NPC Natural Product Communications

2010
Vol. 5
No. 4
519 - 522

Two New Diterpene Phenols from *Calocedrus decurrans*

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Received: September 21st, 2009; Accepted: February 22nd, 2010

Two new p-cymene based diphenols (1-2) were isolated from the heartwood of *Calocedrus decurrans*. Structures were elucidated by 1D and 2D NMR techniques and HRMS. Libocedroquinone (3) was also isolated as a natural product for the first time. A new system of nomenclature is proposed for description of such oligomeric p-cymene derivatives.

Keywords: *Calocedrus decurrans,* Cupressaceae, diterpenes, p-cymene derivatives.

Incense cedar, *Calocedrus decurrans* (Torr.) Florin., is a native tree in Oregon and California which is perhaps best known for its historical role in the manufacture of wooden pencils. Previous studies have identified carvacrol, thymoquinone, various related p-cymene derivatives and tropolones [1-6], although structural data in some cases was inconclusive due to the lack of modem NMR and HRMS techniques. Recent studies have shown the extracts and essential oils from its heartwood have significant antimicrobial activity against *Phytophthora ramorum* (sudden oak death) [7] and biocidal activity against anthropods of public health importance such as fleas, ticks, and mosquitoes [8]. These bioactivities have been attributed to, at least in part, the high amounts of carvacrol and thymoquinone and prompted the further chemical investigations reported here.

We have isolated two new p-cymene based diphenols named calocedrol A (1) and calocedrol B (2) (Figure 1). In addition libocedroquinone (3) was isolated for the first time as a natural product and structures of the previously known libocedrol (4) and heyderiol (5) were confirmed with NMR and MS data. Libocedroquinone (3) was previously reported as the *in vitro* alcoholic ferric sulfate oxidation product of libocedrol 5 [3]. Compounds 1-5 (Figure 1) were isolated from the hexane extract of the heartwood by repeated column

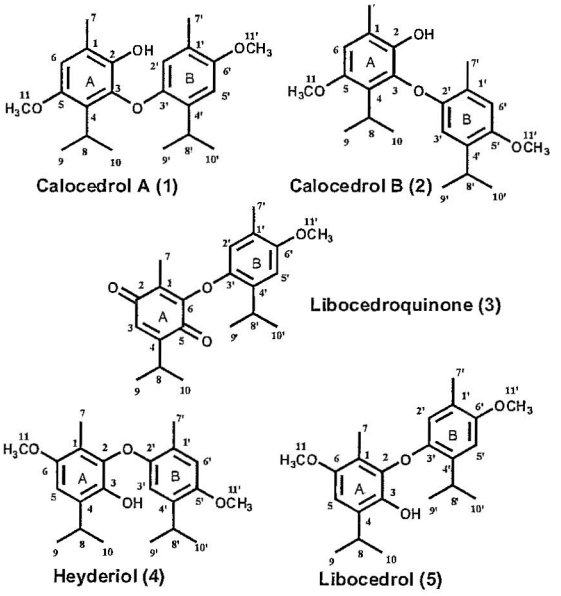


Figure 1: *p*-Cymene based diphenols from Incense cedar heartwood.

chromatography over silica gel with hexane and ether-hexane gradients and monitoring by TLC, GC and NMR to obtain pure compounds.

Compound 1 was obtained as an optically inactive amorphous solid with the molecular formula $C_{22}H_{30}O_4$ as indicated by HRMS and ^{13}C NMR. The NMR spectra

Table 1: ^{1}H NMR spectroscopic data (δ_H) for compounds **1-5** ($CDCl_3$, 400 MHz).

