Synthesis and Antibacterial Activity of α-Methylenecamphor and Isophorone Derivatives

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Abstract: α-Methylenecamphor and α-methylenesisorphorone derivatives were synthesized and their antibacterial activity on Escherichia coli was examined. α-Methylenated camphor and isophorone derivatives showed stronger antibacterial activity than the compounds before α-methylenation, as anticipated. Data of the electronic parameters of the absolute electronegativity (χ), the quantity of electron transfer (ΔN) and the stabilization energy (ΔE) with cysteine strongly supported this finding.


Key words: α-methylenecamphor, α-methylenesisorphorone, α-methylenesisorphorone oxide, antibacterial activity, absolute electronegativity (χ)

1 Introduction

Camphor (1,7,7-Trimethylbicyclo[2,2,1]heptane-2-one) is a constituent of camphor tree (Cinnamonum camphor L.) and due to its typical odor, is known as repellent.

Isophorone (5,5-dimethyl-3-methyl-2-cyclohexenone) has a similar structure to camphor but less strong camphor like odor.

α-Methylenated compounds of both are expected to have stronger antibacterial activity, since the newly formed s-cis vinylketones is contributive to the activity. α-Methylenation of camphor 1 and isophorone 4 as well as isophorone oxide 7 was evaluated for the antibacterial activity. Synthetic route for those is illustrated in Fig. 1.

The absolute electronegativity (χ), the absolute hardness (η) as well as the quantity of electron transfer (ΔN) and the stabilization energy (ΔE) calculated from the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were discussed in relation to the antibacterial activity (1).

2 Experimental

2.1 Synthesis

GC-MS spectra were taken on a Hewlett Packard 5890 Q-Pole spectrometer at 70 eV and major peaks were indicated as m/z (methylsilicone gum, 80-150°C/5°C rate, flow rate He 45 mL/min.) NMR spectra were recorded on a JEOL JNM-MY60 and IR spectra were obtained on a Shimazu IR 400 spectrometer.

α-Methylenecamphor (3)

To a stirred NaH 1.7 g (0.07 mol) in 50 mL ether, was dropped a mixture of (+)-camphor 10.6 g (0.07 mol) and ethyl oxalate 12.3 g (0.084 mol) in 30 mL ethanol at room temperature.

Stirring was continued for 8 hours and left over night. The semi-solid precipitate (ferric chloride test positive) was filtered with celite (5 g) and washed with ether.

The precipitate (Na salt of camphor-ethyl oxalate, 2) with celite was suspended in 100 mL of 10% Na2CO3 and 30 mL THF and treated with 25 mL 37% formaline at ice-cooled temperature. After stirring for 2 hours at room temperature, the mixture was filtered and extract-ed several times with ether. The ether extracts was

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washed with brine and dried over Na₂SO₄.
Evaporation of ether gave 0.5 g of yellowish oil, which
was further purified by silicagel chromatography (ether : hexane = 1:1) to give a colorless oil of 3.

**Na salt of Isophorone-oxide (5)**

To a stirred NaN₃ 1.7 g (0.07 mol) in 50 mL ether,
dropped a mixture of isophorone 9.7 g (0.07 mol)
and ethyl oxalate 12.3 g (0.084 mol) in 30 mL ethanol
at room temperature.

Stirring was continued for 2 hours and left over night. The obtained yellow precipitate (ferric chloride test positive) was filtered and washed with ether to give 5.

Analysis was made after acidification of 5. mp 83-5°C

**α-Methyleneisophorone (6)**

The compound 5 (13 g, 0.05 mol) was dissolved in 100 mL warm 10% Na₂CO₃ and treated with 20 mL 37% formalin at room temperature. After stirring for 1 hour, the mixture was extracted with ether and the extracts was washed with brine and dried over Na₂SO₄.

Evaporation of ether gave 0.5 g of amber oil, which was further purified by silicagel chromatography (ether : hexane = 1:1) to give a colorless oil of 6.

