JOURNAL OF FOOD AND DRUG ANALYSIS 26 (2018) 348–352

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Investigation of borneols sold in Taiwan by chiral gas chromatography

Tsung-Jung Ho [a,b,c], *Chien-Che Hung* [d], *Tzenge-Lien Shih* [e], *Lih-Ming Yiin* [d,*], *Hao-Ping Chen* [f,*]

[a] *Division of Chinese Medicine, China Medical University Beigang Hospital, Yulin, Taiwan*
[b] *College of Chinese Medicine, China Medical University, Taichung, Taiwan*
[c] *Division of Chinese Medicine, An Nan Hospital, China Medical University, Tainan, Taiwan*
[d] *Department of Public Health and Institute of Medical Science, School of Medicine, Tzu Chi University, Hualien, Taiwan*
[e] *Department of Chemistry, Tamkang University, Taipei, Taiwan*
[f] *Department of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan*

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form
19 October 2016
Accepted 19 October 2016
Available online 1 December 2016

Keywords:
borneol
isoborneol
natural borneol

ABSTRACT

Borneol is a monoterpene that is widely used in traditional Chinese medicine. There are two different products sold in Taipei's traditional Chinese medicine market, natural and chemically synthesized borneol. Chemically synthesized borneol contains four stereoisomers, (+)-isoborneol, (−)-isoborneol, (−)-borneol, and (+)-borneol. The ratio of these four isomers in chemically synthesized and natural borneol products was determined by gas chromatography mass spectrometry. A huge variation between these products is highlighted in this survey. The results suggest that the Food and Drug Administrations in Asian countries should establish a regulatory standard regarding the ratio of the four different borneol isomers in both natural and chemically synthesized borneol.

1. Introduction

In traditional Chinese medicine, borneol is used in herbal formulas for alleviating dermal itching and pain [1]. There are two different natural borneol products sold in the market, (+)-borneol and its optical isomer (−)-borneol; both of which have been shown to work on γ-aminobutyric acid receptors [1,2]. The former can be extracted from the resin and essential oil of woody plants of the families Dipterocarpaceae, Lamiaceae, Valerianaceae and Asteraceae [3], and (−)-borneol can be obtained from the herbaceous plant *Blumea balsamifera* [4]. Recent pharmacological studies show that (+)-borneol has anti-inflammatory and neuroprotective effects [5–7]. Animal studies suggest that (−)-borneol has wound-healing and oxidative damage protection activities [8,9]. Borneol can also be chemically synthesized from camphor by reduction methods, but it is not optically pure, containing four different

* *Corresponding authors*. Lih-Ming Yiin, Department of Public Health and Institute of Medical Science, School of Medicine, Tzu Chi University, 701, Section 3, Zhongyang Road, Hualien City 970, Taiwan. Hao-Ping Chen, Department of Biochemistry, School of Medicine, Tzu Chi University, 701, Section 3, Zhongyang Road, Hualien City 970, Taiwan.
E-mail addresses: lmyiin@mail.tcu.edu.tw (L.-M. Yiin), hpchen@mail.tcu.edu.tw (H.-P. Chen).
http://dx.doi.org/10.1016/j.jfda.2016.10.012

stereoisomers, (+)-isoborneol, (−)-isoborneol, (−)-borneol, and (+)-borneol (Figure 1).

Both natural and chemically synthesized borneol are currently used in traditional Chinese medicine. However, the difference in the retail prices between them is huge. (+)-Borneol is mainly obtained from the endangered woody plant, *Dipterocarpus turbinatus*, which is also an important source for the production of aromatic essential oil. Because of its slow growth rate, the price of natural (+)-borneol is the highest among all borneol isomers. It is noteworthy that the white crystal containing (+)-borneol from *D. turbinatus* should be the form in the prescription of ancient traditional Chinese medicine. More recently, (−)-borneol has been found within the perennial herbaceous plant *B. balsamifera*. This optical isomer, found in some formulations of current Chinese medicine, is also administered for external use to alleviate itching and pain. The harvest time of this perennial herbaceous plant is shorter than that of the woody plant, and therefore the price of natural (−)-borneol is lower than that of (+)-borneol. For example, the prices listed on the Sigma−Aldrich (St. Louis, MO, USA) web page for (+)- and (−)-borneol are US$118 per gram and US$122 per 100 g, respectively. By contrast, the retail price of chemically synthesized borneol is about US$30 per kg. It might not be surprising to find that the relationship between the price and quality of borneol sold in the traditional Chinese medicine market is uncertain and unreliable. To demonstrate the quality of borneol sold in the market, five different brands of natural borneol and two of chemically synthesized borneol were purchased from traditional Chinese medicine shops in Taipei City and analyzed by chiral gas chromatography (GC) mass spectroscopy.

