Secondary Metabolites from the Endophytic Fungus *Xylaria cubensis*

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Seven new metabolites, including three sesquiterpenoids, 10-hydroxythujopsene (**1**), akotriol (**2**), and xylaritriol (**3**), one diterpenoid, cubentriol (**4**), one aliphatic derivative, akoenic acid (**5**), one alkaloid, akodionine (**6**), and one isocoumarin, akolitserin (**7**), together with seven known compounds, **8**–**14**, were isolated from the AcOEt-soluble fraction of the fermentation broth of the endophytic fungus *Xylaria cubensis*, derived from the leaves of *Litsea akoensis* HAYATA (Lauraceae). Their structures were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments, and by HR-ESI-MS mass spectrometry. Among the isolates, (−)-(*R*)-7-hydroxymellein showed IL-6 inhibitory activity with an IC_{50} value of 9.41 μM.

Introduction. – The plant endophytes refer to a group of microorganisms, including fungi and bacteria, which live within plants' internal tissues or organs, but typically cause no apparent symptoms of disease in the host plant [1][2]. The interest in endophytic fungi as a source of novel bioactive compounds is increasing, mainly because of the difficulty in finding interesting new lead compounds from comprehensively investigated organisms. Recently, endophytic fungi have been proven to be a good source of structurally diverse and biologically active compounds with huge medicinal and agricultural potential [2–4]. During a program of investigation on potentially bioactive secondary metabolites from Formosan endemic plant endophytes, an endophytic fungal strain, *Xylaria cubensis* (Xylariaceae) BCRC 09F 0035, was isolated from the leaves of a Taiwan endemic plant, *Litsea akoensis* HAYATA (Lauraceae). Previous chemical investigations of the genus *Xylaria* revealed various bioactive natural products [5–7]. Only one known polyketide, cubensic acid, has been isolated from this species [8]. In the course of our search for diverse secondary metabolites from natural fungal sources, and to further understanding of the minor metabolites of the genus *Xylaria*, we examined an AcOEt-soluble fraction of *X. cubensis*, which showed inhibitory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) release and interleukin-6 (IL-6) production in RAW 264.7 murine

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macrophages, as determined by our primary screening (*ca.* 88% inhibition at a concentration of 10 μg/ml). Current phytochemical investigation of the abovementioned fungus has led to the isolation of three new sesquiterpenoids, 10-hydroxythujopsene (**1**), akotriol (**2**), and xylaritriol (**3**), one new diterpenoid, cubentriol (**4**), one new aliphatic derivative, akoenic acid (**5**), one alkaloid, akodionine (**6**), and one new isocoumarin, akolitserin (**7**) (*Fig. 1*), together with seven known compounds, **8**–**14**. The structures of compounds **1**–**7** were established by spectroscopic analysis, and inhibitory effects of some isolates on NO and IL-6 production by macrophages are described herein.

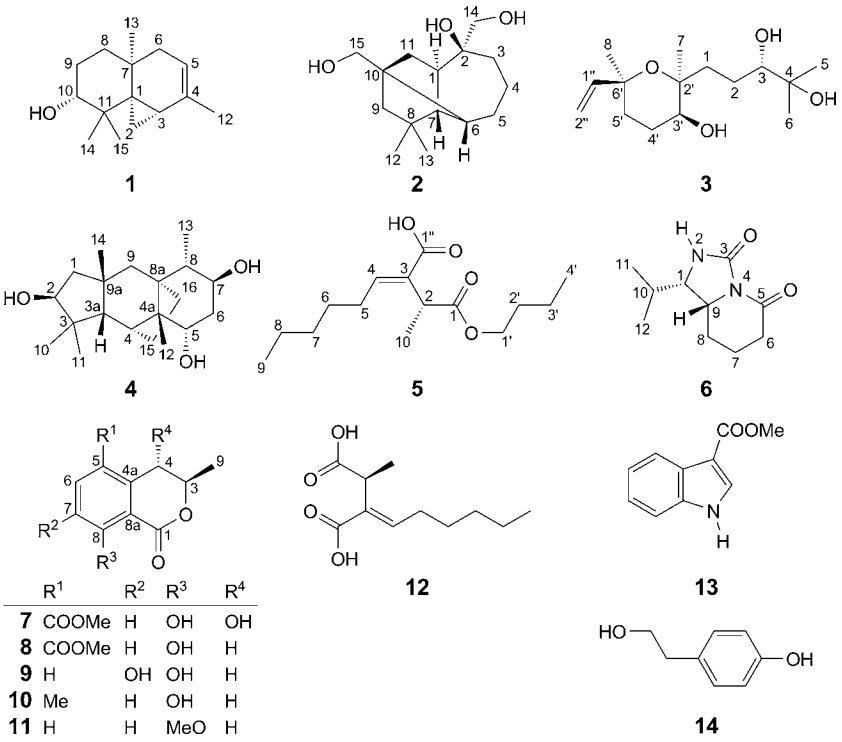

Fig. 1. *New and known compounds,* **1**–**7** *and* **8**–**14**, *respectively, isolated from* Xylaria cubensis

Results and Discussion. – *Structure Elucidation*. The BuOH-soluble fraction obtained from the endophytic fungus *X. cubensis* BCRC 09F 0035 was partitioned to afford an AcOEt-soluble fraction, then the AcOEt fraction was fractionated by a combination of column chromatography (silica gel, *RP-18* columns, *Sephadex LH-20*) and preparative TLC to furnish 14 compounds, **1**–**14**, the structures of which were elucidated by 1D- and 2D-NMR spectra and comparison with literature data.

10-Hydroxythujopsene (**1**) was obtained as optically active pale yellowish oil with $[\alpha]_D^{25} = -7.9$ ($c = 0.03$, $CHCl_3$). The molecular formula was established as $C_{15}H_{24}O$ by ^{1}H- and ^{13}C-NMR, and HR-ESI mass spectra, with four degrees of unsaturation. The IR spectrum showed absorption at 3424 cm^{-1} for a OH group. The ^{1}H- and ^{13}C-NMR

data of **1** were similar to those of (+)-thujopsene [9], except that a OH group (δ(H) 1.30 (br. *s*, HO–C(10); D_2O exchangeable)), and one O-bearing CH group (δ(H) 3.34 (br. *d*, $J = 10.2$, H–C(10))) of **1** replaced the CH_2(10) moiety of (+)-thujopsene. This was supported by pertinent ^{1}H,^{1}H-COSY (*Fig. 2*), HMBC (*Fig. 2*), and NOESY (*Fig. 3*) correlations. The NOESY spectrum (*Fig. 3*) showed correlations between H–C(2) and Me(13), Me(15); and Me(14) and H–C(3), H–C(10). The H-atoms of CH_2(2), Me(13), and Me(15) were positioned at the same side of the *cis*-octaline system, H–C(3), H–C(10), and Me(14) were on the opposite side, and there were no correlations between the H-atoms of two groups. Thus, the relative configuration of **1** could be determined as *rel*-(1*R*,3*S*,7*S*,10*R*). The ^{1}H- and ^{13}C-NMR, HMBC (*Fig. 2*), COSY (*Fig. 2*), NOESY (*Fig. 3*) experiments confirmed the structure

