Menthalactone, a New Analgesic from *Mentha cordifolia* Opiz. Leaves

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Menthalactone, a new long-chain alkene with a bicyclic lactone moiety, was isolated as an analgesic constituent from the leaves of *Mentha cordifolia* Opiz. At a dosage of 100 mg/kg mouse, it decreased the number of squirms induced by acetic acid by 67.3%. Statistical analysis using Kruskall Wallis one-way analysis of variance by ranks showed that menthalactone is different from the solvent control at $\alpha = 0.01$ and approximates the analgesic activity of mefenamic acid at 0.001 level of significance.

Key words: Mentha cordifolia Opiz., Analgesic, Menthalactone

Introduction

Mentha cordifolia Opiz. (Lamiaceae), commonly known as mint, peppermint or marshmint, is cultivated throughout the Philippines and propagated by terminal cuttings. *M. cordifolia* is listed as one of the priority plants under the Department of Science and Technology (DOST), Philippine Council for Health Research and Development (PCHRD), National Integrated Research Program on Medicinal Plants (NIRPROMP). Its powdered dried leaves are presently being produced in tablet form, including pediatric tablets, and have been proven to be analgesic in clinical trial phases I, II, and III (Maramba *et al.*, 1991). It is used against toothache, headache, muscle pain, dysmenorrhea, and in post-operative pain in secondary minor surgery (Maramba *et al.*, 1993).

The mint family is characterized by its volatile oils. The leaves of *M. cordifolia* contain 0.8% volatile oil, consisting mainly of pulgenone, pitoeitone, and limonene (Tan, 1978), menthol, menthene, and menthenone (Quisumbing, 1978). Other constituents include cadinene, 1-carvomenthone, isomenthone, 4,8-epoxy-*p*-menthan-3-one, 2-isopropylcyclopentanone, 3,7-dimethyl-1,6-octadien-3-ol (linalool) (major component of oil), and *p*-menthan-2,5-diol. The present paper reports the bioassay-directed purification and structure elucidation of an analgesic constituent from *M. cordifolia* leaves. The analgesic activity was monitored using the acetic acid-induced writhing test.

Results and Discussion

The hexane (FB) and EtOAc (FD) extracts reduced the number of squirms induced by acetic acid by 81.4% ($\alpha = 0.05$) and 71.0% ($\alpha = 0.15$), respectively. Column chromatography of the hexane extract resulted in ten fractions (FB1–FB10) and the subsequent bioassay showed that fraction FB6, at a dosage of 0.25 mg/g mouse, is analgesic ($\alpha = 0.03$). The analgesic constituents isolated from fractions FB2 and FB10 were β-sitosterol and its glucoside, respectively (Villaseñor *et al.*, 2002).

Fraction FB6F, resulted from normal phase liquid chromatography of FB6, at a dosage of 0.10 mg/g mouse, reduced the number of squirms induced by acetic acid by 60.6% ($\alpha = 0.01$). Repeated and sequential chromatography of FB6F followed by recrystallization afforded white crystals labeled as FB6Fc. Although FB6Fc is not completely soluble in either corn oil or 2% carboxymethylcellulose (CMC) in normal saline solution (NSS), results of the bioassay (Table I) showed that it possesses analgesic activity ($\alpha = 0.01$) at a dosage of 0.10 mg/g mouse.

Isolate FB6Fc is soluble in 30% MeOH/$CHCl_3$ with an Rf value of 0.48 in 10% MeOH/$CHCl_3$. It is detected with iodine crystals and turns into pink upon heating after spraying with vanillin-sulfuric acid but it is UV-inactive. Its ^{13}C NMR and DEPT spectra showed 23 carbon signals with one -CH_3, fifteen -CH_2, six -CH and one quaternary

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Table I. Analgesic activity of menthalactone using the acetic acid-induced writhing test.

Test solution	Dose (mg/g mouse)	No. of squirms ± S.D.	Reduction in no. of squirms (%)
HOAc	0.01 ml	43.0 ± 18.8	
HOAc + mefenamic acid	0.007	11.6 ± 10.1	73.0
HOAc + corn oil	0.01 ml	48.0 ± 10.1	
HOAc + menthalactone dissolved in corn oil	0.1	17.5 ± 13.3	63.5
HOAc + 2% CMC in NSS	0.2 ml	44.0 ± 10.6	
HOAc + menthalactone dissolved in 2% CMC in NSS	0.1	14.4 ± 13.8	67.3

Positive control, HOAc + mefenamic acid; solvent controls, HOAc + corn oil and HOAc + 2% carboxymethylcellulose (CMC) in normal saline solution (NSS).

