Merck) and flash silica gel (230−400 mesh, Merck). TLC was performed on Merck precoated silica gel 60 F_{254} aluminum foil, using ceric sulfate spray reagent for visualization. All reagents used were analytical grade.

Plant Material. The stems and leaves of *Oricia suaveolens* were collected at the Batouri locality in Eastern Cameroon in April 2006 and identified by Mr. Nana Victor of the National Herbarium, Yaoundé, Cameroon, where a voucher specimen (ref 6161 SRF/CAM) was deposited.

Extraction and Isolation. The air-dried and powdered stems (4.5 kg) and leaves (1.5 kg) of *O. suaveolens* were separately extracted with MeOH at room temperature for 72 h. After evaporation under reduced pressure, the dried crude extracts of the stems (OSS, 100.0 g) and the leaves (OSL, 50.0 g) were combined on the basis of similar TLC and immunomodulatory inhibitory properties. The mixed crude extract (150 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 2000 mL) and extracted with *n*-hexane (3 × 500 mL). The aqueous layer was then diluted with 60% MeOH and extracted with CH_2Cl_2 (3 × 500 mL). The aqueous layer was concentrated, and the residue was suspended in H_2O (500 mL) and extracted with *n*-BuOH (3 × 300 mL). The *n*-hexane and CH_2Cl_2 extracts were combined on the basis of similar composition by TLC and immunomodulatory properties. This combined fraction was purified by column chromatography over silica gel 60 (230−400 mesh) and preparative TLC using a gradient system of *n*-hexane, CH_2Cl_2, ethyl acetate, and MeOH. Altogether, 250 subfractions (ca. 250 mL each) were collected and pooled on the basis of TLC analysis, leading to six main fractions (A−F). Fraction A (25.0 g) was the combination of subfractions 1−49, eluted with a mixture of *n*-hexane−CH_2Cl_2 (8:2). Fraction B (7.0 g) was constituted by subfractions 50−70, eluted with a mixture of *n*-hexane−CH_2Cl_2 (6:4). Fraction C (15.0 g) was the combination of subfractions 71−90, eluted with *n*-hexane−CH_2Cl_2 (1:1). Fraction D (8.5 g) was constituted by subfractions 91−110, eluted with pure CH_2Cl_2. Fraction E (13.0 g) was the combination of subfractions 111−160, eluted with CH_2Cl_2−ethyl acetate (9:1), and fraction F (10.0 g) was comprised of subfractions 161−200, eluted with CH_2Cl_2−ethyl acetate (8:2).

Fraction A was chromatographed over a silica gel 60C column with a *n*-hexane−CH_2Cl_2 gradient. A total of 35 fractions of ca. 100 mL each were collected and combined on the basis of TLC. Fractions 1−30 were further chromatographed on silica gel 60H with a mixture of *n*-hexane−CH_2Cl_2 (8:2) for elution to yield lupeol (5.5 mg) and maculine (**11**) (15.5 mg). Fraction B was chromatographed over a silica gel 60C column with a *n*-hexane−CH_2Cl_2 gradient. A total of 25 fractions of ca. 100 mL each were collected and combined on the basis of TLC. Fractions 1−10 were further chromatographed over a silica gel 60H with a mixture of *n*-hexane−CH_2Cl_2 (4:1) to yield 2-methoxyruteacarprine (**3**) (24.0 mg), orisuaveoline B (**2**) (22.0 mg), flindersiamine (**13**) (7.5 mg), and 5-methoxymaculine (**12**) (13.4 mg). Fraction C was chromatographed on a silica gel 60C column with a *n*-hexane−CH_2Cl_2 gradient. Altogether, 25 fractions of ca. 100 mL each were collected and combined on the basis of TLC. Fractions 21−30 were further chromatographed over a silica gel 60H with *n*-hexane−CH_2Cl_2 (1:1) to yield skimmianine (**8**) (10.0 mg), kokusagenine (**10**) (5.0 mg), and orisuaveoline A (**1**) (7.8 mg). Similarly, fraction E was chromatographed on a silica gel 60C column with a *n*-hexane−CH_2Cl_2 (1:3) gradient. As a result, 25 fractions of ca. 100 mL each were collected and combined on the basis of TLC. Fractions 1−10 were further chromatographed over silica gel 60H with *n*-hexane−CH_2Cl_2 (1:3) to yield quinosuaveoline B (**6**) (16.0 mg), quinosuaveoline A (**5**) (12.9 mg), limonin (**14**) (35.5 mg), and tecleaverdoomine (**7**) (9.5 mg). Fractions 11−35 were further chromatographed over silica gel 60H (5−40 μm) with a mixture of CH_2Cl_2−ethyl acetate (1:2) to yield (*S*)-nkolbisine (**4**) (25.0 mg), 7-hydroxydictamine (**9**) (5.5 mg), and oleanolic acid (5.0 mg).

