REGULAR ARTICLE



Resolution of isoborneol and its isomers by GC/MS to identify "synthetic" and "semi-synthetic" borneol products

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Abstract

Borneol is a plant terpene commonly used in traditional Chinese medicine. Optically pure (+)-borneol and (-)-borneol can be obtained by extraction from the plants Dipterocarpaceae and Blumea balsamifera, respectively. "Synthetic borneol" is obtained from the reduction of (+)-camphor to lead to four different stereoisomers: (+)-isoborneol, (-)-isoborneol, (+)-borneol, and (-)-borneol. In contrast, "semi-synthetic borneol" is produced from the reduction of natural camphor, (+)-camphor, to afford two isomers: (-)-isoborneol and (+)-borneol. We established a convenient method to identify them by treating the four stereoisomers with two chiral reagents, (R)-(+)- α -methoxy- α trifluoromethylphenylacetyl chloride ((R)-(+)-MTPA-Cl) and (1S)-(-)camphanic chloride. The resulting derivatives from the above mentioned method were analyzed by gas chromatography. The enantiomers of (+)- and (-)-isoborneol were successfully separated from (+)- and (-)-borneol isomers in this study to make this a useful method in the identification of "synthetic" and "semi-synthetic" borneols. Furthermore, we also examined five different commercial borneols. During this course, a novel and unprecedented partial epimerization from isoborneol-camphanic ester to borneol-camphanic ester was observed. However, this phenomenon did not occur in isoborneol-MTPA esters epimerization to borneol-MTPA case under the same conditions. The DFT calculation of activation energies for both reactions was in a good agreement with the results obtained from GC analysis.

KEYWORDS

borneol, chiral separation, epimerization, GC/MS, isoborneol

1 | INTRODUCTION

Borneol is a plant terpene commonly used in traditional Chinese medicine for restoring consciousness, treating coma due to heat-blockage, or reducing skin irritation and itching.^{1,2} (+)-Borneol used in traditional Chinese medicine can be isolated from the resin and essential oil of woody plants of the *Dipterocarpaceae* family or certain

camphor tree.³ Its optical isomer counterpart, (–)-borneol, can be obtained from the herbaceous plant *Blumea* balsamifera.⁴ Neither (+)-isoborneol nor (–)-isoborneol are present in these natural plants. The interaction between borneol-producing plants and soil bacteria was recently reported.⁵ Both compounds have been shown to affect GABA receptors.^{2,6} Recent animal studies have demonstrated that borneol not only possesses a tissue-

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specific blood-brain barrier opening effect but also increases the concentration of meropenem in brain blood.^{7,8} On the other hand, the so-called "synthetic borneol" is obtained from the reduction of (\pm) -camphor racemates to provide a mixture of four different stereoisomers: (+)-borneol, (+)-isoborneol, (-)-borneol, and (-)isoborneol (Figure 1). The so-called "semi-synthetic borneol" is obtained from the reduction of natural camphor, (+)-camphor, and provides (+)-borneol and (-)isoborneol. Animal oral administration studies have demonstrated the following increasing order of LD50 values: isoborneol < synthetic borneol < (+)-borneol. Therefore, (+)-borneol is the safest one among these samples.⁹ Unfortunately, the retail price of pure (+)-borenol is much higher than that of synthetic borneol.¹⁰

The separation of enantiomers, (+)- and (-)- borneol as well as (+)- and (-)-isoborneol by chiral gas chromatography (GC) was reported by us and other groups previously.¹⁰⁻¹⁴ Generally speaking, the main drawbacks of chiral column are high cost and durability. Our unpublished results also showed that applications of samples extracted from fermentation media or herbal ointment complexes usually led to irreversible damage of the β cyclodextrin-based chiral GC column after several runs.¹⁰ Thus, a more reliable method is strongly needed for routine analysis of traditional Chinese herbal medicine complexes on the market. In order to establish an analytical method, we respectively treated (+)-, (-)-borneol and (+)-, (-)-isoborneol with (R)-(+)- α -methoxy- α trifluoromethylphenylacetyl chloride ((R)-(+)-MTPA-Cl) and (1S)-(-)-camphanic chloride, and analyzed the resulting derivatives by GC. Five different commercial borneols, including natural, synthetic, semi-synthetic borneols, were examined here.

