New Antimicrobial Filicinic Acid Derivatives from Hypericum drummondii

Hiranthi Jayasuriya, Alice M. Clark, and James D. McChesney

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NEW ANTIMICROBIAL FILICINIC ACID DERIVATIVES FROM *HYPERICUM DRUMMONDII*

HIRANTHI JAYASURIYA, ALICE M. CLARK, and JAMES D. MCCHESNEY*

Department of Pharmacognosy and the Research Institute of Pharmaceutical Sciences, University of Mississippi, University, Mississippi 38677

ABSTRACT.—Bioactivity-guided fractionation of the hexane extract of the stems and leaves of *Hypericum drummondii* has afforded four new filicinic acid derivatives: drummondin D [**1**], isodrummondin D [**2**], drummondin E [**3**], and drummondin F [**4**]. The structures of these compounds were established by spectroscopic methods. All compounds possessed strong antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the acid fast bacterium *Mycobacterium smegmatis*.

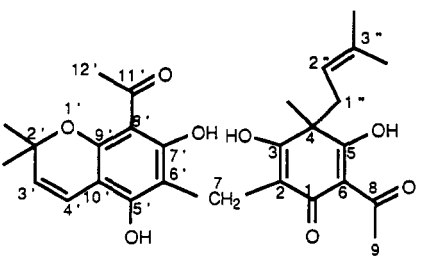
As part of our continuing investigation of potential antimicrobial agents from higher plant sources, we have previously isolated the antibiotics drummondins A, B, and C from the hexane extract of roots of *Hypericum drummondii* (Grev. & Hook.) T. & G. (Hypericaceae) (1). Further study of the hexane extract of the stems and leaves of the plant has now led to the isolation of four additional new antibiotics, drummondin D [**1**], isodrummondin D [**2**], drummondin E [**3**], and drummondin F [**4**]. The filicinic acid portion of these compounds is similar to the chinensins from *Hypericum chinense* L. (2) and could be considered as a hybrid between the albaspidins (3) and the hop bitter principle, colupulone (4).

The residue of the hexane extract of leaves and stems showed much stronger antibiotic activity than the hexane extract of the roots against Gram-positive bacteria using the agar well diffusion assay. The activity of the extract was located by bioautography (see Experimental for details) with *Bacillus subtilis* as the test organism. Two small and one large inhibition zones were detected. The topmost small inhibition zone at R_f 0.37–0.54 corresponded mainly to the previously isolated drummondins A, B, and C (1), which were present in the hexane extract of the roots. However, an additional compound was isolated from this region of the chromatogram and was named drummondin D [**1**].

The second small inhibition zone appeared at R_f 0.31, partially overlapping a large inhibition zone at R_f 0.18. Two compounds were isolated from the second active region of the chromatogram and were named isodrummondin D [**2**] and drummondin E [**3**].

The lower region of the tlc chromatogram responsible for the large inhibition zone was complicated by the presence of at least four compounds. However, the large inhibition zone was centered on a major compound of the extract, which was isolated and named drummondin F [**4**].

The ir spectrum of all isolated compounds suggested the presence of an enolic 1,3-diketo system or a 2-hydroxyaryl ketone (3500–3000 and 1640 cm^{-1}) of the type found



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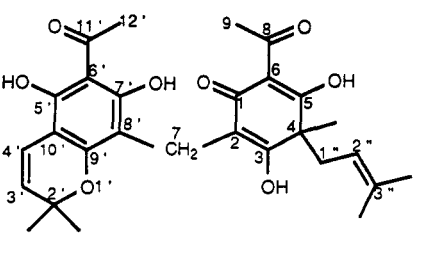

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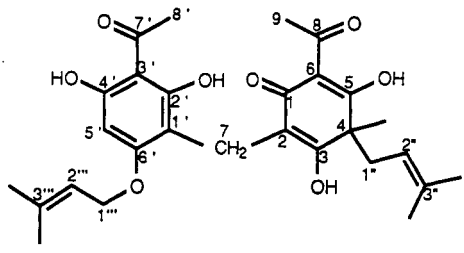

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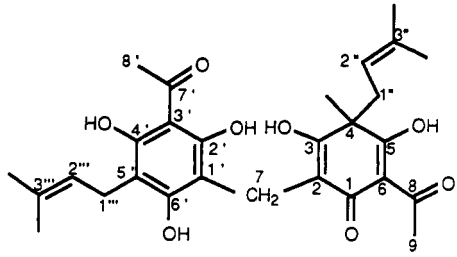