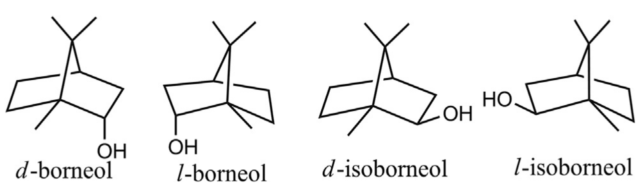

Figure 1 – Chemical structures of borneol stereoisomers. From left to right, (+)-borneol, (−)-borneol, (+)-isoborneol, and (−)-isoborneol.

2. Methods

2.1. Materials

(−)-Borneol and (±)-isoborneol were purchased from Alfa Aesar (Heysham, Lancaster, UK). (+)-Borneol was obtained from Sigma−Aldrich. (+)-Isoborneol was synthesized from (+)-camphor as described previously [10]. A Cydex-B chiral GC column (25 m × 0.22 mm internal diameter; 0.25 μm) was obtained from SGE Analytical Science (Austin, TX, USA).

2.2. GC conditions

Analysis was performed using an Agilent GC system model HP 6890N (Agilent Technologies, Santa Clara, CA, USA) equipped with a split/splitless injector, liner of silanized quartz with a 4 mm internal diameter (effective volume 0.49 mL), and an Agilent 6890 autosampler for 100 vials (Agilent Technologies). Chromatographic conditions were as follows: helium used as a gas carrier; a constant flow of 1.0 mL/min; 2 μL injection volume (splitless model), and a 280°C injector temperature. One milligram of solid borneol was dissolved in 10 mL ethyl ether, and 1 μL of borneol solution was injected into the GC column. The GC temperature program was as follows: 90°C (1 minute), 90–130°C (41 minutes), 130°C (10 minutes), and 200°C (3 minutes). An Agilent 5973N quadrupole mass spectrometer

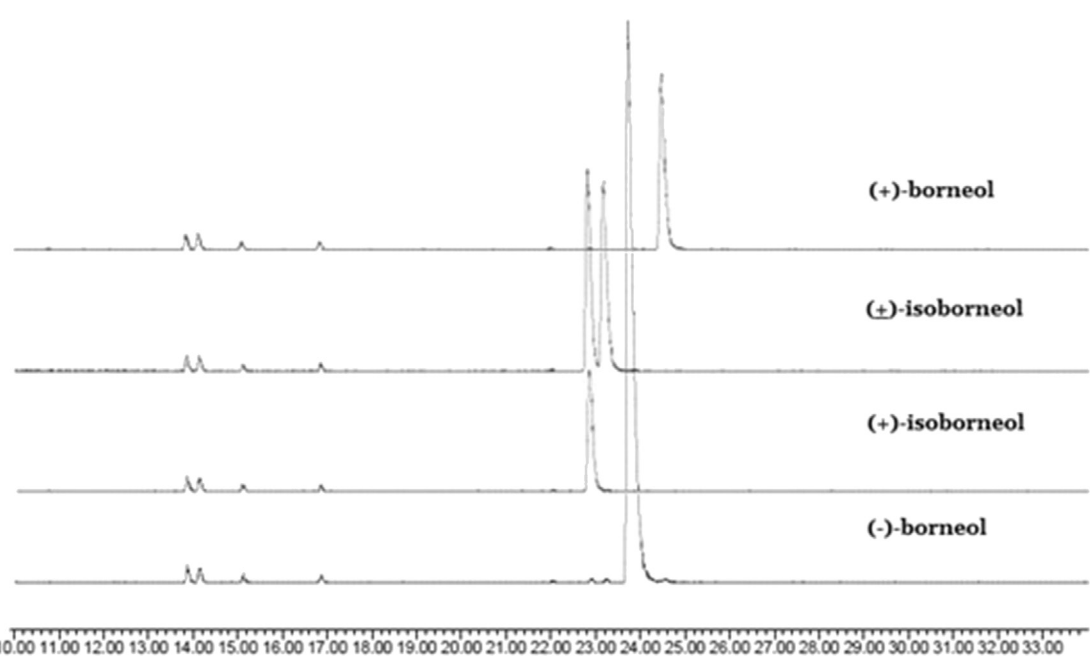

Figure 2 – Gas chromatograms of borneol and isoborneol standards.

