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Chloro Polyketides from the Cultured Fungus (*Aspergillus*) Separated from a Marine Sponge

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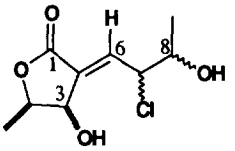
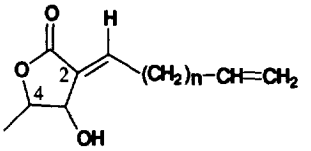
Abstract: The salt water culture of *Aspergillus ?ochraceus* separated from the Indo-Pacific sponge *Jaspis* cf. *coriacea* has yielded two new chlorine containing polyketides, chlorocarolide A (**1a**) and B (**1b**). These compounds have an overall structural analogy to carolic acid whose biosynthesis has been intensely studied. The structures and stereochemical features of the chlorocarolides are reported. Also noted are the similarities of their α-methylene-γ-butyrolactones to those of the litsenolides and mahubenolides, both derived from plants. Copyright © 1996 Elsevier Science Ltd

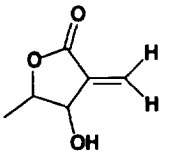
Filamentous fungi have only recently been recognized as regularly occurring on coral reefs.[1,2] The surfaces of submerged wood, algae and nutrient rich sediment are excellent sources for isolating fungi,[3] yet only modest attention has been devoted toward obtaining compounds from the cultures of such fungi. Sponges represent another source for isolating fungi, but the biological literature contains very few descriptions.[4] Nonetheless we have experienced considerable initial success in obtaining fungi from the tissue of sponges, including species that are chemically prolific. New compounds characterized by our laboratory from fungal fermentation broths include chlorinated sesquiterpenes, the chloriolins, from cultures of a fungus derived from *Jaspis* cf. *johnstoni*,[5] and oxygenated polyketides, the nectriapyrones, separated from a *Stylotella* sp. sponge.[6] Four recent reviews have amply covered the very small numbers of other metabolites derived from cultured fungi obtained from other marine substrates.[7] Additional highlights of recent work on this subject have also appeared.[8] Here we describe two new diastereomeric halogenated polyketides named the chlorocarolides. They were obtained from the salt water culture of *Aspergillus ?ochraceus* derived from SCUBA collections of a chemically prolific Indo-Pacific sponge, *Jaspis* cf. *coriacea*.

The sponge used here, while not extremely common, can be found throughout the Indo-Pacific and is a source of ketide-amino acids including the bengamides[9] and bengazoles[10]. After repeated attempts we were finally successful in generating fungal cultures from this organism. The initial fungus culture (id. no. 941026) was obtained by placing the sponge on a solid agar plate[11] under sterile conditions. The EtOAc extracts from four liters of liquid broth,[12] filtered from the mycelium, were concentrated to give an oil (2.1 g) which was subjected to normal phase HPLC (5% MeOH - CH_2Cl_2) and yielded chlorocarolide A (**1a**) (20 mg) and B (**1b**) (20 mg) whose identical molecular formulae were established as $C_9H_{13}O_4Cl$ by a HRFABMS $[M+H]^+$ peak: **1a** *m/z* = 221.0582 (Δ0.1 mmu of calcd.); and **1b** *m/z* = 221.0580 (Δ0.1 mmu of calcd.).

The properties of chlorocarolide A (**1a**)[13] were investigated first. An NMR APT formula of $C_9H_{11}O_2$ was established from the resonances observed for two methyls, a trisubstituted vinyl, four sp^3 methines, and an ester carbonyl. Collectively these data accounted for two of the degrees of unsaturation indicating the presence of one ring. The extremely low-field shift of the vinyl proton (δ 7.51) suggested a conjugated unsaturated ester, while the four methine protons also appeared at relatively low field (between δ 4-5) intimating they were attached to hetero atoms. Two exchangeable alcohol protons (δ 3.24, OH3) and (δ 2.28 OH8) were also identified. The ^1H- ^1H COSY NMR spectrum revealed two separate spin systems consisting of H6-H7-H8(OH8)-Me9 and H3(OH3)-H4-Me5. Two working structures were possible for this data: an α-methylene-γ-butyrolactone with a 2-chloro-3-hydroxy propyl side chain (as in **1**) or a 5,6-dihydro-α-pyrone with a 1,2 dihydroxypropyl attached at C3. Unfortunately the HMBC 2D NMR data obtained did not discriminate







1a† 7*R** 8*R** litsenolide A (**2**), 3*S*, 4*R* (n = 9) **5a** 3*R** 4*R**

1b† 7*S** 8*S** isodihydromahubenolide A (**3**) 3*R*, 4*S* (n = 13) **5b** 3*S** 4*R**

† Can be switched. isodihydromahubenolide B (**4**) 3*S*, 4*S* (n = 13)

between these possibilities. However, the carbonyl shift in **1a** of δ 172.0 was consistent with the data for butyrolactone models.[14] Finally, an *E* double bond geometry was determined by comparing the observed position of H6 (δ 7.51) to calculated data (E_{calc} δ 7.05, Z_{calc} δ 6.43).

