Eco-friendly Pest Management Using Monoterpenoids II — Antifungal Efficacy of Menthol Derivatives

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Ether and ester derivatives of menthol, [5-methyl-2-(1-methylethyl) cyclohexanol], a natural monoterpene are synthesized and evaluated for antifungal potency against *Aspergillus niger*, *Aspergillus oryzae*, *Fusarium oxysporum*, and *Alternaria alternata* to study structure activity relationships. Ethylene dimer ether derivative is observed to be the most potent against all fungal species. Benzyl ether derivative is found to be more active than allyl ether derivative. In simple esters, cinnamate derivative is more activity enhancing against *Aspergillus oryzae*, *Alternaria alternata* and *Fusarium oxysporum*, while benzoate is better against *Aspergillus niger*. Among diesters, malonate is more active against all the test fungi except *F. oxysporum*. Against *A. alternata*, coupling of monoterpene molecules through diester bridge containing one methylene group (malonate) is more active. Among simple ethers and esters, respectively, menthyl benzyl ether and menthyl cinnamate reflects the highest activity against *F. oxysporum*.

Keywords: Eco-friendly pest management, Pest management, Monoterpenoids, Antifungal efficacy, Menthol derivatives

Introduction

Presently, in agriculture, synthetic pesticides are utilized more commonly than botanical pesticides. Previously used several synthetic pesticides were proven problematic due to their persistence in the environment and toxicity to non-target organisms. Certain fungal pathogens have developed resistance to some of the commonly used effective fungicides such as, benomyl and 1-prodione. Concerns about the presence of synthetic chemicals in food supply and in environment has resulted in the withdrawal of number of important fungicides from the market. The most common fungicides such as, captan, benomyl, and 1-prodione have already been banned from some or all post harvest uses in Canada and the US. The future pest management program for sustainable agriculture and public health emphasizes on the plant products and their derivatives in addition to safer chemical pest control agents and biopesticides. Systematic derivatization of natural products, such as, monoterpeneoids, based on structure-activity relationships, warrants evaluation, both as a source and a model for new commercial pest management agents having natural base.

Experimental Methods

Synthesis of Menthol Derivatives

Menthol, alcohols, alkyl halides, acids, and acyl halides were of synthetic grade commercial products and were procured from s d Fine Chem Ltd, Boiser-India. Other acid chlorides were prepared in the laboratory. The synthetic derivatives (Figure 1) were purified by preparative TLC and column chromatographic techniques. All purified ethers and esters were colourless to pale brown in colour and slightly viscous liquids.

Ether Derivatives

Menthyl ethers were synthesized by reacting menthol with various alcohols in the presence of dehydrating agent, i.e., conc. H_2SO_4. Benzene was used as solvent for the reactions with propyl, isopropyl, butyl and benzyl alcohols. A mixture of menthol (0.05 M) and alcohol (methanol or ethanol, 40 mL) was taken in round bottom flask, fitted with reflux condenser. Conc. H_2SO_4 (1.5 mL) was added...
slowly dropwise through condenser with stirring or shaking. The reaction mixture was then refluxed for 4-5 h [for the reaction with other higher alcohols (0.05 M), benzene was used as solvent (40 mL)]. After reflux the reaction contents were cooled and 40 mL of water was added. The oily product was then extracted with ether (3 x 30 mL). The ether extract was washed with water and dried over anhydrous sodium sulphate. The removal of solvent yielded crude menthyl ether in 84-90 per cent yield.

**Dimer Ether Derivatives**

The dimer ether (dimethyl ethylene ether) was synthesized by reacting menthol with 1,2-ethanediol. To a mixture of menthol (0.05 M) and 1,2-ethanediol (0.025 M) in benzene (40 mL), conc. H$_2$SO$_4$ (2 mL) was added carefully dropwise with constant stirring and the mixture was then refluxed for 3-4 h. After reflux the reaction contents were cooled and 40 mL water was added. The oily products were worked up similar ether derivatives. The ether extract was washed. The crude dimethyl ethylene ether was obtained in 78 per cent yield.

