An *In-Silico* Investigation of Menthol Metabolism

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ABSTRACT: Prevalence of mentholated products for consumption has brought great importance to studies on menthol's metabolic pathways to ensure safety, design more potent derivatives, and identify therapeutic benefits. Proposed pathways of (-)-menthol metabolism based on metabolites found experimentally in previous works by Yamaguchi, Caldwell & Farmer, Madyastha & Srivatsan and Hiki *et al* were not in agreement. This *in silico* approach is based on the three *in vivo* studies and aims to resolve the discrepancies. Reactions in the pathways are conjugation with a glucuronic acid /sulfate group, oxidation to alcohol, aldehyde & carboxylic acid, and formation of a four/five-membered ring. Gas-phase structures, the standard Gibbs energies and SMD solvation energies at B3LYP/6-311++G(d,p) level were obtained for 102 compounds in the pathways. This study provides a more complete picture of menthol metabolism by combining information from three experimental studies and filling missing links in pathways previously proposed.

KEY WORDS: Menthol, metabolism, DFT

Introduction

(-)-Menthol or 1*S*,3*R*,4*S*-menthol is a naturally occurring compound found in plants of the *Mentha* genus commonly known as mint. It is the is the most abundant in nature among the 8 possible stereoisomers, comprising of at least 50% of peppermint (*Mentha piperita*) oil and 70-80% of corn mint (*Mentha arvensis*) oil (1). (-)-Menthol, commonly referred to as menthol, has characteristic minty smell and flavor and exerts a cooling sensation when applied to the skin and mucosal membranes (2). Other isomers differ slightly in odor and physical characteristics and do not possess the cooling action (3, 4).

Menthol finds a wide range of applications from personal care products, medications, and confectionery to pesticides and cigarettes. The popularity of the compound as a flavoring agent ranks third most important after vanilla and citrus (5), and the annual production of menthol in India alone is in excess of 200 thousand metric tons.(6) Due to its popularity, mentholated products can be readily purchased as prescribed or over-the-counter medications as alleviators of common cold and respiratory conditions (7), inhibitors of growth of foodborne pathogens (8), and analgesics (9). The cooling sensation and analgesic properties is a result of the activation of transient receptor potential melastatin-8 (TRPM8), an ion channel selective to temperature, voltage, and menthol (10). Chemical derivatives of menthol with enhanced activity have been successfully

synthesized (11). In addition, health effects of mentholated cigarettes is of great concern, not only because the improved taste may facilitate initiation or inhibit quitting but also because metabolism of menthol via this route of administration has not been well studied (12, 13).

For such a well-known compound, relatively few studies have been conducted on toxicological effects of menthol which supports the generally accepted belief that it is safe and nontoxic. No signs of toxicity were observed in rats exposed to continuous doses of up to 800 mg/kg/day for 28 days (5), and chronic exposure to high concentrations of menthol vapor was not reported to have toxic effects in rats (14). *In vitro* studies on various animal tissues report deterioration of biological membranes at concentrations 0.32-0.76 mM (15). The recommended daily intake for humans of 0-0.2 mg/kg proposed by the WHO (16) is not supported by any toxicological data but was set to err on the side of safety knowing that higher doses taken may not have produced adverse side effects.

To the best of our knowledge, three *in vivo* studies by Yamaguchi, Caldwell & Farmer (17), Madyastha & Srivatsan (18) and Hiki *et al* (19) have identified metabolites of menthol in humans and animals. Metabolites were identified by GC/MS from the urinary and biliary metabolites in rats treated with oral doses of 500 (17) and 800 (18) mg menthol/kg of body weight. Over the course of 48 hours, a majority of the doses were excreted in the urine and feces. A more recent randomized, double-blind, placebo-controlled study in human by Hiki *et al* (19) was conducted by directly spraying 0.8% (-)-menthol solution at escalating doses of 10-40 mL onto the gastric mucosa. Blood and urine of the participants were sampled over a 24-hour period and analyzed with GC/MS for menthol metabolites. In total, 72 metabolites were identified or proposed in this human study, compared to 9 and 18 metabolites in the previous two experiments. (See supplementary information for the full list.) *In vitro* investigation of metabolism in human liver microsomes revealed that the same key reactions in the metabolic pathway in rats occur in the microsomes (20, 21).

FIGURE 1 Metabolic pathway of Menthol in rats and in human, an adaptation from Yamaguchi, Caldwell, & Farmer (17), Madyastha & Sirvastan (18) and Hiki *et al* (19). Red, Green, and Blue texts indicate that menthol metabolites were found in both rats and human, only in rats, and only in human respectively. Gray and Black texts indicate menthol metabolites proposed by previous experiments and by this paper respectively. Arrows to the right and to above indicate oxidation reactions +1/2 O_2 and –H_2 respectively. Downward arrows indicate conjugation with a sulfate group. Dashed arrows indicate ring to ring reactions. Diagonal arrows toward top left indicate dehydration reaction. Main pathways are shown on the left and conjugations with a glucuronic acid metabolites with similarly possible connections are shown separately on the right. The full list of compounds is provided in Table 1

This *in silico* investigation is based on the metabolites identified experimentally by the three *in vivo* studies (17-19). We aim to solve discrepancies and missing links found in these three studies by proposing more complete pathways in Figure 1 where all

73 experimentally identified metabolites, 5 previously proposed intermediates and 24 newly proposed intermediates are included. Possible reactions involving in the pathways are conjugation with a glucuronic acid/sulfate group, oxidation to alcohol, aldehyde & carboxylic acid, and formation of a four/five-membered ring at position 3, 7, 8, 9 and 10 of the parent compound. In this paper, we calculated Gibbs energies of reactions and associated them with the type, the position and the step of reaction in the pathways.

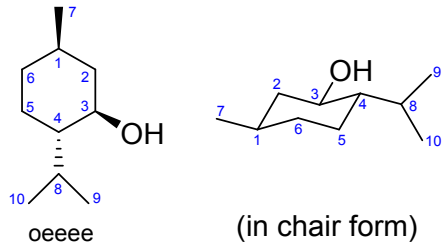
Materials and methods

Gas-phase structures were calculated based on the B3LYP/6-311++G(d,p) level and were confirmed to be at minimum energy on the electronic potential energy surfaces by frequency calculations. The solvation energies in water of the gas-phase structures were calculated with the SMD model (22). The calculation of Gibbs energies in solution phase is the same as in our previous work where there is a special treatment for water (23). (The energies were adjusted to solution standard state conditions – 1M, 1 atm, 298.15 K – with the exception of water where 55.34 M was used.) All quantum chemical calculations were performed using the Q-Chem 5.1 program package (24). (Shell script, spreadsheet templates, and Mathematica (25) notebook used were modified from our previous work (23). All output files and other associated codes to obtain the standard Gibbs energies of the reaction are provided in the electronic supporting information). The abbreviated names for each of the metabolites in this study are as in Figure 1 with the abbreviation explained below in Table 1.

Reaction energies were computed as listed in Table 2 with the relevant additional reagents (oxygen, sulfate group, hydronium ion and glucuronic acid) and product (water and hydrogen) added to the scheme. They may not be the actual compound in the reactions but they serve as simple reference points for the thermodynamic calculations for reactions that we are interested in.

