Myrtucommulones F−I, Phloroglucinols with Thyrotropin-Releasing Hormone Receptor-2 Binding Affinity from the Seeds of *Corymbia scabrida*

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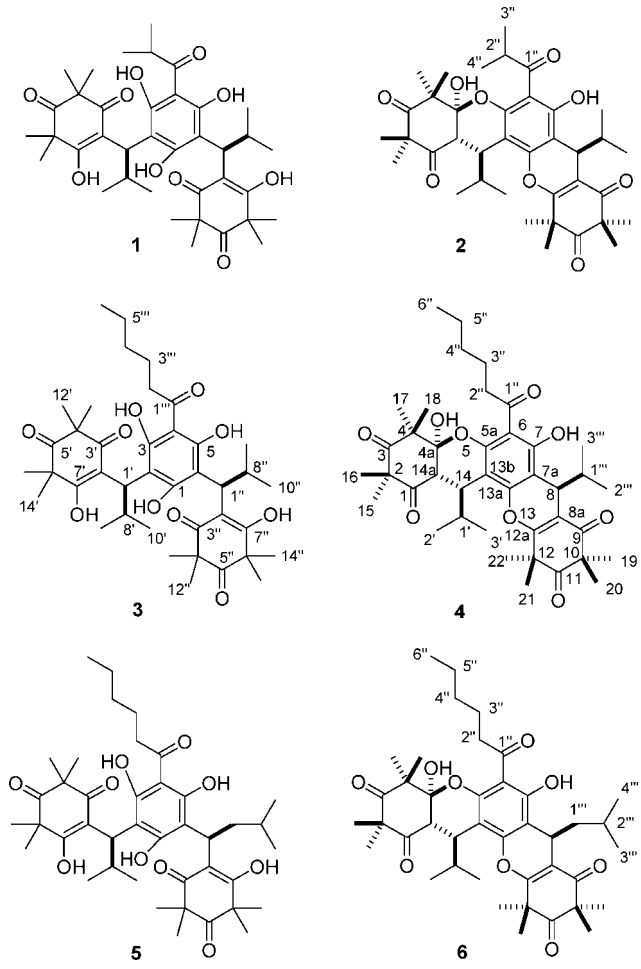
High-throughput screening of a plant and marine invertebrate extract library to find natural products with rat thyrotropin-releasing hormone (TRH) receptor-2 binding affinity led to the isolation of four new, myrtucommulones F−I (**3**−**6**), and two known, myrtucommulones A (**1**) and D (**2**), active acylphloroglucinols from the seeds of the Queensland tree *Corymbia scabrida*. Their structures were assigned from interpretation of 2D NMR and high-resolution ESIMS data. The relative configuration of the stereogenic centers for all six compounds was deduced from ROESY correlations. This is the first time that myrtucommulone A (**1**) has been isolated as a single pure compound. The structure of myrtucommulone D (**2**) has been revised. Myrtucommulones A, D, and F−I showed rat TRH receptor-2 binding affinity with IC_{50} values of 39, 11, 16, 24, 31, and 16 μM, respectively.

We recently discovered two acylphloroglucinols, corymbones A and B, from the flowers of the Australian tree *Corymbia peltata*.[1] Corymbones A and B show binding affinity for recombinant rat thyrotropin-releasing hormone (TRH) receptor-2, a receptor that has been proposed as a potential therapeutic target to treat pain.[2] Corymbones A and B also contained a syncarpic acid (1,1,3,3-tetramethylcyclohexa-2,4,6-trione) residue, and this moiety was responsible for the ^{1}H NMR spectra of these compounds being complex. This moiety was also required for TRH receptor-2 activity since the acylphloroglucinol lacking the syncarpic acid was inactive. Compounds containing syncarpic acid usually possess complex NMR spectra since multiple isomeric forms of this moiety are possible in solution. Acylphloroglucinols containing syncarpic acid residues have been isolated from several other plants from the family Myrtaceae, and these compounds possess a variety of biological activities including insecticidal and antibacterial properties.[3] This paper reports on the chemistry of *Corymbia scabrida* (Brooker & A.R. Bean) K.D. Hill & LAS Johnson (Myrtaceae), which was selected for chemical investigation because CH_2Cl_2 extracts from its seeds possessed THR receptor-2 binding affinity. The TRH receptor-2 activity of *C. scabrida* was ascribed to four new compounds, myrtucommulones F−I (**3**−**6**), together with myrtucommulones A (**1**) and D (**2**). Myrtucommulone A (**1**), a complex acylphloroglucinol isolated as a mixture of homologues and exhibiting strong antibacterial activity against Gram-positive bacteria, contains two syncarpic acid residues, and consequently its structure determination using NMR techniques was hampered by the presence of multiple isomers in solution. The ^{1}H NMR spectra of **1** were highly dependent on pH, temperature, concentration, and solvent.[4,5] A striking feature of the ^{1}H NMR spectrum of **1** was the presence of at least 25 signals between δ_H 10.20 and 16.70, and this was attributed to at least five isomers being present in solution.[4] Myrtucommulone A underwent cyclization and dehydration to produce a symmetrical pentacyclic compound on treatment with acid. A more recent paper reported on the conversion of myrtucommulone A to a silyated cyclized derivative.[6] This derivative was amenable to NMR characterization, revealing that myrtucommulone A cyclized to form a pentacyclic compound lacking the symmetry observed previously.



