Diastereomeric Diterpenes from *Coleus blumei*

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The chloroform extract of the air-dried leaves of *Coleus blumei* afforded a mixture of diastereomers of a new abietane type diterpene whose structures were elucidated by extensive one and two dimensional (1D, 2D) NMR and mass spectrometry. Acetylation of the mixture afforded a single compound. Antimicrobial tests on the diterpene indicate that it is active against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*.

Key words *Coleus blumei*; Labiatae; abietane diterpene; antimicrobial

Coleus blumei is an ornamental medicinal plant found throughout the Philippines. The pounded leaves of the plant are used as cure for headaches, and for the healing of bruises. A decoction of the plant is taken internally for dyspepsia, and dropped into the eyes for opthalmia.[1)] Previous studies on *C. blumei* reported the isolation of flavonoids[2)] and coleon O,[3)] an abietane type diterpenoid, from the plant. Similar abietane type diterpenes have been commonly isolated from several other *Coleus* species, and have been shown to have biological activity. Coleon O from *C. blumei* and *C. scutellarioides* was found to stimulate the rooting of mung bean cuttings by more than 400%.[3)] Barbatusol from *C. barbatus* induced at 3 mg/kg (i.v. rats) potent lowering of blood pressure.[4)] Forskolin from *C. forskohlii* is a potent inotropic, antihypertensive, platelet aggregation inhibitor and adenylate cyclease stimulant.[5)]

We now report the isolation, structural elucidation and antimicrobial test results of a new diterpene (**1**) from *C. blumei*. Analysis of the NMR data from **1** and its tetraacetate (**2**) indicate **1** is an approximately 1 : 1 mixture of diastereomers which differ only by their stereochemistry at C-15.

Results and Discussion

The ^{1}H-NMR (Table 1) and COSY spectral data of **1** indicated resonances for chelated hydroxyl doublet at δ 13.081 (br s) and 13.077 (br s)[6)] and four other hydroxyl groups at δ 6.94 (1H), 5.05 (1H, br) and two closely spaced doublets at δ 11.646 (br s) and 11.667 (br s) and δ 6.081 (s) and 6.061 (s). Methylene protons at δ 2.35 (H-3′, dd, J=15.9, 6.6 Hz) and δ 1.70 (H-3, dd, J=15.9, 2.7 Hz) were coupled to the methine proton at δ 5.41 (H-2, dq), which was in turn coupled to the methylene hydrogens at δ 3.86 (H-1′, m) and δ 1.45 (H-1, m). The methylene proton at δ 3.95 (H-16, m) was geminally coupled to the hydrogen at δ 4.79 (H-16′, m), which was in turn coupled to the proton at δ 3.90 (H-15, m). The latter proton was coupled to the methylene proton at δ 3.99 (H-17′, m) which was geminally coupled to the hydrogen at δ 3.92 (H-17, m). Three methyl groups were indicated by the resonances at δ 1.47 (3H, s), 1.53 (3H, s) and the closely spaced methyl doublet at δ 1.632 (s) and 1.622 (s). The relatively deshielded methyl resonances indicated that they are neighbors to oxygenated groups and olefins. The resonances at δ 2.01 (3H, s) and the closely spaced doublet at δ 2.184 (s) and 2.179 (s) were attributed to two acetate methyls. The signals at δ 1.632, 1.622, 2.184, 2.179, 6.081, 6.061, 11.667, 11.646, 13.077 and 13.081 appeared "doublets" in the 300 MHz spectrum, suggesting the possibility of a mixture of diastereomers. However in no case did the chemical shift difference in the "doublets" exceed 0.02 ppm.

The ^{13}C- and DEPT NMR spectral data of **1** (Table 1) indicated 24 carbon resonances with the following functionalities: the carbonyl of a conjugated ketone at δ 182.7; the carbonyls of two acetates at δ 170.7 and 173.8; five methyl groups at δ 28.5, 29.5, 21.0, 21.4 and closely spaced doublet at δ 24.69 and 24.75; two methylenes at δ 42.4 and the closely spaced doublet at δ 34.02 and 34.07 and two oxygenated methylenes at δ 61.3 and closely spaced doublet at δ 61.69 and 61.62; one methine which appeared as closely spaced doublet at δ 36.62 and 36.57 and one oxygenated methine (δ 68.2); two aliphatic quaternary carbons (δ 40.4, 35.9); and eight olefinic quaternary carbons (δ 105—152). The deshielded resonances (δ 141—152) were assigned to oxygenated carbons, while the shielded resonances (δ 105—133.5) were attributed to non-oxygenated carbons, except that the deshielded resonance at δ 142.1 was assigned to C-5. The deshielding was attributed to the steric effect on C-5 of neighboring groups. This assignment was confirmed by gHMBC. As with the proton NMR, a few of the carbon signals (those at δ 24.69, 24.75, 34.07, 34.02, 36.57, 36.62, 61.62, 61.69) appeared as "doublets" in the 75 MHz ^{13}C spectra, but with less than 0.07 ppm difference between the signals.

