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Maculalactone L and three halogenated carbazole alkaloids from *Kyrtuthrix maculans*

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Abstract

Maculalactone L, a novel tribenzylbutyrolactone secondary metabolite has been obtained from one colony of the epilithic encrusting cyanobacterium *Kyrtuthrix maculans*, during the course of a wide-ranging survey of *Kyrtuthrix* on Hong Kong shores. Maculalactone L may be derived from maculalactone A, described previously from this species, by oxidation reactions. Three halogenated carbazole alkaloids are also described. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Kyrtuthrix maculans*; Cyanobacterium; Maculalactone L; Tribenzylbutyrolactone; Halogenated carbazole alkaloid; Oxidation

1. Introduction

The marine heterocystous cyanobacterium, *Kyrtuthrix maculans* Umezaki (Stigonemataceae) forms a conspicuous blackish-green crust in the high eulittoral of moderately exposed rocky shores in Hong Kong (Kaehler, 1994; Kaehler & Williams, 1996; Nagarkar, 1996; Williams, 1993). A variety of ecological roles have been proposed for many secondary metabolites produced by marine organisms (Hay, Duffy, Pfister & Fenical, 1987; Hay, Kappel & Fenical, 1994) and, in this respect, the chemistry of *K. maculans* is of great interest as compounds produced by this species appear to deter predation by molluscan herbivores (Lee, unpubl. data) and also to cause mortality of barnacle larvae (*Tetraclita* spp. Lee & Chan, unpubl. data). Two novel structural classes of aromatic compounds have been isolated from this species: the tribenzylbutyrolactones, as exemplified by maculalactone A–maculalactone C (Lee & Brown, 1998; Tsui, Williams & Brown, 1996), and the dibenzyldiphenyl-4,5,6,7-tetrahydrobenzofuranones (Lee & Brown, 1998), as exemplified by maculalactone D–maculalactone K.

During the course of an extensive investigation to determine the ecological significance of these compounds, involving quantification of these metabolites from samples collected from a variety of locations around Hong Kong, we consistently noticed an unexpected peak in the gradient HPLC chromatogram of samples from one site, which was entirely absent from other sites. The structural elucidation of this novel compound, maculalactone L, and of three alkaloids also isolated by this technique, is described herein.

2. Results and discussion

Extraction of a *K. maculans* thallus from shores of Ping Chau, Hong Kong with dichloromethane followed by purification by gradient HPLC has yielded the new compound maculalactone L (**1**). The molecular formula of **1** was established as $C_{25}H_{22}O_3$ by high-resolution mass spectrometry. Infrared spectroscopy indicated the presence of a hydroxyl group (3544

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Table 1
^1H and ^{13}C NMR data for compound **1**

Assignment	$^{13}C^a$ (δ mult.)	$^1H^a$ (δ)	Two- and three-bond correlations[b] from ^{13}C to ^1H at:	^1H correlations[c] with ^1H at:
1	177.95 C	–	5.19, 3.15	–
2	76.83 C	–	6.45, 3.15	–
3	135.95 C	–	6.45, 5.19, 3.15	–
4	82.76 CH	5.19	6.45, 2.24, 1.83	6.45, 2.24, 1.83
1′	40.90 CH_2	3.15, 3.15	6.96	–
2′	134.11 C	–	7.27, 3.15	–
3′=7′	130.59 CH	6.96	7.25, 6.96, 3.15	7.27
4′=6′	128.54 CH	7.27	7.27	7.25, 6.96
5′	127.66 CH	7.25	6.96	7.27
1″	131.22 CH	6.45	7.77, 5.19	5.19
2″	134.44 C	–	7.43	–
3″=7″	130.04 CH	7.77	7.77, 7.36, 6.45	7.43
4″=6″	128.59 CH	7.43	7.43	7.77, 7.36
5″	128.49 CH	7.36	7.77	7.43
1‴	41.29 CH_2	2.24, 1.83	7.00	5.19
2‴	136.12 C	–	7.27, 2.24, 1.83	–
3‴=7‴	129.60 CH	7.00	7.23, 7.00, 2.24, 1.83	7.27
4‴=6‴	128.54 CH	7.27	7.27	7.23, 7.00
5‴	126.96 CH	7.23	7.00	7.27

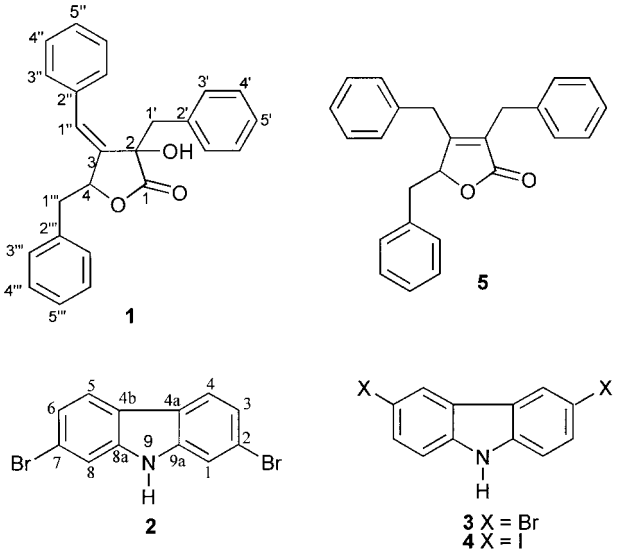
[a] ^{13}C connected to ^1H by a single bond determined from HSQC.
[b] Two- and three-bond correlations determined from HMBC.
[c] ^1H–^1H correlations determined from ^1H–^1H COSY.