Results and Discussion

The ground seeds of *C. scabrida* were extracted exhaustively with CH_2Cl_2, and the extract was resuspended in a small volume of CH_2Cl_2 and filtered. The filtrate was chromatographed on C_{18} HPLC, by elution with 9.9% H_2O/0.1% TFA/90% CH_3CN. Six fractions were collected, and fractions 3, 5, and 6 were bioactive. Analysis of these fractions by (−)-ESIMS and NMR indicated that each fraction was still a mixture of two compounds that differed from each other by 18 Da. Each of these fractions was then

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Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz) HMBC, and COSY NMR Data for Myrtucommulone F (**3**)[a]

	δ_C[b]	δ_H	HMBC	COSY
1	162			
OH-1		14.0–16.0	C-6, C-1, C-2, C-1‴	
2	108			
3	159			
OH-3		12.0		
4	110			
5	160			
OH-5		12.0		
6	108			
1′	40	3.9–4.1, m	C-1, C-6, C-5, C-8′, C-2′, C-3′, C-7′	H-8′
2′	114			
3′	203			
4′	55			
5′	210			
6′	49			
7′	180			
OH-7′		11.0−11.2	C-7′, C-6′, C-2′	
8′	27	3.2, m		H-1′, H-9′, H-10′
9′	18−20	0.8−0.9, m	C-1′, C-8′, C-10′	H-8′
10′	18−20	0.8−0.9, m	C-1′, C-8′, C-9′	H-8′
11′	25−26	1.3−1.5, m	C-3′, C-4′, C-5′, C-12′	
12′	25−26	1.3−1.5, m	C-3′, C-4′, C-5′, C-11′	
13′	25−26	1.3−1.5, m	C-5′, C-6′, C-7′, C-14′	
14′	25−26	1.3−1.5, m	C-5′, C-6′, C-7′, C-13′	
1″	40	3.9–4.1, m	C-8″, C-10″, C-9″, C-2″, C-3″, C-7″	H-8″
2″	114			
3″	203			
4″	55			
5″	210			
6″	49			
7″	178			
OH-7″		11.0−11.2	C-7″, C-6″, C-2″	
8″	27	3.2 m,		H-1″, H-9″, H-10″
9″	18−20	0.8−0.9, m	C-1″, C-8″, C-10″	H-8″
10″	18−20	0.8−0.9, m	C-1″, C-8″, C-9″	H-8″
11″	25−26	1.3−1.5, m	C-3″, C-4″, C-5″, C-12″	
12″	25−26	1.3−1.5, m	C-3″, C-4″, C-5″, C-11″	H-31, H-33
13″	25−26	1.3−1.5, m	C-5″, C-6″, C-7″, C-14″	H-32, H-34
14″	25−26	1.3−1.5, m	C-5″, C-6″, C-7″, C-13″	H-33, H-35
1‴	208			
2‴	45	3.2, m	C-2, C-1‴, C-3‴, C-4‴	H-3‴
3‴	25	1.6, m	C-1‴, C-2‴, C-4‴, C-5‴, C-6‴	H-2‴, H-3‴
4‴	32	1.3, m	C-2‴, C-3‴, C-5‴, C-6‴	H-3‴, H-5‴, H-6‴
5‴	22	0.8, m	C-3‴, C-4‴, C-6‴	H-4‴
6‴	22	0.8, m	C-3‴, C-4‴, C-5‴	H-4‴

[a] Spectra recorded in d_6-benzene at 30 °C. [b] ^{13}C chemical shifts obtained from gHSQC and gHMBC spectra.

partitioned between heptane/CH_2Cl_2/CH_3CN (10:3:7); the upper phase from fraction 3 contained myrtucommulone A (**1**) and the lower phase contained myrtucommulone D (**2**), the upper phase from fraction 5 contained myrtucommulone F (**3**) and the lower phase contained myrtucommulone G (**4**), and the upper phase from fraction 6 contained myrtucommulone H (**5**) and the lower phase contained myrtucommulone I (**6**). Mass ion peaks for the $[M - H]^-$ ions for myrtucommulones D, G, and I were not present in the (−)-ESIMS of the crude CH_2Cl_2 extract, and this suggested that these compounds were artifacts produced by acid-catalyzed dehydration during evaporation of the HPLC solvents.

