ELSEVIER

Food Chemistry 78 (2002) 163–166

Food Chemistry

www.elsevier.com/locate/foodchem

Identification of 2-acetylpyridine in Xiangjing-8618 rice and in Yahonkaoluo leaves

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Received 21 August 2001; received in revised form 21 November 2001; accepted 21 November 2001

Abstract

2-Acetylpyridine was established, for the first time, as the characteristic aroma compound of two plants, Yahonkaoluo leaves (a spice, *Acanthaceae strobilanthus* sp.), wild in Mengla county of Yunnan province of China and Xiangjing-8618 rice (a scented rice, *Oryza sativa* L.), cultivated in the south of Jiangsu province of China, by means of ethylene glycol pre-extraction, porapak Q trapping, GC–MS identification, sniffing technique after capillary column and verification by authentic compound. Ethylene glycol, as a pre-extracting solvent, efficently extracted a micro-basic fraction containing the "scented rice"-like aroma compound in Yahonkaoluo leaves. © 2002 Published by Elsevier Science Ltd.

Keywords: 2-Acetylpyridine; Scented rice; Spice; Aroma

1. Introduction

Over the past 20 years many articles analysing "scented rice"-like aroma compounds of plants have been published. Yajima, Yanai, Nakamura, Sakakibara, and Hayashi (1979) analysed the headspace vapours containing the volatiles of cooked Kaorimai (scented rice, *Oryza sativa* japonica) by cold trapping under reduced pressure, ethyl ether extraction of the condensate, and separating into acidic, basic, and neutral fractions. After GC (100 m long capillary column) and GC–MS, 114 compounds were identified, and though the neutral and basic fractions were small in quantity, they appeared to be relatively important to the characteristic odours. However, characteristic flavour compounds were not found.

2-Acetyl-1-pyrroline, a "popcorn"-like compound, was identified as a key aroma compound in cooked rice (*Oryza sativa* L.), particularly highly aromatic rice of Asia (e.g. Basmati rice), and in Pandan leaves (*Pandanus amaryllifolius* Roxb.), by a 150 m long capillary column, coupled with MS, vacuum steam-distillation continuous extraction apparatus (SDE), and a sniffing technique, after capillary column technique, (Buttery, Juliano, & Ling, 1983; Buttery, Ling, & Juliano, 1982).

2-Acetyl-1-pyrroline was also establised as an important but non-key contributor to the aroma of Xiangjing-8618 (scented rice, *Oryza sativa* L.) cultivated in the south of Jiangsu, China, by Porapak Q trapping of headspace vapours containing the aroma, isolating the basic fraction, sniffing after column and GC–MS identification *(25* m long capillary column; Gu, 1999).

Yahonkaoluo, named by the local Dai community, also called Nuomixiangcao, is a spice, wild in Mengla county of Yunnan province, China, situated near the Sino-Burmese border and emits a "Xiangjing-8618 rice"-like aroma. Owing to its aroma, the leaves are incorporated into common tea to make a local special tea named "Nuomixiangcha" with "scented rice"-like aroma. Up to now, the characteristic aroma compound of Yahonkaoluo leaves and Xiangjing-8618 has not been reported. This work was aimed at identifying the characteristic aroma compound, and trying to find a new method to extract, both efficiently and conveniently, micro-volatile organic nitrogen-containing heterocyclic compounds from the plant.

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0308-8146/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0308-8146(01)00394-6

2. Materials and methods

2.1. Materials

Samples of Yahonkaoluo tender shoots were obtained from a local hilly region (ca. 500 m above sea level) a gloomy, damp valley and shrubbery, at the foot of a limestone hill in May, dried in air, and sent by train to the laboratory.

Xiangjing-8618 rice was purchased from the Seeds Company of Yixin County, and de-husked.

Porapak Q was from Waters Associations, Inc., for gas chromatography column packing material, continuously washed with redistilled diethyl ether as solvent in a Soxhlet apparatus for 12 h before use, after diethyl ether was removed, it was packed into a glass tube (100 × 6 mm) by vacuum, whose ends were filled with silanised glass wool, and stored in an airtight container. Diethyl ether was freshly distilled through a 60 cm long Pyrex helical packed column and stored in an explosion-proof refrigerator.

