Calbistrin E and Two Other New Metabolites from an Australian Isolate of *Penicillium striatisporum*

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An Australian isolate of *Penicillium striatisporum* collected near Shalvey, New South Wales, exhibited selective antifungal activity against *Candida albicans* versus *Saccharomyces cerevisiae*. Bioassay-directed fractionation yielded members of the rare class of fungal metabolites known as the calbistrins. These included a new example of this structure class, calbistrin E (**1**), as well as the known polyenes calbistrin C (**2**) and deformylcalbistrin A (**3**). Also recovered from *P. striatisporum* were new triene and butenolide acids, striatisporin A (**4**) and striatisporolide A (**5**), together with the known fungal metabolites versiol (**6**) and (+)-hexylitaconic acid (**7**). Structures for all metabolites were determined by detailed spectroscopic analysis.

During a search for novel bioactive fungal metabolites we examined an unusual *Penicillium* species (MST-F9530) isolated from a construction site in the western suburbs of Sydney, Australia. Identified as *P. striatisporum,* this strain had not previously been isolated in Australia, and furthermore, published accounts of its chemistry were limited to a single 1994 Japanese patent,[1] which reported a pair of neoplasm inhibitors for which molecular structures were not assigned. It is noteworthy that during the taxonomic upheavals that have characterized the genus *Penicillium*, *P. striatisporum* was at one time regarded as a synonym for *P. restrictum*.[2] Although now designated as a distinct species,[3] *P. restrictum* has been reported to produce two unrelated classes of antifungal polyenes, the calbistrins[4] and the restricticins,[5−7] as well as azaphilone-type antibiotics with angiotensin II inhibitory activity.[8,9]

Whereas *Penicillium* species can be difficult to identify without extensive growth and media studies,[10] *P. striatisporum* can be readily differentiated by the unusual striated ornamentation on the spore surface.[11] In our hands *P. striatisporum* displayed selective antifungal activity against *Candida albicans* compared with *Saccharomyces cerevisiae,* a characteristic that proved to be media dependent and correlated with levels of secondary metabolite production. The occurrence of a rare *Penicillium* species with promising bioactivity and metabolite profiles prompted a more detailed chemical investigation.

HPLC analysis of the fermentation extract of MST-F9530 indicated the presence of an extensive family of metabolites containing a polyene moiety. The occurrence of a distinctive chromophore, together with the selective antifungal activity against *C. albicans*, was suggestive of the calbistrins, a rare class of antifungal metabolites first reported in 1993 from *P. restrictum*.[4,12] Limited to only four known examples, the calbistrins exist as pairs of *E*/*Z* isomers about $\Delta^{8',9'}$, with calbistrins A (**8**) and B (**9**) being tricyclic acetals, and calbistrins C (**2**) and D (**10**) being bicyclic reduced analogues. The calbistrins are known to be unstable to light, base, and aprotic solvents, properties that add to the challenge of isolation and characterization. For example, calbistrin A (**8**) is known to undergo a base-mediated retro-aldol degradation to yield deformylcalbistrin A (**3**).[13] The complete absolute stereochemistry of calbistrin A (**8**) (and by inference **2**, **3**, **9**, and **10**) was resolved in 1997 by total synthesis.[14] The calbistrins reportedly display potential as cholesterol-lowering agents,[15] as antifungals,[16,17] and as promoters of nerve growth factor production.[18] This current report represents the first reoccurrence of calbistrins in the primary literature since their isolation in 1993 and synthesis in 1997 and extends the scope of the calbistrin molecular motif to include a new example, calbistrin E (**1**), which is a tricyclic dehydro analogue of calbistrin A (**8**). It also describes the novel polyene diacid striatisporin A (**4**), which can be viewed as a truncated homologue of the calbistrin side chain, and the new butenolide striatisporolide A (**5**).