TABLE 1 List of 102 compounds in this study grouped by molecular formula.

formula: $C_{10}H_{20}O$ (molar mass 156.26, total 1 compound)



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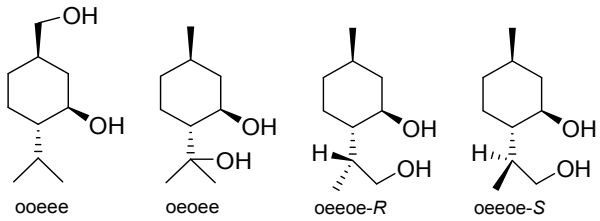

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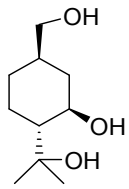
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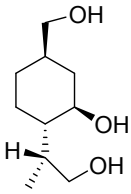
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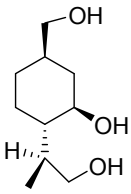
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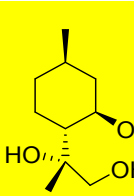
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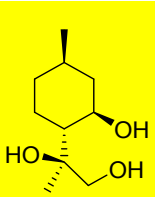
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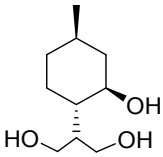
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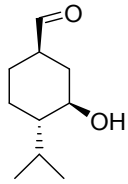
formula: $C_{10}H_{18}O_2$ (molar mass 170.25, total 4 compounds)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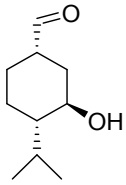
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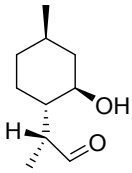
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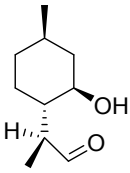
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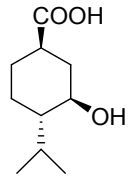
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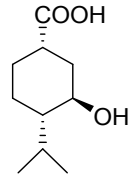
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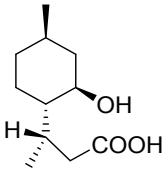
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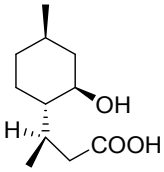
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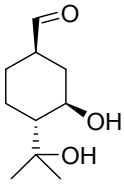
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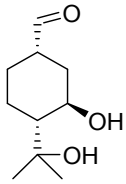
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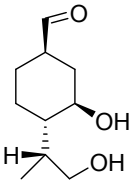
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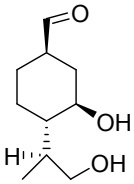
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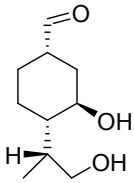
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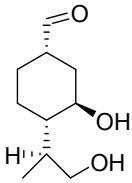
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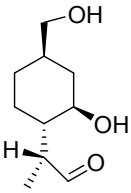
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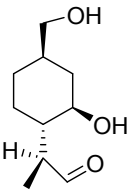
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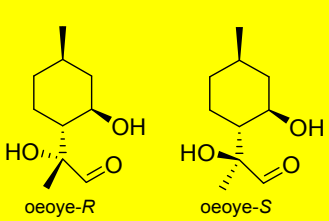
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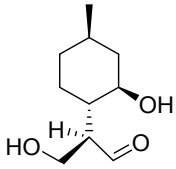
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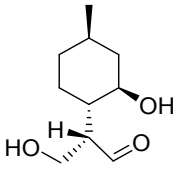
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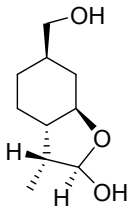
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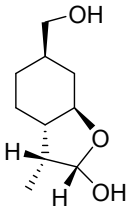
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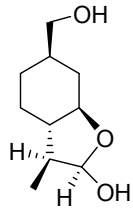
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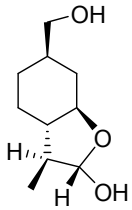
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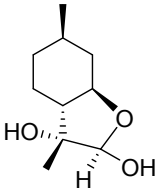
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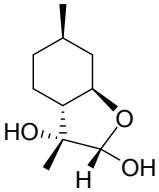
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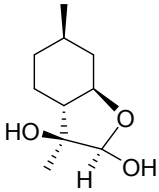
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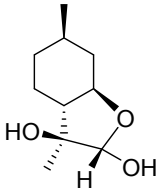
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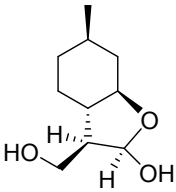
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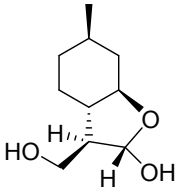
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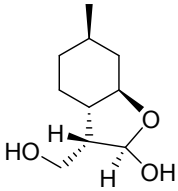
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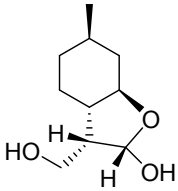
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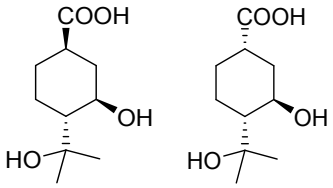

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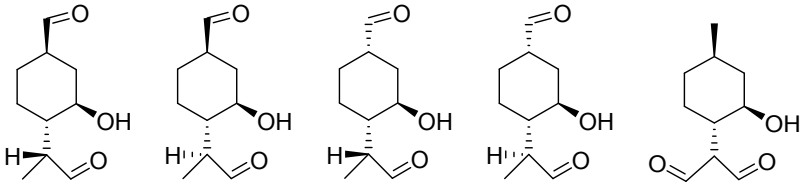
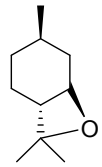


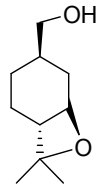
oyeye-*RR* oyeye-*RS* oyeye-*SR* oyeye-*SS* oeeyy

formula: $C_{10}H_{18}O$ (molar mass 154.25, total 1 compound)



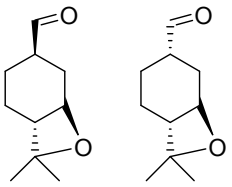
oeoee-4D

formula: $C_{10}H_{18}O_2$ (molar mass 170.25, total 1 compound)



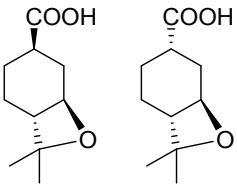
oooee-4D

formula: $C_{10}H_{16}O_2$ (molar mass 168.23, total 2 compounds)



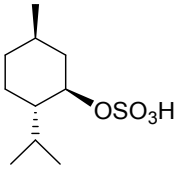
oyoee-4D-*R* oyoee-4D-*S*

formula: $C_{10}H_{16}O_3$ (molar mass 184.23, total 2 compounds)



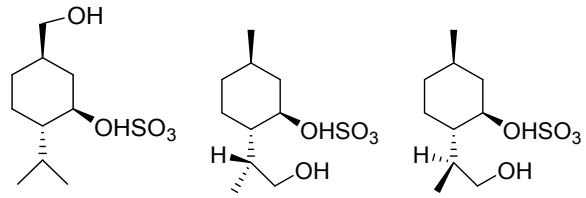
oxoee-4D-*R* oxoee-4D-*R*

formula: $C_{10}H_{20}O_4S$ (molar mass 236.33, total 1 compound)



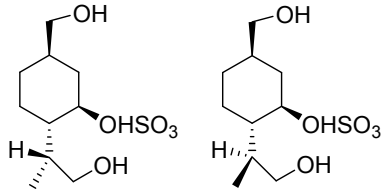
seeee

formula: $C_{10}H_{20}O_5S$ (molar mass 252.33, total 3 compounds)



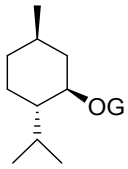
soeee seeoe-*R* seeoe-*S*

formula: $C_{10}H_{20}O_6S$ (molar mass 268.33, total 2 compounds)



