

## A Labdane Diterpene from *Encisanthum congregatum*

Hasnah Mohd. Sirat<sup>1</sup> and A. Hamid A. Hadi\*

Dept. of Chemistry, Faculty of Science,  
Universiti Teknologi Malaysia, KB 791,  
80990 Johor Bahru, Malaysia.

\*Department of Chemistry,  
Universiti Malaya,  
59100 Kuala Lumpur, Malaysia.

Received 25 March 1992

### ABSTRAK

Diterpena labdana telah diasingkan daripada daun kering *Encisanthum congregatum*. Strukturnya telah ditentukan sebagai 8,16-dihidroksi-*ent*-labd-13-en-15-oik lakton berdasarkan data spektroskopi.

### ABSTRACT

A labdane diterpene was isolated from the air-dried leaves of *Encisanthum congregatum*. The structure has been established as 8,16-dihydroxy-*ent*-labd-13-en-15-oic lactone on the basis of spectroscopic data.

**Keywords:** *Encisanthum congregatum*, Annonaceae, Labdane type diterpene

### INTRODUCTION

*Encisanthum congregatum* is one of the species of the Annonaceae family. Chemical screening of the leaf of this plant has shown it to be rich in alkaloids and terpenoids. The alkaloid constituents of *E. congregatum* have been isolated and identified (Ahmad and Hadi 1988) as liriodenine (1) (Bick and Douglas 1964) and O-methylmoschatoline (2) (Bick and Douglas 1965). In continuation of our studies on the biologically active compounds from the plant, the terpenoid constituents of *E. congregatum* were examined and a labdane type diterpene (3) isolated from the leaves. This paper describes the structural elucidation of the newly-isolated compound.

### MATERIALS AND METHODS

Melting point was uncorrected; NMR spectra were recorded using Jeol FX-100 spectrometer in CDCl<sub>3</sub> at 100 MHz for <sup>1</sup>H and 25 MHz for <sup>13</sup>C nuclei, and the chemical shifts are in δ ppm. IR spectrum was recorded on Perkin Elmer 727 B spectrophotometer. Thin-layer chromatography (TLC) was carried out on silica gel plate F<sub>254</sub> (0.25 mm thickness). Column chromatography was performed on silica gel (Merck) 230-400 Mesh.

<sup>1</sup>Author to whom correspondence should be addressed

*Plant Material*

The plant material used was collected at Bukit Bauk, Dungun, Terengganu, Malaysia and identified as *Enicosanthum congregatum*. A voucher specimen was deposited in the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

*Extraction of Enicosanthum congregatum*

Air-dried leaves of *E. congregatum* (1 kg) were extracted with petroleum ether at room temperature for 24 h. The extract was concentrated *in vacuo* and the residue (2.6g) was fractionated by flash column chromatography on silica gel (Clark-Still *et al.* 1978) to give white precipitate. This precipitate was then recrystallized from petroleum ether-ether to yield labdane diterpene butenolide (3) (0.80g) 8,16-dihydroxy-*ent*-labd-13-en-15-oic lactone. M.p. 156-157°C; IR  $\nu_{\max}$  3470(OH), 1780 and 1740 ( $\alpha,\beta$ -unsaturated lactone) and 1630(C=C) $\text{cm}^{-1}$ .  $\lambda_{\max}$  (MeOH) 215 nm (log  $\epsilon$  4.10); M found: 320.2349  $\text{C}_{20}\text{H}_{32}\text{O}_3$  requires: 320.2343.  $^1\text{H}$  NMR:  $\delta$  0.76(6H, s, 2xMe), 0.96(3H, s, Me), 1.10(3H, s, Me), 1.25-1.85(16H, m,  $\text{CH}_2$  and CH), 2.30(2H, m, H-12), 4.75(2H, d,  $J=1.7\text{Hz}$ , H-16), and 5.82(1H, t,  $J=1.7\text{Hz}$ , H-14). MS:  $m/z$ (%) 320,  $\text{M}^+$ (10), 302(49), 287(58), 210(70), 111(100).