The most abundant bioactive compound, myrtucommulone F (**3**), was obtained as an optically active yellow gum. Accurate mass measurement of the pseudomolecular ion at *m/z* 695.3833 in the (−)-HRESIMS allowed a molecular formula of $C_{40}H_{56}O_{10}$ to be assigned to myrtucommulone F (**3**). Infrared absorption bands at 2872−3116, 1658, and 1721 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 295 nm.[7−10]

The ^{1}H NMR spectrum of myrtucommulones F (**3**) (Table 1) in $CDCl_3$ was extremely complex, which suggested the molecule existed as multiple conformations in solution. Variable-temperature studies and changing the NMR solvent to d_6-acetone, d_6-DMSO, C_6D_6, or C_5D_5N did not simplify the spectra. The assignments of the signals were therefore fairly complicated and relied heavily on 2D NMR experiments run in C_6D_6. The ^{1}H and ^{13}C NMR spectroscopic assignments for individual atoms could only be estimated to cover broad chemical shift ranges (approximately 0.2 ppm in ^{1}H and 1 ppm in ^{13}C). It could be deduced that the molecule contained eight quaternary methyl groups, δ_H 1.30−1.50, four tertiary methyl groups, δ_H 0.8−0.9, one secondary methyl, δ_H 0.8, four methylenes, δ_H 3.2, 1.6, 1.3, 0.8, four methines, δ_H 3.00−3.25 (2H), δ_H 3.90−4.20 (2H), and six exchangeable protons (over 20 signals observed between δ_H 11.0 and 16.0). The gHMQC spectrum allowed the protonated carbons to be assigned. The COSY spectrum suggested that the molecule contained two $-CHCH(CH_3)_2$ partial structures and a $CH_2CH_2CH_2CH_2CH_3$ partial structure. Analysis of correlations observed in the HMBC spectrum allowed the structure to be established. The quaternary methyl group region in the ^{1}H NMR spectrum (δ_H 1.30−1.50) showed correlations to carbons at δ_C 210, 203, 178, 180, 55, and 49. This was indicative of 2-enol tautomers of two 1,1,3,3-tetramethylcyclohexatriones.[11] Exchangeable proton signals between δ_H 11.00 and 11.20 could be assigned to the enol exchangeable protons of the cyclohexatriones since these signals correlated to carbons at δ_C 178, 114, and 49. Methine protons at δ_H 3.90−4.20 also showed correlations to carbons at δ_C 203, 178, and 180 as well as to carbons at δ_C 114, 110, 108, 162, 159, 27, and 18–20. This indicated that the two $-CHCH(CH_3)_2$ groups were each attached to a 1,1,3,3-tetramethylcyclohexatrione and to a central 1,3,5-trioxygenated aromatic group. The methylene protons at δ_H 3.20 correlated to a ketone carbonyl carbon at δ_C 208, allowing the partial structure $-CH_2CH_2CH_2CH_2CH_3$ to be extended to $-COCH_2CH_2CH_2CH_2CH_3$. The exchangeable protons signals between δ_H 14.0 and 16.0

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Myrtucommulones D (**2**), G (**4**), and I (**6**)[a]

	myrtucommulone D (**2**)		myrtucommulone G (**4**)		myrtucommulone I (**6**)	
position	δ_C[b]	δ_H (J in Hz)	δ_C[b]	δ_H (J in Hz)	δ_C[b]	δ_H (J in Hz)
1	204.3		204.1		204.1	
2	57.8		58.0		57.9	
3	213.5		213.1		213.2	
4	55.5		54.9		55.0	
4a	100.9		100.5		100.7	
5a	151.9		151.9		151.8	
6	108.8		109.4		103.0	
7	161.5		161.5		161.6	
OH-7		14.08, s		14.31, s		14.30, s
7a	109.2		108.6		110.1	
8	32.5	4.90, d (3.2)	32.1	4.92, d (3.2)	25.9	4.83, t (3.0)
8a	111.9		111.9		114.4	
9	196.8		196.9		196.8	
10	56.1		56.2		56.1	
11	211.0		210.9		210.9	
12	47.6		47.4		47.5	
12a	167.3		167.2		166.2	
13a	153.8		154.0		153.2	
13b	107.9		107.5		104.0	
14	29.7	4.39, dd (3.7, 6.0)	29.1	4.39, dd (3.7, 6.0)	29.7	4.43, dd (3.6, 6.0)
14a	45.3	3.47, d (6.0)	45.4	3.45, d (6.0)	46.0	3.47, d (6.0)
15	22.7	1.40 s	22.6	1.38, s	22.5	1.40, s
16	26.8	1.17, s	26.6	1.14, s	26.6	1.16, s
17	19.4	1.25, s	19.2	1.12, s	19.5	1.19, s
18	24.4	1.34, s	24.4	1.31, s	24.5	1.33, s
19	25.3	1.34, s	25.0	1.35, s	24.5	1.35, s
20	24.2	1.22, s	25.0	1.21, s	24.5	1.22, s
21	25.0	1.53, s	25.0	1.53, s	25.1	1.52, s
22	25.2	1.34, s	24.9	1.34, s	25.2	1.37, s
1′	32.8	2.38, m	32.0	2.40, m	32.6	2.42, m
2′	16.3	0.56, d (6.6)	15.7	0.58, d (6.6)	16.1	0.59, d (6.6)
3′	20.3	0.84, d (6.6)	20.0	0.86, d (6.6)	20.4	0.87, d (6.6)
1″	210.3		205.9		205.9	
2″	40.2	3.77, dqq (7.2, 7.2, 7.2)	45.3	2.91, m	44.8	2.96, m
				2.76, m		2.80, m
3″	18.1	1.12, d (7.2)	23.8	1.68, m	23.9	1.66, m
4″	20.8	1.03, d (7.2)	31.8	1.19, m	31.7	1.19, m
5″			22.4	1.23, m	22.9	1.23, m
6″			13.9	0.82, t (6.6)	14.0	0.84, t (6.6)
1‴	35.0	2.26, m	35.0	2.29, m	45.8	1.80, m
						1.85, m
2‴	18.5	0.81, d (6.6)	18.8	0.86, d (6.6)	25.8	1.67, m
3‴	20.1	0.89, d (6.6)	19.6	0.92, d (6.6)	23.9	0.84, d (6.6)
4‴					24.0	0.97, d (6.6)