A solvent extract obtained from the solid phase culture of *Penicillium striatisporum* (MST-F9530), sourced from a soil sample collected near Shalvey, New South Wales, Australia, displayed potent antifungal activity against *Candida albicans* (assay titer 128) but not *Saccharomyces cerevisiae* (assay titer 0). Bioassay-directed fractionation of this extract employing C_{18} SPE and HPLC yielded the known compound calbistrin C (**2**) (LD_{99} 50 μg/mL), whereas careful analysis of LC/MS (DAD and ESI) and NMR data on associated fractions provided evidence supportive of the presence of all other known calbistrins (**8**, **9**, and **10**). Further fractionation efforts yielded pure samples of deformylcalbistrin A (**3**) as well as a new but unstable calbistrin analogue, calbistrin E (**1**). More polar fractions yielded two *calbistrin-like* components in the form of the new diacid striatisporin A (**4**) and the known polyketide versiol (**6**)[19,20] (LD_{99} 50 μg/mL), as well as the new butenolide striatisporolide A (**5**) and the known plant growth regulator (+)-hexylitaconic acid (**7**).[21]

Structures were assigned to the known compounds **2**, **3**, **6**, and **7** based on spectroscopic comparisons to literature data. Structure arguments for the new compounds **1**, **4**, and **5** are detailed below. It is worthwhile noting that prior to being isolated as versiol from *Aspergillus versicolor,*[19,20] **6** was isolated[22,23] and patented[24] as the antifungal agent LL-N313ζ from *Spororamia affinis*.

High-resolution ESI(+)MS analysis of **1** revealed a pseudomolecular ion (M + Na) supporting a molecular

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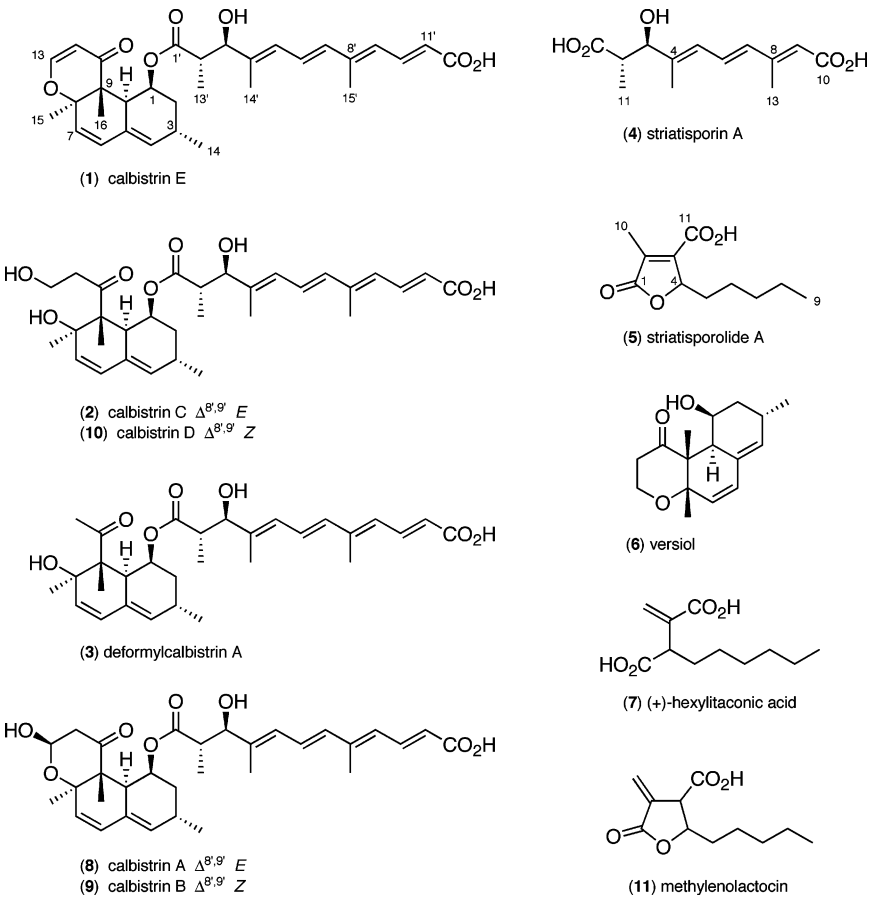
Chart 1



