New Cytotoxic Isomalabaricane Triterpenes from the Sponge *Jaspis* Species

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Three new isomalabaricane triterpenes, 29-hydroxystelliferin E (**1**), 29-hydroxystelliferin A (**2**), and stelliferin G (**3**), together with the known triterpene 3-*epi*-29-hydroxystelliferin E (**4**), were isolated from the organic extract of the sponge *Jaspis* sp. collected in the South Pacific. All the compounds isolated showed antiproliferative activity against melanoma cells (MALME-3M).

The organic extract of *Jaspis* sp. collected in the South Pacific showed cytotoxic activity in the NCI 60-cell line screen.[1] The differential cytotoxicity profile of the extract was similar to that of the stellettins[2,3] and schweinfurthins[4] by COMPARE analysis,[5] indicating the possible presence of compounds sharing a similar mechanism of cytotoxicity. In the search for new antiproliferative metabolites we undertook a bioassay-directed study of the *Jaspis* extract.

Constituents previously isolated from the sponge *Jaspis* include cyclic peptides,[6,7] amino acid derivatives,[8,9] macrolides,[10] and terpenes such as sterols[11,12] and isomalabaricane triterpenes.[13−16] The isomalabaricane skeleton has been reported from three sponge genera: *Jaspis*,[13−16] *Stelleta*,[2,17−20] and *Rhabdastrella*.[21] Several of the reported isomalabaricane derivatives have cytotoxic activity.[2,13,18−20]

The organic extract of the sponge *Jaspis* was subjected to solvent−solvent partitioning,[22] and the MeOtBu and EtOAc fractions were subjected to gel permeation on Sephadex LH-20. The active fractions obtained were initially difficult to purify, since pure compounds rapidly produced isomeric mixtures. Analysis of the ^{1}H NMR spectra showed signals characteristic of the isomalabaricane triterpenes. Because of the tendency of this type of compound to photoisomerize,[2,20] isolation of the triterpenes 29-hydroxystelliferin E (**1**), 29-hydroxystelliferin A (**2**), stelliferin G (**3**), and 3-*epi*-29-hydroxystelliferin E (**4**) required protection of the compounds by wrapping all glassware in aluminum foil.

The first triterpene, 29-hydroxystelliferin E (**1**), was isolated as a light yellow solid. Its molecular formula was established as $C_{34}H_{50}O_6$ by HRFABMS ($C_{34}H_{50}O_6Cs$ *m/z* 687.2661, calcd 687.2662). The ^{1}H NMR spectrum (benzene-d_6) of compound **1** (Table 1) exhibited signals for four vinylic protons, four protons on oxygenated carbons, and nine methyl groups. Two of these methyl groups were present as part of acetyl groups (δ 1.64, 3H, s; δ 1.59, 3H, s); the remaining seven methyl groups could be accounted for in the triterpene skeleton. This is one methyl group less than the eight methyl groups normally seen in the isomalabaricane skeleton. The presence of additional signals for an oxo-methylene group (δ_H 3.79, 1H, dd, J = 11.0, 0.5 Hz and δ_H 3.52, 1H, br d, J = 11.0; δ_C 64.4, t) suggested that the absent methyl group was present as a hydroxymethylene. All of the NMR data were very similar to those for the known compound 3-*epi*-29-hydroxystelliferin E (**4**),[20] except for the signals belonging to C-3, C-28, and C-29. The absence of a signal at δ 5.14 (1H, t, J = 3.3 Hz, H-3 in **4**) in the ^{1}H NMR spectrum of **1** and the presence of a new signal at δ 4.71 (1H, dd, J = 11.0, 4.0 Hz) suggested that an oxygenated methine was still present at C-3 in **1**. The upfield shift of H-3 could be accounted for by an acetate in the equatorial position instead of axial as in **4**. This stereochemistry was confirmed by the NOE enhancement observed for the signals at δ 1.56 (H-5) and δ 1.09 (CH_3-28) when H-3 was irradiated. The relative stereochemistry at the ring junctions was determined to be *trans*−*syn*−*trans* by NOE correlations between δ 0.65 (CH_3-19) and δ 1.38 (H-9) and between δ 0.94 (CH_3-30) and δ 1.73 (CH_3-18) and δ 1.56 (H-5), consistent with the presence of an isomalabaricane ring system. The relative stereochemistry of C-4 was determined by the correlations observed between δ 0.65 (CH_3-19) and the signals at δ 3.52 (H-29a), δ 3.79 (H-29b), and δ 1.38 (H-9) in NOE experiments. The chemical shifts of CH_3-18 (δ 1.73) and H-15 (δ 8.72) and analysis of the coupling constants of H-15, H-16, and H-17

