Corymbones A and B, Phloroglucinols with Thyrotropin Releasing Hormone Receptor 2 Binding Affinity from the Flowers of *Corymbia peltata*

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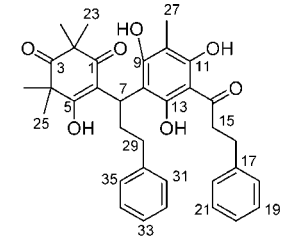
Received November 18, 2007

High-throughput screeing of a plant and marine invertebrate extract library to find natural products with rat thytotropin releasing hormone receptor 2 binding affinity led to the isolation of two new active acylphloroglucinols, corymbones A and B (**1** and **2**) from flowers of the Queensland tree *Corymbia peltata*. Their structures were assigned from interpretation of 2D NMR and high-resolution ESIMS data. Compounds **1** and **2** showed rat TRH receptor 2 binding affinity with IC_{50} values of 23 and 19 μM, respectively.

Thyrotropin-releasing hormone (TRH) is a tripeptide (pyroglutamic acid-histidine-proline-amide) that is found both in the central nervous system and the periphery. TRH has several endocrine functions including stimulation of secretion of thyroxin, growth hormone, and prolactin.[1,2] There is also evidence that TRH acts as a neurotransmitter since it produces a rapid onset, neurotransmitter-like, excitation of spinal lower motor neurons and reduces neurological deficits observed after traumatic spinal cord injury in cats.[3] TRH binding sites have been found in the brain, pituitary, and dorsal and ventral horns of the spinal cord, and this suggested a potential role for TRH in pain control. This idea has been supported by the observation that central iv administration of TRH provides significant short-acting antinociception for chemically and mechanically induced pain.[4] The actions of TRH are mediated by the stimulation of a specific cell surface G-protein coupled receptor, TRH-R. The existence of several subtypes of this receptor has been proposed on the basis of biochemical and electrophysiological experiments and the observation of differential effects of synthetic TRH analogues on endocrine and CNS function.[5–8] Recently a new subtype of the TRH receptor, TRH-R2, was discovered in rat brain tissues.[9,10] A comparison of distribution of TRH-R1 and TRH-R2 in rat brain tissue has shown them to be remarkably complementary. TRH-R1 is highly expressed in neuroendocrine brain regions, while TRH-R2 is expressed in brain regions that are important for the transmission of somatosensory signals and higher brain function.[11] Agonists and antagonists of TRH binding therefore have potential therapeutic value in regulating endocrine function, in controlling pain, and in the treatment of spinal cord injury.

Our interest in finding treatments for pain prompted us to screen extracts against TRH-R2 since it appears to be localized in areas of the brain specific for pain control. High-throughput screening of 20 000 extracts derived from plants and marine invertebrates collected in Queensland led to the discovery that the CH_2Cl_2 extract of the flowers of *Corymbia peltata* (Benth.) K.D. Hill & L. A. S. Johnson (Myrtaceae) inhibited the binding of [^{3}H]-3-methyl-TRH to rat TRH-R2. This paper reports on the isolation, structure determination, and biological activity of the bioactive compounds **1** and **2**, named corymbones A and B, isolated from the flowers of *C. peltata*.

The ground flowers of *C. peltata* were exhaustively extracted with CH_2Cl_2, and the extract was chromatographed on Sephadex LH-20 eluting with CH_2Cl_2/MeOH. TRH-R2 bioactivity was concentrated in a fraction containing a mixture of triterpenes and phloroglucinols. The triterpenes were separated from the phloroglucinols by solubilizing the phloroglucinols in CH_3CN. Separation of lipophilic compounds can be successfully achieved by employing nonaqueous binary or tertiary solvent systems in countercurrent chromatography without loss of material on a solid support.[12,13] Thus the bioactive CH_3CN-soluble material was chromatographed on a centrifugal partition chromatograph (CPC) instrument in ascending mode with a solvent mixture of *n*-heptane/CH_2Cl_2/CH_3CN (10:3:7) employing the upper phase as mobile phase. After 1.5 h the system was switched to descending mode and the mobile phase was changed to the lower phase. Two bioactive compounds were obtained; corymbone A (**1**) eluted first followed by corymbone B (**2**). Both compounds accounted for the activity observed in the crude extract.