formula ($C_{31}H_{38}O_7$) consistent with a dehydro analogue of **8**. As would be expected, the NMR (CD_3OD) data for **1** (see Table 1) and **8** were very similar, with the obvious differences in **1** focusing around loss of the acetal methine (δ_H 5.21; δ_C 92.5) and diastereotopic C-12 methylene (δ_H 2.80 and 2.39; δ_C 48.0) so evident in **8**, and the appearance of an isolated α,β-unsaturated ketone in **1** (δ_H 7.18 and 5.24; δ_C 201.1 (C), 106.8 (CH) and 160.1 (CH)), fully consistent with the dehydro analogue structure for calbistrin E (**1**) as shown. A 2D NMR analysis of **1** (see Table S1, Supporting Information) provided further evidence for the structure assigned to **1** with a combination of COSY and gHMBC correlation sequences clearly defining (a) the side chain C-1′ to C-11′ including C-13′, C-14′, and C-15′ and (b) C-1 to C-13 and including C-14, C-15, and C-16. Note that although the ^{13}C NMR resonance for C-12′ was not observed, the molecular formula for **1**, together with H-11′ multiplicity and chemical shift, clearly support the presence of a CO_2H terminator to this side chain.

The $\Delta^{6',7'}$ and $\Delta^{10',11'}$ configuration for **1** was assigned as *E* on the basis of $J_{6',7'} = 15.2$ Hz and $J_{10',11'} = 15.0$ Hz, while the $\Delta^{4',5'}$ and $\Delta^{8',9'}$ configuration was also assigned as *E* on the basis of the ^{13}C NMR shift for C-14′ (δ_C 11.5) and C-15′ (δ_C 13.0), respectively. ^{13}C NMR comparisons for C-15′ in calbistrins A vs B, and C vs D, clearly revealed a significant *E* (δ_C 13.1) to *Z* (δ_C 21.1) chemical shift difference ($\Delta\delta_C$ 8.0),[4] consistent with theoretical considerations that require olefinic methyl carbons *Z* disposed to substituents to resonate upfield of those that are *E* disposed. Given that **1** was isolated in this study as a co-metabolite with the known metabolite **2**, and given that the complete absolute stereochemistry for **2** has been inferred by association with **8**, which has in turn been assigned by total synthesis,[14]

Table 1. NMR (CD_3OD, 400 MHz) Data for Calbistrin E (**1**)[a]

#	δ_H m, *J* (Hz)	δ_C
1	6.35, m	72.5
2a	2.25, m	36.3
2b	1.34, m	
3	2.54[e], m	28.1
4	5.77, brs	137.5
5		131.2
6	6.10[e]	131.0
7	5.71, d (9.7)	128.4
8		86.9
9		49.2[b,c]
10	2.92, m	41.5
11		201.1
12	5.24, d (6.0)	106.8
13	7.18, d (6.0)	160.1
14	1.04, d (7.2)	21.2
15	1.43, s	20.9
16	1.23, s	17.4
1′		176.4
2′	2.54[e], m	46.1
3′	4.09, d (9.7)	81.0
4′		139.9
5′	6.10[e]	129.8
6′	6.65, dd (11.0, 15.2)	130.8
7′	6.37, d (15.2)	138.5
8′		142.3[c]
9′	6.24, d (11.7)	130.8[c]
10′	7.55, dd (11.7, 15.0)	138.0
11′	5.92, d (14.8)	126.9
12′		*d*
13′	0.90, d (7.1)	14.8
14′	1.75, s	11.5
15′	2.00, s	13.0

[a] ^{13}C assignments were supported by a gHMQC experiment. [b] Signal obscured by solvent peak. [c] Assignments extracted from gHMBC experiment. [d] No assignment could be extracted for C-12′. [e] Overlapping signals.

we tentatively propose a common absolute stereochemistry for **1** on biogenetic grounds.