**RESULTS AND DISCUSSION**

The petroleum ether extract of the leaves of *Enicosanthum congregatum* was evaporated under reduced pressure, followed by fractionation using column chromatography to give white precipitate. Recrystallization of the precipitate with petroleum ether-ether several times furnished compound (3) as white powder. The structure was elucidated by spectroscopic methods.

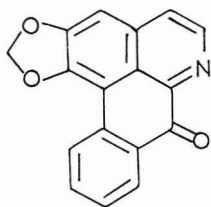
Compound (3) has the molecular formula  $\text{C}_{20}\text{H}_{32}\text{O}_3$  on the basis of the high resolution mass spectrum. In the EI mass spectrum of (3),  $[\text{M}^+]$  appeared at  $m/z$  320 ( $\text{C}_{20}\text{H}_{32}\text{O}_3$ ). The fact that one of the three oxygen atoms of (3) was present as a hydroxyl group and the remaining two were involved in the butenolide ring was confirmed by the presence of peaks at  $m/z$  302  $[\text{M} - \text{H}_2\text{O}]^+$  and  $m/z$  111 (as base peak).

Its infrared spectrum displayed peaks at 1780 and 1740  $\text{cm}^{-1}$ , suggesting the presence of  $\alpha,\beta$ -unsaturated lactone group, a characteristic absorption band for butenolide ring. A prominent band at 3470  $\text{cm}^{-1}$  indicated the presence of a hydroxyl group while the other prominent band at 1630  $\text{cm}^{-1}$  was attributed to carbon double bond stretching.

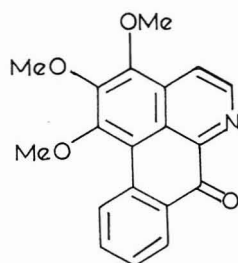
The double bond equivalent (DBE) value of (3) was calculated as five. After assigning a DBE value of three to the butenolide ring, compound (3) had to contain two rings. The molecular formula  $\text{C}_{20}\text{H}_{32}\text{O}_3$  and its bicyclic nature suggested it was a diterpene butenolide possessing a decalin

skeleton presumably belonging to the labdane group (Kimbu *et al.* 1979; Itokawa *et al.* 1980; Lopes and Bolzani 1988).

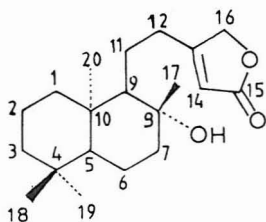
Analysis of the  $^1\text{H}$  NMR spectrum of (3) supported the presence of a labdane moiety and a butenolide ring. Three singlets appearing at 0.76, 0.96 and 1.10 ppm were assigned to the four methyl groups at C-4, C-10 and C-8 respectively. A one-proton triplet at 5.8 ( $J=1.7$  Hz, H-14), indicative of the presence of an  $\alpha$ -olefinic proton, along with a two-proton doublet at 4.7 ( $J=1.7$  Hz, H-16) suggested the presence of an  $\alpha,\beta$ -unsaturated lactone group (Bohlmann *et al.* 1981) which is in agreement with the UV data. The structure of (3) was assigned by comparison of the  $^1\text{H}$  NMR data with *ent*-labda-8 $\beta$ -ol-14-ene (4) (Lopes and Bolzani 1988) and medigenin (5) (Sethi *et al.* 1988).



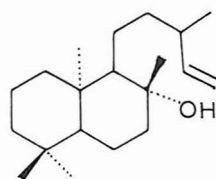
(1)



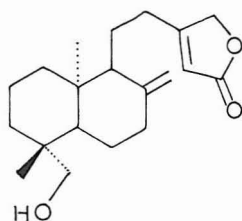
(2)



(3)



(4)



(5)

The structure was also supported by the  $^{13}\text{C}$  NMR spectrum (off resonance and DEPT) which revealed the presence of five quarternary, three methyne, eight methylene and four methyl carbons as shown in Table 1. On the basis of the preceding data, it was concluded that compound (3) is a labdane diterpene butenolide, 8,16-dihydroxy-*ent*-labd-13-en-15-oic lactone.