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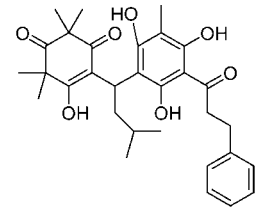

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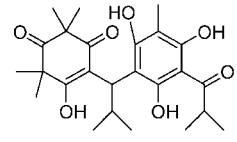

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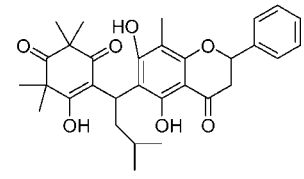

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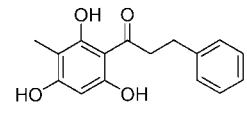

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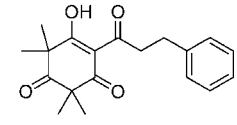

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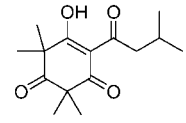

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10.1021/np0706567 CCC: $40.75
Published on Web 04/16/2008

Accurate mass measurement of the pseudomolecular ion in the (−)-HRESIMS at *m/z* 569.2545 allowed a molecular formula of $C_{35}H_{38}O_7$ to be assigned to **1**. Infrared absorption bands at 2870–3370, 1612, and 1722 cm^{-1} suggested the presence of an enolic 1,3-diketo system and a 2-hydroxyaryl ketone, and this was supported by a UV absorbance at 292 nm.[14–17]

The ^1H NMR spectrum of **1** in $CDCl_3$ (see Experimental Section) was complex with a number of resonances being doubled, and this suggested that a 1:3 ratio of isomers was present. The major isomer exhibited signals for four aliphatic quaternary methyls (δ_H1.32, 1.34, 1.41, 1.49), an aromatic methyl (δ_H 2.12), four aliphatic methylenes (δ_H 2.28/2.55, 2.51, 3.05, 3.48), a benzylic methine (δ_H 4.34), 10 aromatic protons that were indicative of two monosubstituted phenyl groups [δ_H 7.24 (d, J = 7.2 Hz, 2H), 7.28 (t, J = 7.2 Hz, 2H), 7.19 (t, J = 7.2 Hz, 1H), 7.09 (d, J = 7.2 Hz, 2H), 7.22 (t, J = 7.2 Hz, 2H), 7.15 (t, J = 7.2 Hz, 1H)], and four phenolic protons, two of which were significantly downfield shifted, suggesting that they either had intramolecular hydrogen bonds or were enols. Partial structures $-CH_2CH_2-$ and $-CH_2CH_2CH-$ were deduced from analysis of the gCOSY spectrum. Analysis of the gHMQC spectrum allowed all of the 20 protonated carbons to be assigned including five methyls (δ_C 7.3, 22.1, 23.9, 26.0, 27.1), four methylenes (δ_C 30.6, 31.2, 34.8, 45.4), one aliphatic methine (δ_C 30.1), and 10 aromatic methines (δ_C 125.3, 125.7, 128.2 × 6, 128.3 × 2). It could also be deduced from this analysis that **1** also had a further 15 quaternary carbons and four protons not attached to carbons. The gHMBC spectrum had a large number of correlations, and these were crucial to establish the structure of **1**. Correlations from the methylene protons 29-CH_2 (δ_H 2.51) to the aromatic carbons C-30 (δ_C 141.4) and C-31/C-35 (δ_C 128.3) extended one partial structure to $-CHCH_2CH_2Ph$. The benzylic methine H-7 (δ_H 4.34) from this partial structure correlated to aromatic oxygenated carbons C-9 (δ_C 162.0) and C-13 (δ_C 159.9) and an upfield aromatic carbon C-8 (δ_C 108.0). The upfield chemical shift of C-8 suggested that it was flanked by the two oxygenated aromatic carbons C-9 and C-13, and this was confirmed by the observation of a $^3J_{CH}$ correlation from H-28b (δ_H 2.28) to C-8. A HMBC correlation from the aromatic methyl protons 27-CH_3 (δ_H 2.12) to C-9 indicated that it too was *ortho* to C-9. The aromatic methyl 27-CH_3 also showed a $^3J_{CH}$ correlation to another oxygenated aromatic carbon, C-11 (δ_C 156.0), and a $^2J_{CH}$ correlation to an upfield aromatic carbon, C-10 (δ_C 103.0). These correlations indicated that the partial structure $-CHCH_2CH_2Ph$ was attached to a methylphloroglucinol at C-8. The benzylic methine H-7 also correlated to C-6 (δ_C 114.2) and C-5 (δ_C 176.8) and to a carbonyl carbon C-1 (δ_C 202.9). The downfield exchangeable proton 5-OH (δ_H 10.34) and two of the aliphatic quaternary methyls, 25-CH_3 (δ_H 1.32) and 26-CH_3 (δ_H 1.49), also correlated to C-5, suggesting that C-1, C-5, and C-6 were an enolized β-diketone. The two methyl signals 25-CH_3 and 26-CH_3 also correlated to another ketone carbonyl carbon, C-3 (δ_C 211.8), and the aliphatic quaternary carbon C-4 (δ_C 48.5). The remaining two quaternary methyls 23-CH_3 (δ_H 1.41) and 24-CH_3 (δ_H 1.34) also correlated to C-3 as well as to the ketone carbonyl carbon C-1 and the aliphatic quaternary carbon C-2 (δ_C 55.0). These data indicated **1** contained an enolized 1,3,5-cyclohexatrione attached to C-7. Finally, a 3-phenyl-1-propionoyl partial structure was deduced from HMBC correlations between the methylene protons 16-CH_2 (δ_H 3.05) and the aromatic carbons C-17 (δ_C 141.2) and C-18/C-22 (δ_C 128.2) and to a ketone carbon, C-14 (δ_C 205.1). The chemical shift of C-14 suggested that it was a phenyl ketone, and this dictated that the 3-phenyl-1-propionoyl group was attached to the only remaining unassigned aromatic quaternary carbon, C-12, thus forming a dihydrochalcone. The observation of three sharp and significantly downfield shifted exchangeable proton signals in the ^1H NMR spectrum in $CDCl_3$ suggested that a network of intramolecular hydrogen bonds was present in the molecule, creating an ordered structure that favored conformations of the dioxocyclohexaenol that were hydrogen bonded to either 7-OH or 13-OH of the phloroglucinol. The structure of corymbone A was therefore assigned as **1**.