Table 1 – Borneol products analyzed in this study.

Brand name	Source	Place of origin	Price (NT dollars)	Package size (g)	Unit price (NT dollars per gram)
A. Qingshan	Synthesis	Taiwan	350	450	0.78
B. Chenyi	Synthesis	Taiwan	389	450	0.86
C. Baishoutang	Natural	Hong Kong	750	150	5
D. Baichangtang	Natural	Hong Kong	800	150	5.3
E. Baochangtang	Natural	Hong Kong	150	37.5	4
F. Generic 1	Natural	Sumatra	1000	75	13.3
G. Generic 2	Natural	Southeast Asia	2500	18.75	133.3

(Agilent Technologies) was operated in selective ion monitoring mode, with ionization source by electron impact at 70 eV, transfer line at 280°C, ion source at 280°C, and quadrupole at 150°C.

3. Results and discussion

3.1. GC separation of borneol and isoborneol standards

The application of cyclodextrin in chiral separation is well established [11]. The Cydex-B chiral GC column employs β-cyclodextrin, consisting of seven glucose units bonded through α-1,4-linkages, as the chiral stationary phase selector. It is specifically designed for separations of chiral or position isomers. The enantiomeric separation of (+)- and (−)-borneol has been reported previously [12]. However, the separation of (+)- and (−)-isoborneol is not included in that study.

(+)-Isoborneol, synthesized as previously described, eluted as a single peak as shown in Figure 2. No other isomer was observed. Figure 2 also shows that the four different borneol stereoisomers were well resolved. The hydroxyl group of isoborneol occupies the less sterically hindered equatorial position. By contrast, borneol has a hydroxyl group in the axial position. Borneol eluted only slightly later than isoborneol, suggesting that the hydroxyl group in the axial position of borneol has stronger affinity with the stationary phase β-cyclodextrin.

3.2. GC analysis of borneol sold in traditional Chinese medicine markets in Taipei

Three different retail borneol products were analyzed in this study: (1) brand-name chemically synthesized borneol; (2) brand-name natural borneol; and (3) generic natural borneol. The details of the seven borneol products are listed in Table 1 and Figure 3.

Figure 4 shows the GC chromatograms of the borneol products analyzed in this study. The corresponding peak area was integrated and used to calculate the ratio of (+)-borneol, (−)-borneol, (+)-isoborneol, and (−)-isoborneol present in the borneol samples (Table 2). It was clear that the ratio of (+)-borneol to (−)-borneol and the ratio of (+)-isoborneol to (−)-isoborneol were close to 1:1 in Samples A and B of chemically synthesized borneol. However, the ratio of (±)-borneol to (±)-isoborneol was not the same between two different chemically synthesized borneol brands.

Three different name brands of natural borneol were analyzed. As shown in Figure 4, it was clear that (+)-borneol was the major component in the Baishoutang brand (Sample C), and (−)-borneol in the Baichangtang brand (Sample D). However, the composition of four different borneol stereoisomers in the Baochangtang brand (Sample E) was almost identical to that of chemically synthesized borneol.

Figure 3 – Chemical synthesized and natural borneol brand-name products sold in Tapei's traditional Chinese medicine market. (A) Qingshan, (B) Chenyi, (C) Baishoutang, (D) Baichangtang, and (E) Baochangtang.