[a] Spectra recorded in d_6-benzene at 30 °C. [b] ^{13}C chemical shifts obtained from gHSQC and gHMBC spectra.

showed two- and three-bond correlations to aromatic carbons at δ_C 108 and 162 and a small four-bond correlation to the carbonyl carbon C-1‴ at δ_C 208. The extreme downfield chemical shifts of these exchangeable protons was in agreement with strong hydrogen bonding between the phenolic proton and the adjacent carbonyl carbons, C-1‴, C-3′, and C-3″. In total these data suggested structure **3** for myrtucommulone F, and the complexity of the ^{1}H NMR spectrum is derived from the ability of the molecule to form a multitude of keto−enol tautomers and hydrogen bonds. Myrtucommulone F (**3**) differs from myrtucommulone A (**1**) by the replacement of an isobutanoyl with a *n*-hexanoyl moiety.

A small proportion of myrtucommulone F (**3**) underwent dehydration and cyclization during evaporation of the fraction after HPLC separation, producing myrtucommulone G (**4**). Myrtucommulone G was therefore an artifact. The (−)-HRESIMS of myrtucommulone G (**4**) displayed a molecular ion peak at *m*/*z* 677.3727, allowing a molecular formula of $C_{40}H_{54}O_9$ to be assigned.

The ^{1}H NMR spectrum of myrtucommulone G (**4**) (Table 2) was considerably simpler than that of myrtucommulone F (**3**) since only one conformer was observed in solution. Analysis of the ^{1}H NMR and COSY spectra indicated the presence of eight quaternary methyl groups, two $-CHCH(CH_3)_2$, and one $-COCH_2CH_2CH_2CH_2CH_3$ partial structure. Correlations from methyl singlets at δ_H 1.31 (H-18) and 1.12 (H-17) to carbons at δ_C 213.1 (C-3), 54.9 (C-4), and 100.5 (C-4a), from the methyl singlets at δ_H 1.38 (H-15) and 1.14 (H-16) to carbons at δ_C 204.1 (C-1), 213.1 (C-3), and 58.0 (C-2), and from the methine proton doublet at δ_H 3.45 (H-14a) to carbons δ_C 58.0, 54.9, 100.5, and 204.1 in the HMBC spectrum suggested that a 6-hemiketal of a 1,1,3,3-tetramethylcyclohexa-2,4,6-trione was present in the molecule. The carbon at 100.5 ppm was a hemiketal formed by cyclization of one of the phenolic oxygens, OH-3, onto the ketone carbon, C-3′, of one of the cyclohexatriones in myrtucommulone F (**3**). COSY correlations from H-14 (δ_H 4.39) to H-14a and the isopropyl methine proton H-1′ (δ_H 2.40) indicated that the 1,1,3,3-tetramethylcyclohexa-2,4-dione-6-hemiketal was attached to an isobutyl group. HMBC correlations were observed from H-14 to three aromatic carbons, δ_C 107.5 (C-13b), 151.9 (C-5a), and 154.0 (C-13a), demonstrating that the isobutyl was also attached to an oxygenated aromatic group. Correlations from methyl singlets at δ_H 1.21 (H-20) and 1.35 (H-19) to carbons at δ_C 210.9 (C-11), 56.2 (C-10), and 196.9 (C-9), from the methyl singlets at δ_H 1.34 (H-22) and 1.53 (H-21) to carbons at δ_C 210.9 (C-11), 167.2 (C-12a), and 47.4 (C-12), and from an isobutyl methine proton doublet at δ_H 4.92 (H-8) to carbons at δ_C 167.2 (C-12a) and 111.9 (C-8a) in the HMBC spectrum indicated that myrtucommulone G (**4**) also contained a 1,1,3,3-tetramethylcyclohexa-2,6-dione-4,5-enol ether attached to a second isobutyl group. The isobutyl methine proton H-8 also showed HMBC correlations to three aromatic carbons at δ_C 108.6 (C-7a), 154.0 (C-13a), and 161.5 (C-7), while a hydrogen-bonded phenolic proton at δ_H 14.31 (OH-7) also correlated to C-7 as well as to δ_C 109.4 (C-6). These data indicated that the two isobutylcyclohexyl groups were cyclized onto a