TABLE 1  
 $^{13}\text{C}$  NMR data of labdane diterpene (3)

$\delta$	DEPT	Assignment
174.3(s)	$\begin{array}{c}   \\ -\text{C}- \\   \end{array}$	C15
171.7(s)	$\begin{array}{c}   \\ -\text{C}- \\   \end{array}$	C13
114.7(d)	CH	C14
75.2(s)	$\begin{array}{c}   \\ -\text{C}- \\   \end{array}$	C8
73.2(t)	$\text{CH}_2$	C16
41.4(s)	$\begin{array}{c}   \\ -\text{C}- \\   \end{array}$	C10
40.2(d)	CH	C9
38.6(s)	$\begin{array}{c}   \\ -\text{C}- \\   \end{array}$	C4
36.3(d)	CH	C5
35.5(t)	$\text{CH}_2$	C1 and C3
31.5(t)	$\text{CH}_2$	C7
27.0(t)	$\text{CH}_2$	C12
24.4(q)	$\text{CH}_3$	C19
22.3(t)	$\text{CH}_2$	C11
22.0(t)	$\text{CH}_2$	C6
21.1(t)	$\text{CH}_2$	C2
18.1(q)	$\text{CH}_3$	C17
17.4(q)	$\text{CH}_3$	C20
16.0(q)	$\text{CH}_3$	C18

### ACKNOWLEDGEMENTS

The authors thank Prof. R. Hodges of Massey University, New Zealand for recording the mass spectrum and Assoc. Prof. Dr. Satapah Ahmad of Chemistry Department, Universiti Teknologi Malaysia for recording the NMR spectra.

### REFERENCES

- AHMAD, F. and A.H.A. HADI. 1988. The alkaloids of *Enicosanthum congregatum*. In *Proceedings of the Unesco Sub Regional Seminar on the Systematic Identification of Natural Products*, ed. M.S. Ikram and B. Din Laily. p.93. UKM, Bangi, Malaysia
- BICK, I.R.C. and G.K. DOUGLAS. 1964. Yellow alkaloids of *Atherosperma moschatum* Labill. *Tet. Lett.* **25**: 1629.
- BICK, I.R.C. and G.K. DOUGLAS. 1965. The structures of atheroline and moschatoline. *Tet. Lett.* **51**: 4655.
- BOHLMANN, F., W.R. ABRAHAM, R.M. KING and H. ROBINSON. 1981. Diterpenes from *Koanophyllon* species. *Phytochemistry* **20**: 1903.
- CLARK-STILL, W., M. KHAN and A. MITRA. 1978. Rapid chromatographic techniques for preparative separations with moderate resolution. *J. Org. Chem.* **43**: 2923.
- ITOKAWA, H., M. MORITA and S. MIHASHI. 1980. Labdane and bisnorlabdane type diterpene from *Alpinia speciosa* K. Schum. *Chem. Pharm. Bull.* **28**: 3452.
- KIMBU, S.F., T.K. NJIMI, B.L. SONDEGAM, J.A. AKINNYI and J.D. CONOLLY. 1979. The structure of a labdane dialdehyde from *Aframomum danielli* (Zingiberaceae). *J. Chem. Soc., Perkin I*: 1303.
- LOPES, L.M.X. and V.DA S. BOLZANI. 1988. Lignans and diterpenes of three *Aristolochia* species. *Phytochemistry* **27**: 2265.
- SETHI, A., A. KHARE and M.P. KHARE. 1988. A labdane diterpene and its glycoside from *Melodinus monogynus*. *Phytochemistry* **27**: 2255.