

Biomimetic Synthesis of Thiaplidiaquinones A and B

Iman M. Khalil, David Barker, and Brent R. Copp*

School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

Supporting Information



ABSTRACT: A biomimetic synthesis of the biologically active ascidian metabolites thiaplidiaquinones A and B is described. Reaction of geranylbenzoquinone with Et_3N in CH_2Cl_2 yielded two isomeric quinones, comprising the benzo[*c*]chromene-7,10dione core of the natural products. Subsequent reaction with hypotaurine yielded the title compounds and their dioxothiazino regioisomers.

While the majority of metabolites biosynthesized by ascidians are alkaloids or peptide related,¹ ascidians of the genus *Aplidium* are known as a rich source of prenylated quinone and hydroquinone natural products.² Derived from geranylated or farnesylated hydro- or benzoquinone (e.g., 1),³ these typically bioactive metabolites can embody intramolecular (e.g., conicol, 2^4) or intermolecular ring closures (e.g., longithorone A, 3^5), yielding complex architectural scaffolds.



More recently, meroterpenoids bearing a benzo[c]chromene-7,10-dione skeleton have been reported from geographically remote species of *Aplidium* ascidians. In 2005, Fattorusso's group isolated thiaplidiaquinones A and B (**4**, **5**) from a Mediterranean ascidian, *Aplidium conicum*, determining that both natural products induced apoptosis in Jurkat cells by a mechanism involving the intracellular production of reactive oxygen species.⁶ In contrast, the related metabolite scabellone B (**6**), isolated from a New Zealand collection of *Aplidium scabellum*, was found to be a relatively nontoxic antimalarial lead compound.⁷ As part of our interest in exploring the structure–activity relationships of benzo[c]chromene-7,10dione natural products,⁸ herein we report a biomimetic synthesis of both thiaplidiaquinones A (4) and B (5) and their anticipated natural product dioxothiazine regioisomers. While this paper was in preparation, Carbone et al. reported a synthesis of thiaplidiaquinone A.⁹



We speculated that the biosynthetic origin of the thiaplidiaquinones could stem from hypotaurine addition to one of two tricyclic pyranoquinones (7, 8), in turn derived from oxa- 6π electrocyclization¹⁰ of *ortho*-quinone methide tautomers (9, 10) of bis-benzoquinones (11, 12) (Scheme 1).

However, while Carbone et al. constructed bis-benzoquinones 11 and 12 via a Suzuki–Miyaura reaction, we speculated that such coupling could be achieved simply by allowing geranylbenzoquinone (13) to tautomerize in the presence of triethylamine, undergo Michael reaction with another equivalent of quinone, and then follow a cascade of oxidation and



© 2012 American Chemical Society and American Society of Pharmacognosy

Received: November 12, 2012 Published: December 10, 2012





spontaneous ring closure to form the more advanced thiaplidiaquinone precursors 7 and 8 (Scheme 2).

Scheme 2. Tautomerization and Dimerization of Geranylbenzoquinone



 $R = CH = CCH_3(CH_2)_2CH = C(CH_3)_2$

Scheme 3. Synthesis of Pre-thiaplidiaquinones A and B

Geranylbenzoquinone (13) was prepared in a two-step sequence by reaction of 4-methoxyphenol with geranylbromide to afford 2-geranyl-4-methoxyphenol,¹¹ oxidation of which with CAN yielded 13^{11} (Scheme 3). Initial assessment of the ability of geranylbenzoquinone to undergo base-induced tautomerization and dimerization was performed in an NMR tube, whereupon addition of Et_3N (1 equiv) to a solution of quinone 13 in CDCl₂ caused a rapid change in color of the solution from pale yellow to dark brown, with ¹H NMR spectra of the reaction mixture indicating rapid consumption of starting material and the production of a complex mixture. An HSQC NMR spectrum of the mixture identified the generation of a new oxymethine fragment, with a correlation observed at $\delta_{
m H}$ 6.18, $\delta_{\rm C}$ 68.4 [cf. scabellone B (6) H-5/C-6 ($\delta_{\rm H}$ 6.00 (1H, d, J = 9.3 Hz, H-5); $\delta_{\rm C}$ 67.6)],⁷ suggestive of successful formation of a benzo [c] chromene ring system. The reaction between quinone 13 and Et_3N (5 equiv) was repeated on a larger scale, with a short reaction time of 2 min, followed by purification using silica gel flash column chromatography. The fraction eluting with 15% MeOH/CH2Cl2 was left exposed to silica gel overnight, whereupon a further color change, this time from brown to dark purple, was observed. Further purification of the purple products yielded 7 and 8 in yields of 5.7% and 2.4%, respectively (Scheme 3). Albeit with low yields, the biomimetic transformation of 13 to tricycles 7 and 8 is unprecedented.