**Ester Derivatives**

Methyl esters were synthesized by reacting menthol with various acids or acid chlorides. To a mixture of menthol (0.05 M) and acid [acetic, benzoic and cinnamic acid, (0.05 M)] in benzene (40 mL), conc. H$_2$SO$_4$ (1.5 mL) was added dropwise and the mixture was refluxed for 3-4 h and then cooled to room temperature. In another route, menthol (0.05 M) in benzene (40 mL) was slowly reacted with acid chlorides (0.06 M) at 5-10 °C and then reaction mixture was stirred for about 3 h at room temperature. After adding 40 mL of water, the products were worked up in usual way, which gave crude methyl esters, in 85-92 per cent yields.

**Dimer Ester Derivatives**

Menthyl diesters were prepared by reacting menthol with various dicarboxylic acids. To a mixture of menthol (0.05 M) and dicarboxylic acid [malonic, succinic, glutaric and adipic (0.025 M)] in benzene (40 mL), conc. H$_2$SO$_4$ (2 mL) was added drop-wise and mixture was refluxed for 3-5 h and then cooled to room temperature. In another route, menthol (0.05 M) in benzene (40 mL) was slowly reacted with acid dichlorides [malonyl, succinyl, glutaryl and adipyl (0.03 M)] at 5-10 °C and then reaction mixture was stirred for about 3 h at room temperature. The products were worked up in usual way, which gave methyl diesters in 85-90 per cent yields.

**Bioassay of Menthol Derivatives**

The fungal strains were subcultured on Sabouraud’s broth and filter paper disc agar method was used for the evaluation of antifungal activity. To each petriplate, 20 mL of sterilized medium was added. After the agar had set, inoculum (suspension culture) was added to each petriplate and spread thoroughly by rotatory motion of the plate. Sterilized Whatman No. 1 filter paper discs (6 mm diam) were thoroughly moistened with 20 mg/mL solution of the menthol derivatives in cyclohexane, were placed on the surface of seeded petriplates and paper disc moistened with cyclohexane as a control. The plates were incubated at 27 °C for 2-4 d. The relative susceptibility of the fungi to the synthesized derivatives was demonstrated by a clear zone of inhibition around the paper disc. The fungicidal potency is proportional to the diam (in mm) of the zone of inhibition. The experiments were performed in duplicate and the average of the measured zones of inhibition was considered and are summarized in Table 1.

Per cent change in antifungal activity = 100 [(D-P)/P]

where, $D$ = Zone of inhibition for derivatives, $P$ = Zone of inhibition for parent compound (menthol).

The results of percentage change in antifungal activity are summarized in Table 1.

**Results and Discussion**

Figure 1 presents the antifungal activity of menthol and its derivatives against the microbial fungal Aspergillus niger, Aspergillus oryzae, Alternaria alternata, and Fusarium oxysporum. Figure 2 illustrates the influence of derivatization in the form of per cent change in antifungal activity in comparison with parent monoterpeneoid, menthol. In general, against all test of fungi species the structural modifications of menthol were found to be better and effective.

**Aspergillus niger**

Among all derivatives, ethylene diether (III-h) showed the highest antifungal activity against A. niger. In others, it was followed in descending order by benzyl (II-f), propyl (II-c), allyl (II-g), isopropyl.
(II-d), methyl (II-a), butyl (II-e), and ethyl (II-b) ethers. Benzoate (IV-j) showed higher activity in esters was in descending order by acetate (IV-i) and cinnamate (IV-k). Among diesters, malonate (V-m), glutarate (V-n), succinate (V-o) and adipate (V-o) was the activity sequence in descending order. Simple esters (IV-I to IV-k) were found more active than dimmer esters (V-I to V-o).

Figure 3 indicates that coupling of methyl groups with the ethylene diether bridge (III-h) reflected the highest enhancement in antifungal activity against\textit{A. niger}. In ethers, introduction of \textit{Aspergillus Oryzae}