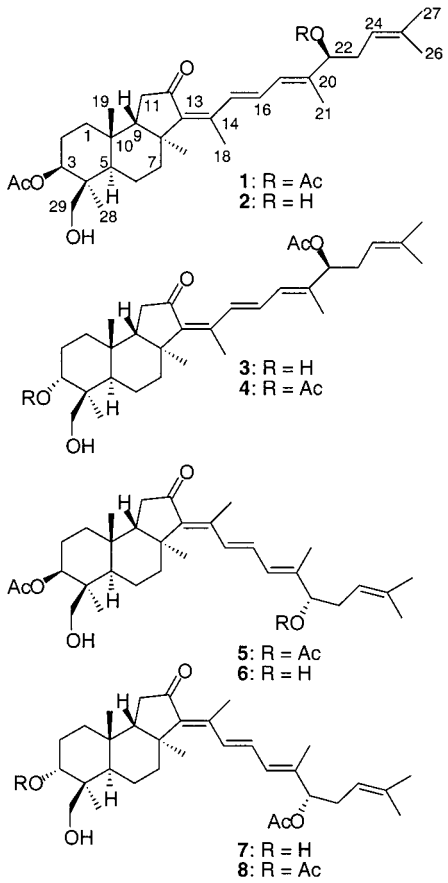

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Table 1. NMR Assignments of 29-Hydroxystelliferin E (**1**) in Benzene-d_6

position	δ_C mult	δ_H mult (J in Hz)	HMBC
1a	32.9 t	0.88 br d (13.0)	H-9, H-19
b		1.15 dd (13.0,4.5)	
2a	25.8 t	1.52 m	
b		1.75 m	
3	81.8 d	4.71 dd (11.0,4.0)	H-2a, H-28, H-29
4	43.3 s		H-3, H-28, H-29
5	47.5 d	1.56 d (12.5)	H-19, H-28, H-29b
6a	19.7 t	1.25 m	H-5
b		1.55 m	
7a	38.6 t	1.58 m	H-5, H-30
b		1.71 m	
8	44.4 s		H-6, H-11b, H-30
9	49.8 d	1.38 dd (16.5,7.5)	H-11, H-19, H-30
10	35.2 s		H-19
11a	36.7 t	1.90 t (16.5)	H-9
b		1.97 dd (16.5,7.5)	
12	204.6 s		H-11b
13	146.4 s		H-7b, H-18, H-30
14	139.0 s		H-16, H-18
15	133.7 d	8.72 d (15.0)	H-16, H-17, H-18
16	129.7 d	6.86 dd (15.0,11.0)	
17	128.0 d[a]	6.49 d (11.0)	H-15, H-16, H-21
18	15.7 q	1.73 s	H-15
19	21.9 q	0.65 s	H-1, H-5, H-9
20	139.9 s		H-16, H-21
21	13.6 q	1.76 s	H-17, H-22
22	78.4 d	5.36 t (7.0)	H-17, H-21, H-23, H-24
23a	32.3 t	2.29 ddd (15.0,7.0,6.5)	H-22
b		2.43 ddd (15.0,7.0,6.5)	
24	119.7 d	5.12 br t (6.5)	H-22, H-23, H-26, H-27
25	134.2 s		H-26, H-27, H-23
26	25.3 q	1.58 br s	H-24, H-27
27	17.7 q	1.46 br s	H-24, H-26
28	23.7 q	1.09 s	H-29
29a	64.4 t	3.52 br d (11.0)	H-3, H-5, H-28
b		3.79 dd (11.0,0.5)	
30	24.9 q	0.94 s	
3-Ac	20.6 q	1.64 s[b]	
	169.4 s		H-3, 3-Ac
22-Ac	20.6 q	1.59 s[b]	
	169.4 s		H-22, 22-Ac