phloroglucinol core. The observation that both methine protons H-8 and H-14 correlated to the oxygenated carbon, C-13a, which did not correlate to the phenolic proton OH-7, indicated that the points of cyclization for the two cyclohexyl groups were the phenolic oxygens attached to C-5a and C-13a. This left the hexanoyl group to be attached at C-6, and the phenolic proton (OH-7) must be hydrogen-bonded to the carbonyl carbon C-1″. The orientation of the two hemiketal cyclizations was therefore as shown in structure **4** for myrtucommulone G. The relative configurations of the four stereogenic centers in **4** were determined from interpretation of correlations observed in a ROESY spectrum. Correlations were observed between H-14a and the two methyl singlets H-16 and H-18, suggesting these three groups were on the β-face of the molecule. A correlation between H-14a and H-2′ indicated that the isopropyl group attached to C-14 was also β. A correlation from H-1′ to the methyl H-21 indicated that H-21 was also β. A correlation from the isopropyl group attached to C-8 was also β since a ROESY correlation was observed between H-21 and the isopropyl methyl proton H-2‴. The hydroxyl group OH-4a was α since the correlations described above together with a correlation between H-18 and H-2″ were possible only with this configuration. The relative configuration assigned to **4** was therefore 4a*S**, 8*R**, 14*S**, 14a*R**, the same as that assigned to the silyated cyclized derivative of myrtucommulone A.[6] The structure of myrtucommulone G (**4**) provided further evidence in support of the proposed structure of myrtucommulone F (**3**) and suggested that the relative configurations of the two sterogenic centers in **3** are both *R**.

The ^{1}H NMR spectrum of myrtucommulone A (**1**), like that of myrtucommulone F (**3**), was very complicated, suggesting a structural similarity between the two compounds. As small sample size precluded the acquisition of useful 2D heteronuclear NMR spectra, the structure of myrtucommulone A was assigned on the basis of analysis of COSY data and from detailed analysis of the NMR spectra obtained for its dehydration product, myrtucommulone D (**2**). Much of the ^{1}H NMR data for myrtucommulone A (**1**) were identical to those of myrtucommulone F (**3**). In fact, the only difference was related to the ketone side chain attached to C-6. The appearance of a methine C-2″ at δ 3.1 in the ^{1}H NMR spectrum of **1** suggested an isopropyl group was attached directly to the ketone carbonyl carbon. The dehydration product of myrtucommulone A (**1**), myrtucommulone D (**2**), was a more amenable target for structural studies. The (−)-HRESIMS of myrtucommulone D (**2**) displayed a molecular ion peak at *m*/*z* 649.3350 [M − H]$^-$ allowing a molecular formula of $C_{38}H_{50}O_9$ to be assigned. It was clear from analysis of ^{1}H, gCOSY, gHSQC, and gHMBC spectra that myrtucommulone A (**1**) had undergone an analogous set of cyclizations and dehydration to those that had occurred with myrtucommulone F (**3**). This analysis also confirmed that the side chain attached at C-6 was an isobutyl ketone in **2** and by analogy also in **1**. ROESY correlations indicated that myrtucommulone D (**2**) possessed the same relative configuration at C-4a, C-8, C-14, and C-14a as myrtucommulone G (**4**). It therefore followed that the configurations at C-3′ and C-3″ in myrtucommulone A (**1**) were both *R**. A structure for myrtucommulone D, which was epimeric at C-5 and C-14a with that we have determined, was assigned by Shaheen et al. from X-ray crystallographic analysis.[11] Comparison of the published ^{13}C NMR data with what we obtained suggested that our compound was the same structure. However, several of the ROESY correlations we observed were not compatible with the published structure. Closer inspection of the ORTEP plot of the X-ray diffraction analysis presented by Shaheen et al., however, indicated that the structure they assigned to myrtucommulone D was epimeric at C-5 and C-14a with that depicted in their X-ray ORTEP plot and was thus the same as what we determined from ROESY analysis. Therefore, the structure of myrtucommulone D has been revised to **2**.