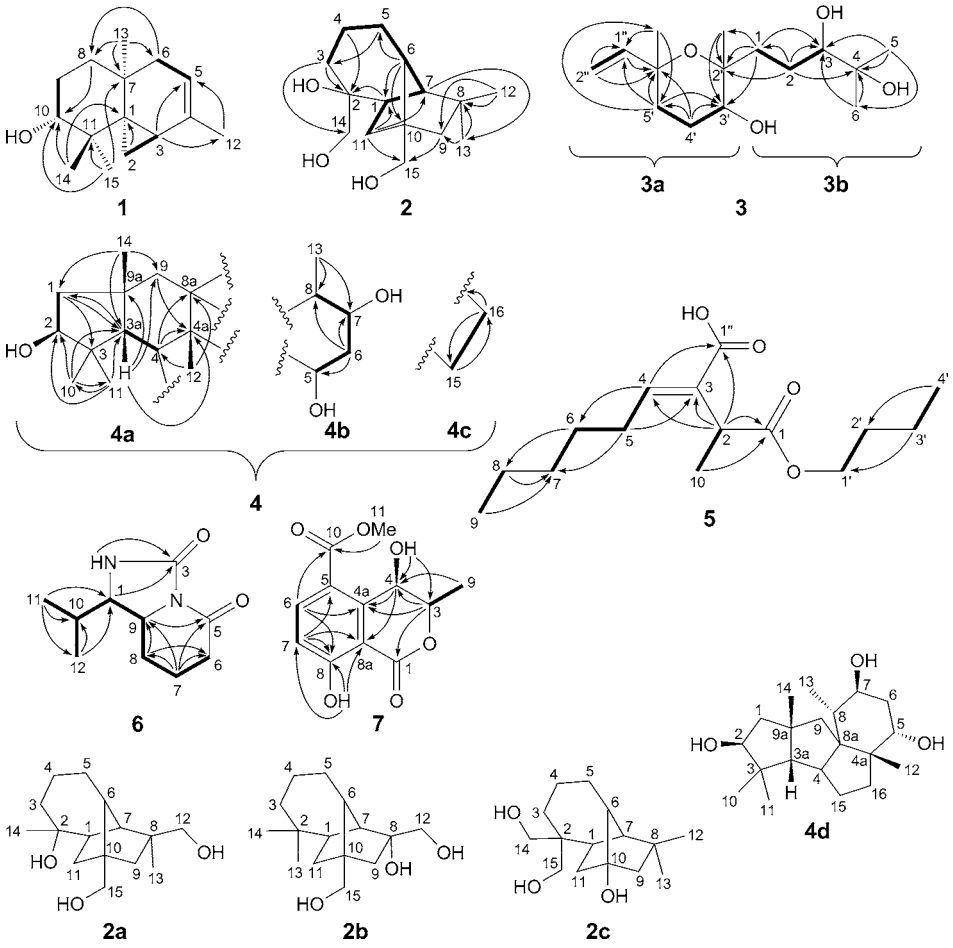

Fig. 2. *Selected HMBCs* (H → C) *and COSY correlations* (bold lines) *of compounds* **1**–**7**, *the structures of fragments* **3a** *and* **3b** *of compound* **3**, *fragments of substructures* **4a**, **4b**, *and* **4c** *of* **4**, *three possible structures* **2a**–**2c** *of* **2**, *and one possible structure* **4d** *of* **4**

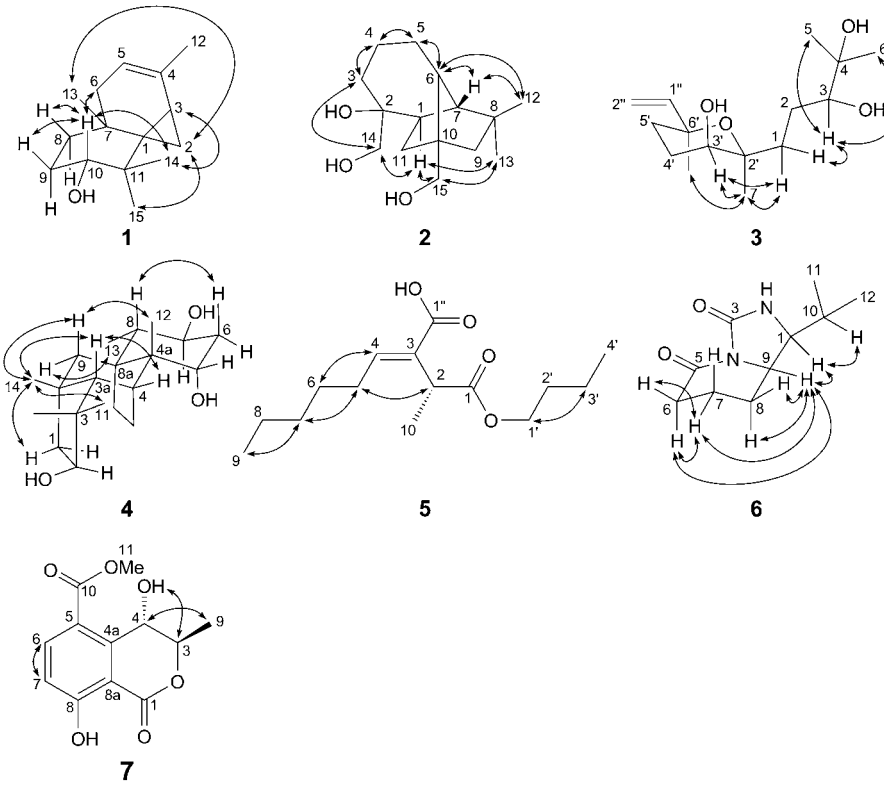

Fig. 3. *Key NOESY correlations of compounds* **1**–**7**

of **1** as (1a*R**,4a*R**,7*S**,8a*S**)-1,1a,4,4a,5,6,7,8-octahydro-2,4a,8,8-tetramethylcyclopropa[*d*]naphthalen-7-ol, named 10-hydroxythujopsene.