The initial fermentation broths were highly enriched in isomer **1a** while later ones yielded equal mixtures of diastereomers, **1a**[13] and **1b**[15] by HPLC. The spectral properties of these compounds were parallel which indicated identical gross structures. The relative stereochemistry at the four chiral centers of both chlorocarolide diastereomers was determined by comparing experimental ^3J's versus values calculated[16] for the eight stereoisomeric possibilities collected in Table 1. The spread in J_{calc} data can be used to assign the *erythro* — *threo* relationship in **1** using: *erythro* C3-C4 J_{calc} = 3.5 - 4.2 Hz; *threo* C3-C4 J_{calc} = 1.0 - 1.6 Hz; *threo* C7-C8 J_{calc} = 3.7 - 3.9 Hz; *erythro* C7-C8 J_{calc} = 1.8 - 2.4 Hz. Both chlorocarolides A and B possess *erythro* C3-C4 and *threo* C7-C8 configurations which is justified by measured $J_{3,4}/J_{7,8}$ (Hz) of 4.5/4.5 for A and 4.0/4.0 for B outlined in Table 2. Furthermore, among the eight diastereomers summarized in Table 1. only the diastereomers of **1a** and **1b** have the dual *erythro* C3-C4 and *threo* C7-C8 configurations required by the NMR *J* values.

Table 1. Calculated ^3J values for the eight stereoisomeric possibilities of **1**.

	Relative stereochemistry				Calcd. (Hz)		Calcd. (Kcal/mol)
Compound	C3	C4	C7	C8	$^3J_{3-4}$	$^3J_{7-8}$	Energy
1a	*R**	*R**	*R**	*R**	3.81	3.87	9.69
1b	*R**	*R**	*S**	*S**	3.47	3.91	10.42
1c	*R**	*R**	*R**	*S**	4.22	2.25	12.96
1d	*R**	*R**	*S**	*R**	3.66	2.40	12.97
1e	*S**	*R**	*R**	*R**	1.15	3.90	9.82
1f	*S**	*R**	*S**	*S**	1.39	3.73	9.28
1g	*S**	*R**	*R**	*S**	1.64	1.87	12.32
1h	*S**	*R**	*S**	*R**	1.02	1.80	11.14

Table 2. Experimental NMR *J*'s (Hz).

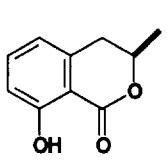
Compound	C3	C4	C7	C8	$^3J_{3-4}$	$^3J_{7-8}$	Reference
1a†	*R**	*R**	*R**	*R**	4.5	4.5	This work
1b†	*R**	*R**	*S**	*S**	4.0	4.0	This work
2	*S*	*R*	-	-	2.0	-	18
3	*R*	*S*	-	-	2.5	-	20
4	*S*	*S*	-	-	5.0	-	20
5a	*R**	*R**	-	-	≈5	-	21
5b	*S**	*R**	-	-	≈2	-	21

† Can be switched.

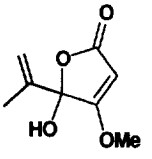
A literature search provided additional α-methylene-γ-butyrolactones for comparison with our experimental data. Particularly relevant are the litsenolides, such as A_2 (**2**), from the roots of *Litsea japonica*;[17,18] the mahubenolides, exemplified by compounds **3** and **4** from the trunk wood of *Clinostemon*

mahuba,[19] and synthetic β-hydroxy-α-methylene-γ-butyrolactones including **5a** and **5b**.[20] The absolute stereochemistry of 3*S*,4*R*-litsenolide A_2 (**2**) has been reported.[17] The $J_{3,4}$ of 2.0 Hz reported in **2** is consistent with its known *erythro* C3-C4 stereochemistry. Likewise, the values of $J_{3,4}$ shown in Table 2. are diagnostic of the relative C3/C4 stereochemistry in the other related known compounds **3** - **5**. Based on a biogenetic assumption we tentatively conclude that the stereochemical difference between **1a** and **1b** occurs at C7/C8.

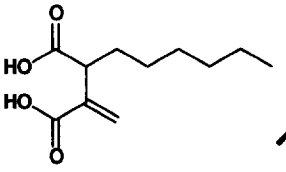
Several other polyketides were isolated from our salt water culture broths in addition to **1a** and **1b**. These include known compounds (-)-(*R*)-mellein[21] (**6**), penicillic acid[21] (**7**), and hexylitaconic acid (**8**)[22]. The configuration of our mellein sample was determined as *R* by comparing the sign of rotation ($[\alpha]_D$ = -41°, c=1.32, MeOH) to the literature data.[21] It is noteworthy that compounds **6** - **8** have been isolated from the terrestrial fungus *A. ochraceus*. Both enantiomers of mellein have been obtained from terrestrial fungi and the (+) form recently reported from the marine fungus *Helicascus kanaloanus*.[23] The reculture of the fungus in malt extract distilled water broths produced mellein (**6**) and penicillic acid (**7**), while no chlorocarolides (**1**) or hexylitaconic acid (**8**) were detected.



