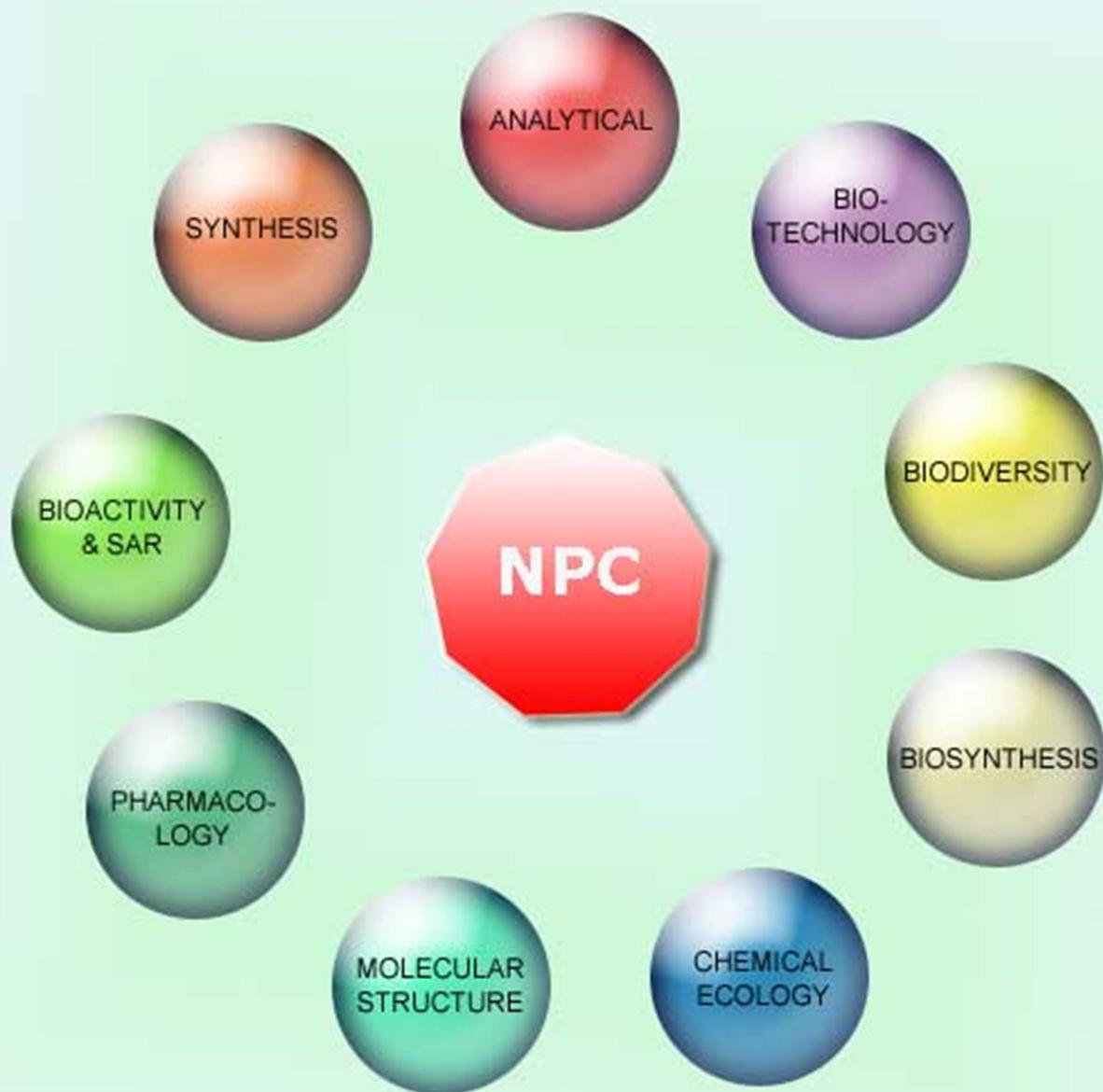


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Dr. Pawan K. Agrawal
On the Occasion of his 60th Birthday**