Akotriol B (**2**) was obtained as optically active, colorless, amorphous powder with $[\alpha]_D^{21} = -20.1$ ($c = 0.04$, $CHCl_3$). Its molecular formula was deduced as $C_{15}H_{26}O_3$, with three degrees of unsaturation, from HR-ESI-MS. The IR spectrum exhibited an absorption at 3384 cm^{-1} for OH groups. The ^1H- and ^{13}C-NMR, and DEPT spectra indicated the presence of two Me, seven CH_2, and three CH groups, and three quaternary C-atoms, of which two CH_2 groups and a quaternary C-atom were O-bearing. The NMR signals suggested a sesquiterpenoid structure for compound **2**. The HMBCs (*Fig. 2*) from H–C(1) to C(2), from H–C(3) to C(2) and C(14), from H–C(4) to C(2), from H–C(5) to C(3), from H–C(6) to C(1), C(5), and C(10), from H–C(7) to C(13), from H–C(9) to C(15), from H–C(11) to C(1) and C(15), from H–C(12) to C(8) and C(9), and from H–C(13) to C(8) and C(9), in combination with the COSY correlations (*Fig. 2*), led to the establishment of the constitutional formula for compound **2** as (1*R**,3a*R**,4*S**,5*R**,8a*R**)-decahydro-1,5-bis(hydroxymethyl)-3,3-dimethyl-1,4-methanoazulen-5-ol. Careful analysis of the NMR data indicated that there were three additional possible structures, **2a**–**2c** (*Fig. 2*), for **2**. According to the main HMBCs (see above) from H–C(3) to one CH_2–O moiety (C(14), δ(C) 64.8), and from

both H–C(9) and H–C(11) to another CH_2–O moiety (C(15), δ(C) 70.0), structures **2a** – **2c** could be ruled out. The NOESY spectrum (*Fig. 3*) showed correlations between H–C(1) and H–C(13)/H–C(14), H–C(15), and between H–C(6) and H–C(12). Thus, H–C(1), H–C(13), and H–C(14) occupied axial orientations, and H–C(12) and H–C(15) were in equatorial orientation. Attempts to grow appropriate crystals of **2** for X-ray crystallography were unsuccessful due to the limited amount of this compound. The lability of this compound further prevented configurational investigation. The ^{1}H- and ^{13}C-NMR, HMBC (*Fig. 2*), COSY (*Fig. 2*), and NOESY (*Fig. 3*) data confirmed the structure of **2**, which we named akotriol.

For xylaritriol (**3**), the molecular formula $C_{15}H_{28}O_4$ was deduced from the HR-ESI-MS and NMR data. The IR absorption at 3313 cm^{-1} indicated the presence of a OH group. The ^{1}H- and ^{13}C-NMR, and DEPT spectra exhibited signals corresponding to 15 C-atoms, which were assigned to four Me, five CH_2 (one olefinic), three CH (one olefinic and two O-bearing) groups, and three O-bearing quaternary C-atoms. The ^{1}H-NMR data of **3** showed signals of four quaternary Me groups (δ(H) 1.13 (*s*, Me(5)), 1.16 (*s*, Me(7)), 1.27 (*s*, Me(6)), and 1.29 (*s*, Me(8))), two O-bearing CH groups (δ(H) 3.59 (*dd*, J = 10.8, 2.4, H–C(3′)) and 3.82 (*t*, J = 7.2, H–C(3))), three olefinic H-atoms (δ(H) 5.05 (*dd*, J = 10.8, 1.2, H_b–C(2″)), 5.23 (*dd*, J = 17.4, 1.2, H_a–C(2″)), and 5.91 (*dd*, J = 17.4, 10.8, H–C(1″))), and four aliphatic CH_2 groups (δ(H) 1.32 – 1.38, 1.56 – 1.62 (2*m*, CH_2(4′), each 1 H), 1.64 – 1.70, 1.79 – 1.83 (2*m*, CH_2(5′), each 1 H), 1.49 – 1.54, 2.09 – 2.16 (2*m*, CH_2(1), each 1 H), and 1.89 – 1.95 (*m*, CH_2(2)). The structure of fragment **3a** with a C_9 moiety was determined on the basis of the COSY correlations (*Fig. 2*) along with the HMBCs (*Fig. 3*). In combination with the COSY correlations H–C(2) (δ(H) 1.92) ↔ H–C(3) (δ(H) 3.82) ↔ H–C(1) (δ(H) 1.49 – 1.54/2.09 – 2.16), this led to the establishment of fragment **3b** (*Fig. 2*). Finally, the HMBCs (*Fig. 2*) from H–C(1) to C(2′), and from H–C(7) to C(3′) indicated that fragments **3a** and **3b** were linked *via* C(2′), indicating **3** to be a sesquiterpenoid derivative. The orientations of H–C(7), H–C(8), and H–C(3′) were determined as α according to the NOE correlations H–C(7) ↔ H–C(8), and H–C(7) ↔ H–C(3′) (*Fig. 3*). The other key NOESY correlations H–C(3) ↔ H–C(5), H–C(3) ↔ H–C(6), H–C(1a) ↔ H–C(3), H–C(1b) ↔ H–C(7), H–C(7) ↔ H–C(3′) were also observed. Based on these evidences, the structure of **3**, named xylaritriol, was determined as (3*R**)-5-[(2*R**,3*R**,6*R**)-6-ethenyltetrahydro-3-hydroxy-2,6-dimethyl-2*H*-pyran-2-yl]-2-methylpentane-2,3-diol.

Compound **4** was obtained as optically inactive, whitish powder ($[\alpha]_D^{23} = \pm 0$ (c = 0.048, $CHCl_3$)). The HR-ESI-MS exhibited a *quasi*-molecular-ion peak at *m*/*z* 345.2406 ($[M + Na]^+$) corresponding to the molecular formula of $C_{20}H_{34}O_3$ with four degrees of unsaturation. The IR absorption at 3390 cm^{-1} indicated the presence of OH groups. The ^{1}H-NMR spectrum of **4** exhibited signals of five Me groups (δ(H) 0.94 (*s*, Me(10)), 0.99 (*s*, Me(11)), 1.07 (*d*, J = 6.6, Me(13)), 1.16 (*s*, Me(12)), and 1.30 (*s*, Me(14)), five sets of nonequivalent CH_2 groups at δ(H) 1.36 (*ddd*, J = 12.0, 8.4, 1.2, H_b–C(16)), 1.45 – 1.53 (*m*, H_b–C(15)), 1.62 (*dd*, J = 13.5, 6.4, H_b–C(1)), 1.72 (*ddd*, J = 13.4, 10.8, 3.6, H_b–C(6)), 1.76 (*d*, J = 15.0, H_b–C(9)), 1.88 (*dt*, J = 13.4, 3.6, H_a–C(6)), 1.93 (*d*, J = 15.0, H_a–C(9)), 1.95 (*qdd*, J = 9.6, 8.4, 1.2, H_a–C(15)), 1.99 (*dd*, J = 13.5, 6.4, H_a–C(1)), 2.06 – 2.12 (*m*, H_a–C(16))), and three O-bearing CH groups (δ(H) 3.42 – 3.52 (*m*, H–C(7)), 3.72 (br. *q*, J = 3.6, H–C(5)), 4.08 (*ddd*, J = 13.5, 8.7, 6.4, H–C(2)), as well as three CH groups at δ(H) 1.46 – 1.54 (*m*, H–C(8)), 1.55 (*d*, J = 9.6,