High-resolution ESI mass spectrometry assigned the molecular formula $C_{32}H_{38}O_4$ [M + Na]⁺ to both 7 and 8, while detailed analysis of NMR data established the presence of 1-oxygeranyl, geranyl, tetrasubstituted benzene, and 2,3disubstituted benzoquinone fragments (see Tables S1 and S2). Close similarity of the NMR data to those observed for scabellone B (6),⁷ also obtained in CDCl₃ solvent, gave confidence that 7 and 8 were indeed isomeric benzo[c]chromene-7,10-diones. The most prominent difference between the two reaction products was the coupling, or lack of it, for the two benzenoid ring protons. The observation of 3.0 Hz coupling between H-1 ($\delta_{\rm H}$ 7.67) and H-3 ($\delta_{\rm H}$ 6.75) defined the higher yielding product to be quinone 7, while the lack of scalar coupling between the two benzenoid protons [$\delta_{\rm H}$ 7.82 (s, H-1); $\delta_{\rm H}$ 6.69 (s, H-4)] of the second reaction product established their relative disposition as being para, i.e., quinone 8.

With precursor quinones 7 and 8 in hand, addition of hypotaurine was undertaken in a manner previously used by us in the synthesis of the anti-inflammatory ascidian metabolite ascidiathiazone A.¹² Thus an aqueous solution of hypotaurine (1 equiv) was slowly added to 7 in CH₃CN/EtOH at 0 °C and stirred for 48 h at room temperature (rt). Chromatographic purification of the reaction product mixture afforded thiaplidiaquinone A (4) and dioxothiazine regioisomer 14 in



Scheme 4. Synthesis of Thiaplidiaquinones A and B



yields of 21% and 40%, respectively (Scheme 4). ¹H and ¹³C NMR data observed for the minor product were in excellent agreement with those reported for the natural product,^{6,9} while data observed for some resonances, in particular H-1 ($\Delta \delta_{\rm H}$ 0.31) and C-6a/C-9/C-10/C-11a ($\Delta \delta_{\rm C}$ 7.9–33), of regiosiomer 14 were markedly different (see Tables S3 and S4). Reaction of the second precursor, quinone 8, with hypotaurine in a similar manner afforded two products, identified as thiaplidiaquinone B (5) (9.3% yield) and its regioisomer 15 (13%). Once again the spectroscopic data observed for the reaction minor product agreed very well with the data reported for the natural product, while those observed for the isomer were quite different (see Tables S5 and S6).⁶ The UV-visible spectra of natural products 4 and 5 were almost superimposable, as were those of the regioisomers 14 and 15, but the two series were markedly different from each other (see Figure S1), suggesting that this classical spectroscopic technique may be a useful tool in assigning dioxothiazine regiochemistry in this compound class.

In conclusion, we have achieved a biomimetic synthesis of the complex dioxothiazino meroterpenoids thiaplidiaquinones A and B by base-mediated dimerization of geranylbenzoquinone, followed by addition of hypotaurine.

EXPERIMENTAL SECTION

General Experimental Procedures. Ultraviolet–visible spectra were run as MeOH solutions on a UV-2102 PC Shimadzu UV–vis scanning spectrophotometer. Infrared spectra were recorded using a Perkin-Elmer Spectrum One Fourier-transform IR spectrometer as a dry film. ¹H and ¹³C NMR spectra were recorded at 298 K, at 400 and 100 MHz, respectively, or at 600 and 150 MHz, respectively, on Bruker DRX spectrometers. NMR experiments were run in CDCl₃ and were referenced to TMS for ¹H and to the central peak of the CDCl₃ signal at 77.16 ppm¹³ for ¹³C. Standard Bruker pulse sequences were utilized. HRMS data were acquired on a Bruker micrOTOF Q II mass spectrometer. Flash column chromatography was performed using Davisil silica gel (40–63 μ m), while analytical thin-layer chromatography was carried out on Merck DC-plastikfolien Kieselgel 60 F254 plates, and products were visualized by UV fluorescence.