High-resolution ESI(+)MS analysis of striatisporin A (**4**) revealed a pseudomolecular ion (M + Na) supporting a molecular formula of $C_{13}H_{18}O_5$ requiring five DBE. Preliminary analysis of the NMR data revealed two trisubstituted (δ_H 6.18; δ_C 142.8 (C), 129.0 (CH) and δ_H 5.80; δ_C 120.3 (CH), 153.9 (C)) and one disubstituted (δ_H 6.92, 6.35; δ_C 131.1 (CH), 137.2 (CH)) olefin, assembled in a conjugated triene, together with a secondary alcohol (δ_H 2.61; δ_C 45.1 (CH)). The absence of additional oxygenated sp^3 carbons in the ^{13}C NMR data for **4** dictated that the remaining four oxygen atoms be incorporated into terminal carboxylic acid moieties. More detailed examination of the NMR data for **4** revealed considerable similarity with the ester side chain evident in all known calbistrins. For example, the ^1H and COSY NMR data for **4** revealed a structural subunit fully consistent with the calbistrin C-1′ to C-8′ subunit, including incorporation of methyl substituents at C-2′, C-4′, and C-8′ and a hydroxyl moiety at C-3′ (calbistrin numbering). Key gHMBC correlations in **4**, between both H-7 (δ_H 6.35) and H-9 (δ_H 5.80) through to C-8 (δ 153.9) (striatisporin A numbering), extended the carbon skeleton to C-9, while further correlations from H-2 (δ_H 2.61) to C-1 (δ_C 179.2), and from H-9 (δ_H 5.80) to C-10 (δ_C 170.4), completed the carbon skeleton and further defined the terminal diacid functionality.

The $\Delta^{6,7}$ configuration for striatisporin A (**4**) was established as *E* by consideration of $J_{6,7}$ = 15.1 Hz, while $\Delta^{4,5}$ and $\Delta^{8,9}$ were also assigned as *E* using the same argument as presented for calbistrin E (**1**), namely, the ^{13}C NMR shifts for C-12 (δ_C 11.8) and C-13 (δ_C 13.9). Finally, the relative stereochemistry about C-2 and C-3 in **4** was assigned as being the same as that for **1** based on comparable values for $J_{2,3}$ (**4** = 9.3 Hz, **1** = 9.7 Hz), while the absolute stereochemistry was assigned tentatively on biogenetic grounds, insofar as **4** is a co-metabolite with calbistrins of known absolute stereochemistry.

High-resolution ESI(+)MS analysis of striatisporolide A (**5**) revealed a pseudomolecular ion (M + Na) supporting a molecular formula ($C_{11}H_{16}O_4$) requiring four DBE. The ^{13}C NMR data for **5** revealed two sp^2 carboxy carbonyls (C-1 and C-11, δ_C 172.7 and 166.6) together with a fully substituted olefin (C-3 and C-2, δ_C 146.7 and 140.1), accounting for three DBE and requiring that **5** be monocyclic. The ^1H and 2D NMR data for **5** revealed an isolated olefinic methyl (C-10; δ_H 2.24; δ_C 11.1) that displayed strong gHMBC correlations to one carboxy carbon (C-1, δ_C 172.7) and both olefinic carbons and a weak correlation to the remaining carboxy carbon (C-11, δ_C 166.6). Further analysis of the ^1H and 2D NMR data for **5** revealed a spin system consistent with an *n*-hexyl chain (C-4 to C-9) terminating in a deshielded oxymethine (C-4, δ_H 5.12; δ_C 81.4), which had gHMBC correlations extending to both olefinic carbons. The 1,1-adequate NMR data for **5** confirmed placement of the olefinic methyl on the upfield olefinic carbon (C-2, δ_C 140.1) and the oxymethine adjacent to the downfield olefinic carbon (C-3, δ 146.7). These observations were only consistent with **5** being the substituted butenolide as shown. Biosynthetically, **5** can be viewed as a new cyclic analogue of the co-metabolite (+)-hexylitaconic acid (**7**) and a double-bond regioisomer of the known antitumor fungal metabolite methylenolactocin (**11**).[25] At this stage the absolute stereochemistry of **5** remains unassigned.

This investigation into an Australian isolate of *P. striatisporum* has shown the species to be a producer of unique secondary metabolites, sharing a common metabolic capability with the related species *P. restrictum*. The opportunity to reisolate and subject examples of the calbistrin structure class, together with biosynthetically related metabolites, to the same antifungal assays has provided preliminary evidence that the antifungal properties are not associated with or indeed influenced by the polyene side chain, but are limited to the bicarbocyclic subunit (i.e., versiol).

Experimental Section

General Experimental Procedures. General experimental procedures are as for previous work.[26] Unless otherwise specified, a constant level of 0.01% TFA was used in all HPLC separations.

Fungal Culture. The isolate of *Penicillium striatisporum* (MST-F9530) was isolated from a soil sample taken from a construction site in Shalvey, in western Sydney, NSW, Australia. The isolate produces white colonies with orange reverse and orange exudate. Minute monverticillate pencilli were observed as large, globose striate conidia. The culture belongs in the subgenus *Aspergilloides*, section *Exilicaulis*.