H–C(3a)), 2.39 (*td*, $J = 9.6$, 5.4, H–C(4))). The ^{13}C-NMR and DEPT spectra of **4** revealed the presence of 20 C-atoms, *i.e.*, five Me groups (δ(C) 13.8 (C(13)), 24.6 (C(10)), 24.9 (C(12)), 25.2 (C(11)), and 33.5 (C(14))), five CH_2 groups (δ(C) 32.1 (C(15)), 39.9 (C(16)), 41.1 (C(6)), 46.1 (C(9)), and 52.3 (C(1))), six CH groups (δ(C) 45.6 (C(8)), 57.7 (C(4)), 70.2 (C(7)), 74.2 (C(3a)), 75.0 (C(5)), and 80.0 (C(2))), and four quaternary C-atoms (δ(C) 46.3 (C(3)), 50.0 (C(9a)), 50.2 (C(4a)), and 62.3 (C(8a))). These data indicated a diterpenoid skeleton for **4**. The above NMR signals and the COSY spectrum (*Fig. 2*) established the presence of three partial substructures: fragments, **a**, **b**, and **c**, for **4** (*Fig. 2*). The fragment **4a**, a C_{13} moiety composed of four Me, two CH_2, three CH groups, and four quaternary C-atoms, was determined on the basis of the COSY (*Fig. 2*) correlations along with the HMBCs (*Fig. 2*). Furthermore, the HMBC spectra showed correlations of H–C(13) with C(7) and C(8), and correlations from H–C(6) to C(5), C(7) and C(8). In combination with the COSY H–C(5) ↔ H–C(6) ↔ H–C(7) ↔ H–C(8), thus led to the establishment of fragment **4b**. Meanwhile, the HMBCs from H–C(15) to C(16), and from H–C(16) to C(15), in combination with the COSY correlations H–C(15) ↔ H–C(16), led to the establishment of fragment **4c**. The entire skeleton of **4** was constructed on the basis of the above HMBC spectrum. The HMBCs of H–C(13) with C(8a), of H–C(8) with C(4a), C(8a), and C(9), of H–C(9) with C(8), and H–C(4) with C(5), indicated that fragments **4a** and **4b** were linked at C(4a) and C(8a). In addition, the HMBC cross peaks from H–C(3a) to C(15), from H–C(16) to C(4), C(8a), and C(9), as well as from H–C(15) to C(4a) and C(8a), suggested that fragments **4a** and **4c** were linked at C(4) and C(8a). Thus, the constitutional formula of **4** was determined. Considering all the spectral evidence, another structure, **4d**, was possible for **4** (*Fig. 2*). However, the HMBC spectrum showed a significant ^3J-correlation between H–C(3a) (δ(H) 1.55) and one quaternary C-atom C(4a) (δ(C) 50.2); thus, structure **4d** could be ruled out. The orientations of H–C(14), H–C(3a), and H–C(12) were determined as β according to the NOESY correlations H–C(14) ↔ H_β–C(1), H–C(14) ↔ H_β–C(9), H–C(14) ↔ H_β–C(9), H–C(14) ↔ H–C(3a), and H–C(12) ↔ H_β–C(9) (*Fig. 3*). The other key NOESY correlations, H–C(6) ↔ H–C(8), H–C(13) ↔ H_α–C(9), H_α–C(1) ↔ H_α–C(2), and H–C(3a) ↔ H–C(4), were also observed. Based on the above evidence, the structure of **4**, named cubentriol, was established.

Akoenic acid (**5**) was obtained as optically active, pale-yellowish oil. The molecular formula of **5** was established as $C_{15}H_{26}O_4$, with three degrees of unsaturation, by HR-ESI-MS. The IR spectrum showed absorptions at 3464 and 1723 cm^{-1} for a OH and C=O groups, respectively. The ^{1}H- and ^{13}C-NMR data of **5** showed signals of two terminal Me groups (δ(H) 0.89 (*t*, $J = 7.2$, Me(9) and Me(4′)); δ(C) 13.9 (C(9)), 13.6 (C(4′))), one tertiary Me group (δ(H) 1.33 (*d*, $J = 7.2$, Me(10)); δ(C) 15.7 (C(10))), one O-bearing CH_2 group (δ(H) 4.01–4.12 (*m*, H–C(1′)); δ(C) 64.7 (C(1′))), one CH group (δ(H) 3.58 (*q*, $J = 7.2$, H–C(2)); δ(C) 37.5 (C(2))), twelve aliphatic CH_2 groups (δ(H) 1.29–1.33 (*m*, CH_2(7)), 1.30–1.34 (*m*, CH_2(8)), 1.30–1.36 (*m*, CH_2(3′)), 1.42–1.54 (*m*, CH_2(6)), 1.49–1.63 (*m*, CH_2(2′)), 2.13–2.29 (*m*, CH_2(5)); δ(C) 19.1 (C(3′)), 22.4 (C(8)), 28.2 (C(6)), 28.7 (C(5)), 30.5 (C(2′)), 31.5 (C(7))), and one olefinic H-atom (δ(H) 6.96 (*t*, $J = 7.6$, H–C(4)); δ(C) 146.5 (C(4))). The ^{13}C-NMR spectrum exhibited, in addition to the signals corresponding to the above-mentioned H-atoms, signals of three quaternary C-atoms (δ(C) 131.5 (C(3)), 171.0 (C(1″)), and 173.7

(C(1))), completing the remaining structure of **5**. Three partial structures, Me–C(2)/C(4)–C(5)–C(6)–C(7)–C(8)–C(9)/C(1′)–C(2′)–C(3′)–C(4′), were established by analysis of its 2D-NMR spectra, especially the COSY correlations (*Fig. 2*). The HMBCs (*Fig. 2*) verified the junctions of C(1) to C(9), of C(1″) to C(3), and of C(1′) to C(4′). Compound **5** showed levorotatory optical activity, $[\alpha]_D^{23} = -24.7$ ($c = 0.07$, $CHCl_3$). By comparinson with (*R*,*E*)-3-(methoxycarbonyl)-2-methylpent-3-enoic acid ($[\alpha]_D^{25} = -107$, $CHCl_3$) [10], the absolute configuration at C(2) in **5** could be tentatively proposed as (*R*). The (4*E*)-configuration was supported by the absence of a NOESY correlation between H–C(4) and H–C(2). Based on spectral evidence, the structure of **5**, named akoenic acid, was elucidated as (2*E*)-2-[(2*R*)-1-butoxy-1-oxopropan-2-yl]oct-2-enoic acid, which was confirmed by HMBC (*Fig. 2*), COSY (*Fig. 2*), and NOESY (*Fig. 3*) experiments.