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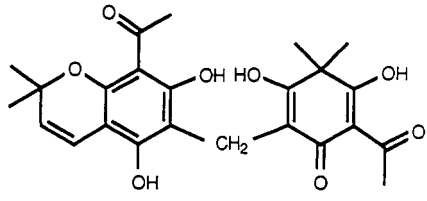

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in drummondins A, B, and C. The presence of methylene protons H-7 (δ 3.5, brs) suggested that the structures of these compounds are closely analogous to the main skeleton of drummondins A, B, and C. The acyl groups proved to be acetyl groups in both moieties of the compounds, and the acetyl methyl protons appeared from δ 2.6 to 2.7 in the ^{1}H-nmr spectrum.

Drummondin D [**1**] was isolated as a yellow oil. The hrms showed a molecular ion peak at *m/z* 496.2105 consistent with the formula $C_{28}H_{32}O_8$. The ^{1}H and ^{13}C nmr (Table 1) of drummondin D were very similar to those of previously isolated drummondin C [**5**]. In addition to the characteristic signals of the 2, 2 dimethylchomene moiety common to both compounds, drummondin D [**1**] exhibited extra signals for a 3-methylbut-2-enyl (isoprenyl) side chain. In the ^{1}H-nmr spectrum these signals appeared as two 3H singlets at δ 1.38 and δ 1.33 for C-3″-vinylic methyls and a broad 1H triplet at δ 4.65 for H-2″. The doublet expected for H-1″ overlapped with the acetyl methyl signals at δ 2.7. The ^{13}C-nmr signals for the isoprenyl side chain were evident at δ 26.5 (C-3″ methyl) and δ 17.6 (C-3″ methyl), δ 136.5 (C-3″ quaternary), δ 118.2 (C-2″ methine), and δ 38.9 (C-1″ methylene).

The ^{13}C-nmr resonance of C-4 of drummondin D (δ 49.6) is about 5 ppm downfield from that in drummondin C. ^{1}H- and ^{13}C-nmr spectra of drummondin D showed signals for only one methyl group at C-4 (δ 1.53, 3H, s; δ 23.3, methyl); hence the isoprenyl side chain was placed at C-4 together with one methyl group. All the other carbon signals of the filicinic moieties of both compounds were similar. Accordingly the filicinic moiety of drummondin D was proved to be modified and resembles that of recently reported chinensins from *H. chinense* (2).

Isodrummondin D [**2**] was crystallized from hexane as yellow prisms, mp 121°. The hrms showed a molecular ion peak at *m/z* 496.2099, which was consistent with the formula $C_{28}H_{32}O_8$. Hence, compound **2** is an isomer of compound **1**. The ^{1}H- and ^{13}C-nmr spectra of the two compounds were very similar, the only differences in the ^{1}H nmr being the chemical shifts of the hydroxyl resonances (Table 1). By direct comparison of the hydroxyl resonances of compounds **1** and **2** with those observed for the isomers drummondin C [**5**] and isodrummondin C, of which structures were established by SINEPT experiments (5), the linear and angular fusions of the pyran rings were es-

TABLE 1. ^{13}C- and ^{1}H-nmr Spectral Data of Drummondin D [**1**][a] and Isodrummondin D [**2**][b].