C signal. The FTIR spectrum showed the presence of a primary amine at 3332 and 3221 cm^{-1} and a C-N stretching vibration at 1278 cm^{-1}. The ^{13}C NMR signals were therefore characteristic of an amine and three ether linkages from δ_C 60.6 to δ_C 75.1, δ_H 3.28 to δ_H 3.86 and a C-O stretching vibration at 1121 cm^{-1}. A long-chain alkene was apparent from signals for the doubly bound carbon atoms at δ_C 130.3, δ_C 129.5, δ_H 5.15, and a C=C stretching vibration at 1623 cm^{-1}; the allylic carbon atoms at δ_C 32.2, δ_C 32.0, δ_H 1.76 and δ_H 1.71; and the aliphatic carbon atoms at δ_C 29 and δ_H 1.01 clusters. The signal at δ 175.5 and a C=O stretching vibration at 1756 cm^{-1} indicate the presence of an ester. The positive ion mode ESI-mass spectrum showed a molecular ion peak at *m/z* 380 $[M+H]^+$ giving the molecular formula of $C_{23}H_{41}NO_3$.

Cross peaks in the COSY spectrum between the geminal protons at δ_H 3.55 and 3.49, the geminal protons and δ_H 3.86, δ_H 3.86 and δ_H 3.29, and δ_H 3.29 and δ_H 1.17 gave fragment **1**. HMBC cross peaks between the geminal protons and δ_C 51.2 (^2J) and δ_C 75.1 (^3J) further supported the structure of fragment **1**. The structure of fragment **2** was derived from COSY cross peaks between the geminal protons at δ_H 1.33 and 1.53, and δ_H 1.33 and δ_H 3.79 together with HMBC cross peaks between δ_H 1.33 and δ_C 24.7 (^3J) and δ_C 71.6 (^2J).

A symmetrical fragment **3** was postulated to account for the similar chemical shifts of the doubly bound carbon atoms, the allylic and the aliphatic carbon atoms. The HMBC spectrum established the bonds between carbon atoms at δ_C 13.3 and δ_C 22.2 (^2J δ_C 22.2, δ_H 0.60); δ_C 22.2 and δ_C 31.5 (^3J δ_C 31.5, δ_H 0.60). The assignments of all the other carbon atoms with similar chemical shifts in the fragment were interchangeable. Infrared spectrum peaks at 1623 (C=C str., lit. value 1665–1635 cm^{-1}) and 723.0 cm^{-1} (=CH oop., lit. value 725–675 cm^{-1}) were indicative of a *cis* configuration.

The long-range correlations (Fig. 1) between δ_C 51.2 and δ_H 1.33 (^3J), δ_C 71.6 and δ_H 3.29 (^3J), δ_C 175.5 and δ_H 3.79 (^3J), δ_C 175.5 and δ_H 1.01 cluster (^2J), and δ_C 29 cluster and δ_H 1.17 (^2J) gave the structure of menthalactone (Fig. 2). This structure would also explain the multiplicities of the pro-

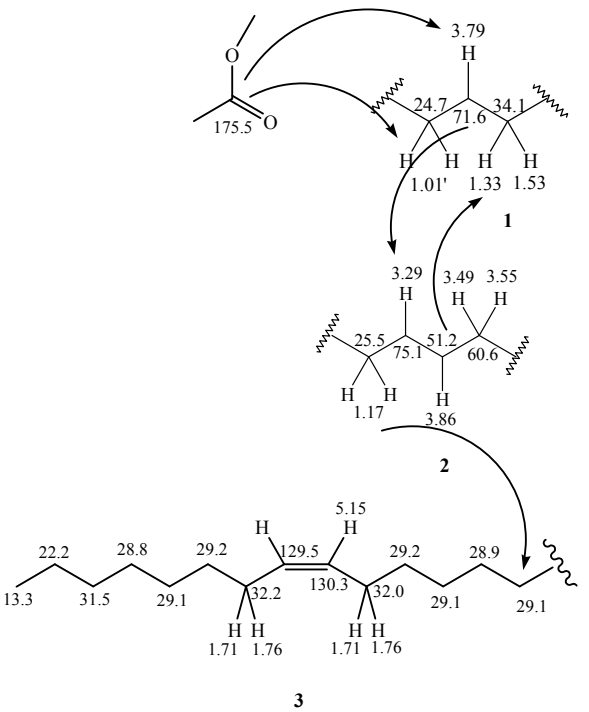

Fig. 1. Long-range correlations among fragments **1**, **2** and **3**.

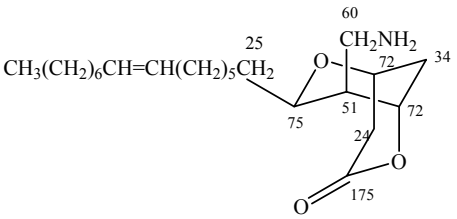

Fig. 2. Chemical structure of menthalactone.

tons at δ_H 1.33 and δ_H 1.53 attached to the carbon atom corresponding to δ_C 34.0. The bulky long-chain alkene moiety **3** would normally occupy an equatorial position and the proton corresponding to δ_H 3.29 would therefore be axial. With a J value of 4 Hz between δ_H 3.29 and δ_H 3.86, the proton at δ_H 3.86 would be equatorial and hence, the -CH_2NH_2 moiety is axial.

Pharmacological studies (Villaseñor *et al.*, 1995) indicated that the hexane extract of *M. cordifolia* exhibits a central nervous system-depressant effect as evidenced by a decrease in motor activity and ataxia which would also account for its analgesic activity.