Akodionine (**6**) was obtained as optically active yellowish needles with $[\alpha]_D^{24} = +11.1$ ($c = 0.16$, MeOH). The molecular formula was determined as $C_{10}H_{16}N_2O_2$, indicating four degrees of unsaturation, on the basis of the $[M + Na]^+$ peak at *m*/*z* 219.1109 (calc. 219.1111 for $C_{10}H_{16}N_2NaO_2^+$) in the HR-ESI-MS. The bands at 3213, 1674, and 1646 cm^{-1} in the IR spectrum revealed the presence of an NH and N–C=O groups, respectively. The ^{1}H-NMR spectrum of **6** showed signals of one ^{i}Pr group (δ(H) 0.91, 1.07 (2*d*, $J = 7.2$, Me(11), Me(12)), 2.63 (*dsept.*, $J = 7.2, 2.4$, H–C(10)), a pair of vicinally coupled ($^3J = 1.8$) aliphatic H-atoms (δ(H) 3.92–3.94 (*m*, H–C(1)), 4.08 (*td*, $J = 6.0$, 1.8, H–C(9))), three CH_2 groups (δ(H) 1.86–1.94, 2.01–2.07 (2*m*, each 1 H, CH_2(7)), 2.01–2.07, 2.35–2.40 (2*m*, 1 H, CH_2(8)), 3.58 (*ddd*, $J = 12.0$, 9.6, 3.0, H_b–C(6)), 3.63 (*ddd*, $J = 12.0$, 7.2, 4.2, H_a–C(6))), as well as one NH group (δ(H) 6.09 (br. *s*, NH; D_2O exchangeable)). The C-atoms of **6** were assigned through ^{13}C-NMR, DEPT, and HSQC experiments as two Me groups (δ(C) 16.0 (C(11)) and 19.2 (C(12))), three CH_2 groups (δ(C) 22.3 (C(7)), 28.5 (C(8)), and 45.1 (C(6))), three CH groups (δ(C) 28.3 (C(10)), 58.8 (C(9)), and 60.4 (C(1))), and two quaternary C-atoms (δ(C) 164.9 (C(3)) and 170.1 (C(5))).

The constitutional formula of **6** was proposed by extensive 2D-NMR experiments, including HSQC, COSY (*Fig.* 2), and HMBC techniques (*Fig. 2*). The HMBC (*Fig. 2*) data allowed us to establish the entire connectivity within the molecule. Correlations between H–C(1) and C(3); NH(2) and C(3); CH_2(7) and C(5), C(6), C(8), and C(9); CH_2(8) and C(6) and C(9); Me(11) and C(1), C(10), and C(12); Me(12) and C(1) and C(10) established the constitutional formula of **6** as 1-isopropylhexahydroimidazo[1,5-*a*]pyridine-3,5-dione. The assignments were verified by significant correlations of both H–C(1) and H_b–C(8) to H–C(9), of Me(11) to H_a–C(8), H_b–C(6) to H_b–C(8), and of H_a–C(6) to H_a–C(7) in the NOESY experiments (*Fig. 2*), and further supported the relative configuration of **6** as (1*S**,9*R**). Based on further spectral evidence, the structure of **6** was elucidated as (1*R**,8a*S**)-hexahydro-1-(propan-2-yl)imidazo[1,5-*a*]pyridine-3,5-dione, designated as akodionine.

Compound **7** was isolated as optically active, colorless needles. $[\alpha]_D^{23} = +126.1$ ($c = 0.05$, $CHCl_3$). The HR-ESI-MS data provided the molecular formula $C_{12}H_{12}O_6$ (*m*/*z* 275.0532 ($[M - H_2O + Na]^+$; calc. 275.0529)). The ^{13}C- and ^{1}H-NMR data of **7** were very similar to those of (−)-(*R*)-5-(methoxycarbonyl)mellein (**8**) [11], except for the presence of a OH group (δ(H) 3.91 (br. *d*, $J = 3.6$, HO–C(4); D_2O exchangeable)) and one CH group (δ(H) 5.10 (*dd*, $J = 3.6$, 1.5, H–C(4)); δ(C) 65.1) in **7**, rather than a CH_2

unit (δ(H) 3.05 (*dd*, J = 17.9, 12.1, H_b–C(4)), 3.88 (*dd*, J = 17.9, 3.3, H_a–C(4)); δ(C) 32.6) in **8**. The relative and absolute configurations of compound **7** was deduced from comparison with similar isocoumarins, **8**–**11**, as (3*R*,4*S*). The assumed relative configuration was verified by pertinent NOESY experiments (see *Fig. 3*).

In addition to the seven new compounds, **1**–**7**, the following seven known compounds were also isolated: (−)-(*R*)-5-(methoxycarbonyl)mellein (**8**) [11], (−)-7-(*R*)-hydroxymellein (**9**) [12], (*R*)-5-methylmellein (**10**) [13], (*R*)-8-methoxymellein (**11**) [14], (+)-(*S*)-piliformic acid (**12**) [15], methyl 1*H*-indole-3-carboxylate (**13**) [15], and 4-(2-hydroxyethyl)phenol (**14**) [16].

The isolates present in sufficient amounts, *i.e.*, **5**, **8**, and **9**, were screened for their abilities to inhibit NO and IL-6 production in LPS-activated RAW 264.7 cells. Among these isolates, only (−)-(*R*)-7-hydroxymellein (**9**) showed IL-6 inhibitory activity with an IC_{50} value of 9.4 μM.

By a comprehensive investigation, 14 pure metabolites with diverse skeletons were obtained from a small amount of AcOEt fraction. Interestingly, the new 10-hydroxythujopsene is the first sesquiterpene with a thujopsane-type skeleton compared to other guaiane- or eremophilone-type sesquiterpenes previously isolated from *Xylaria*. The sesquiterpene and isocoumarins are the major metabolites in this fungus. Cubentriol (**4**) represents a novel structure containing a unique, unprecedented skeleton. This type of compound could stimulate further phytochemical studies.

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Experimental Part

General. TLC: Silica gel *60* F_{254}-precoated plates (*Merck*). Column chromatography (CC): silica gel *60* (70–230 or 230–400 mesh; *Merck*) and *Spherical C18 100A Reversed Phase Silica Gel* (*RP-18*; particle size: 20–40 μm; *Silicycle*). Optical rotations: *Jasco DIP-370* polarimeter; in $CHCl_3$. UV Spectra: *Jasco UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer-2000* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C- and 2D-NMR spectra: *Varian-VNMRS-600* and *Varian-Mercury-400* spectrometers; δ in ppm rel. to Me_4Si; J in Hz. EI-MS: *VG-Biotech Quatro-5022* mass spectrometer; *m*/*z* (rel. %). HR-EI-MS: *Finnigan/Thermo Quest NAT* mass spectrometer; ESI- and HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in *m*/*z*.