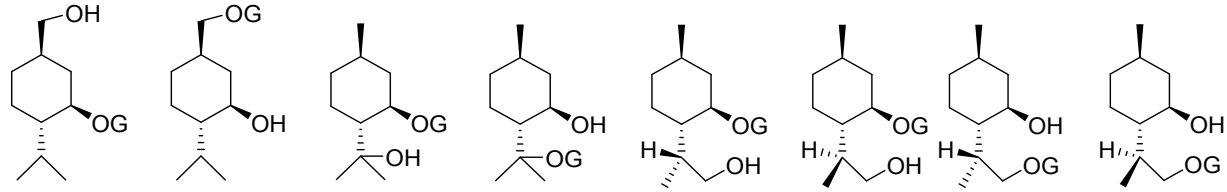
soeoe-*R* soeoe-*S*

formula: $C_{16}H_{28}O_7$ (molar mass 332.39, total 1 compound)



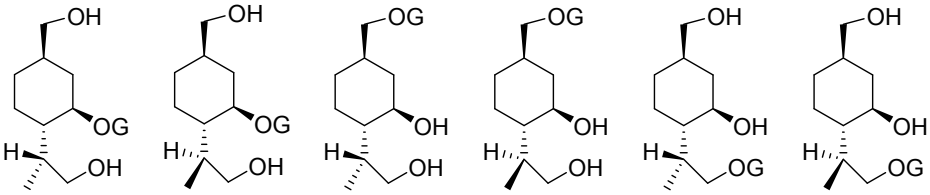
Oeeee

formula: $C_{16}H_{28}O_8$ (molar mass 348.39, total 8 compounds)



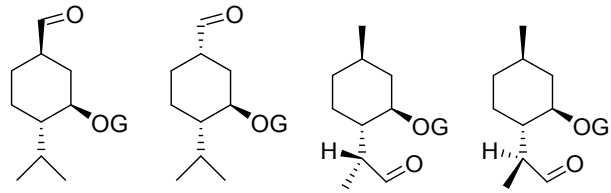
Ooeee oOeee Oeoee oeOee Oeeoe-*R* Oeeoe-*S* oeeOe-*R* oeeOe-*S*

formula: $C_{16}H_{28}O_9$ (molar mass 364.39, total 6 compounds)



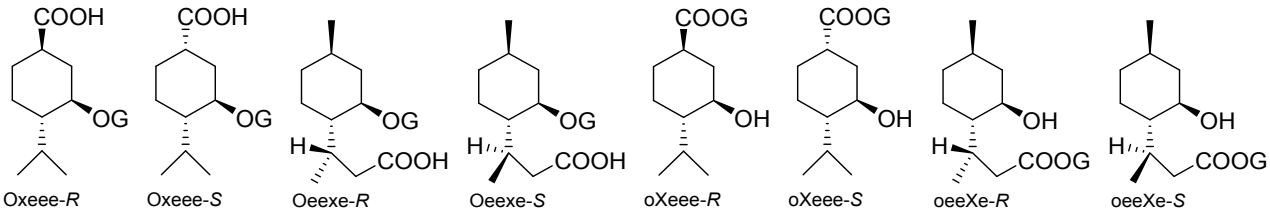
Ooeoe-*R* Ooeoe-*S* oOeoe-*R* oOeoe-*S* ooeOe-*R* ooeOe-*S*

formula: $C_{16}H_{26}O_8$ (molar mass 346.37, total 4 compounds)

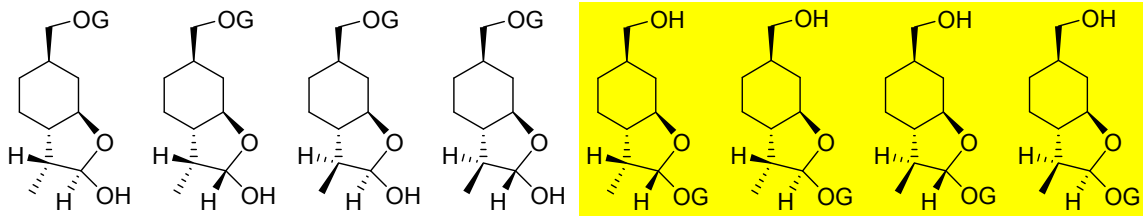


Oyeee-*R* Oyeee-*S* Oeeye-*R* Oeeye-*S*

formula: $C_{16}H_{26}O_9$ (molar mass 362.37, total 16 compounds)

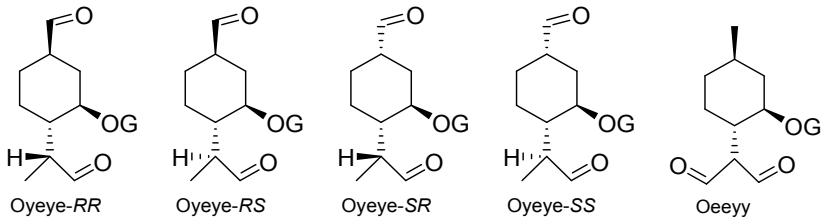


Oxeee-*R* Oxeee-*S* Oeexe-*R* Oeexe-*S* oXeee-*R* oXeee-*S* oeeXe-*R* oeeXe-*S*



oOeye-5A-*RR* oOeye-5A-*RS* oOeye-5A-*SR* oOeye-5A-*SS* ooeYe-5A-*RR* ooeYe-5A-*RS* ooeYe-5A-*SR* ooeYe-5A-*SS*

formula: $C_{16}H_{24}O_9$ (molar mass 360.36, total 5 compounds)



Notes

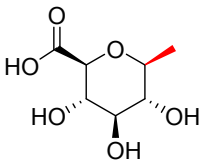
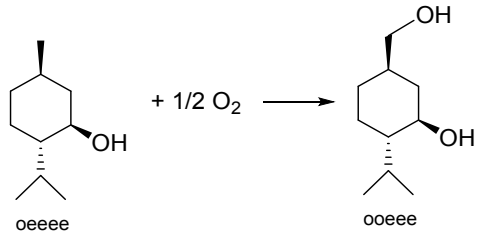
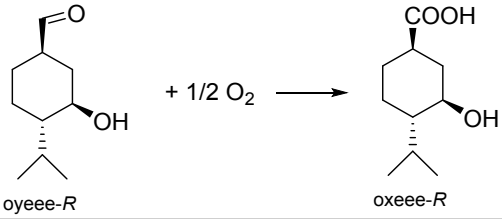
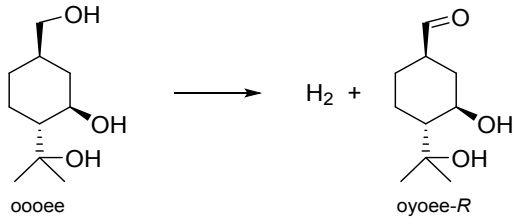
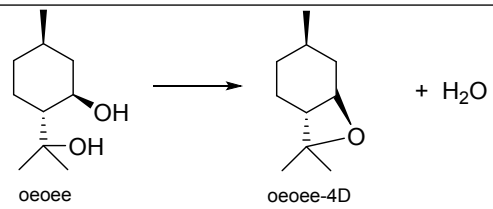
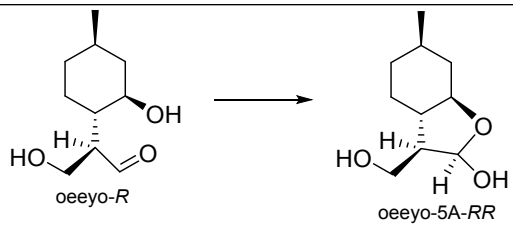
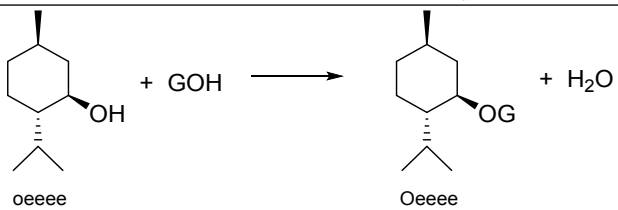
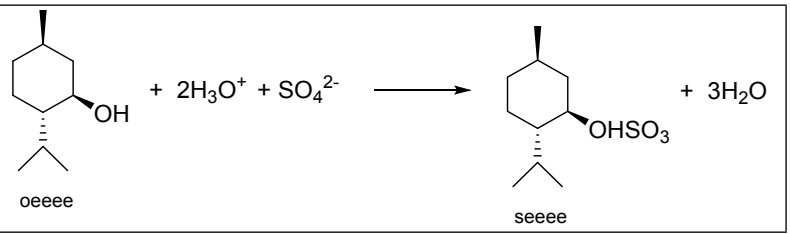
- Compounds are highlighted in yellow when their *RS* designator changes from their parent compounds.
- G stands for 