The (−)-HRESIMS of myrtucommulone H (**5**) displayed a molecular ion peak at *m*/*z* 709.3985 [M − H]$^-$, allowing a molecular formula of $C_{41}H_{58}O_{10}$ to be assigned to myrtucommulone H (**5**). The ^{1}H NMR spectrum of myrtucommulone H (**5**), like that of myrtucommulone F (**3**), was very complicated, indicating myrtucommulone H (**5**) was also structurally related to myrtucommulone F (**3**). The structure elucidation of myrtucommulone H (**5**) followed the same process outlined for myrtucommulone A (**1**). The ^{1}H NMR spectrum of **5** was almost identical to that of myrtucommulone F (**3**). In fact, the only difference related to signals assigned to the side chain attached at C-8. An additional methylene resonance was observed at δ_H 1.9−2.0, and a COSY correlation from this resonance to a methine resonance at δ_H 1.2−1.5, which in turn correlated to methyl resonances at δ_H 0.70−0.95, indicated that the methylene could be placed between C-8 and the isopropyl group. Once again, detailed analysis of the NMR spectra obtained for myrtucommulone I (**6**), the dehydration product of myrtucommulone H (**5**), provided substantiating evidence that an isobutyl group was attached to C-8 in myrtucommulone H. Myrtucommulone I (**6**) was assigned a molecular formula of $C_{41}H_{56}O_9$ from analysis of the [M − H]$^-$ ion at *m*/*z* 691.3844 in the (−)-HRESIMS. Analysis of the ^{1}H, gCOSY, gHSQC, gHMBC, and ROESY NMR data for myrtucommulone I (**6**) (Table 2) confirmed that this compound was very similar to myrtucommulone G (**4**). The spectra were identical except for the resonances associated with substituents attached to C-8. H-8 was a triplet in the ^{1}H NMR spectrum of myrtucommulone I (**6**) compared with a doublet in the spectra of both myrtucommulone G (**4**) and myrtucommulone D (**2**). An upfield shift of ∼6 ppm was observed for C-8 in **6** compared to **2** and **4**, and additional methylene proton resonances observed at δ_H 1.80 and 1.85 that correlated to H-8 and the methine, H-2″, of an isopropyl group in the COSY spectrum of myrtucommulone I suggested its structure was **6**. A similar pattern of ROESY correlations indicated that myrtucommulone I (**6**) possesses the same relative configuration at C-4a, C-8, C-14, and C-14a as myrtucommulone G (**4**). It therefore followed that the configurations at C-3′ and C-3″ in myrtucommulone H (**5**) are both *R**.

Previous papers have reported that myrtucommulone A was isolated with a mixture contaminated by minor homologues.[4−6] This current investigation has successfully separated two homologues, yielding pure compounds that differ from each other by the substituents attached to C-3′ and C-3″. Myrtucommulone A (**1**) is thus reported here as a single compound for the first time. Acid-catalyzed cyclization of myrtucommulone A to yield a single pentacyclic derivative, for which the relative configuration was determined by detailed ROESY correlation analysis, has allowed the relative configuration of the two stereogenic centers in myrtucommulone A to be assigned.

The observation that **1**, **3**, and **5** show optical activity is somewhat surprising since symmetry about the phloroglucinol core suggests that the three compounds should be meso. Their chirality could result from atropisomerism about either the C-2−C-1′ or C-6−C1″ bonds, and this would result in axial chirality. Alternatively, strong hydrogen bonding between the phenolic protons and an adjacent carbonyl carbon could lock the structure into a fixed conformation in which the phloroglucinol core is not symmetrical. That clean cyclization and dehydration of **1**, **3**, and **5** to each yield only one unsymmetrical product is also remarkable. The carbonyl attached to C-4 could form a strong hydrogen bond to either of the adjacent phenols (OH-3 or OH-5). The remaining phenol (OH-1) would then be free to cyclize onto either C-3′ or C-3″ of the adjacent cyclohexatriones. Dehydration of the resultant hemiacetal would then result in any further cyclization yielding only an unsymmetrical product. The optical rotation that we observed for myrtucommolume D (**2**) ([α]$_D$ +13.9) is significantly smaller than that observed by Sharheen et al. ([α]$_D$ +375),[11] suggesting that we isolated **2** as a partial racemate. This would be expected for a purely chemical

transformation and suggests that **4** and **6** were also probably isolated as partial racemates. The large optical rotation observed by Sharheen et al. for **2** however suggests that its production by *Myrtus communis* must be under enzymatic control.

