

## AN ANESTHETIC ALKAMIDE AND FIXED OIL FROM *ACMELLA OLERACEA*

Ampai Phrutivorapongkul\* Aueporn Chaiwon Suwanna Vejabhikul  
Watcharee Netisingha Sunee Chansakaow

Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand 50200

**ABSTRACT:** An anesthetic alkamide, (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide (**1**) and fixed oil were isolated from the arial part of the toothache plant, *Acmella oleracea* (L.) R.K. Jansen. Structure elucidation of **1** was achieved by analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR experiments and comparison of the spectral data with published values. This is the first time to report **1** from *A. oleracea* (L.) R.K. Jansen. Additionally, fatty acids determination in lipid was obtained by GC.

**Keywords:** *Acmella oleracea*, local anesthetic, alkamide, (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide

**INTRODUCTION:** The toothache plant or Paracress (*Acmella oleracea* (L.) R.K. Jansen, family Compositae) is renamed from the two toothache plants, *Spilanthes acmella* (L.) Murray and *S. oleracea* Jacquin. It is an annual herb that scattered around the world's tropic. The plant has shown anti-inflammatory, antibacterial, antifungal, diuretic, sialagogic and larvicidal properties. The flower heads extract was used in India for treatment of gum diseases and dental caries. In Thailand, especially the Northern and North-eastern parts use *A. oleracea* (L.) R.K. Jansen as an indigenous vegetable and a spice for appetizers. This plant contains spilanthol (**2**) which was the principal pungent compound and acts as an anesthetic and analgesic<sup>1-3</sup>. In this paper, phytochemical investigation of the arial parts of *A. oleracea* (L.) R.K. Jansen is reported for isolation of (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide (**1**), an anesthetic alkamide like spilanthol. Compound **1** has been isolated from *A. ciliata*<sup>4</sup>, *S. oppositifolia*<sup>5</sup>, *Asarum forbesii*<sup>6</sup>, *S. alba*<sup>7</sup>, *Salmea scandens*<sup>7</sup>, *Echinacea angustifolia*<sup>5</sup>, *E. pallida*<sup>5</sup>, *E. purpurea*<sup>5,8</sup> and *Leucanthemum hosmariense*<sup>9</sup>. This is the first time to report the isolation of **1** from *A. oleracea* (L.) R.K. Jansen. Additionally, we also investigate fatty acids composition of fixed oil by GC analysis.

## MATERIALS AND METHODS:

### General experimental procedures

The TOF-MS data was obtained from a Q-TOF-2<sup>TM</sup> Micromass UK and NMR data were recorded from a Bruker Avance 400 FT-NMR spectrometer. Fatty acids composition was acquired by GC Shimadzu 2010.

### Plant Materials

The arial parts of *Acmella oleracea* (L.) R.K. Jansen, family Compositae were collected in Muang District, Chiang Mai Province, Thailand in September 2005. Authentication was achieved by comparison with the herbarium specimen at the Herbarium Section, Northern Research Center for Medicinal Plants, Faculty of Pharmacy, Chiang Mai University, Thailand. The herbarium specimen (No.5652) has been deposited in the Faculty of Pharmacy, Chiang Mai University, Thailand.

### Extraction and isolation

A dried and powdered arial parts of *Acmella oleracea* (L.) R.K. Jansen (1.5 kg) was macerated three times for a 3-day-period in 95% ethanol and filtered. The combined filtrate was evaporated under reduced pressure to give a dark green mass (12 gm). This mass (11 gm) was separated by vacuum column chromatography (VCC) of silica gel 60 (Merck, 70-230 mesh

\*To whom correspondence should be addressed.

E-mail: phampai@hotmail.com,

Tel: 0 5394 4342-3, Fax: 0 5322 2741

ASTM) and eluted with gradient technique of  $C_6H_{12}$ -EtOAc- $CH_3OH$  to give 17 fractions. Fraction 1 eluted with hexane is yellow oil, was identified fatty acids composition by GC to give data as shown in Table 1. Fraction 6 eluted with 20-30% EtOAc in hexane, shows the most tingling effect on the tongue (onset = 2 seconds, duration = 20 minutes), was successively chromatographed on a Sephadex LH-20 column with IPA, silica gel column with  $C_6H_{12}$ -EtOAc- $CH_3COCH_3$  (8:1:1) and with  $C_6H_{12}$ -EtOAc- $CH_3COCH_3$  (12:1:1) to give a semipurify fraction. This fraction was then purified by preparative HPLC (Inertsil PREP-ODS, 10 x 250 mm, solvent system, MeOH- $H_2O$  (75:25): flow rate, 5 ml/min: detector, UV 230 nm) to yield **1** (10 mg).