Bioassay Details. Antifungal activity was determined in an agar-based, microtiter plate assay. An aliquot of an overnight fermentation of *C. albicans* (ATCC 10231) or *S. cerevisiae* (ATCC 9763) was applied to the surface of an agar matrix that contained serial 2-fold dilutions of the test compound, which was then incubated at 28 °C. A qualitative assessment of yeast growth was made at 24 h, with the MIC determined as the lowest concentration of the test compound at which no growth was observed.

Extraction and Isolation. A solid fermentation (100 g of wheat, 21 days at 28 °C) was extracted with MeOH (500 mL). This extract was concentrated in vacuo to an aqueous residue that was diluted with H_2O to a final volume of 1 L. Passage through two parallel C_{18} SPE cartridges (2 × 10 g, Varian HF C_{18}), eluting with MeOH (2 × 40 mL), afforded an antifungal active fraction that on concentration in vacuo yielded a combined residue (1.2 g) that was subjected to preparative HPLC (single injection, 60 mL/min with gradient elution of 60% to 40% H_2O/MeCN over 20 min followed by MeCN for 10 min, through a Platinum EPS C_{18} 5 μm 50 × 100 mm column). One hundred 20 mL fractions were collected, then concentrated in vacuo and pooled into 25 fractions on the basis of analytical HPLC. Fraction 12 yielded calbistrin C (**2**) (24.6 mg, 2%), while fraction 15 was purified by isocratic semipreparative C_{18} HPLC (5 mL/min elution, 22% H_2O/MeOH (0.05% TFA), Phenomenex LUNA C_{18} 5 μm 150 × 21.2 mm column) to yield the known deformylcalbistrin A (**3**) (3.2 mg, 0.27%). Further purification of fractions 13 and 14 by semipreparative C_{18} HPLC (2.5 mL/min with gradient elution of 50% H_2O/MeCN to MeCN over 20 min, through a Phenomenex LUNA C_{18} 5 μm 250 × 10 mm column) yielded calbistrin E (**1**) (2.0 mg, 0.17%). Fraction 3 was fractionated by C_{18} SPE (10 g, stepwise gradient of 10% increments of MeCN in H_2O (0.05% TFA) with initial conditions of 80% H_2O/MeCN (0.05% TFA), followed by gradient C_{18} preparative HPLC (21.2 mL/min, gradient of 90% to 50% H_2O/MeCN over 30 min, through a Zorbax SB-C_{18} 5 μm 250 × 21.2 mm column), to yield striatisporin A (**4**) (1.8 mg, 0.15%), versiol (**6**) (2.7 mg, 0.23%), and striatisporolide A (**5**) (7.8 mg, 0.65%). Fraction 5 was purified by gradient C_8 preparative HPLC (21.2 mL/min, gradient of 75% to 65% H_2O/MeCN over 10 min then to 60% over 35 min, through a Zorbax RX-C_8 5 μm 250 × 21.2 mm column) to yield (+)-hexylitaconic acid (**7**) (3.9 mg, 0.33%). All percent yields for purified metabolites were calculated against the combined residue (1.2 g) recovered after elution of the extract from two parallel C_{18} SPE preparative cartridges.

Calbistrin E (1): yellow-orange oil; the compound degraded before optical rotation, IR, and UV measurements could be made; ^1H and ^{13}C NMR (CD_3OD, 400 MHz), see Table 1; ESI(+)MS *m/z* 545 (M + Na); ESI(−)MS *m/z* 521 (M − H); HRESI(+)MS *m/z* 545.2516 [(M + Na), $C_{31}H_{38}O_7Na$ requires 545.2515].