[a] Signal superposed with benzene. [b] Assignments may be interchanged.

allowed the assignment of the double-bond geometries as 13*Z*, 15*E*, 17*E*.

The molecular formula of 29-hydroxystelliferin A (**2**) was determined as $C_{32}H_{48}O_5$ by HRFABMS ($C_{32}H_{49}O_5$, *m/z* 513.3577, calcd 513.3580), which corresponds to C_2H_2O less than 29-hydroxystelliferin E (**1**). The absence in the ^{1}H NMR spectrum of **2** of the triplet at δ 5.36 (H-22) and the singlet at δ 1.64, as observed with compound **1**, and the presence of a new triplet at δ 3.88 (J = 7.0 Hz) together with the presence of a signal at 77.1 ppm in the ^{13}C NMR spectrum suggested that compound **2** was the 22-desacetyl derivative of 29-hydroxystelliferin E. The substitution at C-22 was confirmed by the COSY correlation between H-22 and the C-23 methylene protons at δ 2.19 and 2.25 and by the HMBC correlation between C-22 and H-21. NOE correlations indicated that the stereochemistry for the ring junctions of compound **2** was *trans*−*syn*−*trans* as in 29-hydroxystelliferin E (**1**). The absolute configuration at C-22 was determined to be *S* by Mosher's method[13,23] (Figure 1). The reaction with MTPACl also esterified the primary hydroxyl group (C-29), but this did not interfere with the determination of stereochemistry of the secondary alcohol (C-22).

The molecular formula of stelliferin G (**3**) was determined to be $C_{32}H_{48}O_5$ by HRFABMS ($C_{32}H_{48}O_5Cs$, *m/z* 645.2572, calcd 645.2556), showing that this compound was an isomer of 29-hydroxystelliferin A (**2**). Most of the NMR data (Table 2) recorded for compound **3** were nearly identical to the data for 29-hydroxystelliferin E (**1**), except for the absence of the ^{1}H signal at δ 4.71 (1H, dd, J = 11.0, 4.0 Hz, H-3). The presence of a broad doublet at δ 3.65 (J = 4.5 Hz) together with the ^{13}C signal at 71.1 ppm suggested the presence of a hydroxyl group at C-3. The small coupling constant for H-3 indicated that this substituent was in the axial position. The stereochemistry at this position was confirmed by NOE correlations between H-3 and the signals at δ 0.64 (CH_3-19) and δ 3.18 and δ 3.31 (CH_2-29). The stereochemistries of the ring junctions and at C-4 were the same as those described for **1** based on NOE data.

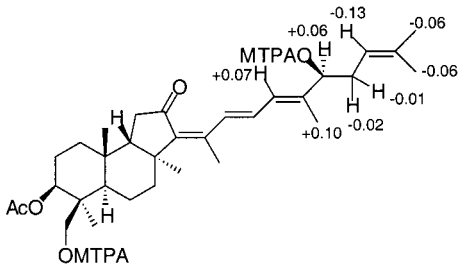

Figure 1. $\Delta\delta$ ($\delta_S - \delta_R$) values obtained from the ^{1}H NMR spectra of the MTPA esters of **2**.

To confirm that the acetate groups were not artifacts due to the use of EtOAc during the isolation process, a second solvent−solvent partition was done without EtOAc. The MeOtBu fraction was further purified to yield 29-hydroxystelliferin E (**1**), 29-hydroxystelliferin A (**2**), and 3-*epi*-29-hydroxystelliferin E (**4**), indicating that the acetate groups are naturally occurring.