Position	^{13}C nmr (75 MHz)[c]		^{1}H nmr (300 MHz)[d]	
	Compound		Compound	
	1	**2**	**1**	**2**
1	198.0 (0)	198.0 (0)	—	—
2	111.0 (0)	110.2 (0)	—	—
3	171.0 (0)	170.0 (0)	—	—
3-OH			9.77 (s)	9.10 (s)
4	49.6 (0)	48.8 (0)	—	—
4-Me	23.3 (3)	23.3 (3)	1.53 (s)	1.63 (s)
5	188.9 (0)	187.9 (0)	—	—
5-OH			17.90 (s)	18.57 (s)
6	114.0 (0)	113.1 (0)	—	—
7	16.8 (2)	16.7 (2)	3.53 (s)	3.59 (s)
8	203.3 (0)	203.0 (0)	—	—
9	29.1 (3)	29.3 (3)	2.69 (s)	2.69 (s)
2′	79.1 (0)	80.6 (0)	—	—
2′-Me	28.0 (3)	27.8 (3)	1.51 (s)	1.57 (s)
			1.50 (s)	1.56 (s)
3′	126.4 (1)	124.2 (1)	5.62 (d, J = 9.9)	5.64 (d, J = 9.9)
4′	117.5 (1)	117.0 (1)	6.66 (d, J = 9.9)	6.67 (d, J = 9.9)
5′	159.8 (0)	160.0 (0)	—	—
5′-OH	—	—	11.38 (s)	14.08 (s)
6′	106.7 (0)	106.7 (0)	—	—
7′	161.6 (0)	160.8 (0)	—	—
7′-OH	—	—	16.04 (s)	11.74 (s)
8′	105.4 (0)	104.9 (0)	—	—
9′	156.7 (0)	154.8 (0)	—	—
10′	104.1 (0)	101.8 (0)	—	—
11′	204.9 (0)	204.9 (0)	—	—
12′	33.0 (3)	33.5 (3)	2.69 (s)	2.70 (s)
1″	38.9 (2)	38.7 (s)	underneath the singlet at δ 2.69	underneath the singlet at δ 2.69
2″	118.2 (1)	117.0 (1)	4.65 (brt, J = 8.4)	4.70 (brt, J = 8.1)
3″	136.5 (0)	136.0 (0)	—	—
3″-Me	17.6 (3)	17.6 (3)	1.38 (s)	1.44 (s)
	25.6 (3)	25.6 (3)	1.33 (s)	1.36 (s)

[a]Nmr data for compound **1** were recorded in Me_2CO-d_6.
[b]^{1}H- and ^{13}C-nmr data for compound **2** were recorded in Me_2CO-d_6 and $CDCl_3$, respectively.
[c]Number of attached protons was determined by APT.
[d]^{1}H-nmr chemical shifts in ppm and coupling constants in Hz.

tablished for compounds **1** and **2**, respectively. The ^{1}H- and ^{13}C-nmr spectral assignments of isodrummondin D [**2**] are given in Table 1.

Drummondin E [**3**] was isolated as a yellow gum. The hrms showed a molecular ion peak at *m/z* 498.2252, which was consistent with the formula $C_{28}H_{34}O_8$. The ^{1}H-nmr spectrum showed signals for two isoprenyl groups. In addition to the signals of the isoprenyl side chain of the filicinic acid moiety at C-4, an additional set of signals, H-1‴ (δ 4.81, d), H-2‴ (δ 5.64, brt), and 3‴-Me (δ 1.86, brs), were present for a second isoprenyl side chain. The downfield shift of the H-1‴ protons of the isoprenyl side chain indicated a prenyl side chain attached to an aromatic ring through an ether linkage. The ^{13}C-nmr signals for the isoprenyl side chain attached to the oxygen were observed at δ 67.1 (C-1‴ methylene), δ 118.1 (C-2‴ methine), δ 142.5 (C-3‴ quaternary), and δ

18.2 and δ 25.9 (C-3‴ methyls). Sarothralin, isolated from *Hypericum japonicum* Thunb., possesses similar *O*-prenylation in the phloroglucinol moiety (6).

Drummondin F [**4**] was crystallized from hexane as yellow rosettes, mp 110°. The hrms showed a molecular ion peak at *m/z* 498.2247, which was consistent with the molecular formula $C_{28}H_{34}O_8$. In addition to the signals observed for the isoprenyl side chain at C-4 of the filicinic acid moiety, an additional set of signals for a second isoprenyl side chain was seen in the ^{1}H-nmr spectrum of compound **4**. The resonances observed in the ^{1}H-nmr spectrum for this second isoprenyl side chain (H-1‴, δ 3.39, d; H-2‴, δ 5.12, brt; 3‴-Me, δ 1.76 s and δ 1.65 s) were different from those observed for the isoprenyl side chain at C-6′ oxygen in compound **3** (Table 2), but were similar to

TABLE 2. ^{13}C- and ^{1}H-nmr Spectral Data of Drummondin E [**3**] and Drummondin F [**4**].