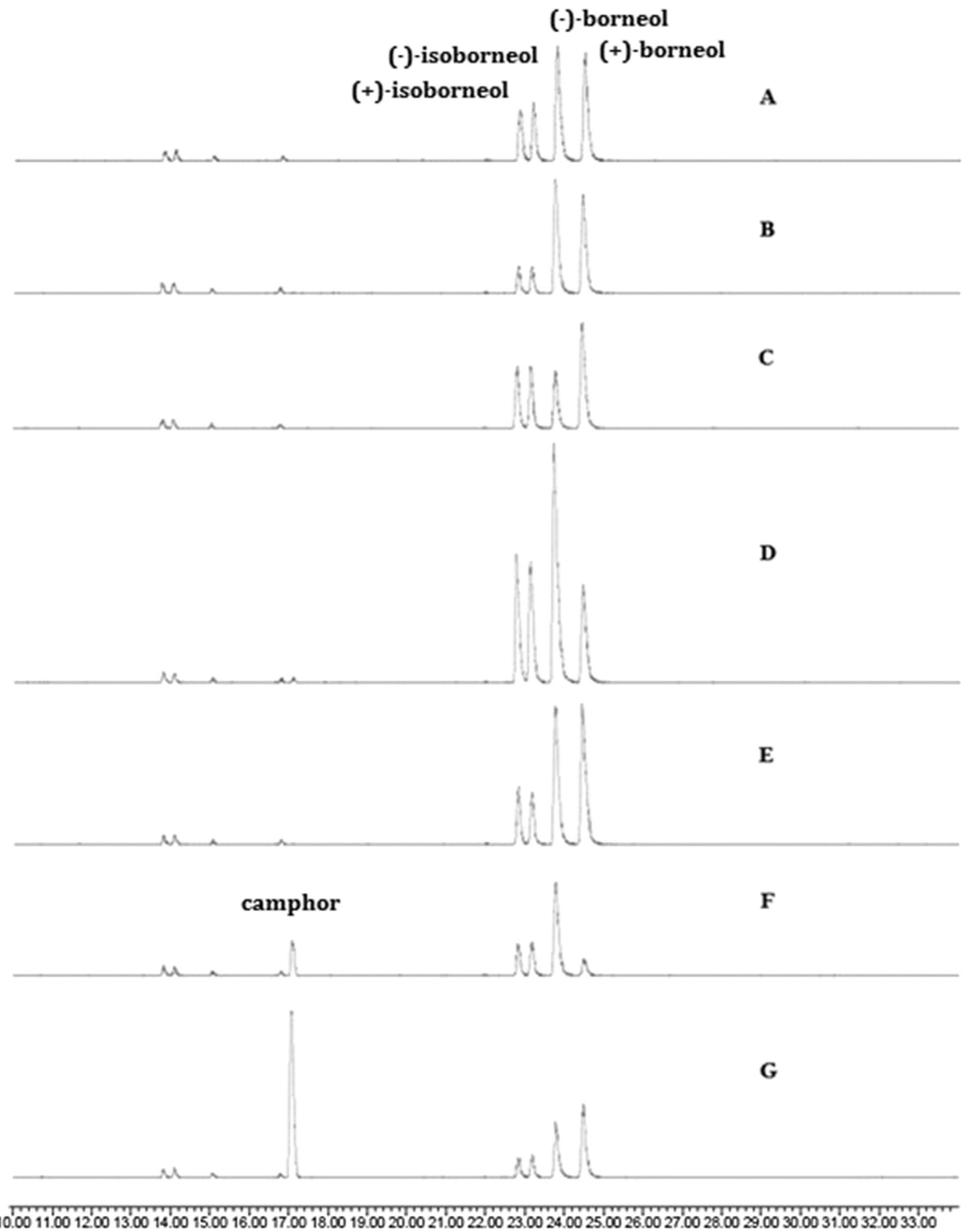

Figure 4 – Gas chromatograms of borneol products sold in Taipei. The four major peaks from 22.0–26.0 minutes are (+)-isoborneol, (–)-isoborneol, (–)-borneol, and (+)-borneol, respectively. The peak appearing at 17.15 minutes is camphor.

Table 2 – Ratio of (+)-borneol, (–)-borneol, (+)-isoborneol, and (–)-isoborneol present in the borneol products analyzed.

Brand name	(+)-borneol	(–)-borneol	(+)-isoborneol	(–)-isoborneol	Camphor:borneol and isoborneol in total
A. Qingshan	34.8	36.1	14.1	15.0	N/A
B. Chenyi	40.2	44.1	7.7	7.9	N/A
C. Baishoutang	41.6	20.1	18.4	19.8	N/A
D. Baichangtang	18.0	45.3	17.7	19.1	N/A
E. Baochangtang	39.7	38.3	10.9	11.1	N/A
F. Generic 1	8.6	59.6	14.7	17.1	1:6.3
G. Generic 2	48.5	32.3	8.8	10.5	1:1.08

Data are presented as %.

Samples F and G were generic natural borneol. Neither of them was well packaged, and they were just stored in bottles in a drug store. The presence of the borneol oxidation product, camphor, was observed. (−)-Borneol was the major product in Sample F, and the amount of (+)-borneol was 15% higher than that of (−)-borneol in Sample G. It is interesting to note that the unit price of Sample G was the highest among all natural borneol products. Accordingly, the percentage of (+)-borneol was also the highest one among them (Table 1 and Figure 4).