2-Geranyl-4-methoxyphenol. Sodium metal (0.71 g, 0.031 mol) was added portionwise to a solution of 4-methoxyphenol (1.91 g, 0.015 mol) in dry Et₂O (65 mL) under a nitrogen atmosphere and then stirred for 3 h at rt. Geranyl bromide (3.34 g, 3.04 mL, 0.015 mol) was then added dropwise, and the solution heated at reflux for 48 h under nitrogen. After the solution cooled to rt, 10% HCl(aq) (5 mL) was added, the aqueous layer was extracted with Et₂O (2 × 10 mL), the combined organic layers were washed with water (2 × 50 mL) and dried (MgSO₄), and the solvent was removed under reduced pressure. Purification by silica gel column chromatography (EtOAc/*n*-hexane, 1:4) gave 2-geranyl-4-methoxyphenol as a pale yellow oil (2.24 g, 56%): R_f (CH₂Cl₂) 0.56; IR (ATR) ν_{max} 3352, 2935, 2834, 1506, 1440,

1220, 1037 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 6.73 (1H, d, *J* = 8.7 Hz, H-6), 6.68 (1H, d, *J* = 2.8 Hz, H-3), 6.65 (1H, dd, *J* = 8.7, 2.8 Hz, H-5), 5.34–5.28 (1H, m, H-2'), 5.10–5.04 (1H, m, H-6'), 4.80 (1H, s, OH), 3.75 (3H, s, H₃-7), 3.33 (2H, d, *J* = 7.2 Hz, H₂-1'), 2.16–2.10 (2H, m, H₂-4'), 2.10–2.05 (2H, m, H₂-5'), 1.76 (3H, s, H₃-9'), 1.68 (3H, s, H₃-8'), 1.60 (3H, s, H₃-10'); ¹³C NMR (CDCl₃, 100 MHz) δ 153.8 (C-4), 148.5 (C-1), 138.7 (C-3'), 132.1 (C-7'), 128.3 (C-2), 124.0 (C-6'), 121.6 (C-2'), 116.5 (C-6), 115.8 (C-3), 112.2 (C-5), 55.8 (C-7), 39.8 (C-4'), 30.1 (C-1'), 26.6 (C-5'), 25.8 (C-8'), 17.8 (C-10'), 16.3 (C-9'); (+)-HRESIMS *m*/*z* 283.1669 [M + Na]⁺ (calcd for C₁₇H₂₄NaO₂, 283.1669].

Geranylbenzoquinone (13). A solution of cerium ammonium nitrate (9.15 g, 0.019 mol) in CH₃CN/H₂O (1:2, 75 mL) was added dropwise to a stirred solution of 2-geranyl-4-methoxyphenol (2.18 g, 9.4 mmol) in CH₃CN (25 mL) at 0 °C. The mixture was stirred overnight at 0 °C, then poured into 10% NaCl(aq) (100 mL) and extracted with Et_2O (2 × 50 mL). The organic extract was then dried (MgSO₄), and the solvent removed under reduced pressure. Purification by silica gel column chromatography (EtOAc/n-hexane, 1:4) gave quinone 13 as a bright yellow oil (1.19 g, 58%): R_f (30%) EtOAc/*n*-hexane) 0.83; IR (ATR) ν_{max} 2966, 2919, 2859, 1656, 1599, 1297 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 6.76 (1H, d, J = 10.1 Hz, H-6), 6.71 (1H, dd, J = 10.1, 2.4 Hz, H-5), 6.55-6.51 (1H, m, H-3), 5.19-5.12 (1H, m, H-2'), 5.11-5.05 (1H, m, H-6'), 3.13 (2H, d, J = 7.2 Hz, H₂-1'), 2.14-2.09 (2H, m, H₂-4'), 2.09-2.05 (2H, m, H₂-5'), 1.70 (3H, s, H₃-8'), 1.63 (3H, s, H₃-9'), 1.60 (3H, s, H₃-10'); ¹³C NMR (CDCl₃, 100 MHz) δ 188.1 (C-4), 187.7 (C-1), 148.7 (C-2), 140.3 (C-3'), 136.9 (C-6), 136.5 (C-5), 132.5 (C-3), 132.0 (C-7'), 124.0 (C-6'), 117.8 (C-2'), 39.8 (C-4'), 27.5 (C-1'), 26.6 (C-5'), 25.8 (C-8'), 17.8 (C-10'), 16.2 (C-9'); (+)-HRESIMS m/z 267.1359 [M + Na]⁺ (calcd for C₁₆H₂₀NaO₂, 267.1356).