(2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide (**1**) white solid, m.p. 63 °C, Micromass Q-ToF MS  $m/z$  (rel. int.): 248.1808 ( $(M^+ + 1)$  5), 167.1154 (100).  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  (ppm): 5.79 (1H, d,  $J$  = 15 Hz, H-2), 7.18 (1H, dd,  $J$  = 15, 10 Hz, H-3), 6.15 (1H, dd,  $J$  = 15, 10 Hz, H-4), 6.07 (1H, dt,  $J$  = 15, 7 Hz, H-5), 2.23-2.28 (2H, m, H-6), 2.23-2.28 (2H, m, H-7), 5.26 (1H, dt,  $J$  = 10, 7 Hz, H-8), 5.97 (1H, br t,  $J$  = 10 Hz, H-9), 6.29 (1H, br t,  $J$  = 12.5, H-10), 5.71 (1H, dq,  $J$  = 15, 7 Hz, H-11), 1.77 (3H, br d,  $J$  = 6.8 Hz, H-12), 3.15 (2H, t,  $J$  = 7 Hz, H-1'), 1.80 (1H, m, H-2'), 0.93 (6H, d,  $J$  = 6.4 Hz, H-3', H-4')

**RESULTS AND DISCUSSION:** The Fatty acids composition of fixed oil identified by GC was shown in Table 1

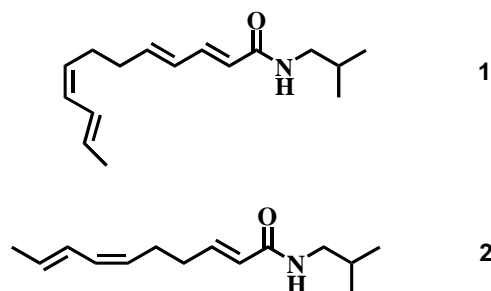
An olefinic alkamide, (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide (**1**), is *N*-isobutylamide that exhibits strongly numbness and tingling effects like those of spilanthol. The tingling effect of these alkamides influence the sensory of trigeminal nerve. Furthermore, the structure of **1** presents the moieties as the 2,4-diunsaturated amide, 2*E* double bond and *N*-isobutylamide which had been studied to be important to this activity<sup>10</sup>. The isolation and structure elucidation of **1** were succeeded by chromatographic and spectroscopic techniques together with comparison of the spectral data with published values. From the

**Table 1** Fatty acids composition of fixed oil

Common name	IUPAC name	%
Caprylic acid (C8:0)	Octanoic acid	0.14
Lauric acid (C12:0)	Dodecanoic acid	0.12
Myristic acid (C14:0)	Tetranic acid	0.28
Palmitic acid (C16:0)	Hexadecanoic acid	25.84
Palmitoleic acid (C16:1 n-7)	cis-9-Hexadecanoic acid	0.93
Stearic acid (C18:0)	Octadecanoic acid	4.54
Oleic acid (C18:1 n-9)	Octadecenoic acid	8.72
Linoleic acid (C18:2 n-6)	cis-9,12-Octadecadienoic acid	56.37
Linolenic acid (C18:3 n-3)	cis-9,12,15-Octadecatrienoic acid	0.72
Arachidic acid (C20:0)	Eicosanoic acid	0.66
Arachidonic acid (C20:4 n-6)	Eicosatetraenoic acid	0.13
Behenic acid (C22:0)	Docosanoic acid	0.43
Lignoceric acid (C24:0)	Tetracosanoic acid	0.26
Erucic acid (other 2C 22:1)	Cis-13-Docosanoic acid	0.18

chemical shift and coupling patterns of protons in  $^1H$ -NMR data, we can assign the geometric isomer of **1** as (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutylamide but not as the other isomers because of these reasons; H-4 and H-5 were at downfield than those of the 4-*cis*. The  $J_{4,5}$  was at 15 Hz instead of 11.5, as expected for a *cis*-double bond. The 10-*trans* geometry has more down field shift of H-10 and H-11 than those the 10-*cis*, and also from the  $J_{10,11}$  of 15 Hz. Furthermore, chemical shifts and coupling constants for H-2, 3, 4, 5, 8, 9, 10 and 11 were similar to those reported for these protons in (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide<sup>4,6,7,9</sup>

Interestingly, the fatty acids found in lipid presented an omega - 6 unsaturated fatty acid, linoleic acid (56.37%). This means that *Acmella oleracea* (L.) R.K. Jansen may be a rich source of essential fatty acid.