Microorganisms, Cultivation, and Preparation of the Fungal Strain. The fungus used in this study was isolated from the leaves of *L. akoensis* and identified as *X. cubensis* on the basis of the rDNA internal transcribed spacer (ITS) gene sequence. Seven-day-old colonies of the *X. cubensis* strain on PDA medium in a 9-cm *Petri* dish were cut into the bottle and blended for 30 s with 100 ml of dist. H_2O to prepare the fungal inoculum for liquid fermentation. Each 500-ml flask contained 150 ml of liquid cultural media (ingredients: corn starch (30 g), corn steep liquor (10 g), yeast extract (5 g), and sea salt (2 g in 1 l dist. H_2O; pH 6)) were loaded with 10 ml of fungal inocula and incubated at 25° for two weeks on a rotary shaker at the speed of 150 circles/min. A total of 10 l of fungal fermented broth were harvested and then filtrated to remove fungal mycelium.

Isolation and Characterization of Metabolites. The broth of *X. cubensis* (10 l) was partitioned with BuOH to produce a BuOH-soluble fraction (20 g). The BuOH-soluble fraction was then partitioned with AcOEt/H_2O 1:1 to produce an AcOEt-soluble fraction (3.8 g), and a H_2O-soluble fraction (14.6 g). The AcOEt-soluble fraction (3.8 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 100:1, gradually increasing the polarity with MeOH) to furnish 16 fractions, *Frs. 1–16*. *Fr. 1* (56.8 mg) was submitted to MPLC (hexane/AcOEt 60:1) to afford five fractions, *Frs. 1-1–1-5*, of which *Fr. 1-1* was composed entirely of **8**

(R_f 0.50; 6.8 mg). *Fr. 1-3* (6.1 mg) was subjected to prep. TLC (*RP-18*; MeOH/H_2O 3:1) to afford **10** (R_f 0.43; 3.1 mg). *Fr. 3* (19.6 mg) was purified by MPLC (hexane/AcOEt 10:1) to yield **1** (R_f 0.73; 1.8 mg). *Fr. 4* (225 mg) was separated by MPLC (hexane/AcOEt 7:1) to give 87 fractions, *Frs. 4-1 – 4-87. Fr. 4-32* (20.9 mg) was purified by prep. TLC (CH_2Cl_2/acetone 30:1) to furnish six fractions, *Frs. 4-32-1 – 4-32-6. Fr. 4-32-1* (1.4 mg) was separated by prep. TLC (*RP-18*; MeOH/H_2O 3:1) to afford **13** (R_f 0.45; 0.7 mg). *Fr. 4-37* (16.9 mg) was purified by prep. TLC (hexane/acetone 3:1) to afford **5** (R_f 0.8; 3.7 mg) and **9** (R_f 0.37; 2.5 mg). *Fr. 4-52* (27.9 mg) was separated by MPLC (CH_2Cl_2/MeOH 100:1) to furnish 50 fractions, *Frs. 4-52-1 – 4-52-50. Fr. 4-52-5* (5.3 mg) was purified by prep. TLC (CH_2Cl_2/MeOH 60:1) to yield **7** (R_f 0.45; 2.4 mg). *Fr. 4-59* (28.6 mg) was subjected to MPLC (CH_2Cl_2/MeOH 100:1) to afford **11** (R_f 0.59; 11.6 mg). *Fr. 5* (491 mg) was subjected to MPLC (*RP-18*; acetone/H_2O 1:2) to afford **6** (R_f 0.58; 8.8 mg). *Fr. 6* (307 mg) was purified by MPLC (CH_2Cl_2/acetone 8:1) to yield nine fractions, *Frs. 6-1 – 6-9. Fr. 6 – 5* (68.0 mg) was subjected to MPLC (hexane/acetone 3:1) to afford eight fractions, *Frs. 6-5-1 – 6-5-8. Fr. 6-5-5* (36.2 mg) was subjected to prep. TLC (*RP-18*; acetone/H_2O 1:1) to afford **14** (R_f 0.60; 21.1 mg). *Fr. 8* (148 mg) was subjected to MPLC (*RP-18*; MeOH/H_2O 1:1) to give **12** (R_f 0.64; 36.1 mg). *Fr. 9* (675 mg) was subjected to MPLC (CH_2Cl_2/acetone 5:1) to furnish ten fractions, *Frs. 9-1 – 9-10. Fr. 9-6* (109 mg) was purified by MPLC (CH_2Cl_2/MeOH 30:1) to furnish 15 fractions, *Frs. 9-6-1 – 9-6-15. Fr. 9-6-3* (24.8 mg) was purified by prep. TLC (CH_2Cl_2/MeOH (15:1) to afford **4** (1.5 mg). *Fr. 9-6-5* (23.3 mg) was separated by MPLC (*RP-18*; MeOH/H_2O 1:1.5) to give nine fractions, *Frs. 9-6-5-1 – 9-6-5-9. Fr. 9-6-5-6* (23.3 mg) was purified by prep. TLC (hexane/acetone 2:1) to afford **3** (R_f 0.27; 2.5 mg). *Fr. 9-6-7* (27.8 mg) was subjected to MPLC (*RP-18*; MeOH/H_2O 1:1) to yield **2** (R_f 0.52; 2.2 mg).

10-Hydroxythujopsene (=*(1a*R**,4a*R**,7*S**,8a*S**)-1,1a,4,4a,5,6,7,8-Octahydro-2,4a,8,8-tetramethylcyclopropa[d]naphthalen-7-ol*; **1**). Pale-yellowish oil. $[\alpha]_D^{25} = -7.9$ ($c = 0.03$, $CHCl_3$). IR (neat): 3424 (OH). ^{1}H-NMR (600 MHz, $CDCl_3$): 5.01–5.03 (*m*, H–C(5)); 3.34 (br. *d*, *J* = 10.2, H–C(10)); 1.78–1.82 (*m*, H_a–C(9)); 1.80 (*s*, Me(12)); 1.75–1.79 (*m*, H_a–C(6)); 1.69–1.73 (*m*, H_b–C(9)); 1.54 (*td*, *J* = 13.5, 4.2, H_a–C(8)); 1.44 (*dd*, *J* = 16.5, 6.9, H_b–C(6)); 1.30 (br. *s*, HO–C(10); D_2O exchangeable), 1.24 (br. *dd*, *J* = 9.3, 5.0, H–C(3)); 1.14 (*dt*, *J* = 13.5, 3.6, H_b–C(8)); 1.11 (*s*, Me(13)); 1.08 (*s*, Me(15)); 0.74 (*s*, Me(14)); 0.72 (*t*, *J* = 5.0, H_a–C(2a)); 0.70 (*dd*, *J* = 9.3, 5.0, H_b–C(2)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 135.3 (C(4)); 114.4 (C(5)); 78.3 (C(10)); 40.7 (C(6)); 39.3 (C(11)); 34.7 (C(1)); 34.3 (C(8)); 31.2 (C(7)); 28.5 (C(9)); 28.4 (C(13)); 24.1 (C(14)); 23.4 (C(12)); 21.9 (C(3)); 20.7 (C(15)); 10.6 (C(2)). ESI-MS: 221 ($[M+H]^+$). HR-ESI-MS: 243.1725 ($[M+Na]^+$, $C_{15}H_{24}NaO^+$; calc. 243.1723).

