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Hydrogen-bonded rotamers of 2′,4′,6′-trihydroxy-3′-formyldihydrochalcone, an intermediate in the synthesis of a dihydrochalcone from *Leptospermum recurvum*

Kamarul'Ain Mustafa,[a] Henrik G. Kjaergaard,[a] Nigel B. Perry[b] and Rex T. Weavers[a,*]

[a]*Department of Chemistry, University of Otago, P.O. Box 56, Dunedin 9001, New Zealand*
[b]*Department of Chemistry, Plant Extracts Research Unit, New Zealand Institute for Crop and Food Research Limited, University of Otago, P.O. Box 56, Dunedin, New Zealand*

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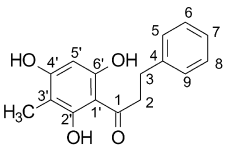
Abstract—Synthesis of 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone, isolated as a natural product for the first time (*ex Leptospermum recurvum*), proceeds through 2′,4′,6′-trihydroxy-3′-formyldihydrochalcone. Two stable rotamers of this formyl derivative have been identified and the problems associated with NMR assignments of this type of compound have been attributed to conformational exchange. Rotamer ratios from molecular modelling and ab initio calculations agree well with those obtained from low temperature NMR studies. There is also excellent correlation between experimental NMR chemical shifts for the hydrogen-bonded hydroxyl protons with those derived from ab initio calculations. This formyl dihydrochalcone showed promising bioactivity in antiviral and antimicrobial assays.

1. Introduction

As an extension of our studies on the bioactive components of the New Zealand manuka, *Leptospermum scoparium*,[1,2] we have screened *Leptospermum* species for a range of biological activities. This study revealed that the ethanol extract of the foliage of *Leptospermum recurvum* Hook. f. (family Myrtaceae) possessed antiviral activity. This plant, endemic to Mt Kinabalu in the States of Sabah, Malaysia has been reported to contain the polyphenols, cyanidin, quercetin, ellagic acid, delphinidin and myricetin,[3] but we could find no reports on medicinal properties. However, *L. recurvum* is almost identical (apart from leaf size) to *L. flavescens*[3] which has been used traditionally in Malaysia to stimulate appetite and relieve stomach disorders and menstrual discomfort.[4]

In this paper we describe the isolation of flavonoid components of *L. recurvum* foliage, including the dihydrochalcone (**1**). Synthesis of **1** was subsequently achieved via the formyl derivative (**2**). NMR studies of **2** produced spectra with some broad peaks and some notable absences of signals. Searching the literature likewise revealed incomplete sets of NMR data. We here detail studies of the solution chemistry of **2**, by low temperature NMR and by molecular mechanics and ab initio studies, that elucidate the conformational exchange responsible for these anomalies.



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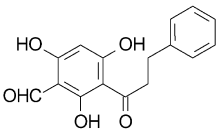

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2. Results and discussion

The dried foliage of *L. recurvum* was extracted with ethanol and, after repeated column chromatography and preparative TLC on silica gel, yielded 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone **1**[5] and an inseparable mixture of 2,5-dihydroxy-6-methyl-7-methoxyflavanone (**3**) and its isomer 2,5-dihydroxy-8-methyl-7-methoxyflavanone (**4**).[6] Further components proved difficult to separate from the major component, the mixture of hydroxyflavanones **3** and **4**. This mixture was dehydrated to convert **3** and **4** to the flavones **5** and **6**. Chromatography yielded 5-hydroxy-6-methyl-7-methoxyflavone (**5**),[7] 5-hydroxy-8-methyl-7-methoxyflavone (**6**),[8] 2′,6′-dihydroxy-3′-methyl-4′-methoxydihydrochalcone (**7**),[9] and 5-hydroxy-6-methyl-7-methoxyflavanone (**8**).[8] HPLC studies revealed that compounds **1**, **3**, **4**, **5**, **7** and **8** were present in the total plant

Keywords: Myrtaceae; conformational exchange; molecular modelling; ab initio calculation; NMR.
* Corresponding author. Tel.: +64-3479-7925; fax: +64-3479-7906; e-mail: rweavers@alkali.otago.ac.nz

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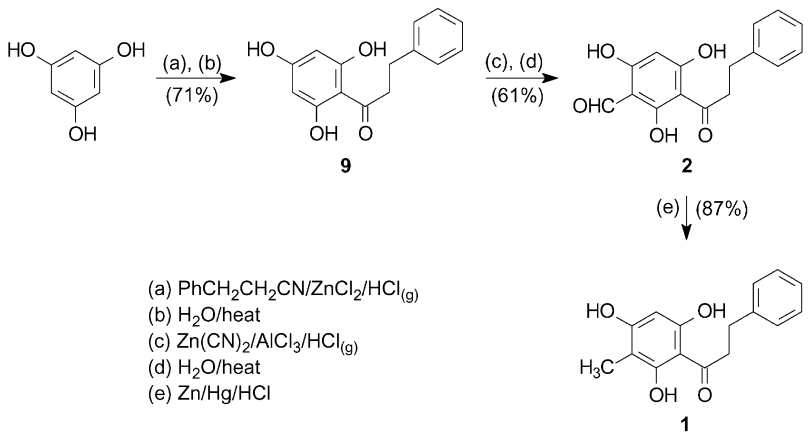
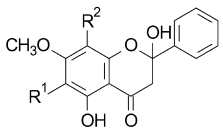
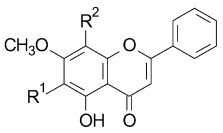


Scheme 1. Synthesis of dihydrochalcone **1**.

extract. Although a significant peak was observed for flavanone **5**, the level of the isomeric flavanone **6** was below detection in the extract.