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New Furanone and Sesquiterpene from the Pericarp of *Calocedrus formosana*Tzong-Huei Lee^a, Ming-Shian Lee^b, Horng-Huey Ko^c, Jih-Jung Chen^d, Hsun-Shuo Chang^e, Mei-Hwei Tseng^f, Sheng-Yang Wang^g, Chien-Chih Chen^{h,†} and Yueh-Hsiung Kuo^{i,j,*}

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One new γ -lactone, namely calolactone (**1**), together with one new drimane-type sesquiterpene, namely caloterpene (**2**), were isolated from the pericarp of *Calocedrus formosana* Florin. Their structures were elucidated by spectroscopic and mass spectrometric analysis.

Keywords: *Calocedrus formosana*, Furanone, Calolactone, Sesquiterpene, Caloterpene.

Calocedrus formosana Florin (= *C. macrolepis* var. *formosana*), a coniferous tree indigenous to Taiwan, is a variable species classified in the family Cupressaceae [1]. Previous studies have demonstrated that the essential oils and extractives of this tree exhibited inhibitory effects against termites, mildew, and fungi, as well as functioning as an antioxidant, an anti-inflammatory agent, and a mosquito larvicide [2a-2f]. However, no prior report focused on the chemical constituents of the pericarp of *C. formosana*. Thus, we set out on a series of extraction, and purification of the extracts, which resulted in the isolation and identification of one new γ -lactone, calolactone (**1**) along with one new sesquiterpene, caloterpene (**2**) (Figure 1).

The molecular formula of **1** was determined to be C₉H₁₄O₄ from the HRESIMS molecular ion peak [M + H]⁺ at *m/z* 187.0965 (calcd. for C₉H₁₅O₄, *m/z* 187.0970), and ¹³C NMR spectrum, indicating that the index of hydrogen deficiency (IHD) of **1** was three. The IR spectrum of **1** indicated the presence of an acetoxy carbonyl (1732 cm⁻¹) and a γ -lactone carbonyl (1766 cm⁻¹). Two lower-shifted resonances observed in the ¹³C NMR spectrum were referred to two carbonyl carbons at δ_C 177.8 (C-1) and 170.6 (C-8), which also indicated that a ring remained for the whole IHD in correspondence with the existence of a γ -lactone ring. The ¹H NMR spectrum (Table 1) of **1** supported by HMQC and DEPT spectra revealed signals for two methyls at δ_H 1.27 (H₃-5) and 1.43 (H₃-7), one acetoxy methyl at δ_H 2.07 (s, H₃-9), three methines at 1.96 (H-3), 2.47 (H-2) and 4.25 (H-4), as well as one oxymethylene with nonequivalent resonances at δ_H 4.13 and 4.22 (H₂-6). Crosspeaks of δ_{H-5} 1.27/ δ_{H-2} 2.47, δ_{H-2} 2.47/ δ_{H-3} 1.96, δ_{H-3} 1.96/ δ_{H-6a} 4.13, δ_{H-6b} 4.22 and δ_{H-4} 4.25, δ_{H-6a} 4.13/ δ_{H-6b} 4.22, and δ_{H-4} 4.25/ δ_{H-7} 1.43 in the COSY spectrum (Figure 1B) coupled with key crosspeaks of δ_{H-5} 1.27/ δ_{C-1} 177.8, δ_{C-2} 38.8 and δ_{C-3} 50.5, δ_{H-6a} 4.13 and δ_{H-6b} 4.22/ δ_{C-2} 38.8, δ_{C-3} 50.5, δ_{C-4} 76.9 and δ_{C-8} 170.6, δ_{H-7} 1.43/ δ_{C-4} 76.9 and δ_{C-3} 50.5 and δ_{H-9} 2.07/ δ_{C-8} 170.6 in the HMBC spectrum

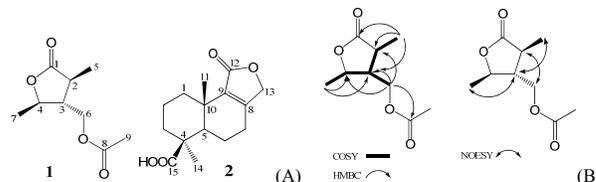


Figure 1: (A) Chemical structures of compounds **1** and **2**; (B) COSY, key HMBC and NOESY of **1**.