As isomalabaricane triterpenes are known to isomerize upon exposure to light,[2,20] all pure compounds were exposed to ambient light while in solution at room temperature to check their stability. The ^{1}H NMR spectra of all four compounds indicated that partial isomerization at C-13 had occurred. Changes in the ^{1}H NMR spectra of each compound included the appearance of a new singlet between δ 2.55 and δ 2.60 assigned to CH_3-18 and a new doublet between δ 6.64 and δ 6.68 corresponding to H-15. After 24 h the ratio between the isomers was approximately 7:3 (13*Z*/13*E*). The 13*E* isomer of compound **2**, 29-hydroxystelliferin B (**6**), was purified from the isomeric mixture, and all of the signals in the ^{1}H and ^{13}C NMR spectra were assigned (Experimental Section).

The isomeric mixtures of the four compounds were tested against melanoma (MALME-3M) and leukemia (MOLT-4) cells.[24] Against MALME-3M the mixtures of 29-hydroxystelliferin A (**2**)/29-hydroxystelliferin B (**6**) and stelliferin G (**3**)/13*E*-stelliferin G (**7**) were the most growth-inhibitory (IC_{50} = 0.11, 0.23, μg/mL, respectively). The other two mixtures, 29-hydroxystelliferin E (**1**)/13*E*-29-hydroxystelliferin E (**5**) and 3-*epi*-29-hydroxystelliferin E (**4**)/13*E*-3-*epi*-29-hydroxystelliferin E (**8**), were approximately 10-fold less potent. A similar trend was seen with the MOLT-4 results (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. NMR experiments were performed on a Varian Inova Unity 500 spectrometer using benzene-d_6 as the solvent. Mass spectra were obtained with a JEOL SX102 mass spectrometer. UV spectra were run on a Beckman DU 640 spectrophotometer, and IR spectra on a Perkin-Elmer Spectrum 2000 FT-IR

Table 2. NMR Assignments of Compounds **2** and **3** in Benzene-d_6

	2		**3**	
position	δ_C mult	δ_H mult (J in Hz)	δ_C mult	δ_H mult (J in Hz)
1a	32.9 t	0.87 br d (12.5)	28.8 t	0.77 dt (12.5,4.5)
b		1.15 dd (12.5,3.5)		1.51 m
2a	25.9 t	1.50 m	26.8 t	1.38 m
b		1.74 m		1.58 m
3	81.9 d	4.72 dd (12.0,5.5)	71.1 d	3.65 br d (4.5)
4	43.4 s		43.3 s	
5	47.6 d	1.51 m	41.6 d	2.01 m
6a	19.8 t	1.22 m	19.3 t	1.08 m
b		1.53 m		1.39 m
7a	38.7 t	1.56 m	38.8 t	1.67 m
b		1.72 dd (13.0,8.5)		1.74 m
8	44.4 s		44.4 s	
9	49.9 d	1.40 dd (16.0,7.0)	50.0 d	1.39 dd (14.5,8.5)
10	35.3 s		34.9 s	
11a	36.9 t	1.93 t (16.0)	36.7 t	2.03 dd (17.0,8.5)
b		2.01 dd (16.0,7.0)		2.04 dd (17.0,14.5)
12	204.5 s		204.9 s	
13	146.2 s		146.6 s	
14	141.6 s		141.0 s	
15	133.0 d	8.75 d (15.5)	133.6 d	8.73 d (15.0)
16	130.3 d	6.93 dd (15.5,11.0)	129.4 d	6.84 dd (15.0,11.0)
17	126.8 d	6.36 d (11.0)	128.0 d[a]	6.49 d (11.0)
18	15.9 q	1.77 s	15.7 q	1.75 s
19	21.9 q	0.65 s	23.9 q	0.64 s
20	143.5 s		138.8 s	
21	12.7 q	1.76 s	13.5 q	1.75 s
22	77.1 d	3.88 br t (7.0)	78.3 d	5.36 t (7.0)
23a	34.8 t	2.19 dt (14.0,7.0)	32.2 t	2.29 m
b		2.25 dt (14.0,7.0)		2.43 ddd (14.5,7.0,6.5)
24	120.9 d	5.15 br t (7.0)	119.6 d	5.12 br t (6.5)
25	134.1 s		134.1 s	
26	25.4 q	1.59 br s	25.7 q	1.58 s
27	18.0 q	1.49 br s	17.8 q	1.49 br s
28	23.8 q	1.10 s	20.5 q	0.99 s
29a	64.4 t	3.52 br d (11.5)	67.4 t	3.18 d (10.7)
b		3.80 d (11.5)		3.31 br d (10.7)
30	25.0 q	0.95 s	24.2 q	1.13 s
Ac	20.6 q	1.59 s	20.5 q	1.64 s
	169.5 s		168.2 s	

