



Actephilol A and epiactephilol A: two novel aromatic terpenoids isolated from *Actephila excelsa*

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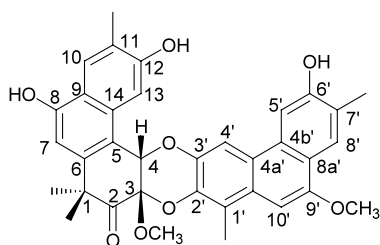
Received 28 March 2001; accepted 30 August 2001

Abstract—An investigation into the chemical constituents of a crude MeOH extract from a specimen of *Actephila excelsa* (family Euphorbiaceae), led to the isolation of two new epimeric aromatic terpenoids, actephilol A **1** and epiactephilol A **2**. The gross structures and relative stereochemistry of **1** and **2** were determined through extensive 2D NMR analysis. © 2001 Elsevier Science Ltd. All rights reserved.

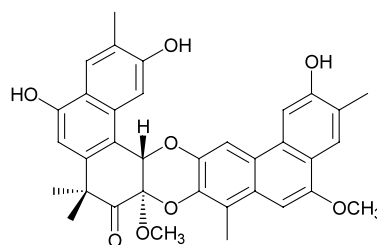
Although chemical investigations into specimens of the plant family Euphorbiaceae are very common, there have been no reports to date from the genera *Actephila*. Our investigations into the chemistry of a specimen of *Actephila excelsa*¹ yielded two new aromatic terpenoids, actephilol A **1** and its C-3 epimer, epiactephilol A **2**.

Ground plant leaf and stems (93 g) were extracted with MeOH (5 L) and concentrated in vacuo. The crude extract was subjected to a modified Kupchan solvent partition sequence,² with the MeOH:CH₂Cl₂ fraction being subjected to reversed phase RP amide C₁₆ and C₁₈ HPLC to yield **1** (3.9 mg, 0.004%) and **2** (4.8 mg, 0.005%).

Actephilol A **1** has a molecular formula of C₃₅H₃₂O₈ [(M+H)⁺, Δ_{mu} −0.6] consistent with 20 degrees of unsaturation.³ It was obvious from the one and two-dimensional NMR data that **1** was a highly substituted aromatic compound. Although NMR data was originally collected in CD₃OD, recollection in C₅D₅N gave better dispersion of resonances (Table 1). Analysis of the ¹³C NMR data led to the identification of a ketone moiety (¹³C: 206.0 ppm), an observation that was supported by a characteristic stretch in the IR spectrum at 1730 cm^{−1}. Further analysis of the one- and two-dimensional NMR data revealed the presence of seven uncoupled aromatic protons (¹³C: 105.2, 125.8, 105.1, 107.5, 107.5, 125.6, 96.3 ppm; ¹H: δ 7.16, 8.68, 7.89, 8.41,



1



2

Keywords: *Actephila excelsa*; Euphorbiaceae; aromatic terpenoids; actephilol A; epiactephilol A.

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Table 1. ^1H and ^{13}C NMR data for **1** and **2** in $\text{C}_5\text{D}_5\text{N}$ and CD_3OD