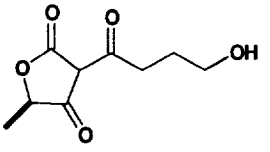
(-)-*R*-mellein (**6**)



penicillic acid (**7**)



hexylitaconic acid (**8**)



(*R*)-carolic acid (**9**)

An appealing structural analogy is evident between the chlorocarolides (**1a**, **1b**) and carolic acid (**9**),[24] a tetronic acid derived from *Penicillium charlessi*. These C_9 compounds have a chain of six contiguous carbons with a C_3 branch at C2, and this parallelism was the basis for the names given to **1**. The pathway for the biosynthesis of **9** has been experimentally shown to involve the condensation of a C_4-dicarboxylic acid, such as oxaloacetate, and a C_6 polyketide, such as a β-ketocaproyl CoA. The resultant C_{10}-intermediate undergoes cyclization by decarboxylation and loss of CoA to produce carolic acid.[24] In the future we plan to initiate experiments designed to explore the extent to which such events are relevant in the biosynthesis of the chlorocarolides. It is also perhaps relevant, that hexylitaconic acid is also an oxaloacetate – β-ketocaprylyl CoA condensation product.[25] In closing we can note that our results differ considerably from the alkaloids recently reported from either *A. ochraceus*[26] or from the sponge source of our fungus *Jaspis coriacea*[9,10]. The new compounds reported above constitute the first example of a chlorinated polyketide to be observed from a marine derived fungus. Chlorine-containing polyketides were hardly known from terrestrial fungi prior to 1990[27,28] and overall the list of fungal products with a chlorine attached to a chiral carbon is very short.[27,29]

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11. Plates made up with 1 g yeast extract, 10 g cellulose, 15 g agar, 100 mg penicillin G, 100 mg streptomycin, 1L 0.2 μm filtered Monterey Bay sea water.
12. Sea water malt media: 15 g/L of malt extract in 0.2 μm filtered Monterey Bay sea water. The broth (8L) was grown on a rotary shaker (120 rpm) at 27°C for 21 days and then harvested. EtOAc extracts were concentrated and partitioned between hexanes and 10% aq. MeOH and then between CH_2Cl_2 and 50% aq. MeOH. The CH_2Cl_2 fractions were purified by sephadex column chromatography and HPLC using 5% MeOH - CH_2Cl_2.
13. Chlorocarolide A (**1a**): Yellow oil; $[\alpha]_D$ = + 78° (c = 2.5, CH_2Cl_2); Assignments based on 2D NMR. ^{13}C NMR (125 MHz) $CDCl_3$ δ 172.0 s (C1); 148.9 d (C6); 134.1 s (C2); 85.5 d (C7); 71.3 d (C3); 67.9 d (C8); 59.2 d (C4); 19.3 q (Me9); 18.9 q (Me5). ^1H NMR (500 MHz) $CDCl_3$ δ 7.51 t, *J* = 1.5 Hz (H6); 4.95 dt, *J* = 4.5, 1.5 Hz (H7); 4.68 br s (H3), 4.46 dq, *J* = 4.5, 7.0 Hz (H4); 4.11 m (H8); 3.24 br s (OH3); 2.28 br s (OH8); 1.48 d, *J* = 7.0 Hz (H_35); 1.34 d, *J* = 6.5 Hz (H_39).
14. Model compound data from Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^1H FT NMR Spectra*, Aldrich Chemical Co., Vol. 1, 1993 has ^{13}C carbonyl shifts as follows: α-methylene-γ-butyrolactone (#1131C) δ 170.7; 2(5*H*)-furanone (#1146C) δ 173.8; 5,6-dihydro-2*H*-pyran-2-one (#1156C) δ 163.7.
15. Chlorocarolide (**1b**): Yellow oil; $[\alpha]_D$ = + 29° (c = 2.2, CH_2Cl_2); ^{13}C NMR (125 MHz) $CDCl_3$ δ 171.8 s (C1); 150.7 d (C6); 133.7 s (C2); 85.6 d (C7); 70.2 d (C3); 67.5 d (C8); 57.6 d (C4); 19.4 q (Me9); 18.9 q (Me5). ^1H NMR (500 MHz) $CDCl_3$ δ 7.62 t, *J* = 1.0 Hz (H6); 4.97 dt, *J* = 4.0, 1.5 Hz (H7); 4.75 dt, *J* = 4.0, 1.5 Hz (H3); 4.30 m (H4); 4.12 m (H8); 2.47 br s (OH); 2.17 br s (OH); 1.34 d, *J* = 6.5 Hz (H_35); 1.25 d, *J* = 7.0 Hz (H_39).
16. MMX-calculations were carried out using the PCMODEL (Version 2.0) program of K.E. Gilbert and J.J. Gajewski available from Serena Software, Bloomington, IN, USA.
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