Evaluating (+)- and (-)- dihydrobovolide in tea flavor.

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Summary

This study was conducted due to sufficient sensory evaluation of enantiomeric isomers (+)- and (-)-dihydrobovolide (3,4-dimethyl-5-pentyl-2(5H)-furanone) for tea infusion. In this study, enantiomer was separated using an HPLC equipped with a chiral column. (+)- and (-)-dihydrobovolide were sniffed to determine odor profiles and threshold levels. The former was a deep green and spicy-herbaceous tea with a note like celery, tobacco, and green tea, and the latter was a light green sweet and spicy-herbaceous tea with a note like celery and green tea. The threshold level of (-)-dihydrobovolide was lower than that of (+)-dihydrobovolide. The quantities of enantiomer in Chinese teas were clarified using a brewed extraction method and GC-MS analysis. The enantiomeric excess of (+)-isomer was a little higher than the (-)-isomer in Yunnan-tuo-tea, Longjing tea, and Darjeeling tea. The optical rotations of (+)- and (-)-dihydrobovolide were respectively $[\alpha]_D$+17.3° and $[\alpha]_D$-17.4°.

Keywords

Dihydrobovolide (3,4-dimethyl-5-pentyl-2(5H)-furanone), Tea aroma, Enantiomer, Chiral, Tea flavor key substance

Introduction

Dihydrobovolide (3,4-dimethyl-5-pentyl-2(5H)-furanone) has a characteristic aroma which reminds us of green tea. It was identified as a flavor component in Ceylon black tea(1), microbial-fermented tea (Toyama-kurocha)(2), Chinese scented green tea(3) and various Chinese teas such as Wuyi-danbing, Da-hong-pao, Longjing tea, and Yunnan-tuo(4). It was identified first in tobacco leaves in 1969(5), and then in many other foods including peppermint oil(6), cooked rice(7), alfalfa(8), bonito(9), beef(10), aged-Nukadoko(11), Rooibos tea(12) and adzuki beans(13). Dihydrobovolide was also reported as a photo-oxidative degradation product in green tea(14) and dried herbs(15). The compound was not noticed as the previous paper reported that dihydrobovolide was an off-flavor component(16). Authors have been paying attention to the compound as the key substance in green tea aroma since flavor research in pan-fired green tea in 1970(17), (18).

Theaspirone was also reported as a key substance in tea aroma(19), but pure theaspirone does not have a tea-like aroma. As theaspirone and dihydrobovolide have almost the same retention time under gas chromatography analysis using wax-column, the authors suppose that was due to dihydrobovolide contamination.

In this research, enantiomeric isomers (+)- and (-)-dihydrobovolide were evaluated by sensory tests. Their threshold levels and optical rotations were determined, and the enantiomeric excess of isomers was observed in tea extracts.