3 $R^1 = CH_3$; $R^2 = H$
4 $R^1 = H$; $R^2 = CH_3$



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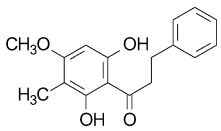
6 $R^1 = H$; $R^2 = CH_3$



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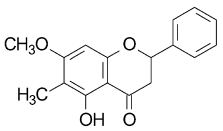

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The 4′-methoxydihydrochalcone **7** was found to be antiviral against Herpes-simplex virus, whereas its demethylated analogue **1** showed no antiviral activity but was mildly cytotoxic. Further studies on the activity of **1** were hampered by lack of sample and the unavailability of further plant material. Synthesis of **1** was conducted by following an established three-step method[10] involving acylation of phloroglucinol, followed by formylation and reduction of the formyl group (Scheme 1).

The NMR spectra of an intermediate from this synthetic work, 2′,4′,6′-trihydroxy-3′-formyldihydrochalcone **2**, showed interesting complexities. This dihydrochalcone has been reported once as a natural product, isolated from the twig and leaf extracts of the Peruvian plant *Psidium acutangulum* (Myrtaceae).[11] This report quoted some ^{13}C NMR data but gave no values for the hydroxylated carbons. Sato et al. reported NMR data for the 5′-methyl substituted analogue, but also did not quote hydroxylated carbon shifts.[10] The reason for this became apparent when the ^{13}C NMR spectrum of **2** was recorded at 25°C; the peaks for C-2′, C-4′, and C-6′ were almost lost in the baseline (Fig. 1). In the ^1H NMR spectrum (Fig. 2) only one broad peak was visible to represent all of the three OH groups.

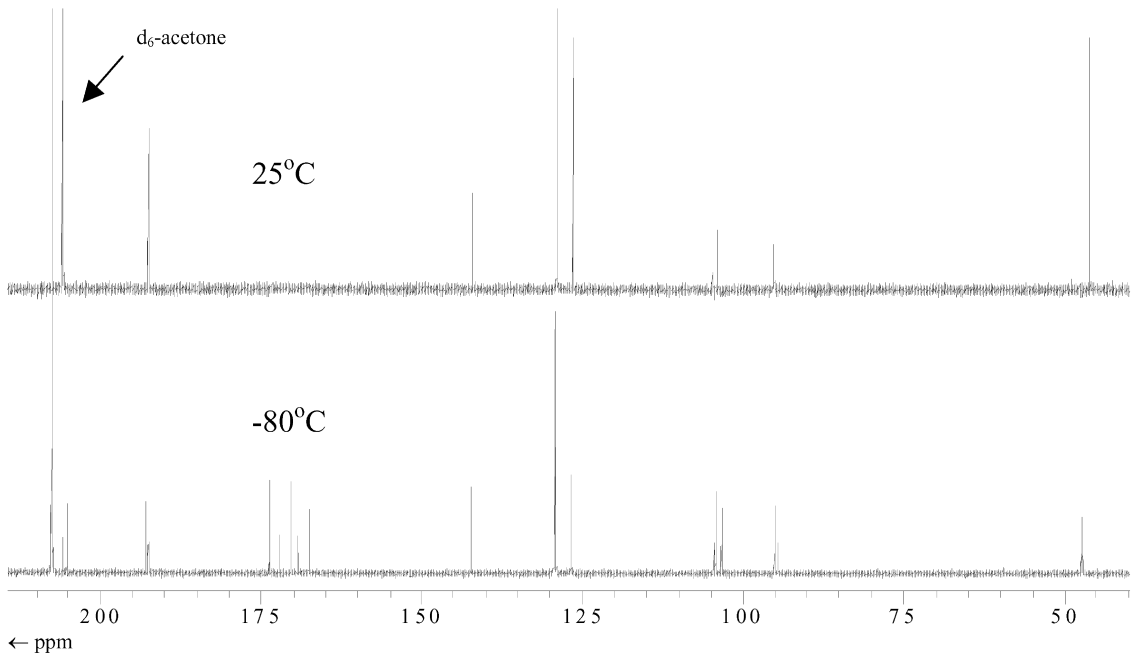


Figure 1. Temperature variance of ^{13}C NMR spectra of dihydrochalcone **2** in d_6-acetone.

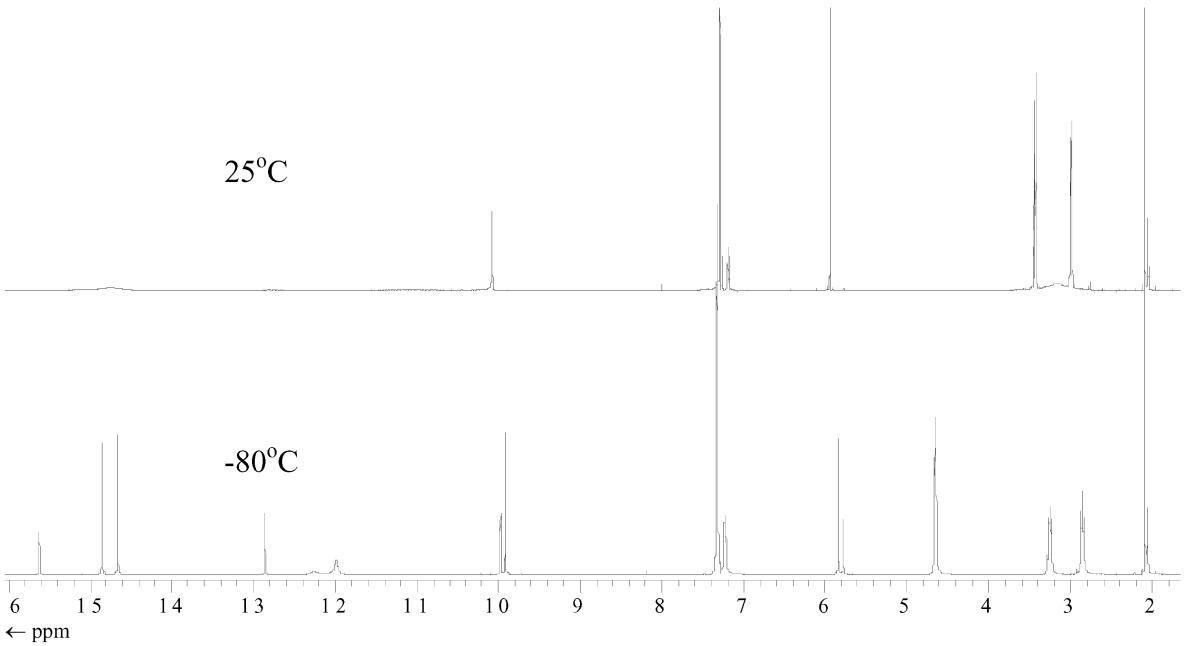


Figure 2. Temperature variance of ^{1}H NMR spectra of dihydrochalcone **2** in d_6-acetone.