The high resolution mass spectrum (HR-MS) of **1** indicated a molecular ion at *m/z* 478.1850 corresponding to a molecular formula of $C_{24}H_{30}O_{10}$. Based on the molecular formula, the index of hydrogen deficiency was ten. With four double bonds and three carbonyl groups in **1**, the rest of the hydrogen deficiency was accounted for by a tricyclic system.

The ^{1}H and ^{13}C assignments for **1** (Table 1) were verified by gHMQC and connectivity was verified by an inverse long-range heteronuclear experiment gHMBC optimized for J=10 Hz (Table 1). The carbon at δ 142.1 (C-5) was long-

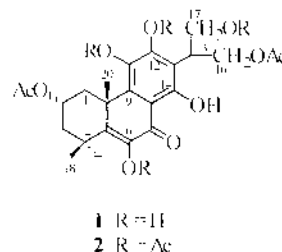

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Table 1. ^{1}H-, ^{13}C-NMR and HMBC Spectral Data of **1** in $CDCl_3$

Carbon No.	^{13}C, δ	^{1}H, δ	HMBC
C-1	34.07[a), 34.02[a)]	1.45 (1H, m, H-1), 3.86 (1H, m, H-1′)	H-3, H-20
C-2	68.2	5.41 (1H, dq, J=6.5, 2.3, H-2)	H-1′
C-3	42.4	1.70 (1H, dd, J=15.9, 2.7 Hz, H-3), 2.35 (1H, dd, J=15.9, 6.6 Hz, H-3′)	H-1
C-4	35.9	—	H-2, H-3, H-3′, H-18, H-19
C-5	142.1	—	H-1, H-18, H-19, H-20
C-6	141.0	—	6-OH
C-7	182.7	—	6-OH
C-8	105.4	—	14-OH
C-9	133.5	—	H-20, 11-OH
C-10	40.4	—	H-1, H-1′, H-20
C-11	135.8	—	11-OH
C-12	150.5	—	11-OH
C-13	109.8	—	14-OH
C-14	152.3	—	14-OH
C-15	36.57[a)], 36.62[a)]	3.90 (1H, m)	—
C-16[c)]	61.3	3.95 (1H, m, H-16), 4.79 (1H, m, H-16′)	
C-17[c)]	61.62[a)], 61.69[a)]	3.92 (1H, m, H-17), 3.99 (1H, m, H-17′)	H-16′
C-18	28.5	1.47 (3H, s, H-18)	H-3, H-19
C-19	29.5	1.53 (3H, s, H-19)	H-3, H-18
C-20	24.69[a)], 24.75[a)]	1.632[b)] (3H, s, H-20), 1.622[b)] (3H, s, H-20)	
OAc (C-2)	170.7	—	2-$CO_2C\underline{H}_3$
	21.0	2.01 (3H, s)	
OAc (C-16)	173.8	—	16-$CO_2C\underline{H}_3$, H-16
	21.4	2.184[b)] (3H, s), 2.179[b)] (3H, s)	
OH (C-6)	—	6.94 (1H)	
OH (C-11)	—	6.081[b)] (1H), 6.061[b)] (1H)	
OH (C-12)	—	11.667[b)] (1H, br s), 11.646[b)] (1H, br s)	
OH (C-14)	—	13.081[b)] (1H, br s), 13.077[b)] (1H, br s)	
OH (C-17)	—	5.05 (1H, br s)	

a) Carbon doublets due to diastereomerism. *b*) Proton doublets due to diastereomerism. *c*) Maybe interchanged in the diastereomers.

range correlated to the methyl protons at δ 1.47 (H-18), 1.53 (H-19) and 1.632 and 1.622 (H-20). This confirms the assignment of the resonance at δ 142.1 to C-5. The carbonyl carbon at δ 173.8 was long-range correlated to the methylene proton at δ 4.79 (H-16) and the acetate methyl at δ 2.184 and 2.179. This confirms the attachment to C-16 of the acetate carbonyl at δ 173.8. The positions of the aromatic hydroxyls were determined by gHMBC. The hydroxyl proton at δ 6.94 (6-OH) was long-range correlated to the carbons at δ 141.0 (C-6) and 182.7 (C-7), while the hydroxyl proton at δ 6.081 and 6.061 (11-OH) was long-range correlated to the carbons at δ 135.8 (C-11), 150.5 (C-12) and 133.5 (C-9). The hydroxyl proton at δ 13.081 and 13.077 (14-OH) was long-range correlated to the carbons at δ 105.4 (C-8), 109.8 (C-13) and 152.3 (C-14).