Calbistrin C (2): yellow-orange oil; $[\alpha]_D$ +71° (c 1.0, MeOH); UV (MeOH), ^1H and ^{13}C NMR (CD_3OD) data were in good agreement with the literature values.[4]

Deformylcalbistrin A (3): yellow-orange oil; $[\alpha]_D$ +65° (c 0.2, MeOH); UV (MeOH) and ^{1}H NMR ($CDCl_3$) data were in good agreement with the literature values.[13]

Striatisporin A (4): yellow oil; $[\alpha]_D$ −40° (c 0.15, MeOH; UV (MeOH) λ_{max} (ϵ) 295 (15450), 212 (7350) nm; ^{1}H NMR (500 MHz, CD_3OD) δ 6.92 (1H, dd, J = 11.1, 15.1 Hz, H-6), 6.35 (1H, d, J = 15.1 Hz, H-7), 6.18 (1H, d, J = 11.1 Hz, H-5), 5.80 (1H, s, H-9), 4.15 (1H, d, J = 9.3 Hz, H-3), 2.61 (1H, dq, J = 7.0, 9.3 Hz, H-2), 2.30 (3H, s, H_3-13), 1.82 (3H, s, H_3-12), 0.97 (3H, d, J = 7.0 Hz, H_3-11); ^{13}C NMR (125 MHz, CD_3OD) δ 179.2 (C, C-1), 170.4 (C, C-10), 153.9 (C, C-8), 142.8 (C, C-4), 137.2 (CH, C-7), 131.1 (CH, C-6), 129.0 (CH, C-5), 120.3 (CH, C-9), 80.8 (CH, C-3), 45.1 (CH, C-2), 14.7 (CH_3, C-11), 13.9 (CH_3, C-13), 11.8 (CH_3, C-12); ESI(+)MS *m/z* 277 (M + Na); HRESI-(+)MS *m/z* 277.1049 [(M + Na), $C_{13}H_{18}O_5Na$ requires 277.1052].

Striatisporolide A (5): pale yellow amorphous solid; $[\alpha]_D$ −24° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (ϵ) 221 (10850) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.12 (1H, brd, J = 7.9 Hz, H-4), 2.24 (3H, s, H_3-10), 2.12 (1H, m, H-5$_a$), 1.60 (1H, m, H-5$_b$), 1.40 (2H, m, H-6), 1.30 (4H, m, H-7, H-8), 0.87 (3H, t, J = 6.5 Hz, H_3-9); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7 (C, C-1), 166.6 (C, C-11), 146.7 (C, C-3), 140.1 (C, C-2), 81.4 (CH, C-4), 32.7 (CH_2, C-5), 31.3 (CH_2, C-7), 24.4 (CH_2, C-6), 22.4 (CH_2, C-8), 13.9 (CH_3, C-9), 11.1 (CH_3, C-10); ESI(+)MS *m/z* 235 (M + Na); HRESI(+)MS *m/z* 235.0942 [(M + Na), $C_{11}H_{16}O_4Na$ requires 235.0946].

Versiol (6): yellow oil; $[\alpha]_D$ −43° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 240 (13150); HRESI(+)MS *m/z* 285.1465 (M + Na, Δmmu = 0.2); ^{1}H and ^{13}C NMR ($CDCl_3$) data were in good agreement with the literature values.[27]

(+)-Hexylitaconic acid (7): orange oil; $[\alpha]_D$ +11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 207 (5630); HRESI(+)MS *m/z* 237.1109 (M + Na, Δmmu = 0.6); ^{1}H and ^{13}C NMR ($CDCl_3$) data were in good agreement with literature values.[21]

