Vaginal Pheromone and Other Compounds in Mung-Bean Aroma

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This paper reports probably a first attempt to identify the molecules which impart a most desirable, characteristic aroma to certain varieties of mung bean of Bengal — a valuable indigenous natural product that may be on the verge of extinction. In boiled, or fried mung bean of the two best strains, strong evidence for the presence of 2-acetyl-1-pyrroline, the molecule responsible for the aroma of Basmati rice1 and one component of tiger pheromone, has been obtained. GCMS analysis and computerized library search further suggested the presence of six volatile compounds in the CCl4 extracts of even the second grade mung bean samples. Two of these were a male-attracting vaginal pheromone and a puberty-delaying pheromone in rodents.

Introduction

Mung bean, Vigna radiata, is of considerable commercial interest in India. Certain strains of unusually good aroma-bearing mung bean (*sona cultivar) were famous in Bengal. Unfortunately, these are rapidly facing extinction or may have already vanished.

From the scientific point of view the aroma components deserve to be analysed or identified. As is well-known, 2-acetyl-1-pyrroline (2AP), the single largest component of basmati rice aroma was identified1 in 1982. It was then traced in Pandanus foetodes2 and then in the tiger pheromone3,5 and Melissa indica/Bassia latifolia flower6 as well. The good mung bean aroma may well be due to some interesting, little known chemicals. Several varieties of mung bean were therefore collected and subjected to chemical analysis. As the aroma components are produced by boiling and frying, heat-dependent Maillard reaction7 was taken into consideration.

With such a perspective the possibility of N-containing molecules (such as 2 AP) was explored. Because of two consecutive yearly floods the best quality mung bean was not available for GC-MS analysis after preliminary investigations, yet certain interesting aroma compounds were detected even in the second-grade samples such as those used in this study.

Materials and Methods

Materials

Several mung bean samples were collected from sources at the point of cultivation the most well-known of which is the *sona moong of Malda; another nearly equally good strain was also available at Lalgora, both in West Bengal. Other samples were purchased from local market in Calcutta.

Extraction and Separation of Aroma Components

Boiling of fried, or non-fried mung seeds was followed by collection of steam distillate and this was shaken with ether, hexane and carbon tetrachloride to extract aroma components in these respective solvents. In preliminary experiments CCl4 appeared
advantageous, therefore, spectroscopic grade CCl₄ was used in subsequent experiments.

Tests for the Aroma Components

Some aliquots of steam distillate were treated with hydrochloric, citric, and picric acids, and tested for disappearance of aroma; and then again treated with alkali for reappearance, if any, of the aroma.

Aqueous and organic solvent-extracts of aroma were treated with NaO₂ or KIO₄ to detect the loss of aroma, if any. Likewise, tonsil powder was also used.

The aqueous aroma-hydrochloride, or citrate was air dried under fan in a cold room and concentrated; the citrate sample was further concentrated by partial lyophilisation.

Paper Chromatographic Separation and Identification of Aroma Impact Components

Aqueous aroma salts were run in paper chromatograms with solvent systems like n-butanol-acetic acid-water, ethanol-acetic acid-water (4:1:1), and water saturated butanol. The position of the aroma-salt in chromatograms was determined after cutting out the paper into several pieces, moistening with alkali, and sniffling. In addition, at least two (out of a panel of five) unbiased sniffers (who were not told which pieces were expected to bear the aroma) were asked for their opinion. In every case, all of them clearly identified the same pieces. Pair-wise and triple sample comparisons were made in paper chromatograms, spotting samples of mung bean aroma citrate, 2-acetyl-1-pyrroline-citrate, Bassia flower aroma-citrate and Pandanus foetzicus aroma-citrate (2 tests with mung and Pandanus aroma, 2 with mung and standard 2 AP, 3 with mung and Bassia, one comparison with 3 aromas, each, namely mung, 2 AP and Bassia).

GC and GC-MS Studies

GLC (Hewlett Packard 5890) analysis was carried out with 20 per cent squalane column at a temperature of 65 and 90°C with oven and FID temperature at 150°C. Anhydrous sodium sulfate-treated CCl₄ extract was used for GC-MS.

GC-MS (Fison’s Instrument) analysis was executed in a DB 5 column (column temperature 40°C), 1 min holding to 60°C at 5°C/min. total run time 30 min. Library search and matching of molecular ion fragments was effected with the help of a computerised library. A blank was run with CCl₄ for comparison.

Results

Of all the mung bean samples tested by boiling and frying, namely from Bihar and Malda, Lalgola, Ranaghat, (all three in West Bengal) and several packets purchased from local market in Calcutta, the most pleasant aroma was that of Malda and Lalgola samples. The aqueous extract of the aroma was treated with diethyl ether, hexane and carbon tetrachloride. The turbid material, especially after crushing mung bean seeds in a blender, as well as pigments contaminated some of the solvents, though the aroma or at least a part of it was transferred to them. Steam distillate of the material collected in CCl₄ turned out to be a satisfactory technique. The lower CCl₄ phase contained aroma components and it was not contaminated with pigments.

Malda and Lalgola strains treated with HCl immediately produced an altered tone of aroma, namely that of sweet-sour, unripe mango. It happened with Bihar and Ranaghat samples also. The acid-water, spotted on filter paper and rendered alkaline with NaOH or KOH, yielded the mung bean smell again. Citric acid or picric acid also led to loss of aroma that reappeared on treatment with alkalis. When NaO₂ or KIO₄ was added to the aroma extract, they very quickly destroyed the aroma, as did the treatment with tonsill.