Akotriol (=*(1*R**,3a*R**,4*S**,8a*R**)-Decahydro-1,5-bis(hydroxymethyl)-3,3-dimethyl-1,4-methanoazulen-5-ol*; **2**). Amorphous powder. $[\alpha]_D^{21} = -20.1$ ($c = 0.04$, $CHCl_3$). IR (KBr): 3384 (OH). ^{1}H-NMR ($CDCl_3$, 400 MHz): 3.82 (*d*, *J* = 11.2, H_a–C(14)); 3.64 (*d*, *J* = 11.2, H_b–C(14)); 3.44 (*d*, *J* = 10.6, H_a–C(15)); 3.34 (*d*, *J* = 10.6, H_b–C(15)); 2.13 (*ddd*, *J* = 13.6, 4.8, 2.0, H_a–C(3)); 2.06 (*dd*, *J* = 12.4, 4.4, H_a–C(11), 1.92–1.96 (*m*, H_a–C(5)); 1.87–1.93 (*m*, H–C(6)); 1.83 (*d*, *J* = 13.6, H_a–C(9)); 1.70–1.75 (*m*, H_a–C(4)); 1.67–1.71 (*m*, H_b–C(5)); 1.50 (*d*, *J* = 13.6, H_b–C(9)); 1.38–1.46 (*m*, H–C(7)); 1.38–1.46 (*m*, H_b–C(11)); 1.38–1.46 (*m*, H_b–C(3)); 1.29–1.33 (*m*, H–C(1)); 1.19–1.26 (*m*, H_b–C(4)); 1.01 (*s*, Me(13)); 0.89 (*s*, Me(12)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 77.5 (C(2)); 70.0 (C(15)); 64.8 (C(14)); 59.9 (C(7)); 59.1 (C(6)); 58.0 (C(1)); 52.8 (C(9)); 48.5 (C(10)); 46.5 (C(8)); 39.6 (C(11)); 39.5 (C(3)); 30.3 (C(5)); 29.0 (C(13)); 26.7 (C(4)); 22.1 (C(12)). ESI-MS: 277 ($[M+Na]^+$). HR-ESI-MS: 277.1780 ($[M+Na]^+$, $C_{15}H_{26}NaO_3^+$; calc. 277.1778).

Xylaritriol (=*(3*R**)-5-[(2*R**,3*R**,6*R**)-6-Ethenyltetrahydro-3-hydroxy-2,6-dimethyl-2*H*-pyran-2-yl]-2-methylpentane-2,3-diol*; **3**). Colorless oil. $[\alpha]_D^{22} = +28.8$ ($c = 0.05$, $CHCl_3$). IR (neat): 3313 (OH). ^{1}H-NMR ($CDCl_3$, 600 MHz): 5.91 (*dd*, *J* = 17.4, 10.8, H–C(1″)); 5.23 (*dd*, *J* = 17.4, 1.2, H_a–C(2″)); 5.05 (*dd*, *J* = 10.8, 1.2, H_b–C(2″)); 3.82 (*t*, *J* = 7.2, H–C(3)); 3.59 (*dd*, *J* = 10.8, 2.4, H–C(3′)); 2.09–2.16 (*m*, H_a–C(1)); 1.89–1.95 (*m*, H–C(2)); 1.79–1.83 (*m*, H_a–C(5′)); 1.64–1.70 (*m*, H_b–C(5′)); 1.56–1.62 (*m*, H_a–C(4′)); 1.49–1.54 (*m*, H_b–C(1)); 1.32–1.38 (*m*, H_b–C(4′)); 1.29 (*s*, Me(8)); 1.27 (*s*, Me(6)); 1.16 (*s*, H–C(7)); 1.13 (*s*, Me(5)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 145.2 (C(1″)); 111.6 (C(2″)); 86.5 (C(2′)); 84.4 (C(3)); 77.3 (C(3′)); 73.0 (C(6′)); 72.1 (C(4)); 38.9 (C(5′)); 31.3 (C(1)); 28.1 (C(8)); 27.6 (C(6)); 26.9 (C(2)); 26.4 (C(4′)); 25.5 (C(5)); 23.8 (C(7)). ESI-MS: 295 ($[M+Na]^+$). HR-ESI-MS: 295.1886 ($[M+Na]^+$, $C_{15}H_{28}NaO_4^+$; calc. 295.1885).

Cubentriol (=*(2*S*,3a*S*,4*R*,4a*R*,5*S*,7*S*,8*S*,8a*S*,9a*S*)-Dodecahydro-3,3,4a,8,9a-pentamethyl-4,8a-ethanocyclopenta[*b*]naphthalene-2,5,7-triol*; **4**). Whitish powder. $[\alpha]_D^{23} = \pm 0$ ($c = 0.048$, $CHCl_3$). IR (KBr): 3390

(OH). ^{1}H-NMR ((D$_6$)acetone, 600 MHz): 4.08 (*ddd*, *J* = 13.5, 8.7, 6.4, H–C(2)); 3.72 (br. *q*, *J* = 3.6, H–C(5)); 3.42 – 3.52 (*m*, H–C(7)); 3.43 (*d*, *J* = 8.7, HO–C(2)); 3.42 (br. *d*, *J* = 3.6, HO–C(5)); 3.24 (*d*, *J* = 6.6, HO–C(7)); 2.39 (*td*, *J* = 9.6, 5.4, H–C(4)); 2.06 – 2.12 (*m*, H$_a$–C(16)); 1.99 (*dd*, *J* = 13.5, 6.4, H$_a$–C(1)); 1.95 (*qdd*, *J* = 9.6, 8.4, 1.2, H$_a$–C(15)); 1.93 (*d*, *J* = 15.0, H$_a$–C(9)); 1.88 (*dt*, *J* = 13.4, 3.6, H$_a$–C(6)); 1.76 (*d*, *J* = 15.0, H$_b$–C(9)); 1.72 (*ddd*, *J* = 13.4, 10.8, 3.6, H$_b$–C(6)); 1.62 (*dd*, *J* = 13.5, 6.4, H$_b$–C(1)); 1.55 (*d*, *J* = 9.6, H–C(3a)); 1.46 – 1.54 (*m*, H–C(8)); 1.45 – 1.53 (*m*, H$_b$–C(15)); 1.36 (*ddd*, *J* = 12.0, 8.4, 1.2, H$_b$–C(16)); 1.30 (*s*, Me(14)); 1.16 (*s*, Me(12)); 1.07 (*d*, *J* = 6.6, Me(13)); 0.99 (*s*, Me(11)); 0.94 (*s*, Me(10)). ^{13}C-NMR ((D$_6$)acetone, 150 MHz): 80.0 (C(2)); 75.0 (C(5)); 74.2 (C(3a)); 70.2 (C(7)); 62.3 (C(8a)); 57.7 (C(4)); 52.3 (C(1)); 50.2 (C(4a)); 50.0 (C(9a)); 46.3 (C(3)); 46.1 (C(9)); 45.6 (C(8)); 41.1 (C(6)); 39.9 (C(16)); 33.5 (C(14)); 32.1 (C(15)); 25.2 (C(11)); 24.9 (C(12)); 24.6 (C(10)); 13.8 (C(13)). ESI-MS: 345 ([*M* + Na]$^+$). HR-ESI-MS: 345.2406 ([*M* + Na]$^+$, $C_{20}H_{34}NaO_3^+$; calc. 345.2405).