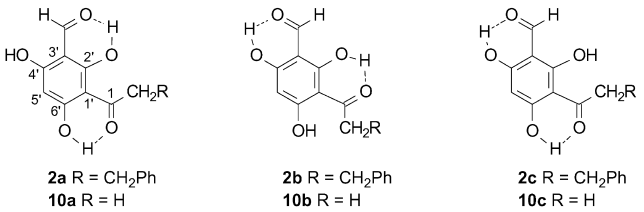


Figure 3. Possible formyl chalcone rotamers.

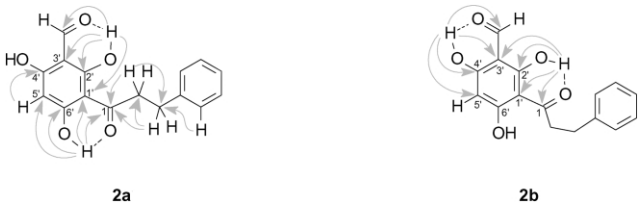
The presence of two carbonyl groups among the three OH groups gives the possibility of three rotamers, each with two strong intramolecular hydrogen-bonds (Fig. 3). Broadening of NMR signals at 25°C could result from being close to the coalescence temperature for exchange between these forms. This was demonstrated by recording the NMR spectra at lower temperatures. At −80°C two sets of peaks in the ratio of 2:1 were clearly observable in slow exchange (Figs. 1 and 2). These corresponded to two of the three possible hydrogen-bonded species. All signals could now be accounted for.

Peaks observed were assignable to structures **2a** (major) and **2b** (minor) by way of 2D NMR spectroscopy at −60°C (HSQC and CIGAR, Table 1). In the major form, C-1 (δ_C 206.0) had a two bond correlation in the CIGAR spectrum via the H-bond[12] to the intramolecular hydrogen-bonded OH proton, resonating at δ_H 14.85. This OH signal also had a three-bond correlation to C-5′ (δ_C 95.1) establishing that this OH was located at C-6′. The other

Table 1. ^{1}H and ^{13}C NMR data and observed CIGAR correlations for **2**

Position	Major rotamer **2a**			Minor rotamer **2b**		
	^{13}C	^{1}H	CIGAR	^{13}C	^{1}H	CIGAR
1	205.3	–	–	205.9	–	–
2	47.0	3.27	1, 3	46.9	3.27	1, 3
3	30.7	2.87	1, 2	30.3	2.87	1, 2
4	142.2	–	–	142.3	–	–
5/6/8/9	129.2	7.30	3, 4, 7	129.2	7.30	3, 4, 7
7	126.7	7.20	5/6/8/9	126.7	7.20	5/6/8/9
1′	103.5	–	–	103.6	–	–
2′	170.4	–	–	172.2	–	–
3′	104.3	–	–	104.6	–	–
4′	167.5	–	–	169.5	–	–
5′	95.1	5.84	1′, 3′ 4′, 6′, 3′-*C*HO	94.6	5.79	1′, 4′, 3′, 3′-*C*HO
6′	173.8	–	–	169.0	–	–
2′-OH	–	14.65	1′, 2′, 3′, 3′-*C*HO	–	15.62	1, 1′, 2′, 3′
4′-OH	–	11.71	Not observed	–	12.85	3′, 4′, 5′, 3′-*C*HO
6′-OH	–	14.85	1, 1′, 5′, 6′	–	11.83	Not observed
3′-CHO	192.9	9.92	2′, 3′	192.5	9.98	3′, 4′, 5′

Recorded at −80°C in acetone-d_6. ^{1}H NMR determined at 500 MHz referenced to solvent (δ 2.05) and ^{13}C NMR at 125 MHz referenced to solvent (δ 29.9). CIGAR spectra were recorded at −60°C and correlations are from the proton signal to the signal of the numbered carbon.



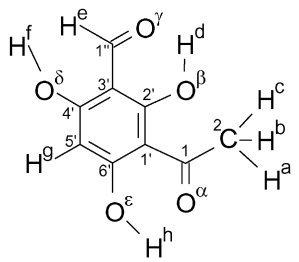
2a **2b**

Figure 4. Selected CIGAR correlations of the major and minor rotamers of **2**.

major hydrogen-bonded OH peak at δ_H 14.65 did not correlate to C-5′, but did correlate to the C-3′ aldehyde carbon signal (δ_C 192.9). These results established the major rotamer as **2a** (Fig. 4).

The identity of the minor form was established similarly. The C-1 carbonyl carbon (δ_C 205.9) had a two-bond correlation to the hydrogen-bonded OH proton signal at δ_H 15.62. This signal also had three-bond correlations to C-1′ (δ_C 103.6) and C-3′ (δ_C 104.6), showing that this OH was located at C-2′. The second hydrogen-bonded OH observed for this minor form, resonating at δ_H 12.85 ppm, had three bond correlations to C-5′ (δ_C 94.6) and C-3′, so it had to be located at C-4′. Thus the minor form was **2b** (Fig. 4). Peaks corresponding to the third form, **2c** (Fig. 3) were not observed in the temperature range −80 to 25°C. This is the first time that the identities of the rotamers of formyl chalcones such as **2** have been established.