cm^{-1}) and a carbonyl group (1772 cm^{-1}), both of which were confirmed by the results of ^{13}C NMR/DEPT spectroscopy (δ_C 76.83 ppm C and δ_C 177.95 ppm C respectively). The relatively upfield chemical shift for this carbonyl group and the presence of a second oxygenated carbon in the ^{13}C NMR spectrum (δ_C 82.76 ppm CH) suggested that the carbonyl resonance was actually part of a lactone group. Twelve aromatic signals were also resolved by ^{13}C NMR — six of which were methine carbons of double intensity (δ_C 130.59, 130.04, 129.60, 128.59, 128.54, 128.54) —suggestive of the presence of three mono-substituted benzene rings in **1**. Further analysis of two-dimensional NMR spectra of **1** (HSQC, HMBC and ^1H–^1H COSY — see Table 1), revealed a modified tribenzylbutyrolactone skeleton (Tsui et al., 1996) in which the double bond had shifted to the 1″–3 position and a hydroxyl group had been introduced at C-2. The *Z*-stereochemistry of the exocyclic double bond was established from NOESY correlations observed between H-1″ and H-1‴ and between H-3″/H-7″ and H-1′.

Gradient HPLC of the same extract also yielded three other UV-active components, which also gave resonances in the aromatic region of the ^1H NMR spectrum, and were proved to be the regioisomeric dibromocarbazole alkaloids **2** and **3** and diiodocarbazole alkaloid **4** by 2D-NMR as above. Although each of the alkaloids **2–4** have been produced previously by organic synthesis (Desbene-Monvernay, Karazoun, Berthelot & Desbene, 1992; Erra-Balsells, 1988; Kovaleva, Reutova, Sarycheva, Sheludyakov, Novikov & Filiminov, 1988; Polivin et al., 1993; Sharma & Sarita, 1988; Smith, James, Mistry, Bye & Faulkner, 1992; Yamato et al., 1991) and 3,6-dibromocarbazole (**3**) is commercially available (*ex*. Aldrich) this is apparently the first report of their occurrence as natural products. Alkaloids are not commonly encountered in marine algae (Hay & Fenical, 1988; Konig & Wright, 1993) and the isolation of three alkaloids from *Kyrtuthrix* may be related to the nitrogen-fixing ability of this species. Chlorine-containing alkaloids which incorporate a carbazole substructure have been reported previously from the cyanobacteria *Tolypothrix tjipanasensis* (Bonjouklian et al., 1991) and *Hyella caespitosa* (Beccalli, Marchesini & Pilati, 1994; Cardellina, Kirkup, Moore, Mynderse, Seff & Simmons, 1979). We are aware of only one report for an alkaloid natural product of related structure which contained bromine (Lam, Schroeder, Veitch, Matson & Forenza, 1991) and of no carbazole-type alkaloids which incorporated iodine.

Compound **1** was present in moderate amounts in *K. maculans* from Ping Chau, but entirely absent from this species collected from Hong Kong shores at several other locations. The major component of the extract from Ping Chau and all other sites studied in this survey was the tribenzylbutyrolactone, maculalactone A (**5**) (Tsui et al., 1996); variable amounts of maculalactones B–K (Lee & Brown, 1998) were also found as minor components from all sites studied. Maculalactones A and L are clearly biogenetically related and one can propose that oxidation of the double

bond in the butyrolactone ring of maculalactone A yields maculalactone L in which this double bond has shifted and a new oxygen atom has been introduced at the 2-position.



3. Experimental

3.1. General

Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. All NMR experiments were run on a Bruker DRX 500 instrument with $CDCl_3$ as solvent. HSQC and HMBC experiments were recorded with 2048 data points in F_2 and 128 data points in F_1. EIMS were recorded at 70 eV on a Finnigan-MAT 95 MS spectrometer; FTIR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8201 PC instrument. TLC plates were developed using *p*-anisaldehyde. HPLC separations were performed using a PREP-SIL 20 mm × 25 cm column, flow rate 8 ml/min, operating with a solvent gradient 18% ethyl acetate hexane → 30% ethyl acetate hexane (90 min).

K. maculans was collected from shorelines of Ping Chau, Hong Kong (22°24′N; 114°40′E; 1.5 m above C.D.). Taxonomic verification was made by Dr. S. Nagarkar (HKU) and a type specimen of *K. maculans* (SML.B20, SML.B21) is available at the Swire Institute of Marine Science of The University of Hong Kong. The sample was air-dried to constant weight (45.1 g) then ground to a fine powder under liq. N_2 and immediately extracted with CH_2Cl_2. The organic extract was then dried and evapd. under red. pres. to yield a green solid (0.379 g; 0.84 % wt/dry wt) which was subjected to gradient HPLC: **1** (6 mg); **2** (4 mg); **3** (4 mg); **4** (3 mg).