Akoenic Acid (= *(2*E*)-2-[(2*R*)-1-Butoxy-1-oxopropan-2-yl]oct-2-enoic Acid*; **5**): Yellowish oil. $[\alpha]_D^{23} = -24.7$ (*c* = 0.07, $CHCl_3$). UV (MeOH): 212. IR (neat): 3464 (OH), 1723 (C=O), 1635 (C=C). ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.96 (*t*, *J* = 7.6, H–C(4)); 4.01 – 4.12 (*m*, CH_2(1′)); 3.58 (*q*, *J* = 7.2, H–C(2)); 2.13 – 2.29 (*m*, CH_2(5)); 1.49 – 1.63 (*m*, CH_2(2′)); 1.42 – 1.54 (*m*, CH_2(6)); 1.33 (*d*, *J* = 7.2, Me(10)); 1.30 – 1.36 (*m*, CH_2(3′)); 1.30 – 1.34 (*m*, CH_2(8)); 1.29 – 1.33 (*m*, CH_2(7)); 0.89 (*t*, *J* = 7.2, Me(9), Me(4′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.7 (C(1)); 171.0 (C(1″)); 146.5 (C(4)); 131.5 (C(3)); 64.7 (C(1′)); 37.5 (C(2)); 31.5 (C(7)); 30.5 (C(2′)); 28.7 (C(5)); 28.2 (C(6)); 22.4 (C(8)); 19.1 (C(3′)); 15.7 (C(10)); 13.9 (C(9)); 13.6 (C(4′)). ESI-MS: 293 ([*M* + Na]$^+$). HR-ESI-MS: 293.1729 ([*M* + Na]$^+$, $C_{15}H_{26}NaO_4^+$; calc. 293.1727).

Akodionine (= *(1*R**,8a*S**)-Hexahydro-1-(propan-2-yl)imidazo[1,5-*a*]pyridine-3,5-dione*; **6**). Yellowish needles (acetone/CH_2Cl_2). M.p. 172 – 174°. $[\alpha]_D^{24} = +11.1$ (*c* = 0.16, MeOH). IR (KBr): 3213 (NH), 1674, 1646 (N–CO). ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.09 (br. *s*, NH(2); D_2O exchangeable); 4.08 (*td*, *J* = 6.0, 1.8, H–C(9)); 3.92 – 3.94 (*m*, H–C(1)); 3.63 (*ddd*, *J* = 12.0, 7.2, 4.2, H$_a$–C(6)); 3.58 (*ddd*, *J* = 12.0, 9.6, 3.0, H$_b$–C(6)); 2.63 (*dsept.*, *J* = 7.2, 2.4, H–C(10)); 2.35 – 2.40 (*m*, H$_a$–C(8)); 2.01 – 2.07 (*m*, H$_b$–C(8)); 2.01 – 2.07 (*m*, H$_a$–C(7)); 1.86 – 1.94 (*m*, H$_b$–C(7)); 1.07 (*d*, *J* = 7.2, Me(12)); 0.91 (*d*, *J* = 7.2, Me(11)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 170.1 (C(5)); 164.9 (C(3)); 60.4 (C(1)); 58.8 (C(9)); 45.1 (C(6)); 28.5 (C(8)); 28.3 (C(10)); 22.3 (C(7)); 19.2 (C(12)); 16.0 (C(11)). ESI-MS: 219 ([*M* + Na]$^+$). HR-ESI-MS: 219.1109 ([*M* + Na]$^+$, $C_{10}H_{16}NaN_2O_2^+$; calc. 219.1111).

Akolitserin (= *(+)-(3*R*,4*S*)-5-Carbomethoxy-3-hydroxymellein* = *Methyl (3*R*,4*S*)-3,4-Dihydro-4,8-dihydroxy-3-methyl-1-oxo-1*H*-isochromene-5-carboxylate*; **7**). Colorless needles (CH_2Cl_2/MeOH). M.p. 144 – 146°. $[\alpha]_D^{23} = +126.1$ (*c* = 0.05, $CHCl_3$). UV (MeOH): 225, 257, 309. UV (MeOH + KOH): 232, 292, 337. IR (KBr): 3410 (OH), 1743 (C=O). ^{1}H-NMR ($CDCl_3$, 600 MHz): 11.98 (*s*, HO–C(8); D_2O exchangeable); 8.13 (*d*, *J* = 8.7, H–C(6)); 7.06 (*d*, *J* = 8.7, H–C(7)); 5.10 (*dd*, *J* = 3.6, 1.5, H–C(4)); 5.05 (*qd*, *J* = 6.9, 1.5, H–C(3)); 3.95 (*s*, Me(11)); 3.91 (br. *d*, *J* = 3.6, HO–C(4); D_2O exchangeable); 1.35 (*d*, *J* = 6.9, Me(9)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 167.9 (C(1)); 167.0 (C(10)); 165.4 (C(8)); 142.1 (C(4a)); 138.5 (C(6)); 120.0 (C(5)); 118.1 (C(7)); 107.6 (C(8a)); 79.5 (C(3)); 65.1 (C(4)); 52.7 (C(11)); 18.8 (C(9)). ESI-MS: 253 ([*M* + H]$^+$). HR-ESI-MS: 275.0532 ([*M* – H_2O + Na]$^+$, $C_{12}H_{12}NaO_6^+$; calc. 275.0529).

Determination of NO and IL-6 Production and Cell Viability Assay. Mouse macrophage cell line (RAW 264.7) was obtained from *Bioresource Collection and Research Center* (*BCRC 60001*) and cultured at 37° in *Dulbecco*'s Modified *Eagle*'s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; *Gibco*), 4.5 g/l glucose, 4 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) in a humidified atmosphere in a 5% CO_2 incubator. The cells were treated with 10, 25, 50 μM natural products in the presence of 1 μg/ml LPS (lipopolysaccharide, *Sigma–Aldrich*) for 20 h. The concentration of NO in culture supernatants was determined as nitrite, a major stable product of NO, by *Griess* reagent assay [17], and cell viabilities were determined using the MTT assay as described in [18]. Levels of IL-6 production were measured in cell-culture supernatants with a *Mouse IL-6 ELISA Ready-SET-Go* kit (*eBiosciencee*) according to the manufacturer's recommendations and quantified with a microplate reader (μ-*Quant*, *Bio-Tek Instruments Inc.*).

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