The identity and assignment of the major and minor forms were supported by both molecular mechanics and ab initio energy calculations done on a simplified structure with a CH_3 group in place of the $-CH_2CH_2C_6H_5$ group, (**10**). An extended labelling system has been used for **10** to identify non-carbon atoms. Molecular mechanics calculations (MMX force field[13]) and conformational searching predicted the two most stable rotamers for **10** as **10a** (71%) and **10b** (29%) at −80°C (Table 2). The **10c** rotamer was predicted to be undetectable by NMR (0.04%). These rotamers are analogous to **2a**, **2b** and **2c**, respectively (Fig. 3).



10

In hydrogen-bonded and conjugated structures, ab initio calculations are more reliable than molecular mechanics and allow prediction of hydrogen bond strengths and NMR shifts.[14] The ab initio calculations were performed with the GAUSSIAN 94 program.[15] The structures of rotamers **10a**, **10b** and **10c**, were fully optimized with the HF/6-31G(d) and B3LYP/6-31G(d) methods. Calculations gave **10a** as having the lowest energy and **10c** the highest, and supported the experimental ratio of 1.0:0.5:0.0 observed for **2a**, **2b** and **2c**. The calculated relative energies and ratios of the rotamers of **10** are compared with the experimental ratios for **2** in Table 2.

To calculate NMR chemical shifts, structures **10a** and **10b** were optimized with the B3LYP/6-31G(d) method.[14] This led to the optimized geometries summarised in Table 3. Calculations of NMR chemical shifts for both the hydrogen-bonded species **10a** and **10b** were done with the HF/6-31G(d) method at the B3LYP/6-31G(d) optimized geometry.[15] The chemical shift values in Table 4 are corrected with respect to calculated TMS values.

Hydrogen bond strengths were compared to the OH bond length. The longer the OH bond the stronger the hydrogen bonded interaction, and therefore the more downfield the signal will be observed in the NMR spectrum. The longest calculated OH bond (Table 3) is that of $O^{\beta}-H^{d}$ (1.012 Å), the 2′-OH, for rotamer **10b**. The ^{1}H NMR chemical shift of the proton of this hydroxyl group in **2b** was the most downfield, observed at δ 15.6 ppm (calculated for **10b** as δ 14.6) (Table 4). The second longest calculated OH bond is that of $O^{\epsilon}-H^{h}$ (1.010 Å), the 6′-OH, for rotamer **10a**. This corresponds to the second most downfield OH proton signal for **2** at δ 14.9 ppm (calculated for **10a** as δ 14.2). In general the calculated chemical shifts for the two rotamers of compound **10** agree well with those found experimentally for **2** (Table 4) except for those of the non-intramolecular hydrogen bonded hydroxyl groups (the 4′-OH of rotamer **a**; and the 6′-OH of rotamer **b**) where the calculated shifts were much smaller than those observed.

Table 3. B3LYP/6-31G(d) optimized geometries of **10a** and **10b**

	10a	**10b**		**10a**	**10b**
Bonded O–H			*H-bonds*		
$r(O^{\epsilon}H^{h})$	1.010	0.971	$r(O^{\gamma}H^{d})$	1.613	–
$r(O^{\beta}H^{d})$	1.009	1.012	$r(O^{\alpha}H^{h})$	1.575	–
$r(O^{\delta}H^{f})$	0.970	1.003	$r(O^{\alpha}H^{d})$	–	1.556
			$r(O^{\gamma}H^{f})$	–	1.668
Bonded C–H			*Bond angles*		
$r(C^{5'}H^{g})$	1.086	1.086	$\angle C^{1}O^{\alpha}H^{h}$	106.2	108.8
$r(C^{2}H^{a})$	1.092	1.091	$\angle C^{1}O^{\alpha}H^{d}$	107.0	106.1
$r(C^{2}H^{b})$	1.094	1.094	$\angle C^{1\prime\prime}O^{\gamma}H^{f}$	109.2	106.7
$r(C^{2}H^{c})$	1.094	1.094	$\angle C^{1\prime\prime}O^{\gamma}H^{d}$	123.9	123.4
$r(C^{1\prime\prime}H^{e})$	1.100	1.100	$\angle C^{1'}C^{1}O^{\alpha}$	119.8	120.0

Bond distances in Å and angles in degrees.

Table 2. Relative energies (kcal mol^{-1}) and ratio of the rotamers of **10** calculated at −80°C

Rotamer	Expt. for **2**	MMX		HF/6-31G(d)		B3LYP/6-31G(d)	
	Ratio	ΔE	Ratio	ΔE	Ratio	ΔE	Ratio
a	1	0	1	0	1	0	1
b	0.5	0.35	0.40	0.22	0.57	0.61	0.24
c	0.0	2.92	5.0×10^{-4}	6.94	1.8×10^{-8}	5.06	2.0×10^{-6}

Table 4. Calculated ^{1}H and ^{13}C NMR chemical shifts for **10a** and **10b** compared with experimental values for **2a** and **2b**

	10a (calc.[a])	**2a** (expt.[b])	**10b** (calc.[a])	**2b** (expt.[b])
H-5′	5.2	5.8	5.3	5.8
2′-O*H*	14.1	14.7	14.6	15.6
4′-O*H*	4.8	11.7	12.9	12.9
6′-O*H*	14.2	14.9	5.0	11.8
3′-C*H*O	10.3	9.9	10.5	10.0
C-1′	98.5	103.5	95.6	103.6
C-2′	177.1	170.4	178.8	172.2
C-3′	94.9	104.3	98.4	104.6
C-4′	168.3	167.5	177.5	169.5
C-5′	86.2	95.1	85.6	94.6
C-6′	181.1	173.8	169.4	169.0
C-1	207.7	205.3	206.8	205.9
3′-*C*HO	193.9	192.9	194.9	192.5

[a] Calculated with the HF/6-31G(d)//B3LYP/6-31G(d) method. Calculated as ($\sigma-\sigma_{TMS}$), where for TMS, σ_H=32.6 ppm and σ_C=200.0, and σ is the GIAO Nuclear Magnetic Shielding Tensor.