Corymbone B (**2**) was assigned the molecular formula $C_{31}H_{38}O_7$ by high-resolution negative electrospray mass measurement of the $[M - H^+]^-$ ion at *m/z* 521.2564 (Δ +3.7 ppm). The ^1H NMR spectrum of **2** (see Experimental Section) was very similar to that of **1**. The major difference was the replacement of the signals associated with one of the phenyl groups and a benzylic methylene in the ^1H NMR spectrum of **1** with an isopropyl group [δ_H 0.87 (d, J = 7.2 Hz, 3H), 0.88 (d, J = 7.2 Hz, 3H), 1.22 (m, 1H)] in **2**. Correlations obtained from a gCOSY experiment indicated that the isopropyl group was vicinal to the methylene 28-CH_2 (δ_H 1.81 and 2.10), which in turn was adjacent to the benzylic methine H-7 (δ_H 4.42). The gHMQC data established the presence of 34 carbon-bound protons (seven methyl, three methylene, two methine, and five aromatic protons). Signals associated with the 1,3,5-cyclohexatrione and the dihydrochalcone were almost identical with those of **1**. A similar pattern of HMBC correlations was observed from H-7 in **1** and **2**, confirming that the isopropyl group in **2** had replaced the benzyl group in **1**. Corymbone B was therefore assigned structure **2**.

The structures of corymbones A and B (**1** and **2**) are related to semimyrtucommulone (**3**), which was recently reported from the Mediterranean tree *Myrtus communis*,[17] and it was observed that **3** existed as a mixture of two isomers in $CDCl_3$. The two isomers observed for corymbones A and B and semimyrtucommulone probably result from a strong hydrogen bond between either 9-OH or 13-OH and the carbonyl attached to C-1, with the isomer containing a hydrogen bond between 13-OH and the C-1 ketone predominating since 13-OH is significantly downfield shifted. Kunzeanones A and B isolated from *Kunzea ambigua*[18] and **4** isolated from *K. ambigua* and *K. baxterii*.[19] are also similar to **1** and **2** except that the dihydrochalcone was replaced by a flavone in **4**. The corymbones contain partial structures that are present in compounds previously isolated from other plants in the family Myrtaceae. Biogenetically the corymbones could therefore be derived from the condensation of 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone (**5**) with appropriately substituted cyclohexatriones. This proposal is supported by the observation that **5**, which has been isolated previously from *Leptospermum recurvum*,[20,21] was also present in the extract, and cyclohexatriones such as grandiflorone (**6**)[22–25] and leptospermone (**7**)[23,26,27] have been isolated previously from various related plants from the family Myrtaceae including *Leptospermum*, *Eucalyptus*, and *Xanthostemon* species.