Position	^{13}C nmr (75 MHz, Me_2CO-d_6)[a]		^{1}H nmr (300 MHz, Me_2CO-d_6)[b]	
	Compound		Compound	
	3	**4**	**3**	**4**
1	198.8 (0)	199.1 (0)	—	—
2	110.9 (9)	111.0 (0)	—	—
3	170.5 (0)	170.6 (0)	—	—
3-OH	—	—	9.09 (s)	9.97 (brs)[c]
4	49.6 (0)	49.8 (0)	—	—
4-Me	23.4 (3)	23.3 (3)	1.52 (s)	1.52 (s)
5	188.2 (0)	188.6 (0)	—	—
5-OH	—	—	18.58 (s)	18.39 (s)[c]
6	113.9 (0)	114.4 (0)	—	—
7	17.2 (2)	17.4 (2)	3.54 (s)	3.55 (s)
8	203.7 (0)	203.7 (0)	—	—
9	buried under acetone signal	29.3 (3)	2.69 (s)	2.69 (s)
1′	105.9 (0)[d]	106.9 (0)[d]	—	—
2′	160.8 (0)[e]	161.5 (0)[e]	—	—
2′-OH	—	—	11.61 (s)	15.80 (brs)[c]
3′	107.3 (0)[d]	106.5 (0)[d]	—	—
4′	161.9 (0)[e]	160.0 (0)[e]	—	—
4′-OH	—	—	13.68 (s)	6.42 (brs)
5′	93.4 (1)	108.2 (0)[d]	6.20 (s)	—
6′	166.1 (0)[e]	159.9 (0)[e]	—	—
6′-OH	—	—	—	11.44 (brs)[c]
7′	204.9 (0)	205.1 (0)	—	—
8′	33.2 (3)	32.8 (3)	2.69 (s)	2.69 (s)
1″	38.6 (2)	38.8 (2)	2.65 (brd, J = 7.2)	buried under signal at δ 2.69
2″	118.2 (1)[f]	118.2 (1)	4.68 (brt, J = 7.2)	4.67 (brt, J = 7.2)
3″	136.4 (0)	136.6 (0)	—	—
3″-Me	17.6 (3)	17.6 (3)	1.44 (s)	1.39 (s)
	25.5 (3)	25.6 (3)	1.38 (s)	1.34 (s)
1‴	67.1 (2)	22.3 (2)	4.81 (d, J = 7.2)	3.39 (d, J = 7.2)
2‴	118.1 (1)[f]	123.9 (1)	5.64 (brt, J = 7.2)	5.12 (brt, J = 7.2)
3‴	142.5 (0)	132.7 (0)	—	—
3‴-Me	18.2 (3)	18.0 (3)	1.86 (brs)	1.76 (s)
	25.9 (3)	25.9 (3)	—	1.65 (s)

[a]Number of attached protons was determined by APT.
[b]^{1}H-nmr chemical shifts (in ppm) and coupling constants in (Hz).
[c]Observed in $CDCl_3$.
[d–f]Assignments bearing the same letter in the same column are interchangeable.

those of uliginosin A (7), which possesses a C-prenylated phloroglucinol moiety. The ^{13}C-nmr signals for the isoprenyl side chain of **4** showed resonances at δ 22.3 (methylene) for C-1‴, δ 123.9 (methine) for C-2‴, δ 132.7 (quaternary) for C-3‴, and δ 18.0 and 25.9 (methyl) for the vinyl methyls. The aromatic proton singlet for H-5′ (δ 6.2) present in compound **3** was absent in the ^{1}H-nmr spectrum of compound **4**. There were signals for three phenolic hydroxyl groups (δ 10.6, 11.4, 15.8) in the phloroglucinol moiety. The above spectral data suggest compound **4** to be the C-prenylated analogue of compound **3**.

Minimum inhibitory concentrations (MIC) determined for all four compounds using the serial broth dilution method previously described (9) are reported in Table 3. All compounds were comparable to or more active than streptomycin against the Gram-positive and the acid fast bacteria used in the assay system. The linear chromene **1** was as active as the angular chromene **2**. Both *C*-prenylated (**4**) and *O*-prenylated (**3**) compounds were active.

TABLE3. Minimum Inhibitory Concentrations of Drummondin D [**1**], Isodrummondin D [**2**], Drummondin E [**3**], and Drummondin F [**4**] (μg/ml).

