Mentha cordifolia, 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, piseoetione, 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 $\beta$-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 $-\text{CH}_3$, fifteen $-\text{CH}_2$, six $-\text{CH}$ and one quaternary...
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 13C NMR signals were therefore characteristic of an amine and three ether linkages from $\delta_C$ 60.6 to $\delta_C$ 75.1, $\delta_H$ 3.28 to $\delta_H$ 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 $\delta_C$ 130.3, $\delta_C$ 129.5, $\delta_H$ 5.15, and a C=C stretching vibration at 1623 cm$^{-1}$; the allylic carbon atoms at $\delta_C$ 32.2, $\delta_C$ 32.0, $\delta_H$ 1.76 and $\delta_H$ 1.71; and the aliphatic carbon atoms at $\delta_C$ 29 and $\delta_H$ 1.01 clusters. The signal at $\delta$ 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 $\delta_H$ 3.55 and 3.49, the geminal protons and $\delta_H$ 3.86, $\delta_H$ 3.86 and $\delta_H$ 3.29, and $\delta_H$ 3.29 and $\delta_H$ 1.17 gave fragment 1. HMBC cross peaks between the geminal protons and $\delta_C$ 51.2 (2$^J$) and $\delta_C$ 75.1 (3$^J$) further supported the structure of fragment 1. The structure of fragment 2 was derived from COSY cross peaks between the geminal protons at $\delta_H$ 1.33 and 1.53, and $\delta_H$ 1.33 and $\delta_H$ 3.79 together with HMBC cross peaks between $\delta_H$ 1.33 and $\delta_C$ 24.7 (2$^J$) and $\delta_C$ 71.6 (3$^J$).

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 $\delta_C$ 13.3 and $\delta_C$ 22.2 (2$^J$ $\delta_C$ 22.2, $\delta_H$ 0.60); $\delta_C$ 22.2 and $\delta_C$ 31.5 (3$^J$ $\delta_C$ 31.5, $\delta_H$ 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 $\delta_C$ 51.2 and $\delta_H$ 1.33 (2$^J$), $\delta_C$ 75.1 and $\delta_H$ 3.79 (3$^J$), $\delta_C$ 175.5 and $\delta_H$ 1.01 cluster (2$^J$), and $\delta_C$ 29 cluster and $\delta_H$ 1.17 (2$^J$) gave the structure of menthalactone (Fig. 2). This structure would also explain the multiplicities of the pro-

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

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

Positive control, HOAc + mefenamic acid; solvent controls, HOAc + corn oil and HOAc + 2% carboxymethylcellulose (CMC) in normal saline solution (NSS).
Menthalactone: White crystals; m.p. (uncorr) 135.1 °C (with decomposition). – FTIR (KBr): ν = 3332 and 3221 (-NH₂), 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. – ¹³C NMR (100 MHz, 30% CD₃OH/CDCl₃) (DEPT) [C-H HETCORR]: δ = 13.3 (-CH₃) [0.60], 22.2 (-CH₂) [1.01', 1.17], 24.7 (-CH₂) [1.01', 1.17], 25.5 (-CH₂) [1.01', 1.17], 28.8 (-CH₂) [1.01], 28.9 (-CH₂) [1.01], 29.06 (-CH₂) [1.01], 29.10 (-CH₂) [1.01], 29.14 (-CH₂) [1.01], 29.16 (-CH₂) [1.01], 29.20 (-CH₂) [1.01], 31.5 (-CH₂) [1.01], 32.0 (-CH₂) [1.71, 1.76], 32.2 (-CH₂) [1.71, 1.76], 34.0 (-CH₂) [1.34, 1.53], 51.2 (-CH₃) [3.86], 60.6 (-CH₂) [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. – ¹H NMR (400 MHz, 30% CD₃OH/CDCl₃) [COSY] [HMBC]: δ = 0.60 (3H, t, J = 6.7 Hz) [1.01] [22.2 (J), 31.5 (J)], 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 (J)], 1.33 (m) [1.53] [24.7 (J), 51.2, 71.5 (J)], 1.53 (m) [1.34, 1.71 [1.01, 5.15] [29 cluster, 129.5 (J), 130.3 (J)], 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 (J), 75.1 (J)], 3.55 (1H, dd, J = 11.5 Hz and 4 Hz) [3.49, 3.86] [51.2 (J), 75.1 (J)], 3.79 (dd, J = 8 Hz and 3.6 Hz) [1.34, 1.01'] [34.1 (J), 175.5], 3.86 (1H, q, J = 4 Hz) [3.55, 3.29], 5.15 (1H, br s) [1.71] [31.99, 129.5 (J)].
**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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