### Table I — Antifungal activities of menthol and its derivatives

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>\textit{Aspergillus niger}</th>
<th>\textit{Aspergillus oryzae}</th>
<th>\textit{Alternaria alternata}</th>
<th>\textit{Fusarium oxysporum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>I</td>
<td>11.3</td>
<td>-</td>
<td>12.8</td>
<td>-</td>
</tr>
<tr>
<td>Ethers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-a</td>
<td>12.6</td>
<td>11.5</td>
<td>12.0</td>
<td>-6.3</td>
</tr>
<tr>
<td>II-b</td>
<td>11.9</td>
<td>5.3</td>
<td>12.5</td>
<td>-23</td>
</tr>
<tr>
<td>II-c</td>
<td>17.9</td>
<td>58.4</td>
<td>13.8</td>
<td>7.8</td>
</tr>
<tr>
<td>II-d</td>
<td>13.5</td>
<td>19.5</td>
<td>13.0</td>
<td>1.6</td>
</tr>
<tr>
<td>II-e</td>
<td>12.0</td>
<td>6.2</td>
<td>11.0</td>
<td>-14.1</td>
</tr>
<tr>
<td>II-f</td>
<td>21.0</td>
<td>85.8</td>
<td>23.0</td>
<td>79.7</td>
</tr>
<tr>
<td>II-g</td>
<td>17.0</td>
<td>50.4</td>
<td>21.0</td>
<td>64.1</td>
</tr>
<tr>
<td>Dimer ethers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-h</td>
<td>25.0</td>
<td>121.0</td>
<td>24.0</td>
<td>91.4</td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-i</td>
<td>18.0</td>
<td>59.3</td>
<td>18.0</td>
<td>44.5</td>
</tr>
<tr>
<td>IV-j</td>
<td>20.0</td>
<td>77.9</td>
<td>19.0</td>
<td>52.3</td>
</tr>
<tr>
<td>IV-k</td>
<td>17.8</td>
<td>57.5</td>
<td>20.0</td>
<td>60.2</td>
</tr>
<tr>
<td>Dimer esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-l</td>
<td>17.0</td>
<td>50.4</td>
<td>18.0</td>
<td>40.6</td>
</tr>
<tr>
<td>V-m</td>
<td>14.5</td>
<td>28.3</td>
<td>12.0</td>
<td>-6.25</td>
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<tr>
<td>V-n</td>
<td>15.7</td>
<td>38.9</td>
<td>15.5</td>
<td>21.1</td>
</tr>
<tr>
<td>V-o</td>
<td>13.0</td>
<td>15.0</td>
<td>16.5</td>
<td>28.9</td>
</tr>
<tr>
<td>A — Zone of inhibition (diam mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B — Per cent change</td>
<td></td>
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</table>

(II-e). In esters, cinnamate (IV-k) exhibited higher activity followed by benzoate (III-j), acetate (IV-i), malonate (V-I), adipate (V-o), glutarate (IV-n) and succinate (IV-m). Figure 3 indicates that coupling of methyl groups with ethylene diether bridge (III-h) resulted in maximum increase. Although most of the structural modifications resulted in increase in activity of parent menthol, some modifications resulted in decrease of antifungal potency against \textit{A. oryzae}. Derivatization of menthol to simple methyl ether (II-a) led to decrease in activity of menthol. The lengthening of ether side chain by introduction of first methylene group (II-b) slightly elevated the activity but remained less active than menthol (I). However, an introduction of second methylene group (II-c) resulted in enhancing activity more than parent compound, again an introduction of third methylene group (II-d) and the branched isopropyl group (II-d) found to be effective. Benzene ring (II-f) and double bond (II-g) in ether side chain reflected activity-enhancing effect. In esters, benzene ring (IV-j) and double bond along with benzene ring (IV-k) showed an increase in antifungal activity. In dimmer esters, increase in length of diester bridge by introduction of second methylene group (V-m) caused fall in activity, however, an introduction of third (V-n) and fourth
I Menthol

![Menthol structure]

Parent monoterpenoid (menthol) \([M], R = H\)

II Ether

\[
M-O-R
\]

(a) Methyl ether \((R = CH_3)\)
(b) Ethyl ether \((R = CH_3-CH_2)\)
(c) Propyl ether \((R = CH_3-CH_2-CH_2)\)
(d) Isopropyl ether \((R = (CH_3)2CH)\)
(e) Butyl ether \((R = CH_3-CH_2-CH_2-CH_2)\)
(f) Benzyl ether \((R = H_3C6-CH_2)\)
(g) Allyl ether \((R = H_2C-CH = CH)\)

III Dimer ethers

\[
M-O(CH_2)n-O-M
\]

(h) Ethylene dimer ether \((n = 2)\)

IV Esters

\[
M-O-C(O)-R
\]

(i) Acetate \((R = CH_3)\)
(j) Benzoate \((R = H_3C6)\)
(k) Cinnamate \((R = H_3C6-CH = CH)\)

V Dimer esters

\[
M-O-C(O)(CH_2)n-C(O)O-M
\]

(l) Malonate \((n = 1)\)
(m) Succinate \((n = 2)\)
(n) Glutarate \((n = 3)\)
(o) Adipate \((n = 4)\)

Figure 1 — Ether and ester derivatives of menthol

Methylene group \((V-o)\) showed enhancing effect on antifungal activity against *A. oryzae*.