Compound	Microorganism		
	Staphylococcus aureus	*Bacillus subtilis*	*Mycobacterium smegmatis*
1	0.78	0.39	1.56
2	0.78	0.20	1.56
3	0.39	0.20	3.12
4	0.78	0.78	1.56
Streptomycin	12.5	0.78	1.56

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Fisher-Johns digital mp analyzer model 355 and were not corrected. Ir spectra were taken as KBr pellets or $CHCl_3$ solutions on a Perkin Elmer 281 B spectrometer. Uv spectra were taken on a Perkin Elmer Lambda 3B UV/VIS spectrophotometer in MeOH solutions. Specific rotations ([α]D) were obtained on a Perkin-Elmer 141 automatic polarimeter using MeOH solutions. Low resolution mass spectra were obtained on a Finnigan 3200 GC/MS mass spectrometer coupled to an INCOS data system operating in ei mode at 70 eV. Hrms analyses were conducted in the Department of Chemistry, University of Kansas, Lawrence. The nmr spectra were obtained on a Varian VXR-300 (300 MHz) instrument. For both ^{1}H and ^{13}C nmr, TMS was used as internal standard, and chemical shifts are expressed in ppm relative to TMS as internal standard (δ units). For ^{1}H nmr, the descriptions are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and brs = broad singlet. The coupling constants are reported in Hz. Cc employed Si gel HF_{254} type 60 or Si gel 60 (230–400 mesh). Tlc analysis was performed by utilizing precoated Silica G-25 UV_{254} plates (Macherey-Nagel Duren), and the detection of compounds was accompanied by spraying with a 1% aqueous solution of fast blue B reagent. All solvents used for chromatography purposes were AR grade.

PLANT MATERIAL.—The plant *H. drummondii* was collected at a farm near Oxford, Mississippi in August 1986. A voucher specimen is lodged in the herbarium, Department of Pharmacognosy, School of Pharmacy, The University of Mississippi. The plant material was air-dried, separated into roots, stems, and leaves, and ground in a Wiley mill.

QUALITATIVE ANTIMICROBIAL SCREENING (AGAR WELL DIFFUSION ASSAY).—Qualitative antimicrobial screening was accomplished using the procedures described by Hufford *et al.* (8), as modified by Clark *et al.* (9). Extracts, crude fractions, and pure compounds were tested for activity against the following microorganisms: *Escherichia coli* (ATCC 15036), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Bacillus subtilis* (ATCC 6633), *Mycobacterium smegmatis* (ATCC 607), *Cryptococcus neoformans* (ATCC 32264), *Saccharomyces cerevisiae* (ATCC 9763), *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (ATCC 26934), and *Trichophyton mentagrophytes* (ATCC 9972). All the above test organisms used are depo-

sited in the University of Mississippi, Department of Pharmacognosy culture collection and were originally from the American Type Culture Collection (ATCC), Rockville, Maryland. Bacterial test organisms were cultured in eugon agar and eugon broth, and fungi and yeast were cultured in mycophil agar and broth.

QUANTITATIVE ANTIMICROBIAL SCREENING.—MIC values were determined using the twofold serial broth dilution technique previously described (9). All compounds were tested within the range 0.20–100 μg/ml. The MIC was taken as the lowest concentration that inhibited growth of the microorganism after 24 or 48 h of incubation. Tubes inoculated with *B. subtilis* were incubated at 30°, while tubes inoculated with *S. aureus* and *M. smegmatis* were incubated at 37°. The minimum inhibitory values were determined after 24 h of incubation for *B. subtilis* and *S. aureus*, and after 48 h of incubation for *M. smegmatis*. Streptomycin sulfate was used as a standard control in the assay system.

BIOAUTOGRAPHY.—The first phases of the isolation of the antimicrobially active materials from the crude extract were followed by bioautography. This was performed by developing a sample of the extract on Si gel tlc plates with 10% Et_2O in hexane with 0.25% of HCO_2H added. After the development, the tlc plate was air-dried and placed face-down on the surface of agar medium (150 mm Petri dish) streaked with the test microorganism, *B. subtilis* (ATCC 6633). After 30 min, the tlc plate was removed and the Petri dish was incubated at 30°. After 24 h the Petri dish was examined for inhibition zones. Subsequent isolation work was followed by tlc using R_f values as guides.

ISOLATION.—The air-dried finely ground stems and leaves of the plant material (3 kg) were exhaustively percolated with hexane at room temperature. The combined hexane extracts were concentrated under reduced pressure to produce a residue (160 g).