Position	Compounds δ_H (*J* in Hz)				
	1	**2**	**3**	**4**	**5**
1					
2					
3			6.54 s		
4					
5				6.66 s	6.63 s
6	6.57 s	6.59 s			
7	2.26 s	2.28 s	1.99 s	1.94 s	1.93 s
8	3.09 hpt	3.04 hpt	3.02 hpt	3.32 hpt	3.36 hpt
	(6.8)	(6.8)	(6.8)	(6.8)	(6.8)
9&10	1.34 d	1.16 d	1.08 d	1.31 d	1.28 d
	(6.8)	(6.8)	(6.8)	(6.8)	(6.8)
11	3.82 s	3.82 s		3.83 s	3.84 s
OH	4.83 s	4.96 s		5.19 s	5.036 s
1'					
2'	6.26 s		6.27 s		6.23 s
3'		6.3 s		6.33 s	
4'					
5'	6.77 s		6.76 s		6.79 s
6'		6.74 s		6.75	
7'	2.05 s	2.42 s	2.08 s	2.44 s	2.05 s
8'	3.48 hpt	3.15 hpt	3.38 hpt	3.14 hpt	3.56 hpt
	(6.8)	(6.8)	(6.8)	(6.8)	(6.8)
9'&10'	1.17 d	1.04 d	1.30 d	1.06 d	1.35 d
	(6.8)	(6.8)	(6.8)	(6.8)	(6.8)
11'	3.8 s	3.82 s	3.81 s	3.85 s	3.83 s

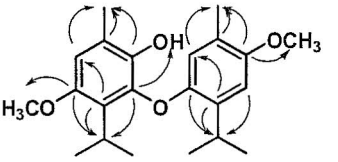
[a]The δ_H values have been established by HSQC and HMBC NMR spectra. Heptet is abbreviated as hpt, doublet as d and singlet as s.

Table 2: ^{13}C NMR spectroscopic data (δ_C) for compounds **1-5** ($CDCl_3$, 100 MHz).

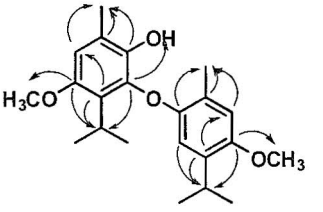
Position	Compounds δ_C				
	1	**2**	**3**	**4**	**5**
1	122.00	121.74	131.28	117.11	117.40
2	141.05	141.16	188.50	140.40	140.35
3	139.68	139.6	130.24	140.64	140.35
4	127.34	127.15	153.4	132.07	132.20
5	151.99	151.50	181.68	105.35	105.21
6	110.78	110.95	152.69	151.55	151.44
7	15.77	15.78	8.89	9.21	9.37
8	25.90	25.92	26.53	27.52	27.33
9&10	22.89	21.37	21.20&21.33	22.48	22.56
11	55.98	56.03		55.93	56.19
1'	124.79	123.02	124.43	123.28	124.81
2'	115.24	149.70	116.33	149.05	114.91
3'	148.07	110.86	147.76	110.85	147.60
4'	133.70	135.46	134.83	135.53	133.83
5'	109.16	151.92	108.89	151.40	109.11
6'	151.99	113.88	153.50	113.78	152.91
7'	15.77	16.20	15.81	16.08	15.86
8'	27.40	26.84	27.35	26.83	27.40
9'&10'	20.81	22.55	22.75	22.48	22.93
11'	55.91	56.01	55.75	56.24	55.91

[a]The δ_C values have been established by HSQC and HMBC NMR spectra.

of compound 1 revealed the presence of 2 isopropyl groups, 2 methyl groups, 2 methoxy groups, one hydroxyl, and 3 aromatic protons in 2 aromatic rings. From the molecular mass of 358 it was concluded that there exists an ether bridge between the two aromatic rings. The proton attachments to the various carbons were revealed by the HSQC and HMBC correlation spectra given in Tables 1 and 2. The aromatic carbons, at 8 122.00 (C-l) and 124.79 (C-l'), showed strong HMBC correlation to the methyl group protons at 8 2.26 (H-7) and 2.05 (H-7'), respectively.



Calocedrol A



Calocedrol B

Figure 2: Key HMBC correlations of calocedrol A and B. To make the figure less crowded only the relevant correlations are included.

Similarly, 8 127.34 (C-4) and 133.70 (C-4') were strongly correlated to the protons on the isopropyl methyl groups, thus helping to assign these quaternary carbons to the corresponding methyl and isopropyl groups.

The position of 8 151.99 as C-5, the aromatic carbon attached to the methoxy group, was assigned on the basis of its HMBC correlations with 8 3.82 (H-ll), 3.09 (H-8) and a very weak correlation with 6.57 (H-6). This suggests that the carbons at 8 127.34, 151.99, and 110.78 are attached successively as C-4, C-5, and C-6.