An interesting issue arose from both the pure (+)- and (-)-isoborneol-camphanic esters being partially



FIGURE 1 Structures of (+)-borneol, (+)-isoborneol, (-)borneol, and (-)-isoborneol. The semi-synthetic borneol is synthesized from natural (+)-camphor to afford two isomers, (+)borneol and (-)-isoborneol. The synthetic borneol is a mixture of four stereoisomers, (+)-borneol, (+)-isoborneol, (-)-borneol, and (-)-isoborneol which are obtained from reduction of (±)-camphor

epimerized to (-)- and (+)-borneol-camphanic esters, respectively, under GC conditions. However, no epimerization occurred while (+)- and (-)-borneol-MTPA esters were under the same conditions. The mechanism is examined based on theoretical calculations in this report.

2 | MATERIALS AND METHODS

2.1 | Chemicals

(+)-Borneol was purchased from Sigma-Aldrich (St. Louis, MO, USA). (–)-Borneol, (+)-isoborneol, and (1*S*)-(–)camphanic chloride were purchased from Alfa Aesar (Lancashire, UK). "Cheng Yi," "Qing Shan," and "Xin Long" brand synthetic borneol, mixture of borneol and isoborneol, was bought from local drug store in Taipei city. (–)-Isoborneol and (+)-isoborneol were synthesized from (+)- and (–)-camphor, respectively, as described previously.¹⁵ Commercial natural borneol, special grade (pure borneol from plant), and common grade (semi-synthetic borneol) were obtained from Taiwan Tekho Camphor Co. Ltd. All chemicals were ACS grade or higher and used without further purification unless otherwise stated.

2.2 | Derivatization

A modified procedure was used in the derivatization of (+)- and (-)-borneol as well as (+)- and (-)-isoborneol.^{16,17}

2.2.1 | Method A ((R)-(+)-MTPA-Cl derivatives)

(*R*)-(+)-MTPA-Cl was dissolved in dry acetonitrile to give a stock solution (268 mM). This stock solution (20 mM) was used for in preparation of (+)-borneol, (–)-borneol, (+)-isoborneol, and (–)-isoborneol derivatives in dry acetonitrile. Equal volumes (20 μ L) of (*R*)-(+)-MTPA-Cl and (+)-borneol solution, for example, were mixed and incubated at 75°C for 90 minutes. Once the reaction completed, anhydrous ethanol (40 μ L) was added to quench the reaction as well as the unreacted (*R*)-(+)-MTPA-Cl reagent. This mixture was incubated at 75°C for another 30 minutes to yield **1**, **2**, **3**, and **4** (Figure 2).

2.2.2 | Method B (camphanic acid derivatives)

The stock solutions of pyridine (20% in dry CH_3CN) and (–)-camphanic chloride (268 mM in dry CH_3CN) were prepared. The stock solutions (20 mM) were used in preparation of (+)-borneol, (–)-borneol, (+)-isoborneol, and (–)-isoborneol derivatives in dry acetonitrile. Equal



FIGURE 2 Derivatization of (+)-borneol, (-)-borneol, (+)isoborneol, and (-)-isoborneol by (*R*)-(+)-MTPA-Cl

volumes (25 μ L) of pyridine, (–)-camphanic chloride, and the various borneol solutions were mixed and incubated at 60°C for 60 minutes to provide compounds **5** to **8** (Figure 3). Upon completion of the reaction, the reaction mixtures were available for GC mass analysis.