**Isophorone oxide (7)**

This compound was prepared by the treatment of isophorone 4 and alkaline H₂O₂ in methanol (2). bp 90-95°C/13mm

**α-Methyleneisophorone oxide (9)**
To a stirred NaH 1.7 g (0.07 mol) in 50 mL of ether, was dropped a mixture of isophorone oxide 7 10.8 g (0.07 mol) and ethyl oxalate 12.3 g (0.084 mol) in 10 mL ethanol at room temperature. Stirring was continued for 2 hours at room temperature and left overnight. The brown colored mixture was poured into 50 mL water. The separated ether layer was extracted with 10% Na₂CO₃ (20 mL × 2) and the extract was combined with the formerly separated water layer. The combined aqueous extract was treated with 20 mL 37% formalin at room temperature. After stirring for 2 hour, the mixture was extracted with ether and the extract was washed with brine and dried over Na₂SO₄. Evaporation of ether gave 0.5 g of amber oil, which was further purified by silicagel chromatography (ether : hexane = 1 : 2) to give a colorless oil of 9.

MS m/z (rel. int.): 166 (M⁺, 10), 138 (3), 123 (7), 109 (26), 95 (100), 81 (47) IR νₜₐₙ (film)cm⁻¹: 2950, 1700, 1685, 1580, 1420, 1400, 1370, 1310, 1280, 1245 ¹H-NMR (CDCl₃): 0.88 (3H, s, -CH₃), 1.0 (3H, s, -CH₃), 1.38 (3H, s, at epoxy-CH₂), 1.80-1.95 (2H, m, -CH₂-), 3.0 (H, s, at epoxy-H), 4.50 (H, s, =CH), 5.35 (H, s, =CH)

2-2 Measurement of Antibacterial Activity
Minimum inhibitory concentration (MIC) of the compounds on Escherichia coli (IFO 3301) was determined by the previous method (1).

2-3 Computer Calculation
The χ, η as well as ΔN and ΔE were similarly calculated by the previous method (1).

3 Results and Discussion
The synthesis of α-methylenecamphor did not proceed smoothly compared with γ-alkyl-γ-butyrolactones (3). α-Ethoxalyl formation by ethyl oxalate and camphor takes longer compared with γ-alkyl-γ-butyrolactones and following α-methylation with formalin, nearly 50% of starting material was recovered. α-Formyl formation of camphor by ethyl formate did not

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Values of χ, η, ΔN and ΔE of α-Methyleneamphor and Isophorone Derivatives and MIC of those on Escherichia coli (IFO3301).</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Camphor (1)</td>
<td>log(MIC)</td>
</tr>
<tr>
<td>α-Methyleneamphor (3)</td>
<td>2.65</td>
</tr>
<tr>
<td>Isophorone (4)</td>
<td>2.88</td>
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<tr>
<td>α-Methyleneisophorone (6)</td>
<td>2.18</td>
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<tr>
<td>Isophorone oxide (7)</td>
<td>3.24</td>
</tr>
<tr>
<td>α-Methyleneisophorone oxide (9)</td>
<td>1.85</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-9.2230</td>
</tr>
</tbody>
</table>

HOMO, LUMO was calculated at EF and AM1 level (eV)  * at cystine

Fig. 2 Relationship between log (MIC) on E.coli and χ, ΔN, ΔE of Camphor Related Compounds.
occurred unexpectedly. One reason for the poor reactivity and low yield of α-methylene camphor may have been caused by the steric hindrance of gem-dimethyl group. In case of isophorone, α-ethoxalyl formation occurred smoothly and Na salt of isophorone oxalate 5 was easily separated from the reaction mixture. Following α-methylation with formaline, nearly 30% of the starting material was recovered. With isophorone oxide, similar in reactivity was observed as in the case of camphor.

The calculation of HOMO and LUMO of those compounds was similarly conducted by the previous method (1) and the χ, η, ΔN and ΔE (at cystine) as well as MIC on E.coli were shown in Table 1.

The χ, ΔN and ΔE were well correlated with log (MIC) and 9 showed the strongest antibacterial activity as shown in Fig.2. The compound 4 having small absolute value in LUMO and rather large value in log (MIC) may be a reason for deviation from the correlation line in Fig. 2. The compounds having exo-methylene group in ketone of α-position are expected to form Michael adducts with amino acid residues in enzyme or protein and the antibacterial activity of the compounds in this case is controlled by χ rather than η as described in 3-methyl-2-cyclopentenones (1). Camphor and isophorone derivatives also suit above case.

Reaction with chloride ion and isophorone oxide 7 did not occurred unexpectedly (1). As expected formerly, α-methylenated camphor and isophorone showed stronger antibacterial activities than before α-methyleneination but the yield of those was unsatisfactorily poor.

References