Corymbones A (**1**) and B (**2**) inhibited the specific binding of [^{3}H]3-methylhistidylTRH to HEK cell membranes expressing recombinant rat TRH receptors 2 with IC_{50} values of 23 and 19 μM, respectively. The IC_{50} value for the positive control TRH was 23 nM. The dihydrochalcone **5** did not inhibit specific binding of [^{3}H]3-methylhistidylTRH up to 1 mM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter (23 °C, 10 cm cell). UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer, and IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian Inova 600 MHz spectrometer. Samples were dissolved in $CDCl_3$, and chemical shifts were calculated relative to the $CDCl_3$ solvent peak (δ_H 7.26 and δ_C 77.0). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences for gCOSY, gHMQC, and gHMBC. HRESIMS were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass spectrometer. Sephadex LH-20 was used during purification. CPC separations were performed on a SANKI LLB-M high-performance CPC system. All solvents used were Omnisolv HPLC grade.

Plant Material. Flowers of *C. peltata* were collected and authenticated by one of the authors (P.I.F.) on February 9, 1996, from the

Newcastle Range in Queensland. A voucher specimen, AQ602692, is deposited at the Queensland Herbarium.

Extraction and Isolation. The air-dried ground flowers of *C. peltata* (15.4 g) were extracted with CH_2Cl_2 (3 × 200 mL), yielding a green gum (5.25 g).

The CH_2Cl_2 extract was chromatographed on Sephadex LH-20, eluting with CH_2Cl_2/MeOH (1:1). Five fractions were collected. Fraction three (1.65 g) was a mixture of triterpenes and phloroglucinols. The triterpenes were separated from the phloroglucinols by solubilizing the phloroglucinols in CH_3CN. The CH_3CN-soluble material was chromatographed on a centrifugal partition chromatograph in ascending mode with a solvent mixture of heptane/CH_2Cl_2/CH_3CN (10:3:7); the upper phase was the mobile phase. After 1.5 h the system was switched to descending mode and the mobile phase was changed to the lower phase. Corymbone A (**1**) (22.4 mg) eluted first followed by corymbone (**2**) (39.1 mg). The fifth fraction from the Sephadex LH-20 separation was pure 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone (**5**) (18.4 mg). The activity in the extract was accounted for by corymbone A (**1**) and corymbone B (**2**).

Corymbone A (1): yellow gum; $[\alpha]_D^{17}$ +12.7 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.36), 233sh (4.16), 292 (4.25), 369 (3.57) nm; IR (KBr) ν_{max} 3370 br, 2937, 1722, 1711, 1692, 1658, 1612, 1462, 1452, 1383, 1094, 754, 699 cm^{-1}; ^1H (600 MHz, $CDCl_3$) δ 1.32 (3H, s, H-25), 1.34 (3H, s, H-24), 1.41 (3H, s, H-23), 1.49 (3H, s, H-26), 2.12 (3H, s, H-27), 2.28 (1H, m, H-28b), 2.51 (2H, t, J = 6.6 Hz, H-29), 2.55 (1H, m, H-28a), 3.05 (2H, t, J= 6.6 Hz, H-16), 3.48 (2H, t, J = 6.6 Hz, H-15), 4.34 (1H, t, J = 6.6 Hz, H-7), 5.61 (1H, bs, 11-OH), 7.09 (2H, d, J = 7.2 Hz, H-31, H-35), 7.15 (1H, t, J = 7.2 Hz, H-33), 7.19 (1H, t, J = 7.2 Hz, H-20), 7.22 (2H, t, J = 7.2 Hz, H-32, H-34), 7.24 (2H, d, J = 7.2 Hz, H-18, H-22), 7.28 (2H, t, J = 7.2 Hz, H-19, H-21), 10.34 (1H, s 5-OH),11.73 (1H, s, 9-OH), 16.10 (1H, s, 13-OH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 7.3 (C-27), 22.1 (C-24), 23.9 (C-26), 26.0 (C-25), 27.1 (C-23), 30.1 (C-7), 30.6 (C-16), 31.2 (C-28), 34.8 (C-29), 45.4 (C-15), 48.5 (C-4), 55.0 (C-2), 103.0 (C-10), 108.0 (C-8), 114.2 (C-6), 125.3 (C-20), 125.7 (C-33), 128.2 (6C, C-18, C-19, C-21, C-22, C-32, C-34), 128.3 (2C, C-31, C-35), 141.2 (C-17), 141.4 (C-30), 156.0 (C-11), 159.9 (C-13), 162.0 (C-9), 176.8 (C-5), 202.9 (C-1), 205.1 (C-14), 211.8 (C-3); (−)-HRESIMS *m/z* 569.2545 $[M - H]^-$ (calcd for $C_{35}H_{37}O_7$, 569.2545).