[b] Recorded at −80°C in acetone-d_6. ^{1}H NMR determined at 500 MHz referenced to solvent (δ 2.05) and ^{13}C NMR at 125 MHz referenced to solvent (δ 29.9).

As the NMR spectra of compound **2** were obtained in deuterated acetone, some intermolecular hydrogen bonded interaction between the free OH groups and the carbonyls of the solvent would be anticipated. Such interaction would account for the discrepancies between the observed and calculated values.

^{1}H NMR spectra of methyl dihydrochalcones **1** and **7** each showed only one strongly hydrogen-bonded signal (δ 13.91 and 13.55, respectively) along with hydroxyl proton signals at higher field. Two and three bond correlations in the HMBC spectra showed that the predominant rotamer in each case is that where the 2′-hydroxyl group is involved in the intra-molecular hydrogen bond (Fig. 5, rotamers **1a** and **7b**).

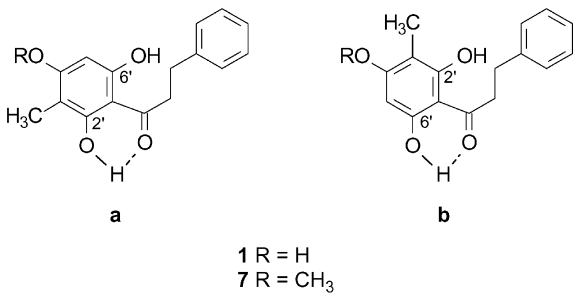
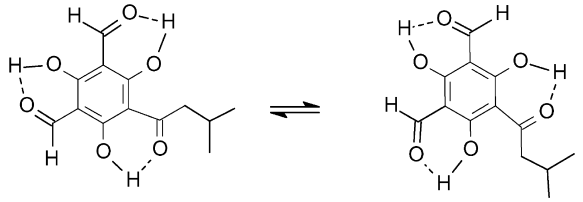


Figure 5. Possible methyl chalcone rotamers.

We propose that this preference may be dictated by optimisation of intermolecular hydrogen bonding interactions involving the free hydroxyl group(s) or by steric interaction distorting the 2′-hydroxyl out of the plane of ring A. The symmetric dihydrochalcone (**9**) showed only one broad signal (δ 11.75) for the 2′ and 6′-hydroxyl groups. This is consistent with fast exchange between the two equivalent hydrogen-bonded rotamers. This conformational exchange should have a lower rotational barrier than that experienced between the rotamers of the formyl derivative **2**, as only one hydrogen bond needs to be broken. By contrast, reported ^{1}H and ^{13}C NMR spectra of jensenone (**12**), which has three hydrogen bonds to break, are consistent with slow exchange at room temperature.[16]



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Assays on synthetic **1** confirmed the biological activity of the natural sample from *L. recurvum*, and showed antimicrobial activity against *Bacillus subtilis* and *Trichophyton mentagrophytes* at 60 μg/disk.

Compound **2** exhibited stronger cytotoxic, antiviral and antimicrobial activities that will be described elsewhere in a paper on structure–activity relationships of dihydrochalcones.

3. Conclusions

Although dihydrochalcone **7**, flavanones **3**, **4** and **8** and flavone **5** have all been reported from natural sources, this is the first report of dihydrochalcone **1** as a natural product. These flavonoids are unusual in that they have C-methylated A-rings and non-oxygenated B-rings. Synthesis of **1** has confirmed the cytotoxicity observed for the natural sample.

The complex NMR spectra of formyl dihydrochalcone **2** have been demonstrated to arise from conformational exchange between hydrogen-bonded rotamers. Low temperature NMR studies have shown clearly that two of the three possible hydrogen-bonded forms **2a** and **2b** are present at the lower exchange limit. The experimentally determined ratio of rotamers for **2** matched well with ratios calculated for the simplified structure **10** from both molecular mechanics and ab initio methods, and NMR chemical shifts calculated for the various forms of **10** corresponded well with the experimentally determined frequencies for **2**. Conformational exchange between these rotamers at room temperature explains the incomplete NMR data previously reported for **2**[11] and for its 5′-methyl analogue.[10] Compound **10**, modelled in this study, has been synthesized previously, and the ^{1}H NMR signals of H-5′, the 3′-C*H*O and 2-CH_3 reported with no mention of broadening. No O*H* signals were reported.[17] We could find no report of the ^{13}C NMR spectrum of **10** which we would anticipate to show broadening.

Simperler and Mikenda[18] have carried out ab initio calculations to derive the relative energies of diacyl phloroglucinols using B3LYP/6-31G(d,p). Of the compounds studied, the methyl derivative (**11**) bears closest resemblance to **10**. Rotamers **11a** and **11b** (Fig. 3) were considered, with **11a** calculated to be the more stable by 0.72 kcal mol^{-1}, similar to our results for **10** (Table 2). However, Simperler and Mikenda did not comment on

rotamer **11c**. Consideration of IR spectra did not enable them to study the rotamer distribution experimentally. Our study shows that employing variable temperature NMR methods may offer a more fruitful approach.

4. Experimental

4.1. General

UV and IR spectra were recorded on a Jasco 7800 UV–Vis spectrometer and a Perkin–Elmer 1600 FTIR instrument respectively. Melting points were determined on a hot bench Leica AG melting point apparatus first calibrated with standard samples of known melting points. NMR spectra were recorded on a Varian INOVA-500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR spectra were recorded on ca. 0.075 M solutions in d_6-acetone (referenced to solvent, δ 2.05 for ^{1}H and δ 29.9 for ^{13}C) or $CDCl_3$ (referenced to solvent, δ 7.25 for ^{1}H and δ 77.7 for ^{13}C). Spectra were assigned with the aid of double-quantum filtered COSY (^{1}H–^{1}H correlations), HSQC (one bond ^{1}H–^{13}C correlations), and HMBC-CIGAR experiments[19] (two and three bond ^{1}H–^{13}C correlations). Preparative TLC was carried out using Merck PLC-glass plated silica gel 60 F_{254}, 20 cm×20 cm (0.5 mm thickness). HPLC equipment consisted of a Waters 717 autosampler, 600 pump and controller, and a 490E UV programmable multi-wavelength detector controlled by Millennium software. Samples were analyzed using an RP-18 analytical column (Merck 100 RP-18, LichroCART 250×4 mm, 5 μm) fitted with a guard column (Merck 100 RP-18, LichroCART 4×4 mm, 5 μm). Samples for analysis were passed through ISOLUTE SPE Columns (100 mg, C-18 EC) primed with MeCN (3×300 μL) prior to filtration and filtered (0.45 μm) and 10 μL injected using the autosampler. Silica gel 60, 200–400 Mesh, 40–63 μm (Merck) was used in the column chromatography and octadecyl-functionalised silica gel (C-18 Aldrich) was used for reversed-phase (RP) chromatography. Biological assays were performed as described previously.[20]