The R_f values obtained from the bioautography were used as guides in isolating the antimicrobially active compounds from the extract. The residue of the hexane extract (40 g) was partially purified by a Si gel column HF_{254} (800 g) eluting with hexane/EtOAc/HCO_2H mixtures consisting of 5–25% EtOAc and 0.25% HCO_2H. Fractions of 300 ml each were collected and pooled into 12 fractions (A–L) on the basis of their tlc patterns.

DRUMMONDIN D [**1**].—Fraction C (2.5 g) was further chromatographed on Si gel 60 (270–430 mesh) using hexane-EtOAc-HCO_2H (98:1.75:0.25) as eluent. Drummondin D [**1**] was obtained as a yellow gum (24.7 mg): $[\alpha]^{23}D$ +110° (c = 3.2, MeOH), ir ν max ($CHCl_3$) cm^{-1} 3350–2800 (broad), 3020 (s), 1640 (s), 1600 (s), 1370 (m), 1220–1200 (s, br); uv λ max (MeOH) nm (log ε) 356 (3.9), 287 (4.1); ^1H and ^{13}C nmr see Table 1; ms *m/z* (relative abundance) 496 (58.5), 428 (16.9), 409 (59.3), 247 (69.3), 231 (80.9), 219 (100); hrms *m/z* calcd for $C_{28}H_{32}O_8$ $[M]^+$ 496.2095, found $[M]^+$ 496.2105.

ISODRUMMONDIN D [**2**] AND DRUMMONDIN E [**3**].—Fraction F (2 g) was chromatographed on a Si gel HF_{254} (200 g) column using hexane-EtOAc-HCO_2H (98:1.75:0.25) to yield isodrummondin D (25.7 mg) and drummondin E (11.9 mg).

Isodrummondin D [**2**] (25.7 mg) was crystallized from hexane as yellow crystals: mp 121°; $[\alpha]^{23}D$ +140° (c = 5.2, MeOH), uv λ max (MeOH) nm (log ε) 357 (4.03), 314 (4.08), 278 (4.33); ir ν max (KBr) cm^{-1} 3600–2400 (broad), 3260 (m), 2985 (m), 2925 (m), 1640 (s), 1600 (s, broad), 1430 (m, br), 1280 (m, br); ^1H and ^{13}C nmr see Table 1; eims *m/z* (relative abundance) 496 (90.2), 428 (28.4), 409 (81.8), 246 (72.4), 231 (83.8), 219 (100), 201 (35.0); hrms *m/z* calcd for $C_{28}H_{32}O_8$ $[M]^+$ 496.2095, found $[M]^+$ 496.2099.

Drummondin E [**3**] (11.9 mg) was obtained as a light yellow gum: $[\alpha]^{23}D$ +30° (c = 3.7, MeOH), ir ν max ($CHCl_3$) cm^{-1} 3600–2500 (broad), 3250 (m, br), 3000 (m, br), 1620 (s, br), 1435 (m), 1365 (m), 1275 (m), 1210 (s, br); uv λ max (MeOH) nm (log ε) 354 (4.2), 294 (4.4); ^1H and ^{13}C nmr see Table 2; ms *m/z* (relative abundance) $[M]^+$ 498 (3.3), 343 (6.8), 247 (6.6), 236 (20), 193 (21.3), 181 (46.5); hrms *m/z* calcd for $C_{28}H_{34}O_8$ $[M]^+$ 498.2252, found $[M]^+$ 498.2262.

DRUMMONDIN F [**4**].—Drummondin F was obtained from fraction H (2.2 g) by cc on a Si gel 60 (270–430 mesh) column using hexane-EtOAc-HCO_2H (98:1.75:0.25) as the eluent. Drummondin F (66.7 mg) was obtained as yellow crystals: mp 106–107°; $[\alpha]^{23}D$ +56.4 (c = 2.5, MeOH), ir ν max (KBr) 3600–2500 (broad), 3520 (w, br), 3150 (m, br), 2930 (m), 1650 (s), 1610 (s, br), 1460 (m), 1440 (m), 1370 (s) cm^{-1}; uv λ max (MeOH) nm (log ε) 350 (4.09), 300 (4.27); ^1H and ^{13}C nmr see Table 2; ms *m/z* (relative abundance) 498 (10), 355 (10), 236 (27), 195 (25), 193 (29), 182 (15), 165 (13); hrms *m/z* calcd. for $C_{28}H_{34}O_8$ $[M]^+$ 498.2252, found $[M]^+$ 498.2247.

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