Experimental

General

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and 2D NMR studies were conducted in 30% CD_3OH/$CDCl_3$ with TMS as internal standard. Normal phase liquid chromatography (NPVLC) and TLC were done on silica gel using gradient ratios of hexane, EtOAc/hexane, and EtOH/EtOAc. Detection included I_2, vanillin-H_2SO_4 spray followed by heating, and UV light. All bioassay data are presented as mean ± standard deviation (S.D.) and were analyzed statistically using Kruskal Wallis one-way analysis of variance by ranks (Walpole, 1997).

Extraction and isolation

M. cordifolia leaves were purchased from the National Research Council of the Philippines (NRCP) in Bicutan, Taguig, MM. Approx. 10 kg of leaves were air-dried and then homogenized in ethanol. The ethanol extract was filtered and then concentrated *in vacuo*. The ethanolic fraction was partitioned between hexane and water. The aqueous layer was further extracted with $CHCl_3$ (6×) and then with EtOAc (6×). The hexane, $CHCl_3$, and EtOAc portions were concentrated *in vacuo* to give fractions FB, FC, and FD, respectively.

Approx. 10 g of the FB fraction were subjected to NPVLC and eluted with hexane, 10% gradient ratios of EtOAc in hexane, EtOAc, 10% gradient ratios of EtOH in EtOAc. The column fractions were combined according to analytical TLC results into 10 fractions, FB1 to FB10.

NPVLC of fraction FB6 (2.7527 g) with 4% gradient ratios of EtOAc in hexane (40% to 80%), 90% EtOAc in hexane, EtOAc, 50% EtOH in EtOAc, and EtOH resulted in 7 fractions, FB6A to FB6G. Fraction FB6F was repeatedly subjected to NPVLC using 4% gradient ratios of 40% to 80% EtOAc in hexane to afford a white crystalline solid labeled as FB6Fc and later renamed as menthalactone.

Menthalactone: White crystals; m.p. (uncorr) 135.1 ºC (with decomposition). – FTIR (KBr): ν = 3332 and 3221 (-NH_2), 2920, 2851, 1756 (C=O), 1623 (C=C), 1543 (N-H bend), 1468, 1362, 1278 (C-N str.), 1121 (C-O str.), 871, 723 cm^{-1}. – ^{13}C NMR (100 MHz, 30% CD_3OH/$CDCl_3$) (DEPT) [C-H HETCORR]: δ = 13.3 (-CH_3) [0.60], 22.2 (-CH_2) [1.01], 24.7 (-CH_2) [1.01', 1.17], 25.5 (-CH_2) [1.01', 1.17], 28.8 (-CH_2) [1.01], 28.9 (-CH_2) [1.01], 29.06 (-CH_2) [1.01], 29.10 (-CH_2) [1.01], 29.14 (-CH_2) [1.01], 29.16 (-CH_2) [1.01], 29.20 (-CH_2) [1.01], 31.5 (-CH_2) [1.01], 32.0 (-CH_2) [1.71, 1.76], 32.2 (-CH_2) [1.71, 1.76], 34.0 (-CH_2) [1.34, 1.53], 51.2 (-CH) [3.86], 60.6 (-CH_2) [3.52], 71.6 (-CH) [3.79], 71.8 (-CH), 75.1 (-CH) [3.29], 129.5 (=CH) [5.15], 130.3 (=CH), 175.5. – ^{1}H NMR (400 MHz, 30% CD_3OH/$CDCl_3$) [COSY] {HMBC}: δ = 0.60 (3H, t, J = 6.7 Hz) [1.01] {22.2 (^2J), 31.5 (^3J) }, 1.01 (br s) [0.60, 1.71] and 1.01' [3.79, 1.17], 1.17 (d, J = 7.1 Hz) [1.01'] {29 cluster, 51.2 (^3J)}, 1.33 (m) [1.53] {24.7 (^3J), 51.2, 71.5 (^2J)}, 1.53 (m) [1.34], 1.71 [1.01, 5.15] {29 cluster, 129.5 (^2J), 130.3 (^2J)}, 1.76 [1.71], 3.29 (1H, d, J = 4.36 Hz) [3.86, 1.17] {71.5}, 3.49 (1H, dd, J = 11.5 Hz and 4 Hz) [3.55, 3.86] {51.2 (^2J), 75.1 (^3J)}, 3.55 (1H, dd, J = 11.5 Hz and 4 Hz) [3.49, 3.86] {51.2 (^2J), 75.1 (^3J) }, 3.79 (dd, J = 8 Hz and 3.6 Hz) [1.34, 1.01'] {34.1 (^2J), 175.5}, 3.86 (1H, q, J = 4 Hz) [3.55, 3.29], 5.15 (1H, br s) [1.71] {31.99, 129.5 (^2J)}.

Analgesic bioassay: Acetic acid-induced writhing test

Swiss Webster albino mice, weighing 20–25 g, were used as test animals. Five mice were used per test sample. Approx. 30 min after oral administration of the test solutions, 0.7% acetic acid was injected intraperitoneally (0.01 ml/g mouse). The number of squirms for each mouse was then counted for 15 min beginning from 5 min after acetic acid injection (Villaseñor *et al*., 2002).

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