[a] Signal superposed with benzene.

Table 3. IC_{50}[a] Values of Isomeric Mixtures **1**/**5**, **2**/**6**, **3**/**7**, and **4**/**8**

mixture	MALME-3M	MOLT-4
29-hydroxystelliferin E (**1**)/13*E*-29-hydroxystelliferin E (**5**)	2.27	19.54
29-hydroxystelliferin A (**2**)/13*E*-29-hydroxystelliferin A (**6**)	0.11	1.62
stelliferin G (**3**)/13*E*-stelliferin G (**7**)	0.23	4.11
3-*epi*-29-hydroxystelliferin E (**4**)/13*E*-3-*epi*-29-hydroxystelliferin E (**8**)	1.20	16.62

[a] Values expressed in μg/mL.

spectrometer. HPLC separations were performed on a Rainin System using a C-18 column (Dynamax, 4.6 mm × 10 cm, MeOH−H_2O, 8:2 and 68:32, flow rate 1.0 mL/min, UV detection at 330 nm).

Material. The sponge *Jaspis* was collected near Tonga (18 40.25 S, 173 59.45 W) in November 1997, by the Coral Reef Research Foundation under contract with the National Cancer Institute. The sponge was frozen immediately after collection and stored frozen until extraction. A voucher specimen has been deposited at the Smithsonian Institution. The taxonomic identification was carried out by Michelle Kelly (NIWA).

Extraction and Isolation. A portion (560 mg) of the organic extract of *Jaspis* Gray (Jaspidae) was subjected to a solvent−solvent partitioning.[22] The MeOtBu and EtOAc fractions were combined and subjected to gel permeation on Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) to give seven fractions (A−G). Two active fractions (C and D) were purified by HPLC (Dynamax C-18, MeOH−H_2O, 80:20) to give 29-hydroxystelliferin E (**1**) (3.5 mg, 0.45% of extract), stelliferin G (**3**) (7.0 mg, 1.14% of extract), 3-*epi*-29-hydroxystelliferin E (**4**) (1.1 mg, 0.20% of extract), and 6.4 mg of an impure fraction containing **2**. This fraction was further purified employing MeOH−H_2O (68:32) as solvent to afford 1.5 mg of 29-hydroxystelliferin A (**2**, 0.27% of extract).

A MeOH solution of 29-hydroxystelliferin A (**2**) (1.2 mg) was kept in the ambient light for 24 h. The resulting mixture was then purified by HPLC (Dynamax C-18, MeOH−H_2O, 7:3) to give 29-hydroxystelliferin A (**2**) (0.5 mg) and 29-hydroxystelliferin B (**6**) (0.3 mg).