Orisuaveoline A (1): yellow, amorphous powder ($CHCl_3$−MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.63), 282 (4.53), 290 (5.70), 326 (4.54), 337 (4.45), 366 (4.60), 390 (4.50) nm; IR (KBr) ν_{max} 3384, 2830, 2832, 1780, 1656, 1663, 1602, 1390, 1012, 750 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-ESIMS (%) *m/z* 321 ($[M + NH_4]^+$, 100); HREIMS $[M]^+$ *m/z* 303.1001 (calcd for $C_{18}H_{13}N_3O_2$, 303.1008).

Orisuaveoline B (2): yellow, amorphous powder ($CHCl_3$−MeOH); UV (MeOH) λ_{max} (log ε) 249 (4.70), 277 (4.50), 295 (4.75), 329 (4.52), 339 (4.20), 342 (3.80), 392 (4.65) nm; IR (KBr) ν_{max} 3384, 2924, 1656, 1602, 1442, 1299, 1217, 1012, 750 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-ESIMS (%) *m/z* 379 ($[M + NH_4]^+$, 100); HREIMS $[M]^+$ *m/z* 361.1062 (calcd for $C_{20}H_{15}N_3O_4$, 361.1063).

(*S*)-Nkolbisine (4): yellow, amorphous powder (MeOH); $[\alpha]^{25}_D$ −35.8 (*c* 0.06, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)] 248 (+3.59), 220 (+0.10); UV (MeOH) λ_{max} (log ε) 229 (3.18), 252 (4.57), 307 (4.19), 321 (4.16), 339 (4.05), 364 (4.10) nm; IR (KBr) ν_{max} 3377, 3126, 2923, 2855, 1976, 1622, 1591, 1448, 1415, 1258 cm^{-1}; ^1H and ^{13}C NMR data;[9] EIMS (%) *m/z* 347 (38) $[M]^+$, 245 (100), 230 (30), 78 (46), 63 (50); HREIMS $[M]^+$ *m/z* 347.1377 (calcd for $C_{18}H_{21}NO_6$, 347.1369).

Quinosuaveoline A (5): yellow, amorphous powder (MeOH); $[\alpha]^{25}_D$ −25.5 (*c* 0.06, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)] 252 (+4.90), 215 (+0.60); UV (MeOH) λ_{max} (log ε) 245 (3.28), 251 (3.35), 308 (3.78), 321 (3.95), 334 (4.05), 364 (4.15) nm; IR (KBr) ν_{max} 3455, 3340, 2975, 2920, 2860, 1670, 1590, 1450, 1415, 1190 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (%) *m/z* 315 (32) $[M]^+$, 268 (45), 230 (100), 85 (46), 76 (65); ESIMS (%) *m/z* 333 ($[M + NH_4]^+$, 100); HREIMS $[M]^+$ *m/z* 315.1015 (calcd for $C_{17}H_{17}NO_5$, 315.1107).

Quinosuaveoline B (6): colorless, amorphous powder ($CHCl_3$); $[\alpha]^{25}_D$ +42.5 (*c* 0.05, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)] 248 (−22.19), 225 (+7.45); UV (MeOH) λ_{max} (log ε) 249 (3.26), 265 (3.53), 329 (3.75), 341 (3.95), 362 (4.05) nm; IR (KBr) ν_{max} 3304, 2930, 2830, 2832, 1680, 1550, 1290, 1045, 850 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (%) *m/z* 315 (10) $[M]^+$, 297 (85), 228 (100), 87 (48), 78 (55); ESIMS (%) *m/z* 333 ($[M + NH_4]^+$, 100) HREIMS $[M]^+$ *m/z* 315.1015 (calcd for $C_{17}H_{17}NO_5$, 315.1107).

Chemiluminescence Assay for Determination of Immunomodulatory Activity. A luminol-enhanced chemiluminescence assay was performed, as described by Helfand et al.[25] In brief, whole blood (diluted 1:200) and neutrophils (1×10^7) suspended in Hank's balance salt solution with calcium and magnesium ($HBSS^{+2}$) were incubated with 50 μL of each test compounds at concentrations of 3.1−50 μg/mL for 30 min. Then, 50 μL (20 mg/mL) of zymosan (Sigma Chemical Co., St. Louis, MO) followed by 50 μL (7×10^5 M) of luminal (G-9382 Sigma) and then $HBSS^{++}$ were added to adjust the final volume to 0.2 mL. $HBSS^{++}$ was used as a control.

Cytotoxicity Assay. Cytotoxic activities of the test compounds were tested against the A549 lung adenocarcinoma cell line by the SRB method according to a reported protocol.[26,27] In brief, freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1×10^4 cells per well with test compounds added from DMSO-diluted stock. After 3 days in the culture, the attached cells were incubated with SRB and subsequently solubilized in DMSO. The absorbance at 550 nm was then measured by using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under experimental conditions, with cisplatin as the positive control (IC_{50} 8.6 μM).

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Supporting Information Available: Graphs of chemiluminescence effect of extract and compounds on oxidative burst by whole blood and neutrophils. This information is available free of charge via the Internet at http://pubs.acs.org.

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