2.3 | Instruments and analysis conditions

The (+)-, (-)-borneol and (+)-, (-)-isoborneol derivatives ((R)-(+)-MTPA-Cl and (-)-camphanic chloride)were investigated by a GC-MS/MS analysis in a positive mode. GC-MS was performed on a Thermo Scientific (USA) GC ultra including a trace and a TSQ triple quadrupole mass spectrometer equipped with electron ionization source. The four derivatives were separated with an HP-5MS column (25 m, 0.2 mm id, 0.33-µm film thickness; J&W Scientific). Data acquisition was performed with Xcalibur 2.2 software. GC oven temperature was initially at 40°C and held at this temperature for 3 minutes, increased to 190°C at 1°C/min and held at this temperature for 3 minutes, and then increased to 290°C at 20°C/min and held at this temperature for 10 minutes. The carrier gas was helium, and the flow rate was 0.7 mL/min. The instrumental parameters used for GC-MS detection of the four derivatives in the positive ion mode were source vaporizer temperature, 290°C; emission current, 50 µA; scan time, 0.3 seconds; Q1 peak width, 0.7; and MS range from m/z 45.0 to m/z 450.0. The sample was injected in PTV splitless mode, and volume was 1 µL. The inlet temperature was 290°C.



FIGURE 3 Derivatization of (+)borneol, (-)-borneol, (+)-isoborneol, and (-)-isoborneol by (-)-camphanic chloride 4 WILEY

2.4 | Calculation

Optimized structures and total energies were obtained by the density functional theory (DFT) method which used the B3LYP functional and 6-31 g (d) basis sets in the Gaussian 09 package.

3 | RESULTS AND DISCUSSION

3.1 | Chromatography of (*R*)-(+)-MTPA-Cl derivatives

The peaks of (R)-(+)-MTPA-Cl derivatives of (+)- and (-)-borneol were not well-resolved (Figure 4A,B). However, (+)- and (-)-isoborneol were successfully separated by 0.36 minutes, and both peaks were sharp and symmetrical (Figure 4C,D). Obviously, there are four corresponding peaks, (+)-, (-)-borneol, (+)-isoborneol, and (-)isoborneol, in chemically synthetic borneol as shown in Figure 4E.

3.2 | GC analysis of borneols sold in traditional Chinese medicine markets in Taipei

Three different synthetic borneols, "Cheng Yi," "Qing Shan," and "Xin Long" brand, common and special grade natural borneol from Taiwan Tekho Camphor Company were examined by using aforementioned method. The results are illustrated in Figure 5. Not surprisingly, four peaks are present in all three synthetic borneol products (Figure 5A,B,C). Only (-)-isoborneol and no (-)-borneol can be detected in common grade natural borneol

product. Meanwhile, only one peak appears in the range of retention time of (+)- and (-)-borneol, suggesting that common grade nature borneols is semi-synthetic borneol (Figure 5D). Neither (+)-isoborneol nor (-)-isoborneol can be detected in special grade natural borneol. Accordingly, the retention time of the only peak in Figure 5E is the same as the major peak in Figure 5D, suggesting that special grade natural borneol is (+)-borneol.

3.3 | Chromatography of (–)-camphanic derivatives

The (-)-camphanic acid derivatives of (+)- and (-)-borneol were separated by 0.20 minutes (Figure 6A,B). It is interesting to note that the derivatives of (-)-isoborneol and (+)-isoborneol split into two peaks (Figure 6C,D). The purity of synthetic (-)- and (+)-isoborneol has been checked by chiral GC.¹⁰ In accordance with this result, only one peak was observed in the chromatograph of the (R)-(+)-MTPA-Cl derivative of (-)-isoborneol (Figure 4C). However, from the results of mass spectra, both the molecule weight and the mass of the fragments of the two peaks that appear in Figure 6C,D are the same. These results suggest that ¹ mass spectrum results cannot be used for differentiation of borneol isomers, and ² these two compounds should be stereoisomers. It seems likely that (-)-isoborneol-camphanic ester was converted into (+)-borneol-camphanic ester through an unprecedented epimerization (Figure 7). This phenomenon also occurred with the (+)-isoborneol-camphanic ester (Figure 7D). The peak area in chromatogram of Figure 6C indicates that 49.1% of (-)-isoborneol-camphanic ester is converted into