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Supporting Information Available: 2D NMR data for calbistrin E (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Morino, T.; Nakamura, K.; Nishikawa, K.; Shimada, K.; Nakagawa, T.; Saito, S. Application: JP Patent JP6172374, 1994
(2) Pitt, J. I. *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*; Academic Press: London, 1979; vi, 634 pp.
(3) Pitt, J. I.; Samson, R. A.; Ahti, T.; Farjon, A.; Landolt, E. *Names in Current Use in the Families Trichocomaceae, Cladonoaceae, Pinaceae and Lemnaceae*; Published for the International Association for Plant Taxonomy by Koeltz Scientific Books: D-61453 Konigstein, Germany, 1993.
(4) Brill, G. M.; Chen, R. H.; Rasmussen, R. R.; Whittern, D. N.; McAlpine, J. B. *J. Antibiot.* **1993**, *46*, 39−47.
(5) Schwartz, R. E.; Dufresne, C.; Flor, J. E.; Kempf, A. J.; Wilson, K. E.; Lam, T.; Onishi, J.; Milligan, J.; Fromtling, R. A.; Abruzzo, G. K. *J. Antibiot.* **1991**, *44*, 463−471.
(6) Hensens, O. D.; Wichmann, C. F.; Liesch, J. M.; VanMiddlesworth, F. L.; Wilson, K. E.; Schwartz, R. E. *Tetrahedron* **1991**, *47*, 3915−3924.
(7) Hensens, O. D.; Liesch, J. M.; Milligan, J. A.; Del Val, S. M.; Schwartz, R. E.; Wichmann, C. US Patent 4952604, 1990
(8) Ishimaru, T.; Harada, S.; Tsuboya, S. Jp Patent 05041994, 1993
(9) Kaneda, K.; Yamauchi, J.; Inagaki, T.; Senba, Y.; Kojima, N. Jp Patent 06080659, 1994
(10) Pitt, J. I.; Research., C. D. o. F. *A Laboratory Guide to Common Penicillium species*; C.S.I.R.O. Division of Food Research: North Ryde, N.S.W., 1985; 184 pp.
(11) Stolk, A. C. *Antonie Van Leeuwenhoek* **1969**, *35*, 261−274.
(12) Jackson, M.; Karwowski, J. P.; Humphrey, P. E.; Kohl, W. L.; Barlow, G. J.; Tanaka, S. K. *J. Antibiot.* **1993**, *46*, 34−38.
(13) Horn, W. S.; Bierilo, K. K.; Bills, G. F.; Dombrowski, A. W.; Helms, G. L.; Jones, E. T.; Linemeyer, D. L.; Sesin, D. F.; Schwartz, R. E.; Wilson, K. E. *J. Nat. Prod.* **1993**, *56*, 1779−1785.
(14) Tatsuta, K.; Itoh, M.; Hirama, R.; Araki, N.; Kitagawa, M. *Tetrahedron Lett.* **1997**, *38*, 583−586.
(15) Helms, G. L.; Linemeyer, D. L.; Horn, W. S.; Dombrowski, A. W.; Jones, E. T. T.; Koupal, L.; Bartizal, K. F.; Rozdilsky, W. Ep Patent 505135, 1992
(16) Agematu, H.; Watanabe, Y.; Chiba, H.; Kaneto, R.; Shibamoto, N.; Yoshioka, T.; Kumamoto, T.; Nishida, H.; Okamoto, R. Ep Patent 514884, 1992
(17) Brill, G. M.; Burres, N. S.; Chen, R. H.; Humphrey, P. E.; McAlpine; Rasmussen, R. R. Patent WO 93/21770, 1993
(18) Nomura, K.; Mizogami, K.; Mizobe, F.; Ito, M.; Hanada, K. Jp Patent 05032656, 1993
(19) Fukuyama, K.; Tsukihara, T.; Katsube, Y.; Hamasaki, T.; Hatsuda, Y. *Tetrahedron Lett.* **1976**, *17*, 189−190.
(20) Fukuyama, K.; Katsube, Y.; Hamasaki, T.; Hatsuda, Y. *J. Chem. Soc., Perkin Trans. 2* **1978**, 683−686.
(21) Isogai, A.; Washizu, M.; Kondo, K.; Murakoshi, S.; Suzuki, A. *Agr. Biol. Chem.* **1984**, *48*, 2607−2609.
(22) McGahren, W. J.; Ellestad, G. A.; Lancaster, J. E.; Morton, G. O.; Kunstmann, M. P. *J. Am. Chem. Soc.* **1974**, *96*, 1616−1617.
(23) McGahren, W. J.; Ellestad, G. A.; Morton, G. O.; Kunstmann, M. P. *J. Org. Chem.* **1976**, *41*, 66−71.
(24) McGahren, W. J. US Patent 3914317, 1975
(25) Park, B. K.; Nakagawa, M.; Hirota, A.; Nakayama, M. *J. Antibiot.* **1988**, *41*, 751−758.
(26) Capon, R. J.; Ratnayake, R.; Stewart, M.; Lacey, E.; Tennant, S.; Gill, J. H. *Org. Biomol. Chem.* **2005**, *3*, 123−129.
(27) Fujii, Y.; Asahara, M.; Ichinoe, M.; Nakajima, H. *Phytochemistry* **2002**, *60*, 703−708.

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