TABLE 2 Representative of oxidation reactions to alcohol, aldehyde and carboxylic acid, ring formation (dehydration reaction and aldol reaction) and conjugations with a glucuronic acid/sulfate group.

Abbreviation/explanation	Example
o1 for oxidation from alkane to alcohol	
o2 for oxidation from aldehyde to carboxylic acid	
o3 for oxidation form alcohol to aldehyde	
4D for dehydration (four-membered ring formation)	
5A for aldol reaction (five-membered ring formation)	
g for conjugations with a glucuronic acid	

s for conjugations with a sulfate group	

Results and discussion

FIGURE 2 The lowest energy structure of (-)-menthol optimized in gas phase at B3LYP/6-311++G(d,p) level of theory

All calculations were completed with no imaginary frequencies, showing that each of the structures obtained from gas-phase calculations were minima on the potential energy surfaces. The lowest energy structure of (-)-menthol is a chair conformer of hexane where all three substituent groups are in equatorial positions as shown in Figure 2. This is consistent with previous computational result at B3LYP/6-31G(d,p) level (26).

Table 2 summarizes the standard Gibbs energies of each of the reactions in the metabolic pathways described in Figure 1. The average energies of each of these types of reactions are summarized in Figure 3. The energies were clustered according to the type of reaction. According to the figure, oxidation reactions (o1 and o2, addition of $1/2O_2$) and conjugation with a sulfate group are the most exergonic and should occur easily. With an exception of conjugation with a sulfate group where charged species are present on the reactant side, relative ranking of average reaction energies in gas-phase and solution-phase are consistent and is always slightly less exergonic in gas-phase. This may be explained by the fact that oxidation tends to introduce polar functional groups whose interactions with water serve to stabilize the compound. The most endergonic reactions are four-membered ring formation (4D) and oxidation from alcohol to aldehyde (o3, removal of H_2). The four-membered ring formation was proposed based on experimental evidence (18) published in 1988 and should be verified in further experiment. Aldehyde products were not found in earlier experiments (17, 18) but identified in the most recent study.(19) It suggests that the oxidation may proceed directly to carboxylic acid metabolites or the aldehyde metabolites may be short-lived.

Difference in reaction energies due to position effect can be mostly explained by steric hindrance (i.e. g-8 has the highest reaction energy.) and inductive effect (i.e. o-8 producing secondary alcohol is the most exergonic.). The first step from the parents compound tends to be the most exergonic with an average at -38.6 kcal/mol and the average reaction energy decreases monotonically to around -3.1 kcal/mol at the fifth step.

TABLE 2 Standard Gibbs energies of reactions in Menthol's metabolic pathway

Reactions	ΔG_{soln}/ kcal mol^{-1}	ΔG_{gas}/ kcal mol^{-1}	Step number	Reaction	Position
oeeee + C6H10O7 → H2O + Oeeee	3.0	-2.9	1	g	3
ooeee + C6H10O7 → H2O + Ooeee	3.4	-0.1	2	g	3

ooeee + C6H10O7 → H2O + oOeee	2.2	-1.7	2	g	7
oeoee + C6H10O7 → H2O + Oeoee	1.2	-3.7	2	g	3
oeoee + C6H10O7 → H2O + oeOee	8.3	1.6	2	g	8
oeeoe-R + C6H10O7 → H2O + Oeeoe-R	4.9	-1.6	2	g	3
oeeoe-S + C6H10O7 → H2O + Oeeoe-S	7.5	-1.6	2	g	3
oeeoe-R + C6H10O7 → H2O + oeeOe-R	5.7	-0.3	2	g	9
oeeoe-S + C6H10O7 → H2O + oeeOe-S	2.3	-4.9	2	g	9
oyeee-R + C6H10O7 → H2O + Oyeee-R	3.0	-0.3	3	g	3
oyeee-S + C6H10O7 → H2O + Oyeee-S	1.9	-1.2	3	g	3
oeeye-R + C6H10O7 → H2O + Oeeye-R	1.2	-7.7	3	g	3
oeeye-S + C6H10O7 → H2O + Oeeye-S	1.5	-7.2	3	g	3
ooeoe-R + C6H10O7 → H2O + Ooeoe-R	7.9	4.7	3	g	3
ooeoe-S + C6H10O7 → H2O + Ooeoe-S	10.7	8.1	3	g	3
ooeoe-R + C6H10O7 → H2O + oOeoe-R	0.8	-3.1	3	g	7
ooeoe-S + C6H10O7 → H2O + oOeoe-S	2.7	-0.9	3	g	7
ooeoe-R + C6H10O7 → H2O + ooeOe-R	2.1	0.0	3	g	9
ooeoe-S + C6H10O7 → H2O + ooeOe-S	1.8	-1.5	3	g	9
oxeee-R + C6H10O7 → H2O + Oxeee-R	7.4	3.9	4	g	3
oxeee-S + C6H10O7 → H2O + Oxeee-S	3.1	-1.7	4	g	3
oeexe-R + C6H10O7 → H2O + Oeexe-R	5.8	2.5	4	g	3
oeexe-S + C6H10O7 → H2O + Oeexe-S	4.9	2.1	4	g	3
oxeee-R + C6H10O7 → H2O + oXeee-R	15.0	10.3	4	g	7
oxeee-S + C6H10O7 → H2O + oXeee-S	15.1	10.2	4	g	7
oeexe-R + C6H10O7 → H2O + oeeXe-R	9.3	5.2	4	g	9
oeexe-S + C6H10O7 → H2O + oeeXe-S	10.0	4.6	4	g	9
ooeye-5A-RR + C6H10O7 → H2O + oOeye-5A-RR	1.5	-2.5	5	g	7
ooeye-5A-RS + C6H10O7 → H2O + oOeye-5A-RS	1.3	-2.2	5	g	7
ooeye-5A-SR + C6H10O7 → H2O + oOeye-5A-SR	1.3	-1.6	5	g	7
ooeye-5A-SS + C6H10O7 → H2O + oOeye-5A-SS	2.6	-0.3	5	g	7
ooeye-5A-RS + C6H10O7 → H2O + ooeYe-5A-RR	7.8	5.4	5	g	9
ooeye-5A-RR + C6H10O7 → H2O + ooeYe-5A-RS	7.5	1.1	5	g	9
ooeye-5A-SS + C6H10O7 → H2O + ooeYe-5A-SR	2.3	-1.1	5	g	9
ooeye-5A-SR + C6H10O7 → H2O + ooeYe-5A-SS	6.3	2.7	5	g	9
oyeye-RR + C6H10O7 → H2O + Oyeye-RR	8.2	3.3	5	g	3
oyeye-RS + C6H10O7 → H2O + Oyeye-RS	2.3	-2.0	5	g	3
oyeye-SR + C6H10O7 → H2O + Oyeye-SR	3.0	0.1	5	g	3
oyeye-SS + C6H10O7 → H2O + Oyeye-SS	4.3	-4.3	5	g	3
oeeyy + C6H10O7 → H2O + Oeeyy	7.7	4.0	5	g	3
oeeee + 2H3O+ + SO4-2 → 3H2O + seeee	-24.0	-428.9	1	s	3
ooeee + 2H3O+ + SO4-2 → 3H2O + soeee	-23.0	-427.7	2	s	3
oeeoe-R + 2H3O+ + SO4-2 → 3H2O + seeoe-R	-19.9	-423.5	2	s	3
oeeoe-S + 2H3O+ + SO4-2 → 3H2O + seeoe-S	-23.4	-427.7	2	s	3
ooeoe-R + 2H3O+ + SO4-2 → 3H2O + soeoe-R	-17.5	-422.3	3	s	3
ooeoe-S + 2H3O+ + SO4-2 → 3H2O + soeoe-S	-19.2	-423.6	3	s	3
oeeee + 1/2O2 → ooeee	-52.1	-45.3	1	o1	7
oeeee + 1/2O2 → oeoee	-56.0	-51.5	1	o1	8
oeeee + 1/2O2 → oeeoe-R	-51.9	-47.2	1	o1	9
oeeee + 1/2O2 → oeeoe-S	-50.4	-45.7	1	o1	9
Oeeee + 1/2O2 → Ooeee	-51.7	-42.5	2	o1	7
Oeeee + 1/2O2 → Oeoee	-57.8	-52.3	2	o1	8
Oeeee + 1/2O2 → Oeeoe-R	-50.0	-45.8	2	o1	9
Oeeee + 1/2O2 → Oeeoe-S	-45.9	-44.4	2	o1	9
ooeee + 1/2O2 → oooee	-55.7	-52.3	2	o1	8
oeoee + 1/2O2 → oooee	-51.8	-46.1	2	o1	7
ooeee + 1/2O2 → ooeoe-R	-50.5	-45.9	2	o1	9
ooeee + 1/2O2 → ooeoe-S	-49.3	-45.0	2	o1	9
oeeoe-R + 1/2O2 → ooeoe-R	-50.7	-44.0	2	o1	7