4-Geranyl-6-(2,6-dimethylhepta-1,5-dienyl)-2-hydroxy-6Hbenzo[c]chromene-7,10-dione (7) and 3-Geranyl-6-(2,6-dimethylhepta-1,5-dienyl)-2-hydroxy-6H-benzo[c]chromene-7,10-dione (8). Triethylamine (0.26 mL, 0.19 g, 1.84 mmol) was added dropwise to a rapidly stirring solution of geranylbenzoquinone (0.09 g, 0.37 mmol) in CH₂Cl₂ (10 mL) under an atmosphere of nitrogen. When the reaction mixture became a dark brown color (approximately 2 min), it was loaded onto a silica gel chromatography column (CH₂Cl₂). The fraction that eluted with 10-15% MeOH/CH2Cl2 was then loaded onto another silica gel chromatography column (CH₂Cl₂) and left exposed to silica overnight. The purple compounds formed were eluted with CH2Cl2 to yield 4-geranyl-6-(2,6-dimethylhepta-1,5dienyl)-2-hydroxy-6H-benzo[c]chromene-7,10-dione (7) as a purple oil (5.13 mg, 5.7%) and 3-geranyl-6-(2,6-dimethylhepta-1,5-dienyl)-2hydroxy-6H-benzo[c]chromene-7,10-dione (8) as a purple oil (2.13 mg, 2.4%):

Compound 7: R_f (CH₂Cl₂) 0.29; IR (ATR) ν_{max} 3266, 2977, 1643, 1390 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.67 (1H, d, J = 3.0 Hz, H-1), 6.75 (1H, d, J = 3.0 Hz, H-3), 6.73 (2H, s, H-8, 9), 6.04 (1H, d, J = 9.5 Hz, H-6), 5.32 (1H, d, J = 9.5 Hz, H-1"), 5.25 (1H, t, J = 7.3 Hz, H-2'), 5.09 (1H, t, J = 6.5 Hz, H-6'), 4.92 (1H, t, J = 6.6 Hz, H-5"), 4.70 (1H, br s, OH), 3.24 (2H, dd, J = 7.3, 3.0 Hz, H₂-1'), 2.11–2.05 (2H, m, H₂-5'), 2.04–2.00 (2H, m, H₂-4'), 2.00–1.97 (2H, m, H₂-4"),

1.97–1.90 (2H, m, H₂-3"), 1.94 (3H, s, H₃-8"), 1.68 (3H, s, H₃-8'), 1.67 (3H, s, H₃-9'), 1.59 (3H, s, H₃-10'), 1.58 (3H, s, H₃-7"), 1.50 (3H, s, H₃-9"); ¹³C NMR (CDCl₃, 100 MHz) δ 187.1 (C-10), 185.2 (C-7), 149.9 (C-2), 147.0 (C-4a), 144.3 (C-2"), 137.2 (C-8/9), 137.0 (C-3'), 135.8 (C-8/9), 134.9 (C-6a), 132.3 (C-4), 131.9 (C-6"), 131.6 (C-7'), 130.5 (C-10a), 124.4 (C-6'), 123.6 (C-5"), 121.6 (C-2'), 120.2 (C-3), 118.4 (C-1"), 117.6 (10b), 112.5 (C-1), 67.3 (C-6), 39.9 (C-3"), 39.8 (C-4'), 28.2 (C-1'), 26.8 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.8 (C-10'), 17.8 (C-9"), 17.4 (C-8"), 16.6 (C-9'); (+)-HRESIMS *m/z* 509.2645 [M + Na]⁺ (calcd for C₃₂H₃₈O₄Na, 509.2662).