Materials and Methods

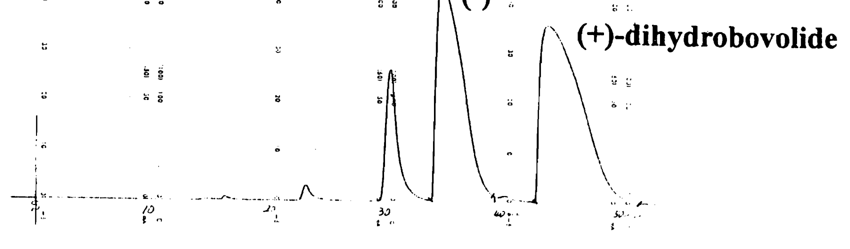
Standard racemic dihydrobovolide was synthesized from ethyl acetate and hexanal using a method with Dutch patent No. 6514140. The racemate was separated by HPLC (Hitachi L-7000) on a chiralpak AS column (20mm i.d.×250mm + gird column 50mm) (Daicel Chem. Co.) eluted with hexane/Isopropanol=95/5(v/v). The system was operated at a flow rate of 5.0ml/min. Column temperature was held at 40°C, and a UV absorbance monitor held at 230 nm. Enantiomers obtained by HPLC separation were used for GC and GC-MS analysis, sensory test, and measuring optical rotation. Optical rotation was measured using a JASCO DIP-370 optical meter equipped with an Na lamp (589 nm) and micro quartz cell (Φ3.5×100 mm), and held at 30°C. The former fraction sample 69.6 mg/2.0 ml hexane and the latter fraction sample 70.2 mg/2.0 ml hexane were held. For GC analysis, a HP5970 gas chromatograph equipped with an FID and a 10 m×0.25 mm i.d. chiraldex G-TA capillary column were used. The oven temperature was held at 120°C, and the helium carrier gas flow rate was 1.8 ml/min (sprit). The injector and detector temperatures were both 200°C. For GC-MS analysis, a HP5972 mass spectrometer interfaced with a HP5890 gas chromatograph was used. The conditions were the same as above. The helium carrier gas flow rate was 1.0 ml/min (sprit-less). The tea aroma concentrates from Yunnan-tuo-tea, Longjing tea, and Darjeeling tea were prepared with a brewed extraction method. One hundred grams of powdered sample was brewed with 700 ml of deionized boiling water for 10 min. After filtration, the filtrate was saturated with NaCl and was extracted by using 200 ml of dichloromethane. The extract was dried over anhydrous sodium sulfate. After filtration, the extract was centrifuged. For concentration, the supernatant was evaporated by a Kunderna-Danish evaporative concentrator.

The sniffing test and judgement of the threshold level were performed by 10 members including 5 flavorists. A sample, 0.01% of (+)- and (-)-dihydrobovolide/triacetin+dipropyleneglycol solution which was diluted with distilled water, and 2 blank samples were tested to determine the threshold level.

Results and Discussion

HPLC chromatogram is shown in Figure 1. The racemic compound was clearly separated into two peaks, (+)- and (-)-dihydrobovolide by HPLC and GC analyses. From the results, optical rotation of (+)- and (-)- dihydrobovolide were respectively $[\alpha]_D$+17.3° and $[\alpha]_D$-17.4°. The order of the fractions order was (-)- and (+)-enantiomer by HPLC analysis, but GC analysis showed that the retention order was (+)- and (-)-enantiomer. The threshold levels were 3.7 ppm of (+)-enantiomer and 1.6 ppm of (-)-enantiomer. The threshold level of (-)-dihydrobovolide was lower than that of (+)-dihydrobovolide. As for evaluation of the aroma profile, (+)-enantiomer is a deep green and metallic spicy-herbaceous tea with a note like celery, tobacco, and green tea, and (-)-enantiomer is a light green sweet and spicy-herbaceous tea with a note like celery and green tea. Both enantiomers seemed to be the most important key compounds for tea flavor. Only a small amount of dihydrobovolide can be imagined to be a green tea.

Mass and IR spectra of synthetic dihydrobovolide are shown in Figure 2. Gas chromatogram and two types of Ion chromatograms of Yunnan-tuo-tea extract and mass spectrum of peak A are shown in Figure 3.