oeeoe-S + 1/2O2 → ooeoe-S	-51.0	-44.6	2	o1	7
oeoee + 1/2O2 → oeooe-R	-48.8	-46.5	2	o1	9
oeoee + 1/2O2 → oeooe-S	-48.9	-43.3	2	o1	9
oeeoe-S + 1/2O2 → oeooe-R	-54.5	-52.3	2	o1	8
oeeoe-R + 1/2O2 → oeooe-S	-53.0	-47.6	2	o1	8
oeeoe-R + 1/2O2 → oeeoo	-50.5	-44.9	2	o1	10
oeeoe-S + 1/2O2 → oeeoo	-52.0	-46.3	2	o1	10
Ooeee + 1/2O2 → Ooeoe-R	-46.1	-41.1	3	o1	9
Ooeee + 1/2O2 → Ooeoe-S	-42.0	-36.8	3	o1	9
Oeeoe-R + 1/2O2 → Ooeoe-R	-47.8	-37.8	3	o1	7
Oeeoe-S + 1/2O2 → Ooeoe-S	-47.8	-34.9	3	o1	7
oOeee + 1/2O2 → oOeoe-R	-52.0	-47.3	3	o1	9
oOeee + 1/2O2 → oOeoe-S	-48.8	-44.2	3	o1	9
oeeOe-R + 1/2O2 → ooeOe-R	-54.4	-43.7	3	o1	7
oeeOe-S + 1/2O2 → ooeOe-S	-51.5	-41.2	3	o1	7
oeoee-4D + 1/2O2 → oooee-4D	-51.9	-45.2	3	o1	7
oyeee-R + 1/2O2 → oyoee-R	-55.8	-51.0	3	o1	8
oyeee-S + 1/2O2 → oyoee-S	-56.4	-51.8	3	o1	8
oyeee-R + 1/2O2 → oyeoe-RR	-49.5	-45.5	3	o1	9
oyeee-R + 1/2O2 → oyeoe-RS	-50.3	-45.6	3	o1	9
oyeee-S + 1/2O2 → oyeoe-SR	-49.4	-44.9	3	o1	9
oyeee-S + 1/2O2 → oyeoe-SS	-50.1	-44.8	3	o1	9
oeeye-R + 1/2O2 → ooeye-R	-51.6	-44.9	3	o1	7
oeeye-S + 1/2O2 → ooeye-S	-51.8	-45.0	3	o1	7
oeeye-S + 1/2O2 → oeoye-R	-49.5	-48.9	3	o1	8
oeeye-R + 1/2O2 → oeoye-S	-51.0	-48.2	3	o1	8
oeeye-R + 1/2O2 → oeeyo-R	-49.0	-46.7	3	o1	10
oeeye-S + 1/2O2 → oeeyo-S	-48.7	-45.7	3	o1	10
oxeee-S + 1/2O2 → oxoee-S	-55.3	-50.5	4	o1	8
oxeee-R + 1/2O2 → oxoee-R	-55.9	-51.0	4	o1	8
oyeee-R + 1/2O2 → oxeee-R	-73.8	-66.7	3	o2	7
oyeee-S + 1/2O2 → oxeee-S	-73.6	-66.8	3	o2	7
oeeye-R + 1/2O2 → oeexe-R	-74.3	-70.4	3	o2	9
oeeye-S + 1/2O2 → oeexe-S	-72.8	-69.3	3	o2	9
Oyeee-R + 1/2O2 → Oxeee-R	-69.4	-62.5	4	o2	7
Oyeee-S + 1/2O2 → Oxeee-S	-72.4	-67.3	4	o2	7
Oeeye-R + 1/2O2 → Oeexe-R	-69.8	-60.2	4	o2	9
Oeeye-S + 1/2O2 → Oeexe-S	-69.4	-60.0	4	o2	9
oyoee-R + 1/2O2 → oxoee-R	-74.0	-66.7	4	o2	7
oyoee-S + 1/2O2 → oxoee-S	-72.6	-65.4	4	o2	7
oyoee-4D-R + 1/2O2 → oxoee-4D-R	-72.9	-67.0	5	o2	7
oyoee-4D-S + 1/2O2 → oxoee-4D-S	-70.2	-63.5	5	o2	7
ooeee → oyeee-R + H2	10.1	5.0	2	o3	7
ooeee → oyeee-S + H2	11.4	5.8	2	o3	7
oeeoe-R → oeeye-R + H2	8.5	5.4	2	o3	9
oeeoe-S → oeeye-S + H2	7.2	4.1	2	o3	9
Ooeee → Oyeee-R + H2	9.7	4.8	3	o3	7
Ooeee → Oyeee-S + H2	9.9	4.8	3	o3	7
Oeeoe-R → Oeeye-R + H2	4.8	-0.7	3	o3	9
Oeeoe-S → Oeeye-S + H2	1.2	-1.4	3	o3	9
oooee → oyoee-R + H2	10.1	6.3	3	o3	7
oooee → oyoee-S + H2	10.7	6.2	3	o3	7
ooeoe-R → oyeoe-RR + H2	11.2	5.4	3	o3	7
ooeoe-R → oyeoe-RS + H2	10.4	5.2	3	o3	7
ooeoe-S → oyeoe-SR + H2	11.2	5.9	3	o3	7
ooeoe-S → oyeoe-SS + H2	10.5	6.0	3	o3	7
ooeoe-R → ooeye-R + H2	7.7	4.5	3	o3	9