Compound 8: R_f (CH₂Cl₂) 0.43; IR (ATR) ν_{max} 3393, 2922, 1647, 1423 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.82 (1H, s, H-1), 6.72 (2H, s, H-8, 9), 6.69 (1H, s, H-4), 5.99 (1H, d, J = 9.5 Hz, H-6), 5.35 (1H, d, J = 9.5 Hz, H-1"), 5.30 (1H, t, J = 7.1 Hz, H-2'), 5.11–5.05 (1H, m, H-6'), 4.98 (1H, br s, OH), 4.94-4.90 (1H, m, H-5"), 3.33 $(2H, dd, J = 7.1, 4.0 Hz, H_2-1'), 2.16-2.11 (2H, m, H_2-5'), 2.11-2.07$ (2H, m, H₂-4'), 2.03-1.95 (2H, m, H₂-4"), 1.97-1.92 (2H, m, H₂-3"), 1.92 (3H, s, H₃-8"), 1.73 (3H, s, H₃-9'), 1.69 (3H, s, H₃-8'), 1.59 (3H, s, H₃-7"), 1.59 (3H, s, H₃-10'), 1.50 (3H, s, H₃-9"); ¹³C NMR (CDCl₃, 100 MHz) & 187.2 (C-10), 185.1 (C-7), 149.2 (C-4a), 149.1 (C-2), 144.0 (C-2"), 139.0 (C-3'), 137.0 (C-8/9), 135.9 (C-8/9), 133.8 (C-6a), 133.4 (C-3), 132.1 (C-7'), 131.9 (C-6"), 130.2 (C-10a), 124.0 (C-6'), 123.7 (C-5"), 120.7 (C-2'), 118.6 (C-4), 118.2 (C-1"), 115.8 (C-10b), 115.1 (C-1), 67.6 (C-6), 39.8 (C-3"), 39.8 (C-4'), 29.7 (C-1'), 26.6 (C-5'), 26.2 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.9 (C-9"), 17.8 (C-10'), 17.4 (C-8"), 16.4 (C-9'); (+)-HRESIMS m/z509.2649 $[M + Na]^+$ (calcd for $C_{32}H_{38}O_4Na$, 509.2662).

Thiaplidiaquinone A (4) and Regioisomer 14. A solution of hypotaurine (1.2 mg, 0.01 mmol) in H_2O (0.10 mL) was added dropwise to a solution of 4-geranyl-6-(2,6-dimethylhepta-1,5-dienyl)-2-hydroxy-6H-benzo[c]chromene-7,10-dione (7) (4.02 mg, 0.01 mmol) in CH₃CN/EtOH (1:1, 0.8 mL) at 0 °C. The resulting mixture was stirred for 2 days, after which H_2O (3 mL) was added. The aqueous solution was then extracted with CH₂Cl₂ (2 mL × 2) and dried (MgSO₄), and the solvent removed under reduced pressure. The crude reaction product was purified using silica gel chromatography (EtOAc/*n*-hexane, 1:1) to yield thiaplidiaquinone A (4) as a red oil (1.02 mg, 21%) and regioisomer 14 as a dark blue oil (1.93 mg, 40%).

Thiaplidiaquinone Å (4): R_f (EtOAc) 0.63; UV (MeOH) λ_{max} (log $\varepsilon)$ 331 (3.04) nm; IR (ATR) $\dot{\nu}_{\rm max}$ 3304, 2926, 1683, 1622, 1587, 1445, 1348, 1282, 1120 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) δ 7.54 (1H, br s, H-1), 6.90 (1H, br s, NH-11), 6.71 (1H, br s, H-3), 6.08 (1H, d, J = 9.5 Hz, H-6), 5.28-5.23 (1H, m, H-1"), 5.23-5.19 (1H, m, H-2'), 5.08 (1H, tt, J = 7.0, 1.4 Hz, H-6'), 4.98–4.87 (1H, m, H-5"), 4.19– 4.04 (2H, m, H₂-10), 3.43-3.31 (2H, m, H₂-9), 3.28 (1H, dd, J = 15.5, 7.1 Hz, H-1'a), 3.17 (1H, dd, J = 15.5, 7.4 Hz, H-1'b), 2.10–2.04 (2H, m H₂-5'), 2.04–1.99 (2H, m, H₂-4'), 1.99–1.96 (2H, m, H₂-4"), 1.96-1.92 (2H, m, H₂-3"), 1.92 (3H, s, H₃-8"), 1.68 (3H, s, H₃-8'), 1.66 (3H, s, H₃-9'), 1.59 (3H, s, H₃-10'), 1.59 (3H, s, H₃-7"), 1.50 (3H, s, H₃-9"); ¹³C NMR (CDCl₃, 100 MHz) δ 179.3 (C-12), 175.3 (C-7), 149.7 (C-2), 146.2 (C-4a), 145.6 (C-2"), 143.9 (C-11a), 138.8 (C-6a), 137.1 (C-3'), 132.5 (C-4), 131.9 (C-6"), 131.6 (C-7'), 128.2 (C-12a), 124.4 (C-6'), 123.7 (C-5"), 121.4 (C-2'), 120.1 (C-3), 117.0 (C-1"), 116.8 (C-12b), 111.6 (C-1), 110.6 (C-7a), 67.5 (C-6), 48.8 (C-9), 40.1 (C-10), 39.9 (C-4'), 39.9 (C-3"), 28.2 (C-1'), 26.8 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.8 (C-9"), 17.8 (C-10'), 17.6 (C-8"), 16.3 (C-9'); (+)-HRESIMS [M + Na]⁺ 614.2536 (calcd for C₃₄H₄₁NNaO₆S, 614.2547).