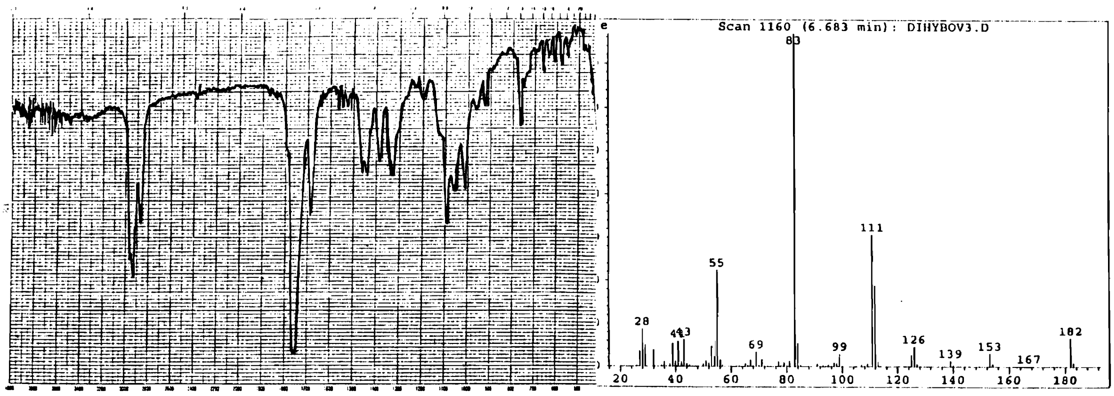
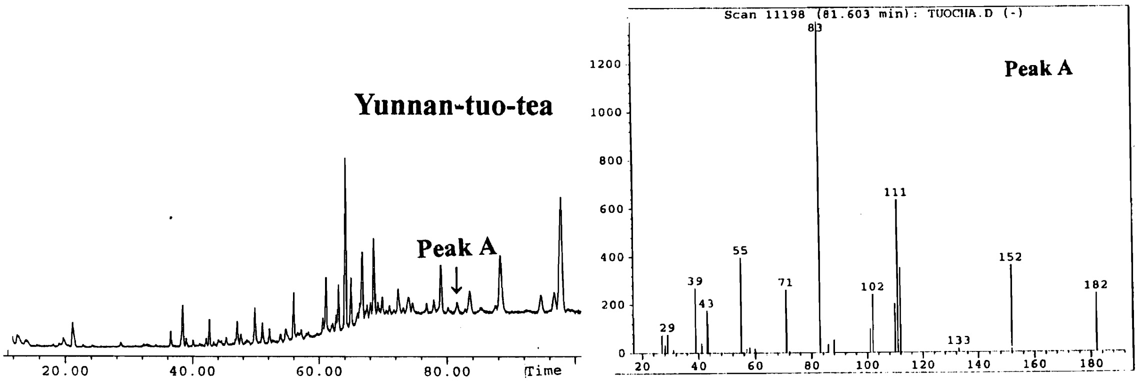
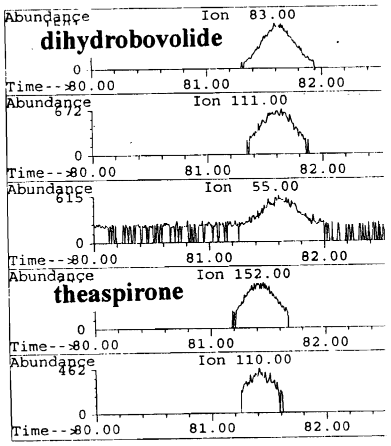


Figure 2. Mass and IR spectra of dihydrobovolide (synthetic).



Ion Chromatogram I (Peak A)



Ion Cromatogram II

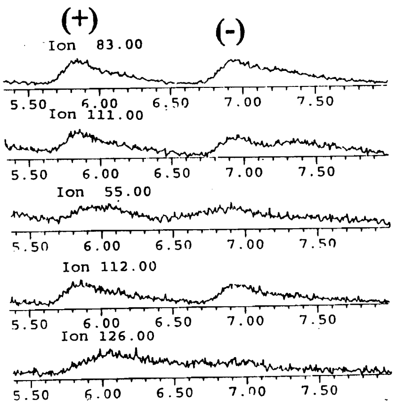


Figure 3. Gas Chromatogram of aroma extract of Yunnan-tuo-tea, Mass spectrum(Peak A), and Ion Chromatogram I (Peak A).: HP-wax 60m×0.25mm. 60℃→ 2℃/min→190℃. Sprit-less. Ion Cromatogram II.: chiraldex G-TA, 10m×0.25mm. 120℃, 1.8ml/min. Sprit.

Table 1. Enantiomeric excess of (+)- & (-)-dihydrobovolide isomers(%)

	(+)-isomer	(-)-isomer
Yunnan-tuo(雲南沱茶)	53	47
Longjing tea(龍井茶)	57	43
Darjeeling tea	59	41

* Calculated by peak height of ion chromatogram

Ion Chromatogram I shows that Peak A consists of 2 compounds, dihydrobovolide and theaspirone. Ion Chromatogram II shows that the retention time of (+)- and (-)-dihydrobovolide are respectively 5.85 min. and 6.90 min.. The ratio of (+)- and (-)-enantiomer is shown in Table 1. Three samples showed almost the same results. Enantiomeric excess of (+)-isomer was a little higher than that of (-)-isomer in Yunnan-tuo-tea, Longjing tea, and Darjeeling tea. On the formation mechanisms, it was reported that dihydrobovolide was a photooxidative degradation product from precursor, fatty acid. Since the enantiomer excess is not that large, it seems the enzyme does not participate in the reaction.