FIGURE 4 Extracted ion chromatograms (EIC) of (R)-(+)-MTPA-Cl derivatives of borneols and isoborneols: A, (+)-borneol standard; B, (-)-borneol standard; C, (-)-isoborneol standard; D, racemic isoborneol standard; E, synthetic borneol. Very partial separation of borneol enantiomers and good separation of isoborneol enantiomers were observed



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FIGURE 5 Gas chromatograms of commercial borneols sold in Taipei. A, "Qing Shan" brand synthetic borneol; B, "Cheng Yi" brand synthetic borneol; C, "Xin Long" brand synthetic borneol; D, common grade natural borneol from Taiwan Tekho Camphor company; and E, special grade natural borneol from Taiwan Tekho Camphor company



FIGURE 6 Extracted ion chromatograms (EIC) of (–)-camphanic chloride derivatives of borneols and isoborneols: A, (+)-borneol standard; B, (–)-borneol standard; D, (+)-isoborneol standard



FIGURE 7 Epimerization between **6** and **5** under GC condition. This phenomenon does not occur between **3** and **1**

(+)-borneol-camphanic ester, and similarly 48.7% of (+)isoborneol-camphanic ester is converted into (-)-borneol-camphanic ester under GC conditions. However, no corresponding epimerization was observed in the case of (R)-(+)-MTPA-Cl derivatives. We have to point out

Energy Diagram-Reaction A

that the optical values in front of the derivatives indicate the original value of each borneol.

3.4 | Calculation of epimerization reactions for MTPA and camphanic acid derivatives

Isoborneol-camphanic ester was epimerized into borneolcamphanic ester, but isoborneol-MPTA ester was not. To address this point, calculation of both epimerization reactions was carried out. As shown in Figure 7, the transition states, TS1 and TS2, were the most likely to occur during the course of the reactions. Based on this assumption, the calculated activation energy by the DFT method for the epimerization of MTPA derivatives of (+)-isoborneol (**3** to **1**, 125.6374 kcal/mol) is about 1.8-fold higher than that of camphanic acid derivatives of (+)-isoborneol (**6** to **5**, 69.8504 kcal/mol). The stability between **6** and **5** is almost equal (Figure 8B, -2.5883 kcal/mol), but not for **3** and **1** in which compound **1** is less stable than **3** (Figure 8A,





Energy Diagram-Reaction B



FIGURE 8 Calculations of the free energy and activation energy in the epimerization reaction of A, (*R*)-MTPA derivatives of (–)-isoborneol; B, (–)-Camphanic acid derivatives of (–)isoborneol

+9.32 kcal/mol). The larger activation energy might explain why the epimerization did not occur from **3** to **1** under GC conditions.

4 | CONCLUSION

The enantiomers of isoborneol can be clearly differentiated from four synthetic borneol isomers by using chiral reagent (R)-(+)-MTPA-Cl in this study. It is noteworthy that (+)-isoborneol is only present in "synthetic borneol," and not in "semi-synthetic borneol." This method is successfully used to examine commercial borneols on the market and could become a standard analytic method to identify commercial borneols. The enantiomers of borneol were only partially separated by the (R)-(+)-MTPA-Cl derivatives, and other chiral reagents might be needed to achieve better separation. We are delighted to find that epimerization from (+)-, (-)-isoborneol-camphanic esters to (-)-, (+)-borneol-camphanic esters occurred. This epimerization is very unusual that either (+)- or (-)isoborneol is assisted by only camphanic acid but not (R)-(+)-MTPA-Cl at 290°C under GC conditions. In accordance with our finding, epimerization from (+)-borneol to (+)-isoborneol was reported previously by using half-sandwich ruthenium catalysts, and the diasteromeric ratio of (+)-borneol to (-)-isoborneol was 2.42 to 1.¹⁸ In contrast, the reactions reported here proceeded in the absence of inorganic catalyst.

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