Although most coleons have an oxygenated carbon at C-3 rather than C-2 as proposed for **1**, C-2 oxygenation is known, for example xanthanthusin A,[7)] which was isolated from *Coleus xanthanthus*.

The relative stereochemistry of **1** in ring A was determined by NOESY which indicated correlation through space of the ^{1}H nuclei in the molecule (Table 2). Thus, the methine proton at δ 5.41 (H-2) shows correlation in space with the methyl protons at δ 1.632 and 1.622 (H-20), indicating that H-2 is in the axial position. This makes the acetate attached to C-2 in the equatorial position. The methyl protons at δ 1.632 and 1.622 (H-20) are close in space to the methyl protons at δ 1.47 (H-18). Thus, these methyl groups are in the axial positions and the remaining methyl group at δ 1.53 is in the equatorial position.

Table 2. NOESY Correlation Data of **1** in $CDCl_3$

^{1}H	NOESY correlation
H-1′	H-1, H-2
H-2	H-1′, H-3′, H-20
H-3′	H-2, H-3, H-18, H-20
H-18	H-3′
H-16	H-16′

As noted above, the observation that several of the ^{1}H and ^{13}C signals appeared as closely spaced “doublets” rather than the expected singlets suggested that **1** was an approximate 1 : 1 mixture of diastereomers, rather than a single compound. Of the three chiral centres in **1**, it did not appear reasonable that inversion of stereochemistry at either C-2 or C-10 would produce diastereomeric compounds which differed in ^{13}C chemical shift by less than 0.07 ppm. We therefore propose that **1** is approximately a 1 : 1 mixture of the two diastereomers formed by inversion of the stereochemistry at C-15. Such a mixture is likely if the acetylating enzyme cannot distinguish the diastereotopic hydroxyls at C-16 and C-17.

Table 3. Antimicrobial Test Results on **1**

Sample	Concn. (μg)	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus niger*		*Trichophyton mentagrophytes*	
		C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z.	A.I.
1	60	11, 11	0.1	—	0	13, 13	0.3	14, 14	0.4	15, 15	0.5	12, 12	0.2	—	0
Standard		20	1.0	25	1.5	25	1.5	20	1.0	30	2.0	30	2.0	30	2.0
Antibiotic		Chloramphenicol		Tetracycline		Tetracycline		Chloramphenicol		Chlotrimazole		Chlotrimazole		Chlotrimazole	

C.Z.; clearing zone, A.I.; antimicrobial index.

Given that **1** is almost planar, except for the area around the C-1 and C-2 carbons, this would not be surprising. Hydrogen-bonding interactions from the C-16/C-17 oxygens through the C-11 and C-12 hydroxyls also provides a pathway by which the difference in C-15 stereochemistry can be "transmitted" to C-1 and C-20.

To test this proposal, **1** was acetylated to the tetraacetate (**2**) in acetic anhydride/pyridine overnight, thereby removing the C-15 stereocentre. ^{1}H and ^{13}C spectra of **2** showed considerable simplification relative to **1**, and no appearance of "doublets" in either ^{1}H- or ^{13}C-NMR. We therefore conclude that **1** is diastereomeric at C-15.

Compound **1** was tested for its antimicrobial potential, and the results of the study are presented in Table 3. Among the seven microorganisms tested, **1** was found to be active against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans* with an average antimicrobial index of 0.4, 0.3 and 0.5, respectively, at a concentration of 60 μg. For the antibiotics chloramphenicol for *B. subtilis*, tetracycline for *P. aeruginosa* and chlotrimazole for *C. albicans*, the average antimicrobial index are 1.0, 1.5 and 2.0, respectively, at a concentration of 30 μg. Thus, **1** has a low antimicrobial activity.