29-Hydroxystelliferin E (1): light yellow solid; $[\alpha]^{25}_D$ −40° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 342 (4.52), 232 (4.23) nm; IR (NaCl) ν_{max} 3446, 2923, 2853, 1730, 1685, 1556 cm^{-1}; ^1H and ^{13}C NMR in benzene-d_6, see Table 1; HRFABMS (magic bullet matrix/CsI) *m*/*z* $[M + Cs]^+$ 687.2661, calcd for $C_{34}H_{50}O_6$-Cs, 687.2662; FABMS (magic bullet matrix/CsI) *m*/*z* 687 $[M + Cs]^+$ (76), 652 (45), 495 (22), 419 (33), 393 (99), 299 (44), 133 (100).

29-Hydroxystelliferin A (2): bright yellow solid; $[\alpha]^{25}_D$ −37° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 348 (4.04), 232 (3.77) nm; IR (NaCl) ν_{max} 3398, 2925, 2856, 1727, 1679, 1566 cm^{-1}; ^1H and ^{13}C NMR in benzene-d_6, see Table 2; HRFABMS (magic bullet matrix) *m*/*z* 513.3577, calcd for $C_{32}H_{49}O_5$, 513.3580; FABMS (magic bullet matrix) *m*/*z* 535 $[M + Na]^+$ (22), 513 $[M + H]^+$ (24), 495 (61), 443 (32), 329 (35), 309 (100).

Stelliferin G (3): bright yellow solid; $[\alpha]^{25}_{D}$ −17° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 342 (4.43), 232 (4.13) nm; IR (NaCl) ν_{max} 3448, 2924, 2852, 1684, 1636, 1595, 1565 cm^{-1}; ^1H and ^{13}C NMR in benzene-d_6, see Table 2; HRFABMS (magic bullet matrix/CsI) *m*/*z* $[M + Cs]^+$ 645.2572, calcd for $C_{32}H_{48}O_5$-Cs, 645.2556; FABMS (magic bullet matrix/CsI) *m*/*z* 645 $[M + Cs]^+$ (54), 558 (98), 542 (61), 475 (73), 419 (100), 393 (95).

29-Hydroxystelliferin B (6): ^1H NMR (benzene-d_6, 500 MHz) δ 6.94 (1H, dd, J = 15.0, 11.0 Hz, H-16); 6.67 (1H, d, J = 15.0 Hz, H-15); 6.47 (1H, d, J = 11.0 Hz, H-17); 5.13 (1H, br t, J = 7.0 Hz, H-24); 4.72 (1H, dd, J = 12.0, 5.5 Hz, H-3); 3.92 (1H, br t, J = 7.0 Hz, H-22); 3.80 (1H, d, J = 12.0 Hz, H-29b); 3.50 (1H, m, H-29a); 2.60 (3H, s, CH_3-18); 2.23 (2H, m, H-23); 2.01 (1H, m, H-7b); 2.01 (1H, dd, J = 16.0, 7.0 Hz, H-11b); 1.96 (1H, m, H-7a); 1.93 (1H, t, J = 16.0 Hz, H-11a); 1.76 (1H, m, H-2b); 1.63 (3H, s, CH_3-21); 1.58 (1H, m, H-5); 1.58 (6H, s, CH_3-26, Ac); 1.55 (2H, m, H-6); 1.51 (1H, m, H-2a); 1.47 (3H, s, CH_3-27); 1.42 (1H, dd, J = 16.0, 7.0 Hz, H-9); 1.15 (1H, m, H-1b); 1.11 (3H, s, CH_3-28); 1.05 (3H, s, CH_3-30); 0.88 (1H, m, H-1a); 0.63 (3H, s, CH_3-19); ^{13}C NMR (benzene-d_6, 125 MHz) δ 203.4 (s, C-12); 169.0 (s, CH_3CO); 146.5 (s, C-13); 144.4 (s, C-20); 140.6 (s, C-14); 135.9 (s, C-25); 132.2 (d, C-15); 131.4 (d, C-16); 125.4 (d, C-17); 120.6 (d, C-24); 81.6 (d, C-3); 76.2 (d, C-22); 64.1 (t, C-29); 49.6 (d, C-9); 47.4 (d, C-5); 44.8 (s, C-8); 43.8 (s, C-4); 39.7 (t, C-7); 36.5 (t, C-11); 35.3 (s, C-10); 34.8 (t, C-23); 32.6 (t, C-1); 25.7 (q, C-30); 25.6 (q, C-26); 25.0 (t, C-2); 23.6 (q, C-28); 21.6 (q, C-19); 20.3 (q, CH_3CO); 19.5 (t, C-6); 17.6 (q, C-27); 14.4 (q, C-18); 13.1 (q, C-21).