Authentic 2-acetylpyridine was from Fluka, purum: > 98% (GC).

2.2. Isolation of the volatiles

Air-dried shoots of Yahonkaoluo (500 g) were ground into powder, placed into glassware, and immersed in ethylene glycol for 2 days. The mixture was filtered with four-layer gauze, the filtrate was poured into a two-neck flask (water bath at 50 °C), adjusted to pH10 with sodium hydroxide solution (3.0 M). Airflow (80 ml/min, 4 h) allowed the volatiles to pass through a Porapak Q trap after purification by molecular sieve, activated carbon, and Porapak Q. Redistilled diethyl ether washed the Porapak Q trap (10 ml × 3). The ether solution was extracted with dilute hydrogen chloride solution (1.5 M, 10 ml × 3), the water layer was adjusted to pH10 with sodium hydroxide solution (3.0 M), and extracted with redistilled diethyl ether (10 ml × 3) to get the basic fraction of the volatiles (ca. 30 ml, according to Johnson, Waller, & Burlingame, 1971). The volatiles were divided into neutral, acidic, and basic fractions. The aroma of Yahonkaoluo was found in the basic fraction. The ether solution was kept in an explosion-proof refrigerator (−18 °C, 24 h) for removal of ice, then anhydrous sodium sulfate (2 g) was added for removal of trace water, it was concentrated to 5 ml in a 50 ml flask with Vigreux column (30 × 1 cm i.d.) and evaporated to 50 μl (water bath at 42 °C) in a 5 ml Junk-A tube (Junk et al., 1974).

Six-hundred grams of Xiangjing-8618 rice (× 15, total 9 kg) and 600 ml of water were placed in a two-neck flask (water bath at 100 °C), airflow (100 ml/min, 3 h) took the headspace vapour into Porapak Q trap. Procedure to extract the basic fraction and make sample for analysing was the same as for Yahonkaoluo leaves.

2.3. Capillary GLC–MS

The capillary GLC column was a PEG-20M, 30 m × 0.25 mm i.d., Pyrex glass capillary column, wall-coated with Carbowax 20M, temperature programmed at 2 °C/min from 70 to 170 °C, and then held at this temperature for 25 min. The injector (1/20 split for Yahonkaoluo sample, 1/1 split for Xiangjing-8618 rice sample) temperature was 200 °C and average carrier gas (He) flow velocity was 1 ml/min. The GLC instrument was directly coupled to Finnigan MAT 4610 mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV, the ion source temperature was 200 °C and the filament emission current was 0.25 mA. Kováts' index were calculated against *n*-paraffins as references (Guiochon, 1964; Kováts, 1958).

2.4. Sniffing after capillary column

The apparatus, supplied by the Central Research Institute of Wuxi University of Light Industry, was a modified GC, equipped with controllable electric heater, heat shield covering the apparatus and protecting nose against heat, and sniffing port. The outlet of the capillary column from the GC chamber, passed through the centre of a spiral wire heater, inserted into the sniffing port. The GC, capillary column, and operating conditions were the same as used in the previous section. When analysing the retention times, the odour descriptions of each peak were recorded.

3. Results and discussion

3.1. Characteristic aroma component of Yahonkaoluo leaves

A component (PEG-20M column, peak 15; Kováts'GLC Index 1618) was noted to possess a strong cooked "scented rice" or "popcorn" like aroma, very like that of Yahonkaoluo leaves. The major mass spectrum data (MS/El) at m/z 121 (51%), m/z 93 (36%), m/z 79 (90%), m/z 78 (100%), m/z 52 (49%), m/z 51(68%), m/z 43 (72%), m/z 39 (26%), and the Kováts' Index, were the same as the authentic data.

3.2. Characteristic eroma component of Xiangjing-8618 rice

A component (PEG-20M column, peak 12; Kováts' GLC Index 1618), emitted the characteristic aroma of Xiangjing-8618 rice, the mass spectrum data (MS/El) at m/z 121 (62%), m/z 79 (100%), m/z 78 (92%), m/z 52 (54%), m/z 51 (54%), m/z 43 (98%), and Kováts' GLC Index were identical to the authentic data.