References

1) T. Yamanishi, M. Kawatsu, T. Yokoyama, and Y. Nakatani, Methyl jasmonate and lactones including jasmine lactone in Ceylon tea. *Agric. Biol. Chem.*, **37**, 1075-1078 (1973).

2) M. Kawakami, and T. Shibamoto, The volatile constituents of piled tea: Toyama-kurocha. *Agri Biol. Chem.*, **55**, 1839-1847 (1991).

3) T. Yamanishi, S. J. Luo, W. F. Guo, and A. Kobayashi, Aroma of Chinese scented green tea. *Food Sci.* **17**, 181-190 (1988).

4) M. Kawakami, and S. J. Luo, Aroma composition of various Chinese teas using a brewed extraction method. J. *Ibaraki Christian Univ.* **34**, 88-99 (2000).

5) H. Kaneko, and M. Mita, Isolation from cigar tobacco leaves of 2,3-dimethyl-4-hydroxy-2-nonenoic acid lactone. *Agric. Biol. Chem.*, **33**, 1526 (1969).

6) I. Sakata, T. Hashizume, Constituents of Japanese peppermint oil. *Agric Biol. Chem.*,**37**, 2441-2442 (1973).

7) I. Yajima, T. Yanai, M. Nakamura, H. Sakakibara, and T. Habu, Volatile flavor components of cooked rice. *Agric Biol. Chem.*, **42**, 1229-1233 (1978).

8) T. Kami, Composition of the essential oil of alfalfa. *J. Agric. Food Chem.*, **31**, 38-41 (1983).

9) I. Yajima, M. Nakamura, H. Sakakibara, J. Ide, T. Yanai, and K. Hayashi, Volatile flavor components of dried bonito (katsuobushi). *Agric Biol. Chem.*, **47**, 1755-1760 (1983).

10) G. MacLeod, Food Chem., Identification of bovolide and dihydrobovolide in beef. *Food Chem.* **40**, 113-117 (1991).

11) M. Imai, A. Sato, and H. Ishii, Study on the volatile components of 130-year-aged "Nukadoko" for pickling. *Agric. Biol. Chem.*, **55**, 2209-2220 (1991).

12) M. Kawakami, A. Kobayashi, and K. Kator, Volatile constituents of Rooibos tea (*Aspalathus linearis*) as affected by extraction process. *J. Agric Food Chem.*, **41**, 633-636 (1993).

13) Y. Tokitomo, The formation of odor components of cooked adzuki beans. *Mem.Fac.Lib.Arts Educ.*, **39**, 164-1649 (1988).

14) H. Horita, T. Hara, A. Sannai, and T. Fujimori, The light-produced volatile components of green tea. *Agric. Biol. Chem.*, **49**, 3601-3603 (1985).

15) I. A. Sigrist, G. G. G. Manzardo, R. Amado, Furan fatty acid photooxidative degradation products in dried herbs and vegetables. *J. Food Sci.*, **18**, 17-19 (2000).

16) H. Horita, Off-flavor components of green tea during preservation. *JARQ*, **21**, 192-197 (1987).

17) T. Yamanishi, M. Nose (Kawakami), and Y. Nakatani, Further investigation of flavor constituents in manufactured green tea. *Agric. Biol. Chem.*, **34**, 599-608 (1970).

18) M. Nose (Kawakami), Y. Nakatani, and T. Yamanishi, Identification and composition of intermediate and high boiling constituents in green tea flavor. *Agric. Biol. Chem.*, **35**, 261-271 (1971).

19) K. Ina, Y. Sakato, and H. Hukami, *Tetrahedron Lett.*, 23, 2777 (1968).