**Alternaria Alternata**

Among all derivatives, ethylene ether \((III-h)\) showed the highest antifungal potency against *A. alternata*. In ethers, it was followed in descending order by benzyl \((II-f)\), allyl \((II-g)\), propyl \((II-c)\), isopropyl \((II-d)\), methyl \((II-a)\), ethyl \((II-b)\) and butyl \((II-e)\) ethers. In esters, cinnamate \((IV-k)\) showed higher activity, followed descending order for acetate \((IV-i)\), malonate \((V-l)\), adipate \((V-o)\), glutarate \((V-n)\), benzoate \((IV-j)\), and succinate \((V-m)\). Malonate \((V-l)\) and acetate \((IV-i)\) exhibited similar activity. Figure 2 shows that the highest increase was seen due to coupling of methyl groups with ethylene diether bridge \((III-h)\). In ethers, although methyl ether \((II-a)\) showed increase in activity over parent menthol, every further introduction of methylene group in ether side chain, except second methylene group \((II-c)\), showed negative effect on activity. Normal propyl ether side chain \((II-c)\) enhanced activity must more than branched isopropyl \((II-d)\). An introduction of benzene in ether side chain enhanced the activity
(II-f) and also the double bond exhibited the enhancement (II-g). In esters, contrary to the ethers, introduction of benzene ring in ester side chain (IV-j) strongly reduced the activity to the level below the parent (I). However, benzene ring along with the double bond (IV-k) again enhanced the antifungal potency to a greater extent. In dimmer esters, although bridging of two methyl groups with malonate ester bridge (V-I) resulted in increase the lengthening. Lengthening of diester bridge by introduction of second methylene group (V-n) resulted in fall of activity, less than parent (I). Further introduction of third and fourth methylene (V-n and V-o) showed enhancement of antifungal potency against A. alternata.

**Fusarium Oxysporum**

Among all derivatives, ethylene dimmer ether (III-h) showed the highest antifungal activity against F. oxysporum. In ethers, it followed descending order by benzyl (II-f), allyl (II-g), propyl (II-c), ethyl (II-b), isopropyl (II-d), butyl (II-e) and methyl (II-a).
ethers. Cinnamate (IV-k) showed higher activity among esters, followed descending order by acetate (IV-i), benzoate (IV-j), glutarate (V-n), adipate (V-o), succinate (V-m) and malonate (V-i). Except benzyl (II-f), all simple ethers (II, a-g) and all dimmer esters (V, l-o), were less active than simple esters (IV, i-k). Figure 3 shows that coupling of methyl groups with ethylene ether bridge (III-h) resulted in the highest increase in activity. In ethers, an introduction of methylene group in ether side chain showed enhancing effect up to normal propyl ether, however, further introduction reversed the action. Normal propyl ether (II-e) side chain showed more enhancements in activity than the branched isopropyl (II-d). An introduction of benzene ring ether side chain (II-f) and double bond (II-g) showed positive effect on activity. Introduction of benzene along with double bond (IV-k) was found to be more enhancing than introduction of benzene (IV-j) in the ester side chain. In dimer esters, lengthening of diester bridge by introduction of methylene groups exhibited positive effect up to glutarate (V, l-n) however, an introduction of fourth methylene group (V-o) showed slight decrease in antifungal potency against F. oxysporum.

The enhanced antifungal activities shown by most of the derivatives of menthol indicate that optimal structure can be elucidated through bioreational design of the derivatives. Through the establishment of the structure-activity relationships, a moderately or nontoxic new group of antifungal agents is achievable. The main advantages of using monoterpenoids in pest management are their natural occurrence and availability in abundance, their easy derivatization or structural modification, their potent bioactivity and biodegradability and thus ecofriendlyness. The Integrated Pest Management (IPM) is now an important strategy in agriculture and public health pest management. The chemical compounds such as, monoterpenoids that have better and wide pest management activity, thus may play important role in IPM. The growing consciousness of pesticide safety among consumers and tougher regulatory policies are widening the opportunity for moderately toxic, environmentally safer, effective natural compounds such as, monoterpenoids and their derivatives.

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