Preparation of MTPA Esters. 29-Hydroxystelliferin A (**2**) (0.6 mg) was dissolved in 160 μL of pyridine-d_5, and 5 μL of *R*-(−)-MTPACl was added to this solution. The reaction was monitored by ^1H NMR, and after 5 min there was virtually 100% conversion to the corresponding (*S*)-MTPA ester. Preparation of the (*R*)-MTPA ester derivative of **2** was carried out in a similar manner, but *S*-(+)-MTPACl was used and the reaction time was 1 h.

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References and Notes

(1) Boyd, M. R. In *Cancer Drug Discovery and Development, Drug Development: Preclinical Screening, Clinical Trial and Approval*; Teicher, B., Ed.; Humana Press: Totowa, NJ, 1997; Vol. 2, pp 23−42.
(2) McCormick, J. L.; McKee, T. C.; Cardellina, J. H., II; Leid, M.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 1047−1050.
(3) McKee, T. C.; Bokesh, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 431−438.
(4) Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 1509−1512.
(5) Boyd, M. R.; Paull, K. D. *Drug. Dev. Res.* **1995**, *34*, 91−109.
(6) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123−3124.
(7) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797−2800.
(8) Adamczeski, M.; Quiñoà, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110*, 1598−1602.
(9) D'Auria, M. V.; Giannini, C.; Minale, L.; Zampella, A.; Debitus, C.; Frostin, M. *J. Nat. Prod.* **1997**, *60*, 814−816.
(10) Kobayashi, J.; Murata, O.; Shigemori, H.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 787−791.
(11) Theobald, N.; Wells, R.; Djerassi, C. *J. Am. Chem. Soc.* **1978**, *100*, 7677−7684.
(12) Cho, J.-H.; Djerassi, C. *J. Org. Chem.* **1987**, *52*, 4517−4521.
(13) Tsuda, M.; Ishibashi, M.; Agemi, K.; Sasaki, T.; Kobayashi, J. *Tetrahedron* **1991**, *47*, 2181−2194.
(14) Ravi, B. N.; Wells, R. J.; Croft, K. D. *J. Org. Chem.* **1981**, *46*, 1998−2001.
(15) Ravi, B. N.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 39−50.
(16) Zampella, A.; D'Auria, M. V.; Debitus, C.; Menou, J.-L. *J. Nat. Prod.* **2000**, *63*, 943−946.
(17) McCabe, T.; Clardy, J.; Minale, L.; Pizza, C.; Zollo, F.; Riccio, R. *Tetrahedron Lett.* **1982**, *23*, 3307−3310.
(18) Su, J. Y.; Meng, Y. H.; Zeng, L. M.; Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1994**, *57*, 1450−1451.
(19) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 512−514.
(20) Oku, N.; Matsunaga, S.; Wada, S.-I.; Watabe, S.; Fusetani, N. *J. Nat. Prod.* **2000**, *63*, 205−209.
(21) Rao, Z.; Deng, S.; Wu, H.; Jiang, S. *J. Nat. Prod.* **1997**, *60*, 1163−1164.
(22) Meragelman, K. M.; McKee, T. C.; Boyd, M. R. *J. Nat. Prod.* **2000**, *63*, 427−428.
(23) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(24) Bokesch, H. R.; Blunt, J. W.; Westergard, C. K.; Cardellina, J. H., II; Johnson, T. R.; Michael, J. A.; McKee, T. C.; Hollingshead, M. G.; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 633−635.

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