Experimental

General Experimental Procedures IR spectra were recorded on a Perkin-Elmer 1600 Fourier Transform IR spectrometer and UV spectra on a HP 8452A diode array spectrometer. NMR spectra were recorded on a Bruker AMX Fourier Transform 300 in $CDCl_3$ at 300 MHz for ^{1}H and 75 MHz for ^{13}C. The high- and low-resolution EI-MS were recorded on a Micromass AutoSpec mass spectrometer. Column chromatography was performed with Silica gel 60 (70—230 mesh); TLC was performed with plastic backed plates coated with Silica gel F_{254} TLC; plates were visualized by spraying with vanillin–H_2SO_4 and warming.

Plant Material *Coleus blumei* was collected from Ilo-ilo, Philippines in November 1996. It was identified as *Coleus blumei* BENTH (Labiatae) at the Philippine National Museum, and a voucher specimen DLSUCD #028 is kept at the Chemistry Department of De La Salle University.

Extraction and Isolation Air-dried leaves of *Coleus blumei* (600 g) were ground in an osterizer, then extracted with $CHCl_3$ (2.5 l) at room temperature for 2 d. The mixture was filtered and the filtrate concentrated *in vacuo* to afford a crude extract (40 g). This extract was dissolved in EtOH (750 ml), then placed in an ice bath. To the solution was added 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components.[8] The mixture was then filtered and the filtrate concentrated *in vacuo* until a mixture of water and oily residue remained. The concentrate was extracted with $CHCl_3$, and the extract was dried with anhydrous Na_2SO_4, then filtered. The filtrate was concentrated *in vacuo* to afford the treated extract (11.7 g), which was fractionated in a column of silica gel (70—230 mesh) using increasing proportions of Me_2CO in $CHCl_3$ (10% increments, 100 ml) as eluents. The $CHCl_3$ fraction was rechromatographed in 10% Me_2CO in $CHCl_3$ (2×), followed by rechromatography in CH_2Cl_2 : CH_3CN : Et_2O (18 : 1 : 1) to afford **1** (yellowish oil, 15 mg). IR ν_{max} ($CHCl_3$) cm^{-1}: 3394 (OH), 2957, 2957, 2928, 2858, 1735 (C=O), 1623 (C=O), 1598 (C=C), 1463, 1420, 1386, 1369, 1327, 1287 (C–O–C), 1268 (C–O–C), 1251 (C–O–C), 1178 (C–O), 1162 (C–O), 1145 (C–O), 1038 (C–O), 968, 757; UV λ_{max} ($CHCl_3$) nm: 271, 294 (sh), 324, 391; ^{1}H- and ^{13}C-NMR data are listed in Table 1; EI-MS *m/z* 478 [M^+] (27), 403 (15), 374 (14), 358 (32), 344 (35), 343 (100); HR-EI-MS *m/z* 478.1850 [M^+] ($C_{24}H_{30}O_{10}$).

Acetylation **1** (2 mg) was acetylated in acetic anhydride/pyridine overnight to afford the tetraacetate **2** (oil, 1 mg). ^{1}H-NMR ($CDCl_3$): δ 1.26 (3H, s), 1.58 (2×3H, s), 1.66 (1H, m, H1), 1.78 (1H, m, H3), 1.99 (3H, s), 2.00 (3H, s), 2.02 (3H, s) 2.29 (1H, m H3), 2.34 (3H, s), 2.36 (3H, s), 2.37 (1H, m, H1) 2.39 (3H, s), 3.68 (1H, m, H15), 4.36—4.58 (4H, m, H16, H17), 5.34 (1H, m, H2),13.5 (1H, br s, OH). ^{13}C-NMR ($CDCl_3$): δ 20.3, 20.8, 20.9, 21.3, 22.7, 29.3, 29.3, 31.2, 31.9, 36.5, 42.1, 42.5, 62.8, 62.8, 67.0, 115.6, 119.2, 148.7, 160.6, 167.2, 168.5, 170.6, 171.0, 185.6. HR-EI-MS *m/z* 646.2256 (Calcd for $C_{32}H_{38}O_{14}$, 646.2262).

Antimicrobial Test Microbial suspension containing approximately 6×108 cells/ml was prepared for each test organism for 24-h culture of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* and from a 5 d old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water.

One-tenth milliliter of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* were transferred into pre-poured nutrient agar (NA) plates. While *A. niger* and *T. mentagrophytes* were transferred into pre-poured potato dextrose agar (PDA) plates. About 5 ml of corresponding medium, melted and cooled to about 45 °C was poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. One-centimeter wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Sixty micrograms of samples and 30 μg of standard agent was used. Antimicrobial index (AI) was computed by subtracting the diameter of the well from the clearing zone divided by the diameter of the well.

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