**ACKNOWLEDGEMENTS:** The authors would like to acknowledge their thanks to Faculty of Pharmacy, Chiang Mai University for research fund and to Northern Research Center for Medicinal Plants Chiang Mai University for facility used in instrumentation.

#### REFERENCES:

1. Nakatani N, Nagashima M. 1992. Pungent alkamides from *Spilanthes acmella* L. var. *oleracea* Clarke. Biosci Biotech Biochem 56(5): 759-62.
2. Ratnasooriya WD, Pieris KPP, Samaratunga U, Jayakody JRAC. 2004. Diuretic activity of *Spilanthes acmella* flowers in rats. J Ethno-pharmacol 91: 317-20.
3. Ramsewak RS, Erickson AJ, Nair MG. 1999. Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 51: 729-32.
4. Martin R, Becker H. 1985. Amides and other constituents from *Acmella ciliata*. Phytochemistry 24(10): 2295-300.
5. Molinartorres J, Garciglia RS, Chavez ER, Rio RED. 1996. Purely olefinic alkamides in *Heliopsis longipes* and *Acmella (Spilanthes) oppositifolia*. Biochem Sys Ecol 24(1): 43-7.
6. Zhang F, Chu CH, Xu Q, Fu SP, Hu JH, Xiao HB, Liang XM. 2005. A new amide from *Asarum forbesii* Maxim. J Asian Nat Prod Res 7(1): 1-5.
7. Herz W, Kulanthaivel P. 1985. An amide from *Salmea scandens*. Phytochemistry 24(1): 173-4.
8. Raner GM, Cornelious S, Moulick K, Wang Y, Mortenson A, Cech NB. 2007. Effects of herbal products and their constituents on human cytochrome P450<sub>2E1</sub> activity. Food Chem Toxicol 45: 2359-65.
9. Bohlmann F, Fritz U, Dutta L. 1980. Neue acetylenverbindungen aus *Leucanthemum* Arten und revision der struktur eines germacranolids. Phytochemistry 19: 841-4.
10. Ley JP, Krammer G, Reinders G, Bertram HJ. 2005. 11th Weurmann Flavour Research Symposium, Roskilde, Denmark.

#### อัลคาไมด์ที่มีฤทธิ์ชาและน้ำมันจากผักคราดหัวแหวน

อำไพ พฤติวรพงศ์กุล\* เอื้อพร ไชยวรรณ สุวรรณหา เวชอภิกุล วชิรี เนติสิงหะ สุนีย์ จันทร์สกา

คณะเภสัชศาสตร์ มหาวิทยาลัยเชียงใหม่ อ. เมือง จ. เชียงใหม่ 50200

**บทคัดย่อ:** การศึกษาองค์ประกอบทางเคมีที่มีฤทธิ์ชาเฉพาะที่จากส่วนเหนือดินของผักคราดหัวแหวน สามารถแยก (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide (1) ซึ่งเป็นสารกลุ่มเอไมด์ที่ไม่อิ่มตัวจากผักคราดหัวแหวนเป็นครั้งแรก การพิสูจน์โครงสร้างทางเคมีของ 1 ได้จากการวิเคราะห์ข้อมูลของ <sup>1</sup>H-NMR และ <sup>13</sup>C-NMR ร่วมกับการเปรียบเทียบโครงสร้างของสารที่มีรายงานไว้ในอดีต นอกจากนี้ได้ทำการวิเคราะห์หากรดไขมันในน้ำมันที่แยกได้จากส่วนเหนือดินของผักคราดหัวแหวนด้วย GC

**คำสำคัญ:** ผักคราดหัวแหวน ฤทธิ์ชาเฉพาะที่ อัลคาไมด์ (2*E*,4*E*,8*Z*,10*E*)-*N*-isobutyl-2,4,8,10-dodecatetraenamide

\*ติดต่อได้ที่ phampai@hotmail.com โทรศัพท์ 0 5394 4342-3 โทรสาร 0 5322 2741