It is interesting to note that although myrtucommulones A (**1**), F (**3**), and H (**5**) should be more polar than myrtucommulones D (**2**), G (**4**), and I (**6**), since they contain two additional phenols, they partition onto the upper (less polar) phase of the trisolvent mixture of heptane/CH_2Cl_2/CH_3CN, while **2**, **4**, and **6** partition into the lower (more polar) phase. The ^{1}H NMR spectra of **1**, **3**, and **5** provide clues to explain this counterintuitive result. The observation of significantly downfield shifted phenolic signals (δ 10.7−17.8) suggests that very strong intramolecular hydrogen bonds between the phenolic protons and the adjacent carbonyl oxygens are present in **1**, **3**, and **5**. These hydrogen bonds would prevent the phenols from forming hydrogen bonds with solvent molecules, thus making them nonpolar. Myrtucommulones D (**2**), G (**4**), and I (**6**), on the other hand, each contain a hemiacetal hydroxyl, which can interact with the solvent, and therefore they are more polar than **1**, **3**, and **5**.

Myrtucommulones A (**1**) D (**2**), and F−I (**3**−**6**) inhibited the specific binding of [^{3}H]3-methylhistidylTRH to HEK cell membranes expressing recombinant rat TRH receptors-2 with IC_{50} values of 39, 11, 16, 24, 31, and 16 μM, respectively. The IC_{50} value for the positive control TRH was 23 nM.

Experimental Section

General Experimental Procedures. All solvents used were Omnisolv HPLC grade. Optical rotations were measured on a JASCO P-1020 polarimeter (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 Varian Inova 600 MHz NMR spectrometer. Samples were dissolved in d_6-benzene, and chemical shifts were calculated relative to the d_6-benzene solvent peak (δ_H 7.10 and δ_C 128.0). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences gCOSY, gHMQC, gHSQC, gHMBC, and ROESY. HRESIMS were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass spectrometer. HPLC separations were achieved using a Rainin Microsorb C_{18} semipreparative column (3 μm, 10 mm × 50 mm).

Plant Material. Seeds of *C. scabrida* were collected by one of the authors (P.I.F.) in April 1996, 81 km from Springsure in central Queensland. A voucher specimen, AQ602588, is deposited at the Queensland Herbarium.

Extraction and Isolation. The air-dried, ground seeds of *C. scabrida* (11.0 g) were extracted with CH_2Cl_2 (4 × 300 mL), yielding a green gum (5.25 g). The CH_2Cl_2 extract was resuspended in a small volume of CH_2Cl_2 and filtered. The filtrate was chromatographed on C_{18} HPLC, by elution with 9.9% H_2O/0.1% TFA/90% CH_3CN. Six fractions were collected, and fractions 3, 5, and 6 were bioactive. Analysis of these fractions by (−)-ESIMS and NMR indicated that each fraction was still a mixture of two compounds that differed from each other by 18 Da. These fractions were then partitioned between heptane/CH_2Cl_2/CH_3CN (10:3:7); the upper phase from fraction 3 contained myrtucommulone A (**1**) (2.3 mg, 0.02%) and the lower phase contained myrtucommulone D (**2**) (1.5 mg, 0.014%), the upper phase from fraction 5 contained myrtucommulone F (**3**) (21 mg, 0.19%) and the lower phase contained myrtucommulone G (**4**) (4 mg, 0.036%), and the upper phase from fraction 6 contained myrtucommulone H (**5**) (12.5 mg, 0.11%) and the lower phase contained myrtucommulone I (**6**) (1.5 mg, 0.014%).

Myrtucommulone A (1): yellow gum; $[\alpha]_D^{17.6}$ +42.1 (*c* 0.066, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.34), 233 (4.21), 272 (4.25), 295 (4.27) nm; IR (KBr) ν_{max} 3139 br, 2970, 2872, 1738, 1722, 1711, 1642, 1468, 1443, 1403, 1051, 758 cm^{-1}; ^{1}H NMR (600 MHz, C_6D_6) δ 0.8−1.8 (multiple signals, 14 × CH_3), 3.0−3.4 (multiple signals, 2 × CH), 3.90−4.20 (multiple signals, 3 × CH) 10.7−17.8 (multiple singlets, 5 × OH); (−)-HRESIMS *m/z* 667.3515 [M − H]$^-$ (calcd for $C_{38}H_{51}O_{10}$, 667.3488).

Myrtucommulone D (2): yellow gum; $[\alpha]_D^{17}$ +13.9 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.50), 270 (4.29), 297 (4.41) nm; IR (KBr) ν_{max} 3121 br, 2969, 2934, 2873, 1722, 1710, 1658, 1642, 1619, 1599, 1467, 1451, 1442, 1402, 1384, 1157, 1136, 1092, 1023, 757 cm^{-1}; ^{1}H (600 MHz, C_6D_6) and ^{13}C NMR (150 MHz, C_6D_6), Table 2; (−)-HRESIMS *m/z* 649.3350 [M − H]$^-$ (calcd for $C_{38}H_{49}O_9$, 649.3382).