The tertiary C-6 carbon attached to proton 8 6.57 (H-6) shows a strong correlation to 8 2.26 (H-7), leading to the conclusion that 8 122.00 (C-l) is the carbon adjacent to 110.78 (C-6). The H-6 proton showed strong correlation with 8 15.77 (C-7), 127.34 (C-4) and 141.05 (C-2), confirming that 122.00 (C-l) is attached to 141.05 (C-2). Since the hydroxyl proton appearing at 8 4.83 shows strong correlations to 8 139.68 (C-3) and 122.00 (C-l), it was concluded that the hydroxyl is bonded to 8 141.05 (C-2) and the carbon containing the ether bridge is at 8139.68 (C-3).

In the B ring, the carbon resonating at 8 148.07 (C-3') has to be attached to the ether bridge because of its 8 value and strong HMBC correlations with the proton at 8 3.48 (H-8') and the aromatic proton at 8 6.77 (H-5'). It has already been established that 8 27.40 (C-8') is attached to 8 133.70 (C-4') on the aromatic ring. The proton at H-5' shows strong HMBC correlation to 8 27.40 (C-8'), 148.07 (C-3') and 124.79 (C-l'). Hence the carbons at 8 148.07, 133.70 and 109.16 are consecutively bonded to each other as C-3', C-4', C-5'. The aromatic carbon bonded to the methoxy group, resonates at 8 151.99, with correlations to the

methyl proton H-7' at 8 2.05. The remaining aromatic proton at 8 6.26 reveals a strong correlation with H-7'. Hence it can be concluded that the carbons 8 109.16, 151.99, 124.79 and 115.24 are positioned in a successive manner as *C-5', C-6',* C-l', and *C-2',* respectively. With this evidence, compound 1 was established as a new diterpene phenol named calocedrol A, composed of two p-cymene units (A, p-methoxycarvacrol and B, p-methoxythymol) linked through an ether bridge.

Compound 2 was also isolated as an optically inactive amorphous solid with a molecular formula of $C_{22}H_{30}O_4$ as indicated by HRMS and NMR. The carbon and proton NMR spectra were very similar to those of 1, indicating an isomer. The key difference was in the positions of the ether bridge and the methoxy group on ring B that was established by HMBC correlation spectra. As shown, the B-ring is a p-methoxycarvacrol unit and the ether bridge was between the phenolic oxygen at 2' and the A-ring at *C-3.*

NMR data was also assigned for compounds 3-5 in Tables 1 and 2, since assignments were not comprehensive in earlier research. Compound 3, clearly exhibits a thymoquinone A-ring linked to a p-methoxythymol B-ring. Compounds 4 and 5 are clearly isomers, both with p-methoxythymol A-rings, but with a p-methoxycarvacrol B-ring in 4 and a p-methoxythymol B-ring in 5.

Obviously, the nature of compounds 1-5 suggests their formation through oxidative coupling reactions involving p-methoxycarvacrol, p-methoxythymol and thymoquinone monomers, that have all been isolated earlier by Zavarin *et at.* [1,2]. Verification of these monomeric components can be elucidated by signals of their isopropyl methine protons as shown for compounds 1-5 in Figure 3. Those protons belonging to p-methoxythymol are all downfield to those of *p*-methoxycarvacrol and thymoquinone rings.

In addition to common names, a new and simple system of nomenclature analogous to that used for proanthocyanidins and polysaccharides [9,10] is proposed for oligomeric p-cymene/p-menthane compounds, described in this paper and for future compounds to be discovered. This system uses the common monomer unit names and IUPAC numbering for each p-cymene/ p-menthane unit. Linkages indicate bond location and direction between the monomer units. Common monomer names are retained such as *p*-methoxycarvacrol, p-methoxythymol, thymoquinone etc. This method would give a simple and accurate description of the oligomer structure. For example,