HCl gas from Matheson Gas was dried by passing through conc. H_2SO_4. Hydrocinnamonitrile (99% purity) from AJAX Chemicals was used without further purification. Phloroglucinol dihydrate (97% purity) from BDH was dehydrated by heating under reflux in toluene using a Dean–Stark apparatus.[21] The residual material after solvent removal was crystallized from EtOAc and cyclohexane.

4.2. Computational methods

Molecular mechanics calculations were performed with PCModel version 7.[13] Conformational searches used the MMX force field, with the mixed Monte Carlo coordinate movements/bond rotations strategy[22] for the generation of initial structures. The default cut-off criteria were employed.

The structures of rotamers **10a**, **10b** and **10c**, were fully optimized with the HF/6-31G(d) and B3LYP/6-31G(d) methods. NMR shielding tensors were calculated with the gauge-including atomic orbitals (GIAO) method.[23] The theoretical ^{1}H and ^{13}C chemical shifts were calculated as the isotropic magnetic shielding in tetramethylsilane (TMS) minus that of the compound. The structure of TMS was fully optimized with the B3LYP/6-31G(d) method. For both the compounds and the TMS reference the GIAO shielding tensors were calculated with the HF/6-31G(d) method on the B3LYP/6-31G(d) optimized structure.[24,25] All calculations were performed by using Gaussian94.[15]

4.3. Plant material

L. recurvum was collected from Lincoln Landcare Research Garden, New Zealand in November 1997. A voucher specimen (voucher code 971103-13) is kept at the Plant Extracts Research Unit, University of Otago, Dunedin, New Zealand.

4.4. Extraction and isolation

Foliage of *L. recurvum* (60 g) was dried, ground, extracted (EtOH, 1×700 mL, 2×350 mL) and fractionated by RP column chromatography (50 g, C-18) by gradient elution from H_2O through CH_3CN to $CHCl_3$. The fractions were pooled into three groups: A, B and C based on their UV-active spots on TLC silica (4:4:1, cyclohexane/EtOAc/EtOH). The first group, A (78 mg) was subjected to flash chromatography on silica gel (2 g), developed in steps from cyclohexane to EtOAc. Fractions eluted with 4:1, cyclohexane/EtOAc, gave an inseparable mixture of isomeric hydroxyflavanones **3** and **4** (8 mg). Fractions eluted with 4:1 and 3:1, cyclohexane/EtOAc gave dihydrochalcone **1** (11 mg). Fraction B (63 mg) consisted of **3** and **4**. Fraction C (136 mg) containing **3**, **4**, **5**, **7** and **8**, was dehydrated as follows.[26]

A solution of fraction C in AcOH (8 ml) containing six drops of 96% H_2SO_4 was refluxed for 30 minutes. The precipitate obtained by dilution with H_2O was filtered off and washed until neutral. It was then subjected to flash chromatography on silica gel (13 g). Fractions eluted with 19:1 cyclohexane/EtOAc gave flavanone **8** (7 mg) and a mixture of two components which, after preparative TLC (toluene), gave flavones **5** (5 mg) and **6** (3 mg). Fractions eluted with 17:1 cyclohexane/EtOAc gave dihydrochalcone **7**.

Analytical RP HPLC showed that compounds **1**, **3**, **4**, **5**, **7** and **8**, but not flavone **6**, were present in the crude extract.

4.4.1. 2′,4′,6′-Trihydroxy-3′-methyldihydrochalcone 1.[5] Pale yellow oil. UV (MeOH) λ_{max} (log ε): 322 (3.62), 290 (4.23); IR (film) ν_{max} cm^{-1}: 3353 broad, 3015, 2974, 1620 broad, 1523, 1426, 1215. ^{1}H NMR (acetone-d_6): 13.81 (1H, s, 2′-OH), 9.68 (1H, br. s, 6′-OH), 9.08 (1H, s, 4′-OH), 7.27 (2H, m, H-5/H-9), 7.17 (3H, m, H-6/H-7/H-8), 6.07 (1H, s, H-5′), 3.39 (2H, t, J=8 Hz, H-2), 2.98 (2H, t, J=8 Hz, H-3), 1.96 (3H, s, 3′-Me). ^{13}C NMR (acetone-d_6): 205.2 (C-1), 165.1 (C-2′), 162.8 (C-4′), 160.4 (C-6′), 142.8 (C-4), 129.1 (C-5/C-9), 126.9 (C-6/C-7/C-8), 104.9 (C-1′), 103.3 (C-3′), 94.8 (C-5′), 46.2 (C-2), 31.3 (C-3), 8.0 (3′-Me).