ooeoe-S → ooeye-S + H2	6.4	3.7	3	o3	9
oeooe-R → oeoye-R + H2	12.2	7.5	3	o3	9
oeooe-S → oeoye-S + H2	10.5	4.8	3	o3	9
oeeoo → oeeyo-R + H2	9.9	3.6	3	o3	9
oeeoo → oeeyo-S + H2	10.5	4.8	3	o3	9
oooee-4D → oyoee-4D-R + H2	9.9	5.1	4	o3	7
oooee-4D → oyoee-4D-S + H2	11.0	6.0	4	o3	7
oyeoe-RR → oyeye-RR + H2	7.2	4.3	4	o3	9
oyeoe-RS → oyeye-RS + H2	7.1	4.4	4	o3	9
oyeoe-SR → oyeye-SR + H2	7.6	3.8	4	o3	9
oyeoe-SS → oyeye-SS + H2	8.0	4.6	4	o3	9
ooeye-R → oyeye-RR + H2	10.7	5.2	4	o3	7
ooeye-R → oyeye-SR + H2	12.4	6.1	4	o3	7
ooeye-S → oyeye-RS + H2	9.8	5.1	4	o3	7
ooeye-S → oyeye-SS + H2	12.1	7.0	4	o3	7
oeeyo-R → oeeyy + H2	10.6	7.4	4	o3	10
oeeyo-S → oeeyy + H2	10.0	6.3	4	o3	10
oeoee → oeoee-4D + H2O	15.6	15.6	2	d	
oooee → oooee-4D + H2O	15.5	16.6	3	d	
oyoee-R → oyoee-4D-R + H2O	15.3	15.4	4	d	
oyoee-S → oyoee-4D-S + H2O	15.8	16.3	4	d	
oxoee-R → oxoee-4D-R + H2O	16.3	15.1	5	d	
oxoee-S → oxoee-4D-S + H2O	18.1	18.2	5	d	
ooeye-R → ooeye-5A-RR	3.3	1.2	4	a	
ooeye-R → ooeye-5A-RS	4.2	1.8	4	a	
ooeye-S → ooeye-5A-SR	2.8	-0.4	4	a	
ooeye-S → ooeye-5A-SS	1.2	-1.5	4	a	
oeoye-R → oeoye-5A-RR	-2.7	-1.0	4	a	
oeoye-R → oeoye-5A-RS	-2.6	-2.9	4	a	
oeoye-S → oeoye-5A-SR	-0.7	-2.3	4	a	
oeoye-S → oeoye-5A-SS	-0.5	-0.6	4	a	
oeeyo-R → oeeyo-5A-RR	0.5	-0.5	4	a	
oeeyo-R → oeeyo-5A-RS	-1.9	0.3	4	a	
oeeyo-S → oeeyo-5A-SR	0.8	1.7	4	a	
oeeyo-S → oeeyo-5A-SS	2.3	2.0	4	a	

Concluding remarks and future directions

FIGURE 3 Average standard Gibbs energies in solution phase and gas phase for oxidation from alkane to alcohol (o1), oxidation from aldehyde to carboxylic acid (o2), oxidation from alcohol to aldehyde (o3), dehydration or four-membered ring formation (4D), aldol reaction or five-membered ring formation (5A), conjugation with a glucuronic acid (g) and conjugation with a sulfate group (s) at five different positions of (-)-menthol.

FIGURE 4 Relative stability of 102 (-)-menthol metabolite compounds in solution phase

In this study, gas-phase structures of menthol and its metabolites (a total of 102 compounds and 151 reactions) were obtained by quantum calculations at B3LYP/6-311++G(d,p) level. The standard Gibbs energies of their respective

reactions in solution were calculated with the SMD solvation model and corrected for standard state conditions. As summarized in Figures 3 and 4, slight differences in calculated standard Gibbs energies in the gas-phase and solution phase may indicate stabilizing/destabilizing effects by the solvent. Information obtained in this study open possibilities for further investigation of the pharmacological effects of menthol and its metabolites. Given that oxidation pathways of menthol are energetically favorable, potency and toxicity of these oxidized derivatives should be evaluated in future research.

ASSOCIATED CONTENT

Supporting Information.

All output files and other associated codes to obtain the standard Gibbs energies of reaction are provided.

AUTHOR INFORMATION

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Author Contributions: T.L., the principal investigator, performed the calculation and wrote the manuscript. K.C. and W.L. assisted T.L. to compose the manuscript.

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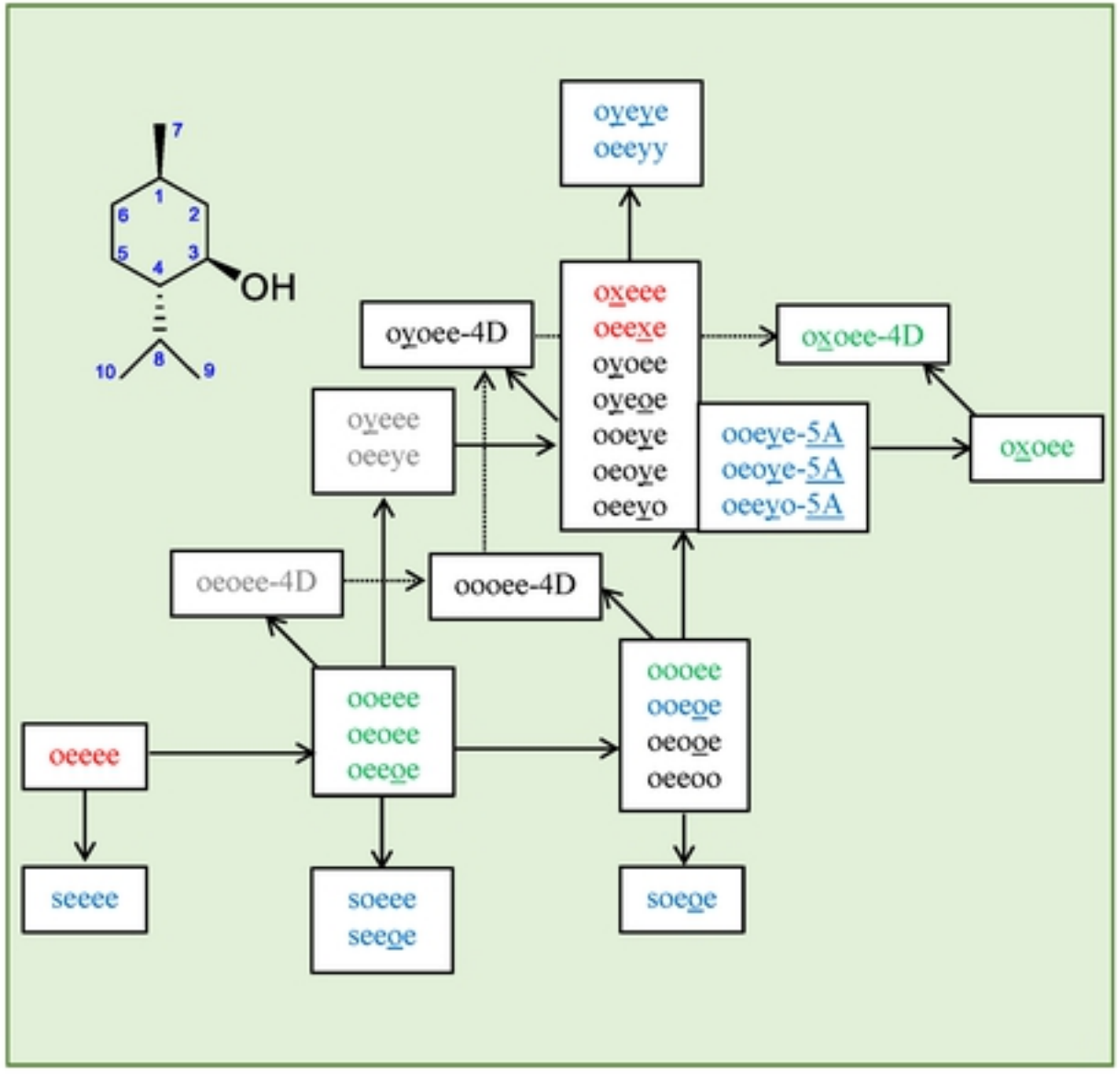