3.2. Maculalactone L (*1*)

$[\alpha]_D$ +12.5° ($CHCl_3$, *c* 0.04); HREIMS *m/z* (rel. int.): 370.1559 ([M^+], calc 370.1569 for $C_{25}H_{22}O_3$) (6), 279 (60) [M^+-$C_6H_5CH_2$], 261 (25), 233 (57), 215 (29), 205 (18), 202 (9), 155 (24), 91 (100). IR ν_{max} $CHCl_3$ cm^{-1}: 3544, 2928, 1772. ^{1}H NMR: δ 7.77 (2H, d, J = 8.2 Hz), 7.43 (2H, dd, J = 8.2, 8.2 Hz), 7.36 (1H, dd, J = 8.2, 8.2 Hz), 7.27 (4H, dd, J = 8.2, 8.2 Hz), 7.25 (1H, dd, J = 8.2, 8.2 Hz), 7.23 (1H, dd, J = 8.2, 8.2 Hz), 7.00 (2H, d, J = 8.2 Hz), 6.96 (2H, d, J = 8.2 Hz), 6.45 (1H, d, J = 1.8 Hz), 5.19 (1H, ddd, J=8.3, 5.2, 1.8 Hz), 3.15 (2H, s), 3.07 (1H, br s, —OH), 2.24 (1H, dd, J = 14.2, 5.2 Hz), 1.83 (1H, dd, J = 14.2, 8.3 Hz). ^{13}C NMR/DEPT – see Table 1. A NOESY experiment showed a strong correlation between δ_H 6.45 (H-1″) and δ_H 7.77 (H-3″/H-7″) and δ_H 2.24 (H-1‴); and also between δ_H 7.77 (H-3″/H-7″) and δ_H 7.43 (H-4″/H-6″), δ_H 6.45 (H-1″) and δ_H 3.15 (H-1′).

3.3. 2,7-Dibromocarbazole (*2*)

HREIMS *m/z* (rel. int.): 327/325/323 ([M^+], 322.8939, calc 322.8945 for $C_{12}H_7NBr_2$) (48/100/49), 246/244 [M^+-Br] (16/16), 165 [M^+-Br_2] (19), 164 (16). IR ν_{max} $CHCl_3$ cm^{-1}: 3245 (br), 3056, 2969, 1585, 1482, 1442, 1311, 1209, 1167, 1137, 1094, 854, 745, 693. ^{1}H NMR: δ 8.08 (1H, br s, H-9), 7.87 (2H, d, J=8.3 Hz, H-4/5), 7.57 (2H, d, J = 1.5 Hz, H-1/8), 7.36 (2H, dd, J = 8.3, 1.5 Hz, H-3/6). ^{13}C NMR/DEPT: δ 140.4 C (C-8a/9a), 123.4 CH (C-3/6), 121.9 C (C-4a/4b), 121.5 CH (C-4/5), 119.8 C (C-2/7), 113.9 CH (C-1/8).

3.4. 3,6-Dibromocarbazole (*3*)

HREIMS *m/z* (rel. int.): 327/325/323 ([M^+], 322.8938, calc 322.8945 for $C_{12}H_7NBr_2$) (48/100/51), 246/244 [M^+-Br] (25/26), 165 [M^+-Br_2] (26), 164 (21). ^{1}H NMR: δ 8.12 (2H, d, J = 1.9 Hz, H-4/5), 8.11 (1H, br s, H-9), 7.51 (2H, dd, J = 8.6, 1.9 Hz, H-2/7), 7.30 (2H, d, J = 8.6 Hz, H-1/8). ^{13}C NMR/DEPT ($CDCl_3$): δ 138.5 C (C-8a/9a), 129.4 CH (C-2/7), 124.2 C (C-4a/4b), 123.3 CH (C-4/5), 112.7 C (C-3/6), 112.2 CH (C-1/8).

3.5. 3,6-Diiodocarbazole (*4*)

HREIMS *m/z* (rel. int.): 418.8671 ([M^+], calc 418.8668 for $C_{12}H_7NI_2$) (100), 292 [M^+-I] (21), 165 [M^+-I_2] (12), 164 (13). ^{1}H NMR: δ 8.33 (2H, d, J = 1.6 Hz, H-4/5), 8.12 (1H, br s, H-9), 7.68 (2H, dd, J = 8.5, 1.6 Hz, H-2/7), 7.21 (2H, d, J = 8.5 Hz, H-1/8). ^{13}C NMR/DEPT ($CDCl_3$): δ 138.6 C (C-8a/9a),

134.9 CH (C-2/7), 129.5 CH (C-4/5), 124.7 C (C-4a/4b), 112.7 CH (C-1/8), 82.5 C (C-3/6).

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