Corymbone B (2): yellow gum; $[\alpha]_D^{17}$ +11.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.23), 232sh (4.02), 289 (4.07), 362sh (3.48) nm; IR (KBr) ν_{max} 3401 br, 2941, 2871, 1721, 1620, 1492, 1452, 1384, 1162, 1043, 755, 699 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.87 (3H, d, J = 7.2 Hz, H-30), 0.88 (3H, d, J = 7.2 Hz, H31), 1.22 (1H, m, H-29), 1.33 (6H, s, H-24, H-25), 1.39 (3H, s, H-23), 1.50 (3H, s, H-26), 1.81 (1H, m, H-28a), 2.10 (1H, m, H-28b), 2.10 (3H, s, H-27), 3.03 (2H, t, J = 6.6 Hz, H-16), 3.47 (2H, t, J = 6.6 Hz, H-15), 4.42 (1H, t, J = 6.6 Hz, H-7), 5.80 (1H, bs, 11-OH), 7.19 (1H, t, J = 7.2 Hz, H-20), 7.25 (2H, d, J = 7.2 Hz, H-18, H-22), 7.29 (2H, t, J = 7.2 Hz, H-19, H-21), 10.41 (1H, s, 5-OH), 11.71 (1H, s, 9-OH), 16.10 (1H, s, 13-OH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 7.3 (C-27), 21.4 (C-24), 21.6 (2C, C-30, C-31), 23.0 (C-26), 25.4 (C-25), 26.2 (C-23), 26.3 (C-29), 26.7 (C-7), 29.9 (C-16), 37.5 (C-28), 44.5 (C-15), 47.9 (C-4), 54.2 (C-2), 102.2 (C-10), 107.8 (C-8), 114.0 (C-6), 125.7 (C-20), 127.7 (2C, C-18, C-22), 128.0 (2C, C-19, C-21), 141.6 (C-17), 155.0 (C-11), 159.2 (C-13), 161.3 (C-9), 175.7 (C-5), 202.4 (C-1), 204.4 (C-14), 211.3 (C-3); (−)-HRESIMS *m/z* 521.2564 $[M - H]^-$ (calcd for $C_{31}H_{37}O_7$, 521.2545).

TRHR-2 Receptor Binding Assay. Assays were performed in 50 mM Tris buffer containing 3 mM $MgCl_2$ 1 mg/mL BSA, pH 7.4, with HEK2935 cell membranes expressing recombinant rat TRH receptors 2 (supplied by AstraZeneca R&D Montreal) (~10 μg of protein as determined by the Pierce BCA method), and [^{3}H]3-methylhistidylTRH (1 nM equivalent to 50 000 dpm) in a total volume of 210 μL. Controls included 3 μM TRH for nonspecific binding. Compounds were tested at a final concentration of 2% DMSO. Reactions were initiated by the addition of membranes and then continuously mixed for 90 min at 23 °C prior to rapid filtration and washing over GF/B filtermats (Tomtec 96 Mach 2). Mats were dried and counted for 1 min per assay by liquid scintillometry (Betaplate, Wallac). IC_{50} values for the isolated compounds were obtained by testing three wells per concentration.

Acknowledgment. We thank AstraZeneca for financial support. We thank H. T. Vu (Griffith University) for obtaining accurate mass measurements. We thank Ms. S. Kidd for help with biological assays.

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NP0706567