Group \ Position	3	7	8	9	10
Original form	o	e	e	e	e
Alkane	-	e	e	e	e
Alcohol	o	o	o	o	o
Aldehyde	y	y	-	y	y
Carboxylic acid	-	x	-	x	-
Dehydration	-4D for four-membered ring formation at positions 3 and 8				
Aldol reaction	-5A for four-membered ring formation at positions 3 and 9				
Glucuronic acid	O	O,X	-	O,X,Y	-
sulfonation	s	-	-	-	-

- An underlined indicates that there are *R* and *S* stereoisomers due to the substitution.
- Substitution at position 9 leads to a new chiral center if it is not the same as 10.
- Substitution at position 10 is forced to have lower or the same oxidation state for the carbon atom when compared to position 9.

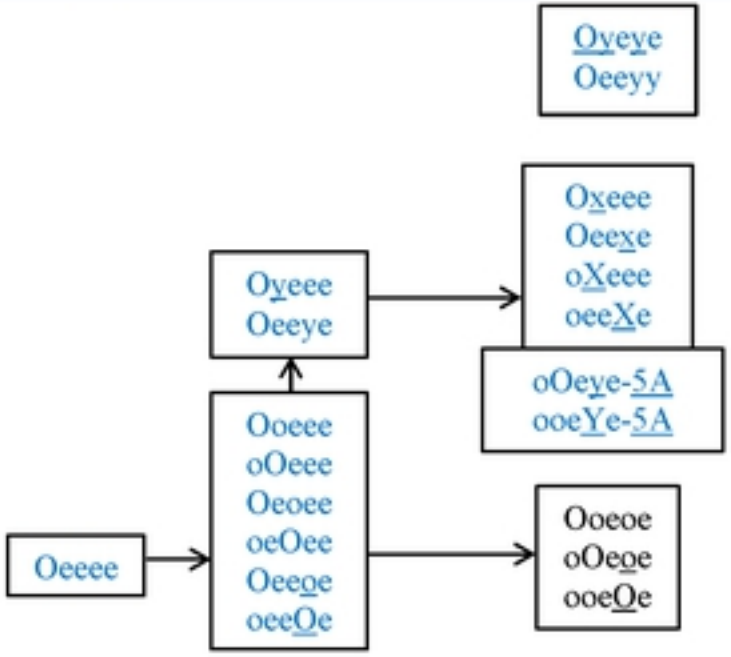


Figure 1

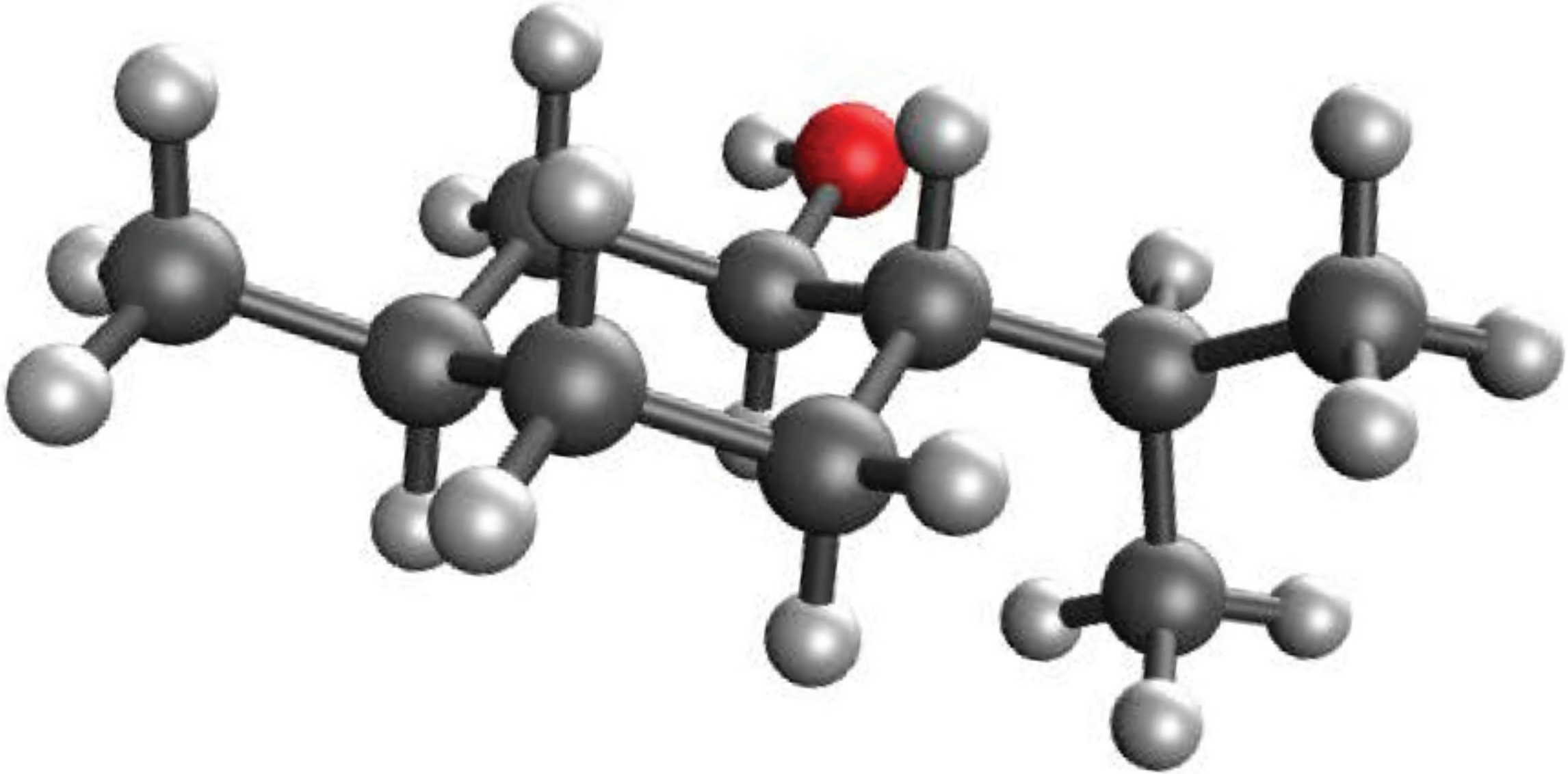
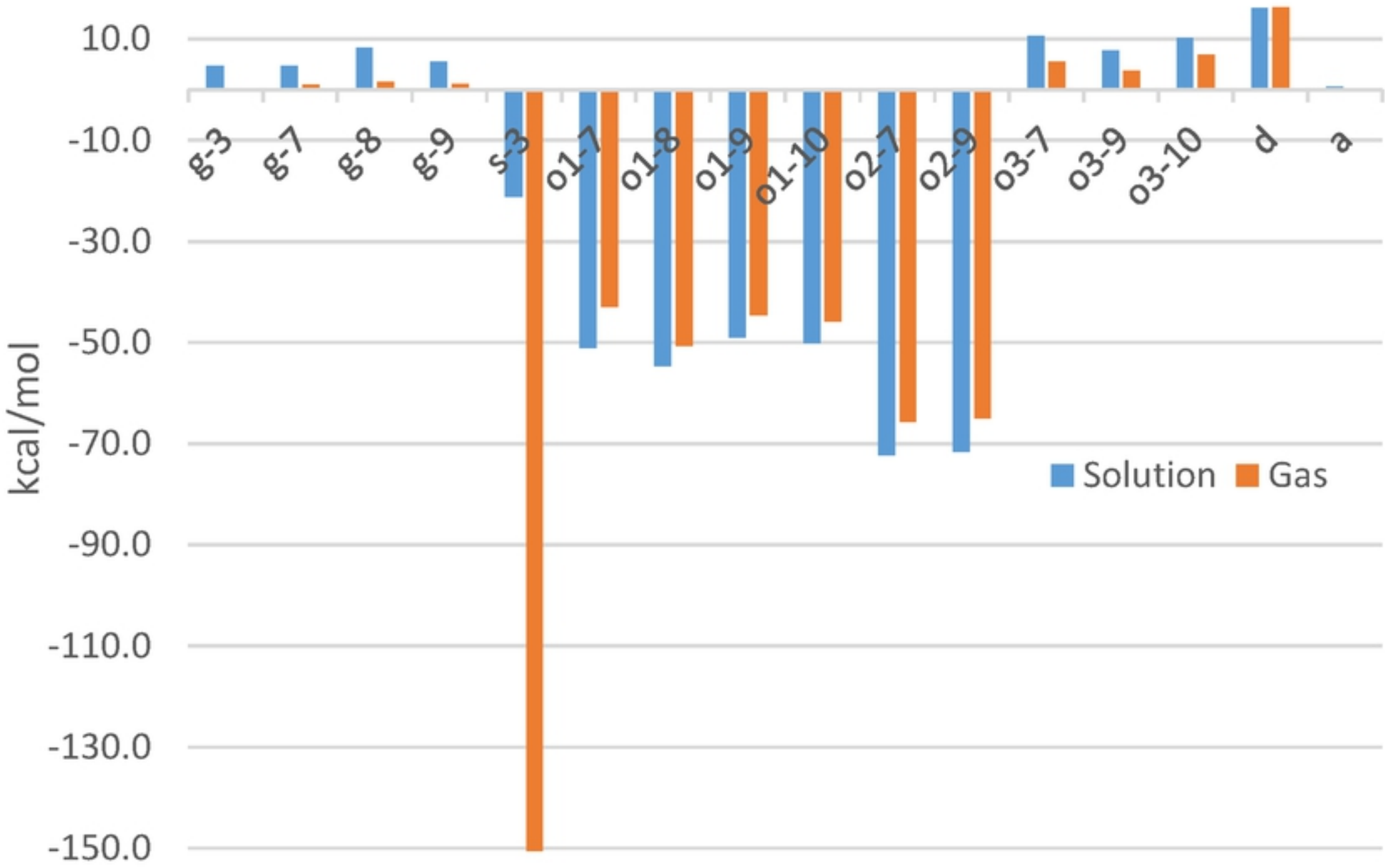