3.3. *Conclusion*

Borneol is widely used in traditional Chinese medicine. According to the Pharmacopoeia of the People's Republic of China, isoborneol cannot be detected in natural borneol by the thin layer chromatography method specified by it. Five different natural borneol products sold in Taipei's traditional Chinese medicine stores were examined in this study. In all five samples of natural borneol, the content of (±)-isoborneol was > 20%. In other words, none of them were authentic natural borneol.

In addition, a huge variation in the ratio of borneol isomers was found among all the natural borneol samples examined. Because the chemical structure and source of (+)- and (−)-borneol are different, it is necessary to specify these differences on the packages of those products. Because it has the lowest selling price, chemically synthesized borneol is the major form currently used in traditional Chinese medicine. However, even the ratio of (±)-borneol to (±)-isoborneol significantly varied between the two chemically synthesized borneol products examined. All of these results suggest that the Food and Drug Administrations in Asian countries should establish a regulatory standard regarding the content ratio of the four different borneol isomers in natural and chemically synthesized borneol.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by Grant CMUBH R105-009 from China Medical University Beigang Hospital, Yulin, Taiwan to H.P.C. and J.T.H.

REFERENCES

[1] Granger RE, Campbell EL, Johnston GA. (+)-And (−)-borneol: efficacious positive modulators of GABA action at human recombinant α 1 β 2 γ 2L GABA(A) receptors. Biochem Pharmacol 2005;69:1101–11.

[2] Hall AC, Turcotte CM, Betts BA, Yeung W-Y, Agyeman AS, Burk LA. Modulation of human GABA A and glycine receptor currents by menthol and related monoterpenoids. Eur J Pharmacol 2004;506:9–16.

[3] Chen L, Su J, Li L, Li B, Li W. A new source of natural D-borneol and its characteristic. J Med Plants Res 2011;5:3440–7.

[4] Wang YH, Tian HY, Wang AM, Wang HX, Zou CL. A green and solvent-free process for preparation of high-purity (−)-borneol from leaves of *Blumea balsamifera* (L) DC. Trop J Pharmaceut Res 2014;13:41–6.

[5] Zhong W, Cui Y, Yu Q, Xie X, Liu Y, Wei M, Ci X, Peng L. Modulation of LPS-stimulated pulmonary inflammation by borneol in murine acute lung injury model. Inflammation 2014;37:1148–57.

[6] Liu QS, Pang ZR, Liu R, He GR, Cui J, Yin XY. Effective compounds group of Mongolian prescriptions BAIMAI-SAN protect against peripheralneuropathy in lower limbs of rats through neuro protective effect. J Ethnopharmacol 2011;135:786–91.

[7] Liu R, Zhang L, Lan X, Li L, Zhang TT, Sun JH, Du GH. Protection by borneol on cortical neurons against oxygen-glucose deprivation/reperfusion: involvement of anti-oxidation and anti-inflammation through nuclear transcription factor κ B signaling pathway. Neuroscience 2011;176:408–19.

[8] Barreto RS, Quintans JS, Barreto AS, Albuquerque-Júnior RL, Galvão JG, Gonsalves JK, Nunes RS, Camargo EA, Lucca-Júnior W, Soares RC, Feitosa VL, Quintans-Júnior LJ. Improvement of wound tissue repair by chitosan films containing (−)-borneol, a bicyclic monoterpene alcohol, in rats. Int Wound J 2016;13:799–808.

[9] Horváthová E, Kozics K, Srančíková A, Hunáková L, Gálová E, Ševčovičová A, Slameňová D. Borneol administration protects primary rat hepatocytes against exogenous oxidative DNA damage. Mutagenesis 2012;27:581–8.

[10] Soai K, Ohi K. Stereoselectivity in the reduction of cyclic ketones with sodium borohydride. Effect of the ratio of the components of a mixed solvent of tetrahydrofuran and methanol. Bull Chem Soc Jpn 1985;58:1601–2.

[11] Schurig V. Separation of enantiomers by gas chromatography. J Chromatogr A 2001;906:275–99.

[12] Ravid U, Putievsky E, Katzir I. Stereochemical analysis of borneol in essential oils using permethylated β-cyclodextrin as a chiral stationary phase. Flavour Fragr J 1996;11:191–5.