Regioisomer 14: R_f (50% EtOAc/*n*-hexane) 0.14; UV (MeOH) λ_{max} (log ε) 304 (3.03), 368 (3.36), 442 (2.54), 575 (2.46) nm; IR (ATR) ν_{max} 3304, 2970, 1661, 1594, 1571, 1441, 1350, 1282, 1124, 1065 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.82 (1H, d, J = 2.7 Hz, H-1), 6.78 (1H, d, J = 2.7 Hz, H-3), 6.73 (1H, br s, NH-8), 5.98 (1H, d, J = 9.4 Hz, H-6), 5.28 (1H, d, J = 9.4 Hz, H-1″), 5.20 (1H, t, J = 7.4 Hz, H-2′), 5.08 (1H, t, J = 6.4 Hz, H-6′), 4.92 (1H, t, J = 6.1 Hz, H-5″), 4.19–4.05 (2H, m, H₂-9), 3.42–3.34 (2H, m, H₂-10), 3.23 (1H, dd, J = 15.8, 7.0 Hz, H-1′a), 3.15 (1H, dd, J = 15.8, 7.3 Hz, H-1′b), 2.11– 2.04 (2H, m, H₂-5′), 2.03–1.95 (2H, m, H₂-4′), 2.03–1.95 (2H, m, H₂-4″), 1.94–1.91 (2H, m, H₂-3″), 1.90 (3H, s, H₃-8″), 1.67 (3H, s, H₃-8'), 1.64 (3H, s, H₃-9'), 1.59 (3H, s, H₃-10'), 1.59 (3H, s, H₃-7"), 1.51 (3H, s, H₃-9"); ¹³C NMR (CDCl₃, 100 MHz) δ 177.7 (C-12), 177.5 (C-7), 150.4 (C-2), 148.4 (C-4a), 144.8 (C-2"), 143.8 (C-7a), 137.1 (C-3'), 133.6 (C-12a), 132.3 (C-4), 132.0 (C-6"), 131.7 (C-7'), 130.9 (C-6a), 124.3 (C-6'), 123.5 (C-5"), 122.1 (C-3), 121.4 (C-2'), 118.2 (C-1"), 117.5 (C-12b), 113.7 (C-1), 111.4 (C-11a), 67.1 (C-6), 49.2 (C-10), 40.2 (C-9), 39.9 (C-4'), 39.7 (C-3"), 28.2 (C-1'), 26.8 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.8 (C-9"), 17.8 (C-10'), 17.4 (C-8"), 16.2 (C-9'); (+)-HRESIMS [M + Na]⁺ 614.2531 (calcd for C₃₄H₄₁NNaO₆S, 614.2547).

Thiaplidiaquinone B (5) and Regioisomer 15. A solution of hypotaurine (2.55 mg, 0.02 mmol) in H_2O (0.15 mL) was added dropwise to a solution of 3-geranyl-6-(2,6-dimethylhepta-1,5-dienyl)-2-hydroxy-6H-benzo[c]chromene-7,10-dione (8) (11.39 mg, 0.02 mmol) in CH₃CN/EtOH (1:1, 1 mL) at 0 °C. The resulting mixture was stirred for 2 days, after which H_2O (3 mL) was added. The aqueous solution was then extracted with CH_2Cl_2 (3 mL × 2) and dried (MgSO₄), and the solvent removed under reduced pressure. The crude reaction product was purified using silica gel chromatography (EtOAc/*n*-hexane, 1:1) to yield thiaplidiaquinone B (5) as a light purple oil (1.29 mg, 9.3%) and regioisomer 15 as a dark blue oil (1.84 mg, 13%).