No.	CD_3OD				$\text{C}_5\text{D}_5\text{N}$			
	1		2		1		2	
	^{13}C (δ)	^1H (δ , m)	^{13}C (δ)	^1H (δ , m)	^{13}C (δ)	^1H (δ , m)	^{13}C (δ)	^1H (δ , m)
1	49.6		48.6		48.5		48.4	
2	206.9		206.9		206.0		205.9	
3	98.0		98.4		98.0		98.3	
4	74.1	5.61 (s)	73.9	5.59 (s)	74.4	5.93 (s)	74.0	5.95 (s)
5	115.0		115.1		114.5		114.6	
6	144.4		144.3		144.3		144.1	
7	104.5	6.72 (s)	104.4	6.72 (s)	105.2	7.16 (s)	104.8	7.10 (s)
8	156.4		156.4		157.4		157.3	
9	120.2		120.2		120.6		120.3	
10	125.5	8.00 (s)	125.1	7.99 (s)	125.8	8.68 (s)	125.7	8.66 (s)
11	127.2		127.2		127.5		127.5	
12	157.7		157.6		158.6		158.5	
13	104.4	7.30 (s)	104.6	7.31 (s)	105.1	7.89 (s)	105.5	7.91 (s)
14	135.5		135.4		136.0		136.0	
1'	120.7		120.1		120.4		120.0	
2'	141.6		141.4		141.6		141.5	
3'	141.6		141.3		141.7		141.5	
4'	107.4	8.01 (s)	107.8	7.78 (s)	107.5	8.41 (s)	108.1	8.11 (s)
4a'	120.8		120.6		120.8		114.6	
4b'	132.6		132.6		132.5		132.5	
5'	106.7	7.82 (s)	106.5	7.66 (s)	107.5	8.41 (s)	107.4	8.25 (s)
6'	156.8		156.8		157.8		157.7	
7'	126.7		126.5		127.2		127.1	
8'	125.1	7.98 (s)	125.1	7.96 (s)	125.6	8.45 (s)	125.6	8.45 (s)
8a'	123.0		122.1		122.8		120.5	
9'	154.2		154.3		154.0		154.1	
10'	96.1	6.83 (s)	96.2	6.94 (s)	96.3	6.95 (s)	96.4	7.12 (s)
10a'	128.6		129.4		128.4		129.4	
1 α -CH ₃	29.5	1.45 (s)	29.4	1.45 (s)	29.8	1.60 (s)	29.8	1.56 (s)
1 β -CH ₃	28.7	1.66 (s)	28.7	1.67 (s)	28.8	1.72 (s)	28.9	1.72 (s)
11-CH ₃	16.8	2.38 (s)	16.9	2.37 (s)	17.7	2.67 (s)	17.7	2.68 (s)
1'-CH ₃	11.1	2.37 (s)	11.3	2.67 (s)	11.6	2.29 (s)	11.9	2.82 (s)
7'-CH ₃	16.7	2.37 (s)	16.7	2.35 (s)	17.7	2.69 (s)	17.7	2.68 (s)
3-OCH ₃	52.2	3.59 (s)	52.3	3.49 (s)	52.3	3.48 (s)	52.3	3.48 (s)
9'-OCH ₃	55.6	4.02 (s)	55.6	4.07 (s)	55.8	4.05 (s)	55.8	4.07 (s)

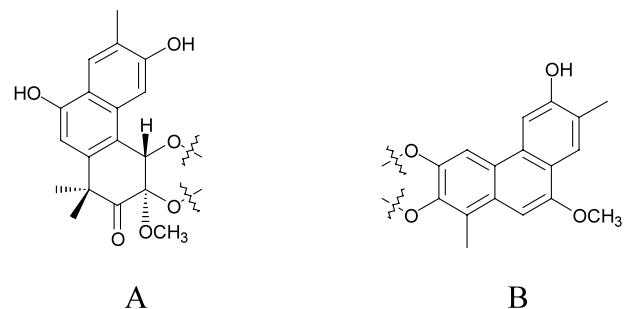
8.41, 8.45, 6.95), six oxygenated aromatic carbons (^{13}C : 157.4, 158.6, 141.6, 141.7, 157.8, 154.0 ppm), an aromatic methoxy group (^{13}C : 55.8 ppm; ^1H : δ 4.05), a methyl ketal moiety (^{13}C : 98.0, 52.3 ppm; ^1H : δ 3.48), an oxymethine (^{13}C : 74.4 ppm; ^1H : δ 5.93), as well as three aromatic methyls (^{13}C : 17.7, 17.7, 11.6 ppm; ^1H : δ 2.67, 2.69, 2.29) and an aliphatic *gem* dimethyl group (^{13}C : 29.8, 28.8 ppm; ^1H : δ 1.60, 1.72).