Table 1: ¹H NMR and ¹³C NMR data (in CDCl₃) for compounds **1** and **2**.

	1 ^a		2 ^a	
	¹³ C	¹ H	¹³ C	¹ H
1	177.8		34.5	2.59 brd (13.3) 1.15 td (13.3, 4.0)
2	38.8	2.47 m	18.7	1.85 m 1.55 m 2.22 m 1.07 m
3	50.5	1.96 m	37.6	
4	76.9	4.25 m	43.3	
5	14.0	1.27 d (7.1)	53.1	1.41 dd (12.3, 1.6) 2.15 m
6	62.2	4.13 dd (12.7, 5.7) 4.22 dd (12.7, 4.5)	19.9	1.95 m 1.78 m
7	19.8	1.43 d (6.1)	25.6	2.38 dd (18.6, 5.5)
8	170.6		159.3	
9	20.7	2.07 s	134.4	
10			35.4	
11			17.6	1.06 s
12			172.2	
13			70.6	4.60 d (17.0) 4.54 d (17.0)
14			28.7	1.29 s
15			181.4	

^a ¹H and ¹³C NMR acquired at 500 and 125 MHz, respectively.

(Figure 1B) established the gross structure of **1**. Key crosspeaks of δ_{H-5} 1.27/ δ_{H-3} 1.96, δ_{H-7} 1.43/ δ_{H-3} 1.96 and δ_{H-6a} 4.13 and δ_{H-6b} 4.22/ δ_{H-2} 2.47 in the NOESY spectrum (Figure 2) corroborated the relative configurations of H₃-5, H₃-7 and the methylene acetoxy moiety attached at C-3 to be β -, β - and α -oriented. Accordingly, the structure of **1** was deduced to be as shown in Figure 1, and was named calolactone.

Compound **2**, a white solid, was determined as $C_{15}H_{20}O_4$, by HRESIMS. The IR absorptions at 3300–2500, 1691 and 1745 cm^{-1} indicated the presence of a carboxylic acid and an α,β -unsaturated γ -lactone carbonyl functionality, respectively. Fifteen carbon resonances observed in the ^{13}C NMR spectrum coupled with the DEPT spectrum of **2** were attributable to two methyls at δ_C 17.6 and 28.7, six methylenes at δ_C 18.7, 19.9, 25.6, 34.5, 37.6 and 70.6, one methine at δ_C 53.1 and six quaternary carbons at δ_C 35.4, 43.3, 134.4, 159.3, 172.2 and 181.4 (Table 1). The molecular formula, $C_{15}H_{20}O_4$, indicated that the IHD of **2** was six, including one carboxylic acid and an α,β -unsaturated γ -lactone, as evidenced by four quaternary carbon signals at δ_C 134.4, 159.3, 172.2 and 181.4 in the ^{13}C NMR spectrum and IR interpretations. Thus, the number of rings remaining in the structure of **1** should be two. The 1H -NMR spectrum coupled with the HSQC spectrum exhibited signals for two methyl groups [δ_H 1.06 (H_3 -11) and 1.29 (H_3 -14)], six methylene groups [δ_H 1.07 (H_b -3), 1.15 (H_b -1), 1.55 (H_b -2), 1.78 (H_a -7), 1.85 (H_a -2), 1.95 (H_b -6), 2.15 (H_a -6), 2.22 (H_a -3), 2.38 (H_b -7), 2.59 (H_a -1), 4.54 (H_b -13) and 4.60 (H_a -13)] and one methine [δ_H 1.41 (H -5)] (Table 1). The planar structure of **2** was further deduced by key COSY (H_2 -1/ H_2 -2; H_2 -2/ H_2 -3; H -5/ H_b -6; H_a -6/ H_b -7) and key HMBC (H_3 -11/ C -1, -5, -9 and -10; H_3 -14/ C -3, -4, -5 and -15; H_2 -7/ C -8 and -9; H_2 -13/ C -8, -9 and -12) correlations (Figure 2). The above assignments were characteristic of drimane-type sesquiterpenes containing a *trans*-decalin moiety [3], with two methyls located at C-4 and -10, one carboxylic acid attached to C-4, and one α,β -unsaturated γ -lactone moiety located at C-8 and -9. The orientations of H-5, H_3 -11 and H_3 -14 were determined to be axial, axial, and equatorial, respectively, judged from the coupling constants of H-5/ H_{ax} -6 (12.3 Hz), as well as the key cross-peaks of H_3 -14/H-5, H-5/ H_{ax} -1 and H_{eq} -1/ H_3 -11 in the NOESY spectrum of **2** (Figure 2). Conclusively, the structure of **2** was assigned as shown in Figure 1A, and the compound was named caloterpene.