Thiaplidiaquinone B (5):. R_f (EtOAc) 0.59; UV (MeOH) λ_{max} (log ϵ) 324 (3.00), 362 (sh, 2.84), 558 (2.17) nm; IR (ATR) ν_{max} 3447, 3301, 2928, 1618, 1586, 1424, 1282, 1164, 1122 cm⁻¹; ¹H NMR $(CDCl_2, 600 \text{ MHz}) \delta 7.69 (1H, br s, H-1), 6.85 (1H, br s, NH-11),$ 6.69 (1H, br s, H-4), 6.06 (1H, d, J = 9.6 Hz, H-6), 5.31–5.25 (1H, m, H-1"), 5.31-5.25 (1H, m, H-2'), 5.07 (1H, t, J = 6.7 Hz, H-6'), 4.94 $(1H, t, J = 6.5 Hz, H-5''), 4.14-4.07 (2H, m, H_2-10), 3.37-3.29 (2H, m)$ m, H₂-9), 3.37-3.29 (2H, m, H₂-1'), 2.14-2.10 (2H, m H₂-5'), 2.10-2.06 (2H, m, H₂-4'), 2.01-1.97 (2H, m, H₂-4"), 1.97-1.92 (2H, m, H₂-3"), 1.91 (3H, s, H₃-8"), 1.73 (3H, s, H₃-9'), 1.69 (3H, s, H₃-8'), 1.60 (3H, s, H_3 -10'), 1.60 (3H, s, H_3 -7"), 1.51 (3H, s, H_3 -9"); ¹³C NMR (CDCl₃, 150 MHz) δ 179.2 (C-12), 175.3 (C-7), 149.0 (C-2), 148.1 (C-4a), 145.4 (C-2"), 143.9 (C-11a), 139.3 (C-3'), 137.5 (C-6a), 133.1 (C-3), 132.2 (C-7'), 132.0 (C-6"), 127.9 (C-12a), 123.9 (C-6'), 123.7 (C-5"), 120.4 (C-2'), 118.8 (C-4), 116.6 (C-1"), 114.8 (C-12b), 114.3 (C-1), 110.2 (C-7a), 67.7 (C-6), 48.7 (C-9), 40.0 (C-10), 39.9 (C-3"), 39.8 (C-4'), 29.8 (C-1'), 26.5 (C-5'), 26.1 (C-4"), 25.9 (C-8'), 25.8 (C-7"), 17.9 (C-10'), 17.8 (C-9"), 17.6 (C-8"), 16.4 (C-9'); (+)-HRESIMS m/z 614.2538 $[M + Na]^+$ (calcd for C₃₄H₄₁NNaO₆S, 614.2547).

Regioisomer 15: R_f (50% EtOAc/n-hexane) 0.17; UV (MeOH) λ_{\max} (log ε) 303 (3.17), 370 (3.39), 450 (2.69), 587 (2.68) nm; IR (ATR) $\nu_{\rm max}$ 3457, 3335, 2930, 1659, 1613, 1584, 1552, 1425, 1277, 1214, 1123 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) δ 7.98 (1H, s, H-1), 6.67 (1H, br s, NH-8), 6.64 (1H, s, H-4), 5.94 (1H, d, J = 9.4 Hz, H-6), 5.33 (1H, d, J = 9.4 Hz, H-1"), 5.27 (1H, t, J = 7.0 Hz, H-2'), 5.08 (1H, t, J = 6.8 Hz, H-6'), 4.96-4.91 (1H, br m, H-5"), 4.15-4.05 (2H, m, H₂-9), 3.38–3.33 (2H, m, H₂-10), 3.31 (2H, d, J = 7.0 Hz, H₂-1'), 2.14-2.09 (2H, m, H₂-5'), 2.09-2.04 (2H, m, H₂-4'), 2.02-1.96 (2H, m, H₂-4"), 1.96-1.91 (2H, m, H₂-3"), 1.88 (3H, s, H₃-8"), 1.69 (3H, s, H₃-9'), 1.68 (3H, s, H₃-8'), 1.60 (3H, s, H₃-7"), 1.60 (3H, s, H₃-10'), 1.51 (3H, s, H₃-9"); ¹³C NMR (CDCl₃, 150 MHz) δ 177.9 (C-12), 177.2 (C-7), 150.7 (C-4a), 149.5 (C-2), 144.4 (C-2"), 143.7 (C-7a), 138.7 (C-3'), 136.2 (C-3), 133.3 (C-12a), 132.0 (C-7'), 132.0 (C-6"), 129.5 (C-6a), 124.1 (C-6'), 123.6 (C-5"), 120.4 (C-2'), 118.4 (C-4), 118.0 (C-1"), 116.0 (C-1), 115.6 (C-12b), 111.3 (C-11a), 67.3 (C-6), 49.2 (C-10), 40.1 (C-9), 39.9 (C-4'), 39.8 (C-3"), 29.4 (C-1'), 26.6 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.8 (C-7"), 17.9 (C-9"), 17.8 (C-10'), 17.4 (C-8"), 16.3 (C-9'); (+)-HRESIMS m/z 614.2529 [M + $Na]^+$ (calcd for $C_{34}H_{41}NNaO_6S$, 614.2547).