3.3. Bound form of acylpyridine

The leaves (I) growing in the rainy season (April to December) were hardly scented (cell sap pH 5.90–6.10); both the leaves (II) growing in winter (January to March) and air-dried leaves (III) in the rainy season can emit stronger "scented rice"-like aroma (cell sap pH 6.60–6.80). The volatile oil, isolated from any one of the above three types of leaves (ground; 500 g each), using simultaneous steam-distillation/solvent extraction apparatus (SDE) and the same concentrating and analysing procedures as mentioned above, contained too little acetylpyridine to be tested with GC–MS (cf., ethylene glycol extraction method above). Owing to the warm and humid climate (April to December) in Mengla lying in border of the tropics and the subtropics, Yahonkaoluo leaves were growing well, synthesizing organic acids (e.g. tartaric acid, citric acid, malic acid, etc.) which led the pH of the cell sap to drop. These weak acids reacted with weak organic bases (e.g. 2-acetylpyridine; Klingsberg, 1960) to form unstable organic salts, which decomposed once the pH of the cell sap rose to release free organic bases. Larger amounts of acetylpyridine exist as bound form in the leaves.

3.4. Solvents of extraction

Common solvents' solubilizing powers for organic nitrogen-containing heterocyclic compounds are in the order: chloroform > propanone > alcohols > ethyl acetate > diethyl ether > benzene > hexane. Due to their polarity and ability to form hydrogen bonds, alcoholic solvents extract both free and bound acetylpyridine. Among these alcohols, ethylene glycol is suitable for extracting volatile nitrogen-containing heterocyclic aroma compounds because of its odourlessness, high boiling point (198 °C), moderate viscosity (19.9×10^{-3} Pa s, 20 °C), strong penetration into plant tissue, lack of adsorbability by Porapak Q, and poor solubility in diethyl ether. Extracted from only 500 g of the leaves, the volatile oil can be used for the identification of acetylpyridine. In general, nitrogen-containing heterocyclic aromas of plants are low in concentration (ppm or ppb) but high in odour threshold, and mixed with a large number of other volatiles, so an efficient and selective extraction solvent is very important for isolation.

3.5. Adsorbent

If Porapak Q adsorbent was replaced by Tenax GC, the volatile oil isolated was not used to identify acylpyridines. Tenax GC {poly [P- (2,6-diphenyl)phenylene oxide]}, has a weaker acidity due to its phenol-like structure; at weakly bonds with basic acylpyridines in headspace vapour so that the acylpyridines are not washed away from Tenax GC adsorbent. (Sakodynskii, Palina, & Kliniskaya, 1974). Porapak Q (poly ethyl vinyl benzene/divinylbenzene), which has a larger adsorbing capacity and wider scope, is a better adsorbent for volatile pyridine derivatives (Butler & Burke, 1976).

4. Conclusions

2-Acetylpyridine was identified as the characteristic aroma compound of Yahonkaoluo leaves and Xiangjing-8618, in the basic fraction of the volatile oil. Ethylene glycol, as a preextracting solvent, efficiently extracted the basic fraction of the volatiles. Porapak Q adsorbent is suitable for the trapping volatile micro-basic fraction in the headspace vapour. 2-Acetylpyridine is related to a group of "popcorn-like" odour compounds which includes 2-acetyl-1,4,5,6-tetrahydropyridine, 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, 2-propionyl-3,4,5,6-tetrahydropyridine (Hofmann & Schieberle, 1998), 2-acetylpyrazine, 2-acetyl-2-thiazoline (Cerny & Grosch, 1992), 2-acetylthiazole, 5-acetyl-2,3-dihydro-1,4-thiazine and 5-propionyl-2,3-dihydro-1,4-thiazine (Hofmann, Hassner, & Schieberle, 1995), 2-propionylpyridine (Gu et al., 1996).

Acknowledgements

I thank Professor TangJian of the Central Research Institute, Wuxi University of Light Industry, for helpful discussion and some instrument modification, and also thank Professor Wu Weisong of Yunnan Tropical Crops Institute, for help in collecting Yahonkaoluo leaves. The samples analysis was done in the Central Research Institute, Wuxi University of Light Industry, Ministry of Light Industry, PR China.

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