Figure 2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o1-7
o1-8
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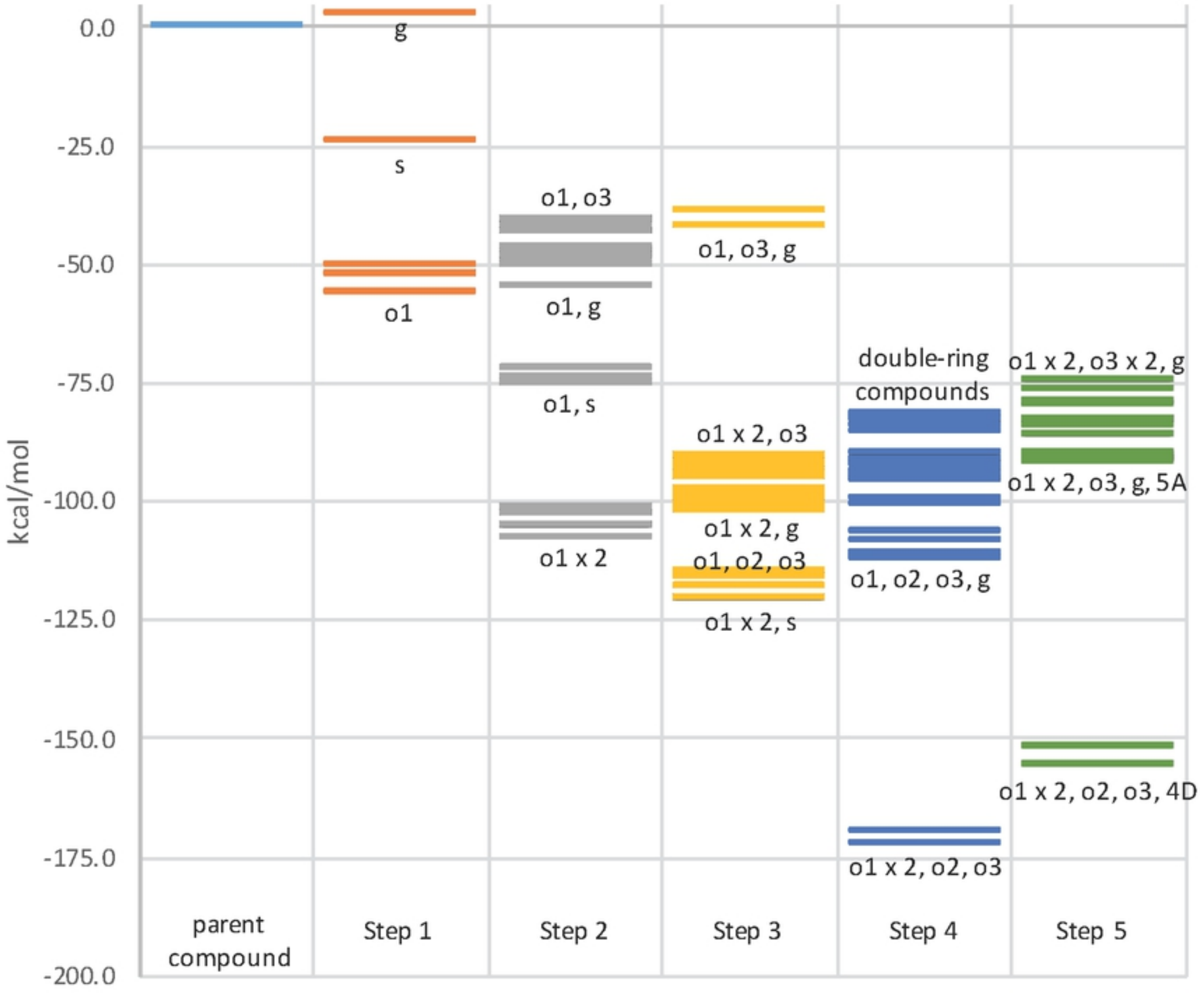
o2-7
o2-9
o3-7
o3-9
o3-10
d
a
Solution
Gas

Figure 3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o1 x 2, g
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o1 x 2, s
double-ring compounds
o1, o2, o3, g
o1 x 2, o2, o3
o1 x 2, o3 x 2, g
o1 x 2, o3, g, 5A
o1 x 2, o2, o3, 4D
parent compound
Step 1
Step 2
Step 3
Step 4
Step 5

Figure 4