Detailed analysis of the gHMBC ($^nJ_{\text{CH}}$ 8 Hz) data allowed for the elucidation of two partial structures, A and B (Fig. 1), with two oxygen atoms and one degree of unsaturation remaining. From the ^{13}C chemical shifts of the four remaining points of attachments (98.0, 74.4, 141.6 and 141.7 ppm) it became obvious that the link between the two part structures was a 1,4-dioxane moiety. All that remained was to determine the regiochemistry of **1**. However, no correlation was observed in the gHMBC from H-4 to C-3'.

In an effort to discriminate between the two possible regioisomers, a two-dimensional gROESY NMR was

obtained. Observed correlations in the gROESY spectrum (H-4 to H-13 and 3-OCH₃, H-10 to 11-CH₃, H-8' to 7'-CH₃, H-10' to 1'-CH₃ and 9'-OCH₃) gave further evidence for partial structures A and B (Fig. 1). However, no correlation was observed that allowed distinction between the two possible regioisomers.

In a final effort to obtain a spectroscopic solution to the problem, the gHMBC data was recollected, this

**Figure 1.** Proposed part structures for **1** and **2**.

time optimised for 4 Hz coupling. As well as the observation of additional correlations that confirmed the partial structures A and B (Fig. 1), a weak three bond correlation between H-4 and C-3' allowed the regiochemistry to be established and hence the gross structure of **1** to be determined as shown. The relative stereochemistry about the C-3/4 ring junction was determined to be *cis* through the observation of a correlation in the gROESY spectrum between H-4 and 3-OCH₃.

Epiactephilol A **2** has a molecular formula of C₃₅H₃₂O₈ [(M+H)⁺, Δ_{mmu} +3.2] identical to that of **1**.⁴ Comparison of the ¹H and ¹³C NMR data indicated that **2** showed remarkable similarity with **1** (Table 1). Only the ¹H NMR chemical shifts of H-4', H-5', H-10' and 1'-CH₃, and the ¹³C NMR chemical shift of C-4a', showed any notable difference. Analysis of the gHMBC and gROESY data established that **2** contained the same two partial structures present in **1** (Fig. 1). Despite this, no correlations were observed in either the gHMBC or the gROESY for **2** that allowed for the preference of one regioisomer over the other. In order to distinguish between the two possible regioisomers an attempt was made to crystallise **2**. During this attempted crystallisation it was noted by ¹H NMR that **2** was transformed into a 1:1 mixture with **1**. This indicated that the difference between **2** and **1** was stereochemical and not geometrical. The gROESY spectrum showed no correlation between H-4 and 3-OCH₃, which indicated that **2** is the C-3 epimer of **1**.

Both **1** and **2** were biologically evaluated against a mammalian cell line and for antibacterial activity (*Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae*). Although both **1** and **2** displayed activity at a concentration of 250 μg/mL against the mammalian cell line, no antibiotic activity was observed.

It is likely that **1** and **2** are formed by dimerisation of two degraded diterpenes, one with more aromatization than the other. A series of related triterpene dimers have been isolated from the plant family Celastraceae,^{5–7} however **1** and **2** are the first examples to possess this particular substitution pattern about the dioxane core.

Acknowledgements

We would like to thank Chng Bee Lee for mass spectral data, Lee Khai Lin for help in the preparation of extracts, and Assoc. Professor Lai from the School of Chemistry for the generation of the IR spectrum and the measurement of specific rotation. Finally we would like to acknowledge financial support from Glaxo-SmithKline and the Economic Development Board of Singapore.

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4. [α]_D -81 (c 0.4, MeOH); IR (CHCl₃) ν_{max} 3420, 2980, 2930, 1730, 1610 cm⁻¹; UV (PDA) λ_{max} 226, 249, 288 nm; HRESIMS (M+H) 581.2207 (calculated for C₃₅H₃₃O₈ 581.2175)
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