4.4.2. 2,5-Dihydroxy-6-methyl-7-methoxyflavanone 3 and its isomer, 2,5-dihydroxy-8-methyl-7-methoxyflavanone 4.[6] Yellowish oil. $[\alpha]_D^{21}$=+24.2, c 0.1, $CHCl_3$ (lit.,[6]

+20.7, *c* 0.105, $CHCl_3$); $[\alpha]^{21}_{577\ nm}$=−2.84, $[\alpha]^{21}_{546\ nm}$=−27.4, $[\alpha]^{21}_{435\ nm}$=230.4, $[\alpha]^{21}_{405\ nm}$=−300.4 (all *c* 0.1, $CHCl_3$). UV (MeOH) λ_{max} nm (log ε): 289 (4.38), 335 (3.80) (shoulder). IR (film) ν_{max} cm^{-1}: 3500–3100, 2953, 1638–1600, 1448, 1297, 1215, 1125. EIMS *m/z* 300 (M^+ for $C_{17}H_{16}O_5$), 282, 105 (100), 77. ^1H NMR ($CDCl_3$): **3/4**; 11.92/11.97 (2×1H, s, 5-OH), 7.67 (4H, m, H-2′/H-6′), 7.44 (4H, m, H-3′/H-5′), 7.41 (2H, m, H-4′), 6.12/6.11 (2×1H, s, H-8/H-6), 3.84/3.85 (2×3H, s, 7-OMe), 3.03/3.02 (2×2H, s, H-3), 2.00/2.04 (2×3H, s, 6-Me/8-Me); ^{13}C NMR ($CDCl_3$): **3/4**, 194.2/194.7 (C-4), 165.7/166.1 (C-7), 160.2/162.0 (C-5), 157.7/155.5 (C-9), 141.9/142.2 (C-1′), 129.2 (C-4′), 128.8 (C-3′/5′), 125.0 (C-2′/6′), 106.8/92.6 (C-6), 102.3 (C-10), 101.1/101.6 (C-2), 91.6/105.7 (C-8), 55.8/55.9 (7-OMe), 48.4/48.2 (C-3), 6.9/7.8 (6-Me/8-Me).

4.4.3. 5-Hydroxy-6-methyl-7-methoxyflavone 5.[7] Yellowish gum. UV (MeOH) λ_{max} (log ε): 314 (4.00), 273 (4.28), 249 (4.04). IR (film) ν_{max} cm^{-1}: 3440 broad, 3030, 2930, 1652, 1615, 1588, 1492, 1456, 1349, 1216, 1139. ^1H NMR ($CDCl_3$): 12.73 (1H, br. s, 5-OH), 7.89 (2H, m, H-2′/H-6′), 7.54 (1H, m, H-4′), 7.51 (2H, m, H-3′/H-5′), 6.68 (1H, s, H-3), 6.50 (1H, s, H-8), 3.93 (3H, s, 7-OMe), 2.11 (3H, s, 6-Me). ^{13}C NMR ($CDCl_3$): 182.5 (C-4), 163.6 (C-2/C-7), 158.5 (C-5), 155.5 (C-9), 131.5 (C-1′/C-4′), 129.1 (C-3′/C-5′), 126.3 (C-2′/C-6′), 109.3 (C-6), 106.0 (C-3), 105.5 (C-10), 89.5 (C-8), 55.8 (7-OMe), 7.4 (6-Me).

4.4.4. 5-Hydroxy-8-methyl-7-methoxyflavone 6.[8] Pale yellow oil. UV (MeOH) λ_{max} (log ε): 340 (3.70), 335 (3.70), 274 (4.40). IR (film) ν_{max} cm^{-1}: 3456 broad, 3015, 2964, 1651, 1610, 1523, 1476, 1420, 1220. ^1H NMR ($CDCl_3$): 12.76 (1H, br. s, 5-OH), 7.91 (2H, m, H-2′/H-6′), 7.54 (1H, m, H-4′), 7.52 (2H, m, H-3′/H-5′), 6.66 (1H, s, H-3) 6.41 (1H, s, H-6), 3.91 (3H, s, 7-OMe), 2.30 (3H, s, 8-Me). ^{13}C NMR ($CDCl_3$): 183.1 (C-4), 163.8 (C-2), 163.3 (C-7), 160.3 (C-5), 154.6 (C-9), 131.8 (C-1′/C-4′), 129.1 (C-3′/C-5′), 126.3 (C-2′/C-6′), 105.3 (C-10), 105.1 (C-3), 104.1 (C-8), 95.3 (C-6), 56.0 (7-OMe), 7.7 (8-Me).

4.4.5. 2′,6′-Dihydroxy-3′-methyl-4′-methoxydihydrochalcone 7.[9] Pale yellow oil. UV (MeOH) λ_{max} (log ε): 324 (3.55), 288 (4.29). IR (film) ν_{max} cm^{-1}: 3292, 3026, 2974, 1625, 1600, 1517, 1426, 1215. ^1H NMR (acetone-d_6): 13.45 (1H, s, 2′-OH), 9.98 (1H, s, 6′-OH), 7.27 (2H, m, H-5/H-9), 7.17 (3H, m, H-6/H-7/H-8), 6.14 (1H, s, H-5′), 3.81 (3H, s, 4′-OMe), 3.41 (2H, t, *J*=8 Hz, H-2), 2.98 (2H, t, *J*=8 Hz, H-3), 1.92 (3H, s, 3′-Me). ^{13}C NMR (acetone-d_6): 205.8 (C-1), 170.5 (C-2′), 164.6 (C-4′), 161.1 (C-6′), 142.8 (C-4), 129.2 (C-5/C-9), 126.6 (C-6/C-7/C-8), 105.3 (C-1′), 104.4 (C-3′), 91.0 (C-5′), 55.7 (4′-OMe), 46.5 (C-2), 31.3 (C-3), 7.2 (3′-Me).