Myrtucommulone F (3): yellow gum; $[\alpha]_D^{17}$ +18.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.43), 232 (4.36), 270 (4.32), 295 (4.43) nm; IR (KBr) ν_{max} 3116 br, 2968, 2932, 2872, 1721, 1658, 1598, 1468, 1384, 1300, 1259, 1160, 1121, 1049, 1021, 758 cm^{-1}; ^{1}H (600 MHz, C_6D_6) and ^{13}C NMR (150 MHz, C_6D_6), Table 1; (−)-HRESIMS *m/z* 695.3833 [M − H]$^-$ (calcd for $C_{40}H_{55}O_{10}$, 695.3801).

Myrtucommulone G (4): yellow gum; $[\alpha]_D^{17}$ +22.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.49), 271 (4.15), 296 (4.22) nm; IR (KBr) ν_{max} 3427 br, 3130 br, 2959, 2930, 2872, 1722, 1710, 1657, 1619, 1467, 1369, 1260, 1205, 1183, 1160, 1120, 1025 cm^{-1}; ^{1}H (600 MHz, C_6D_6) and ^{13}C NMR (150 MHz, C_6D_6), Table 1; (−)-HRESIMS *m/z* 677.3727 [M − H]$^-$ (calcd for $C_{40}H_{53}O_9$, 677.3695).

Myrtucommulone H (5): yellow gum; $[\alpha]_D^{17}$ +11.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.54), 285 (4.37) nm; IR (KBr) ν_{max} 3130 br, 2959, 2933, 2872, 1721, 1711, 1692, 1658, 1649, 1620, 1612, 1467, 1451, 1402, 1178, 1117, 1047 cm^{-1}; ^{1}H NMR (600 MHz, C_6D_6) δ 0.7−0.95 (multiple signals, 5 × CH_3, + CH_2), 1.2−1.5 (multiple signals, 8 × CH_3 + 2 × CH_2 + CH), 1.9−2.4 (multiple signals 2 × CH_2), 3.0−3.4 (multiple signals, CH + CH_2), 3.90−4.20 (multiple signals, 2 × CH) 10.7−17.8 (multiple singlets, 5 × OH); (−)-HRESIMS *m/z* 709.3985 [M − H]$^-$ (calcd for $C_{41}H_{57}O_{10}$, 709.3957).

Myrtucommulone I (6): yellow gum; $[\alpha]_D^{17}$ +18.8 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.51), 294 (4.22) nm; IR (KBr) ν_{max} 3140 br, 2957, 2931, 2872, 1738, 1619, 1457, 1442, 1302, 1160, 1106, 1077, 1045 cm^{-1}; ^{1}H (600 MHz, C_6D_6) and ^{13}C NMR (150 MHz, C_6D_6), Table 2; (−)-HRESIMS *m/z* 691.3844 [M − H]$^-$ (calcd for $C_{41}H_{55}O_9$, 691.3851).

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 receptor-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, then continuously mixed for 90 min at 23 °C prior to rapid filtration and washing over GF/B filtermats (Tomtec 96 Mach 2, Camden, CT). 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.

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References and Notes

(1) Carroll, A. R.; Urban, S.; Lamb, J.; Moni, R.; Guymer G. P.; Forster, P. I.; Quinn, R. J. *J. Nat. Prod.* **2008**, *71*, 881−883.
(2) Boschi, G.; Desiles, M.; Reny, V.; Rips, R.; Wrigglesworth, S. *Br. J. Pharmacol.* **1983**, *79*, 85–92.
(3) Ghisalberti, E. L. *Phytochemistry* **1996**, *41*, 7–22.
(4) Kashman, Y.; Rotstein, A.; Lifshitz, A. *Tetrahedron* **1974**, *30*, 991–997.
(5) Lounasmaa, M.; Puri, H-S.; Widen, C.-J. *Phytochemistry* **1977**, *16*, 1851–1852.
(6) Appendino, G.; Bianchi, F.; Minassi, A.; Sterner, O.; Ballero, M.; Gibbons, S. *J. Nat. Prod.* **2002**, *65*, 334–338.
(7) Widen, C. J.; Vida, G.; von Euw, J.; Reichstein, T. *Helv. Chim. Acta* **1971**, *54*, 2824–2859.
(8) Forsen, S.; Merenyi, F.; Nilsson, M. *Acta Chem. Scand.* **1964**, *18*, 1208–1221.
(9) Parker, W. L.; Johnson, F. *J. Am. Chem. Soc.* **1968**, *90*, 4716–4723.
(10) Van Klink, J. W.; Brophy, J. J.; Perry, N. B.; Weavers, R. T. *J. Nat. Prod.* **1999**, *62*, 487–489.
(11) Shaheen, F.; Ahmad, M.; Khan, S. N.; Hussain, S. S.; Anjum, S.; Tashkhodjaev, B.; Turgunov, K.; Sultankhodzhaev; Choudhary, M. I.; Rahman, A. *Eur. J. Org. Chem.* **2006**, 2371–2377.

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