ASSOCIATED CONTENT

Supporting Information

Tables of NMR data for 4, 5, 7, 8, 14, and 15, figure of UV spectra of 4, 5, 14, and 15, and copies of ¹H and ¹³C NMR spectra for 2-geranyl-4-methoxyphenol, geranylbenzoquinone,

Journal of Natural Products

and compounds 4, 5, 7, 8, 14, and 15. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +64 9 373 7599, x 88284. Fax: + 64 9 373 7422. E-mail: b.copp@auckland.ac.nz.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge funding from the University of Auckland. We thank the late Prof. E. Fattorusso for copies of NMR data of the natural products, Dr. M. Schmitz for assistance with NMR data acquisition, and Dr. N. Lloyd for MS data.

DEDICATION

This paper is dedicated to the memory of Prof. Ernesto Fattorusso (1937–2012).

REFERENCES

(1) Menna, M.; Fattorusso, E.; Imperatore, C. *Molecules* **2011**, *16*, 8694–8732.

(2) Zubía, E.; Ortega, M. J.; Salvá, J. Mini-Rev. Org. Chem. 2005, 2, 389–399.

(3) Sunassee, S. N.; Davies-Coleman, M. T. Nat. Prod. Rep. 2012, 29, 513–535.

(4) Garrido, L.; Zubía, E.; Ortega, M. J.; Salvá. J. Nat. Prod. 2002, 65, 1328–1331.

(5) Fu, X.; Hossain, M. B.; Schmitz, F. J.; van der Helm, D. J. Org. Chem. 1997, 62, 3810-3819.

(6) Aiello, A.; Fattorusso, E.; Luciano, P.; Macho, A.; Menna, M.; Munoz, E. J. Med. Chem. 2005, 48, 3410–3416.

(7) Chan, S. T. S.; Pearce, A. N.; Januario, A. H.; Page, M. J.; Kaiser, M.; McLaughlin, R. J.; Harper, J. L.; Webb, V. L.; Barker, D.; Copp, B. R. J. Org. Chem. 2011, 76, 9151–9156.

(8) Cadelis, M. M.; Barker, D.; Copp, B. R. Synlett 2012, 23, 2939–2942.

(9) Carbone, A.; Lucas, C. L.; Moody, C. J. J. Org. Chem. 2012, 77, 9179–9189.

(10) Lumb, J.-P.; Trauner, D. Org. Lett. 2005, 7, 5865-5868.

(11) Baeza, E.; Catalán, K.; Peña-Cortés, H.; Espinoza, L.; Villena, J.; Carrasco, H. Quim. Nova **2012**, 35, 523–526.

(12) Pearce, A. N.; Chia, E. W.; Berridge, M. V.; Clark, G. R.; Harper, J. L.; Larsen, L.; Maas, E. W.; Page, M. J.; Perry, N. B.; Webb, V. L.; Copp, B. R. *J. Nat. Prod.* **2007**, *70*, 936–940.

(13) Gottlieb, H. E.; Kotlyar, V.; Nudelamn, A. J. Org. Chem. 1997, 62, 7512-7515.