4.4.6. 5-Hydroxy-6-methyl-7-methoxyflavanone 8.[8] Yellowish gum. $[\alpha]_D^{21.7}$=+34.7, *c* 0.01, MeOH (lit.,[27] +45, *c* 0.02, MeOH); $[\alpha]^{22}_{577\ nm}$=−184.0, $[\alpha]^{22}_{546\ nm}$=−545.3, $[\alpha]^{22}_{435\ nm}$=−2802, $[\alpha]^{22}_{405\ nm}$=−3298 (all *c* 0.01, MeOH). UV (MeOH) λ_{max} (log ε): 290 (4.30), 326 (3.54) (shoulder). IR (film) ν_{max} cm^{-1}: 3015, 2925–2950, 1638, 1580, 1449, 1293, 1205, 1156, 1129. ^1H NMR ($CDCl_3$): 12.05 (1H, s, 5-OH), 7.45 (2H, m, H-2′/H-6′), 7.42 (3H, m, H-3′/H-4′/H-5′), 6.09 (1H, s, H-8), 5.41 (1H, dd, *J*=3, 13 Hz, H-2), 3.83 (3H, s, 7-OMe), 3.07 (1H, dd, *J*=13, 17 Hz, H-3), 2.83 (1H, dd, *J*=3, 17 Hz, H-3), 2.01 (3H, s, 6-Me). ^{13}C NMR ($CDCl_3$): 195.8 (C-4), 165.8 (C-7), 161.1 (C-5), 160.4 (C-9), 138.5 (C-1′), 128.9 (C-3′/C-4′/C-5′), 126.1 (C-2′/C-6′), 106.1 (C-6), 102.8 (C-10), 90.7 (C-8), 79.4 (C-2), 55.8 (7-OMe), 43.5 (C-3), 6.8 (6-Me).

4.4.7. 2′,4′,6′-Trihydroxydihydrochalcone 9.[10] Into a mixture of dry phloroglucinol (2.30 g, 0.0183 mol), hydrocinnamonitrile (3.88 g, 0.0230 mol), and $ZnCl_2$ (0.91 g, 0.00670 mol) in dry Et_2O (46.0 mL) was passed dry HCl gas under vigorous stirring by mechanical stirrer with cooling in an ice–MeOH bath (−10 to −20°C) for 3 h. The reaction mixture was allowed to stand overnight in a freezer, and again dry HCl gas was passed through it for 3 h. After further standing in a freezer, the reaction mixture was decanted and the residual brown viscous syrup was hydrolysed by refluxing in H_2O (69.0 mL) for 2 h. The yellow solid was collected by filtration. A further crop of crystals was obtained as yellow needles by concentration of the filtrate to give a total mass of 3.32 g (71%) of 2′,4′,6′-trihydroxydihydrochalcone **9**. Mp 144°C (lit.,[10] 142–143°C). UV (MeOH) λ_{max} (log ε): 325 (3.68), 287 (4.43) nm. IR (neat) ν_{max} 3293, 3016, 1631, 1605, 1518, 1452 cm^{-1}; ESI (−ve) *m/z* 257 [M−H]; ^1H NMR (acetone-d_6): 11.75 (2H, br. s, 2′-OH/6′-OH), 9.27 (1H, s, 4′-OH), 7.27 (4H, m, H-5/H-6/H-8/H-9), 7.18 (1H, m, H-7), 5.94 (2H, s, H-3′/H-5′), 3.39 (2H, t, *J*=8 Hz, H-2), 2.97 (2H, t, *J*=8 Hz, H-3); ^{13}C NMR (acetone-d_6): 203.3 (C-1), 164.0 (C-4′), 163.6 (C-2′/C-6′), 141.2 (C-4), 127.7 (C-5/C-6/C-8/C-9), 125.2 (C-7), 103.1 (C-1′), 94.1 (C-3′/C-5′), 44.4 (C-2), 29.6 (C-3).

4.4.8. 2′,4′,6′-Trihydroxy-3′-formyldihydrochalcone 2[10]**.** Into a vigorously stirred solution of 2′,4′,6′-trihydroxychalcone (0.20 g, 0.000780 mol), $Zn(CN)_2$ (0.18 g, 0.00155 mol) and $AlCl_3$ (0.21 g, 0.00155 mol) in dry Et_2O (4.0 mL) was passed dry HCl gas with cooling in an ice-MeOH bath (−10 to −20°C) for 3 h. The reaction mixture was allowed to stand overnight in a freezer. This operation was repeated once more. After decantation, the residual dark yellow viscous syrup was heated under reflux in H_2O (4.0 mL) for 1 h. After filtration, the residue was dissolved in EtOAc (4.0 mL). The EtOAc soln was washed with H_2O (3×4 mL), and brine (3×4 mL), dried over Na_2SO_4, and then concentrated using a rotary evaporator to give a reddish brown syrup which was then purified by passing through a silica column (Davisil silica, cyclohexane/EtOAc; 3:1) to give **2** as pinkish powder (0.14 g, 61%). Mp 158°C (lit.,[10] 157–158°C). IR (film) ν_{max}: 3149, 3026, 2975, 2923, 1626 (broad), 1430, 1315, 1215, 758 cm^{-1}; UV (MeOH) λ_{max} (log ε): 325 (3.73), 273 (4.52) nm; ESI (−ve) *m/z*: 285 [M−H]; found: C, 67.2; H, 5.0%; calcd for $C_{16}H_{14}O_5$: C, 67.1; H, 4.9%. ^1H and ^{13}C NMR (Table 1).

4.4.9. 2′,4′,6′-Trihydroxy-3′-methyldihydrochalcone 1.[5] A solution of **2** (0.023 g, 0.08 mmol) in MeOH (0.80 mL) was heated under reflux with Zn–Hg amalgam (0.46 g), conc. HCl (0.4 mL) and H_2O (0.1 mL) for 15 min. After decantation to remove the Zn–Hg amalgam, H_2O (1.0 mL) was added to the supernatant solution. The mixture was extracted with EtOAc (3×4 mL) and the combined extracts were washed with H_2O (3×10 mL) and brine (1×10 mL) and dried over anhydrous Na_2SO_4. The solvent was

removed by rotary evaporation to give **1** as a yellow oil (0.019 g, 87%). A sample for spectral studies and bioassays was further purified by prep. TLC (cyclohexane/EtOAc; 1:1), yield 73%. UV (MeOH) λ_{max} (log ε): 325 (3.73), 273 (4.52) nm; ESI (−ve) *m/z*: 271 [M−H]; IR (neat) ν_{max}: 3394, 3015, 2923, 1718, 1626 cm^{-1}. ^{1}H and ^{13}C NMR (as above).

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