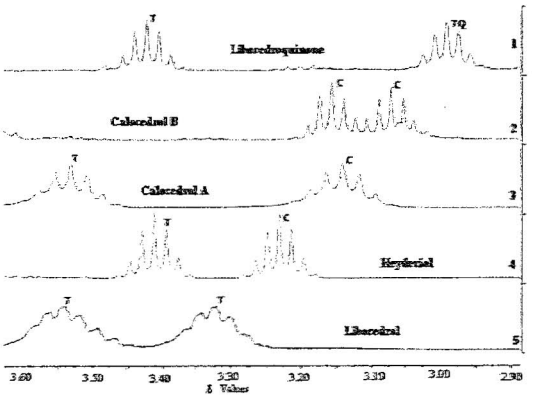


Figure 3: The stacked 1H NMR spectra from δ 2.90 to 3.65 for the isopropyl methine protons of libocedroquinone (1), calocedrol B (2) and A (3), heyderiol (4), libocedrol (5). Peaks marked (T) are those derived from *p*-methoxythymol, those marked with (C) are derived from *p*-methoxycarvacrol and the ones marked (TQ) are from thymoquinone units.

compound 1 is composed of a p-methoxycarvacrol unit (A) and a *p-methoxythymol* (B) unit. They are linked from *C-3* of unit A through the phenolic oxygen of the p-methoxythymol. Compound 1 thus becomes p-methoxycarvacrol-(3--0--3*')-p*-methoxythymol. Compound 2 is p-methoxycarvacrol-(3--0--2')-p-methoxycarvacrol and compound 3, which is composed of thymoquinone and a p-methoxythymol unit, becomes thymoquinone-(6--0--3')-p-methoxythymol.

Experimental

General experimental procedures: TLC analysis was done using silica gel 60 plates F_{254} (250 um, glass or aluminum, Merck) with fluorescent indicator (254 nm) and visualized with UV or aq $KMnO_4$ solution. All NMR spectra were acquired in $CDC1_3$ on a Bruker 400MHz instrument and are reported in ppm relative to tetramethylsilane and referenced internally to the residually protonated solvent. All the 1D and 2D NMR data were processed and analyzed by Spinworks 3 software. The HRMS was acquired with a Jeol MSRoute instrument.

Plant Material: Trees of *Calocedrus decurrens* were collected from a logging operation near Warm Springs, Oregon in June 2005. A voucher specimen is deposited in the Oregon State University Herbarium. The heartwood was removed and shavings made and stored in vacuum sealed containers at -20°C until extracted.

Extraction and isolation: The heartwood shavings (lKg) were soaked in hexane for 72 hours at room temperature and filtered. The solvent was evaporated under reduced pressure to give 12.6g of residue. This was dissolved in hexane and eluted through a silica gel column (360g) with hexane: ether (4: 1). Fractions

containing the compounds of interest were selected by TLC, then pooled together and concentrated. The residue was passed through another silica gel (particle size less than 0.063 mm) column and eluted with increasing polarity of 0.1% to 0.5% ether in hexane. The column fractions were followed by TLC and their purity ascertained by NMR. The initial fractions contained libocedrol (920 mg), followed by elution of heyderiol (100 mg). The subsequent fractions yielded calocedrol A (60 mg) and calocedrol B (10 mg). The later fractions were that of libocedroquinone (500 mg).

Calocedrol A [p-Methoxycarvacrol-(3--O--3′)-*p*-methoxythymol] (1)

^{1}H and ^{13}C NMR: Tables 1 and 2.

HRMS: *m/z* 358.21351 *[M]*$^{+}$, calcd for $C_{22}H_{30}O_4$ 358.21441.

Calocedrol B [p-Methoxycarvacrol-(3--O--2')-p-methoxycarvacrol] (2)

^{1}H and ^{13}C NMR: Tables 1 and 2.

HRMS: *m/z* 358.21351 *[M]*$^{+}$, calcd for $C_{22}H_{30}O_4$ 358.21441.

Libocedroquinone [thymoquinone-(6--O--3')-p-methoxythymol] (3)

^{1}H and ^{13}C NMR: Tables 1 and 2.

HRMS: *m/z* 342.18263 *[M]*$^{+}$, calcd for $C_{21}H_{26}O_4$ 342.18311.

Heyderiol [p-methoxythymol-(2--O--2')-p-methoxy-carvacrol] (4) [4]

^{1}H and ^{13}C NMR: Tables 1 and 2.

Libocedrol [p-methoxythymol-(2--O--3′)-*p*-methoxythymol] (5) [3]

^{1}H and ^{13}C NMR: Tables 1 and 2.

Acknowledgements - We are grateful for the help of Mr. Gene Keane and the Warm Springs Forest Products Industries for their help in providing incense cedar heartwood.

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