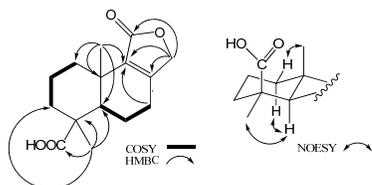


Figure 2: Key COSY, HMBC and NOESY features of **2**.

Experimental

General: Optical rotations, Jasco-DIP-1000 digital polarimeter; IR, Nicolet MAGNA-IR 550 spectrophotometer Series II; UV, Helios

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Beta UV-Visible spectrometer; NMR, Bruker DMX-500SB spectrometers; HRESIMS, High Definition Mass Spectrometry System with an ESI interface and a TOF analyzer; in m/z (rel.%). CC, silica gel Kieselgel 60 (70–230 mesh; Merck).

Plant material: The pericarp of *Calocedrus formosana* Florin was collected in Nan-Tou, Taiwan, in Aug, 1998, and was identified by Prof. Shang-Tzen Chang in the Department of Forestry, National Taiwan University. A voucher specimen (No. 223133) has been deposited in the Herbarium of the Department of Life Science, National Taiwan University, Taipei, Taiwan.

Extraction and isolation: The pericarp of *C. formosana* (5.0 kg) was ground into powder and extracted with methanol (20 L), 3 times (5 days each time) at room temperature. Evaporation of the organic solvent from each solution under reduced pressure gave the crude residue, which was partitioned using ethyl acetate and water. The EtOAc part (500 g) was pre-absorbed into 800 g SiO_2 and then subjected to CC on silica gel (3.5 kg) using *n*-hexane with increasing amounts of EtOAc as an eluent to afford 5 major fractions denoted as fractions I–V by monitoring with TLC. Repeated chromatography of fractions III on a silica gel 60 column (230-400 mesh) with *n*-hexane/EtOAc (7:3, v/v) gave **1** (36 mg). Fraction V was re-chromatographed on a silica gel 60 column (230-400 mesh) with *n*-hexane/EtOAc (3:7, v/v) to afford **2** (56 mg).

Calolactone (1)

Amorphous white powder.

$[\alpha]_{25}^D$: -12.0 (c 0.27, in $CHCl_3$).

IR (KBr): 1766, 1732 cm^{-1} .

1H and ^{13}C NMR: Table 1.

HRESIMS m/z : 187.0965 (calcd. for $C_9H_{15}O_4$, m/z 187.0970).

Caloterpene (2)

Amorphous white powder.

$[\alpha]_{25}^D$: -2.2 (c 0.19, in $CHCl_3$).

IR (KBr): 3300-2500, 1745, 1691 cm^{-1} .

UV λ_{max} (log ϵ) in MeOH: 254 (4.1) nm.

1H and ^{13}C NMR: Table 1.

HRESIMS m/z : 265.1436 (calcd. for $C_{15}H_{21}O_4$, m/z 265.1440).

Acknowledgement - This work was kindly supported by CMU under the Aim for Top University Plan of the Ministry of Education, Taiwan, and Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002).

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