An aliquot of the steam distillate of Malda and Lalgola strains of mung bean concentrated by air drying and then by partial lyophilisation was spotted on paper and rendered alkaline; it emitted a very characteristic, intense aroma, resembling very concentrated 2-AP or the sweet, fresh smell of the just husked indigenous rice varieties. With passage of time, the quality of smell altered in a manner exactly mimicking 2-AP, i.e. changing from sweet to the racy tone. Spotted on Whatman paper 3 in acidic condition (2% acetic acid) and developed in the three solvent systems (methanol : acetic acid : water :: 4 : 1 : 1; n-butanol : acetic acid : water :: 4 : 1 : 1, v/v/v) and water-saturated butanol, the aroma material could be isolated in a specific region by sniffing the pieces of chromatogram cut out in a consecutive series and rendered alkaline. After preliminary results obtained with samples from both Malda and Lalgola, detailed
Figure 1—Comparison of the three chromatograms run in water saturated butanol, B—Mung bean, 2 AP—2 acetyl-1-pyrroline, M—Maduca (bassia) flower; +--indicates smell, ++--indicates strong smell.

study was carried out with samples from the latter. The chromatograms shown are based on this material. In all the three solvents, the Rf values of this region were very close to those of the standard 2 AP and Maduca indica/Bassia latifolia flower extract. Figure 1 shows the three chromatograms run in water saturated butanol, the amount spotted was relatively large. But a narrow strip above the base line was aromaless. A smaller amount and a longer run (Figure 2) resulted into a sharper segregation of aroma material.

Figure 3 shows the comparative chromatograms of Mung bean and Maduca flower aroma run in n-butanol : acetic acid : water (4 : 1 : 1, v/v). Pandanus foetidus/amaryllis leaf extracts in citrate water was also compared in the same PC experiments. Standard 2 AP, P. foetidus, Maduca flower and mung bean aroma (of two strains) Malda and Lalgola were seen to have the same Rf value.

On injecting 2-3 μL CCl₄ extract of ordinary quality mung bean (from Cacutta) into 20 per cent squalane column in GLC experiments at column temperature of 65 and 90 °C, four peaks were recorded with Rf less than that of the CCl₄.

CCl₄ extracted aroma was subjected to GCMS analysis. DB 1 and 2 and DB 5 columns were tried and only DB 5 clearly separated several aroma components — six of which were good peaks. Computerised library search suggested the following identifications (Table 1).
Table 1—The aroma compounds of second grade Mung bean as indicated by GCMS.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>m/e</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108.9</td>
<td>2-5, dimethyl pyrazine</td>
</tr>
<tr>
<td>2</td>
<td>122.0</td>
<td>2-ethyl-5-methyl pyrazine</td>
</tr>
<tr>
<td>3</td>
<td>136.4</td>
<td>2-ethyl-3, 6-di-methyl pyrazine</td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td>2-ethyl-3, 6-di-methylamino ethanol</td>
</tr>
<tr>
<td>5</td>
<td>94.02</td>
<td>dimethyl disulphide</td>
</tr>
</tbody>
</table>

Additional 6, benzaldehyde

* See discussion. Compounds (1-4) were not available, and therefore, these could not be used as standards.

Discussion

Several indirect evidences, viz., periododate treatment immediately removing the aroma, loss of aroma following tonsil powder treatment (which absorbs N-containing molecules), loss of aroma on acidification and then its reappearance on alkali-treatment, extremely sweet, utterly non-mung and 2 AP-like aroma of concentrated aqueous citrate extract, nature of decay-dependent alteration of the aroma tone (from extreme sweet to ricy), and a number of comparative PC runs with standard 2AP and P. foetida/Amaryllitoifolius and Madhuca indica/Bassia latifolia aroma in different solvents strongly suggested that the fragrance of mung beans of Malda and Lalgola was at least partly due to 2AP. This apparently occurred in a much lesser quantity, if at all, in the second grade mung bean samples examined. In some indigenous fragrant rice varieties, characteristic aroma is perceptible before boiling, even in the ripening or pre-ripened stage. But rice, on boiling, again yields aroma due to Maillard reaction. Unboiled rice or even rice plant may develop the aroma either through an enzymatic reaction or Maillard reaction which occurs at a slower rate at a lower temperature but catches up over time. The biosynthetic pathway of 2 AP in the rice plant is not known, but in mung bean there was no aroma before boiling or frying and so it was most likely caused by the Maillard reaction. Some other aroma compounds were also absorbed by tonsil, suggesting the presence of N-atom(s) containing components.

After removing the putative 2AP as citrate or some other salt, another component of the fragrance, sweet-sour-mango smell, very unlike mung bean aroma, was perceptible. Apart from the Malda and Lalgola samples, this was so to a lesser degree with two samples of other strains obtained from West Bengal and Bihar. All these samples were not available again, therefore, the further study could not be carried out. Since 2AP has already been detected in many other vegetable sources, the interest was focused on other aroma molecules. GLC on 20 per cent squalane showed that the first grade mung bean aroma component has also more than one molecule, even apart from the sweet-sour mango smell.

Figure 1—Comparison of Mung bean and Madhuca flower aroma run in isobutanol-acetic acid-water (4:1:1). B—Mung bean, M—Madhuca (Bassia) flower. +—indicates presence of smell.
GCMS study of a second grade sample (from Calcutta) was attempted after successful separation with CCl₄ from the aqueous fraction remained as a clear phase which did not contain any pigment. DB 5 column is suitable for separating the aroma molecules and this indeed, was borne out by the trials with DB 1, 2 and 5 columns.

Of the molecules identified by GCMS analysis (Table 1), only benzaldehyde and dimethyl disulfide are well-known. Others were not available in the market, and inquiry at Alfred Bader library, Aldrich, also failed to elicit any further information. Dimethyl disulfide was also difficult to procure in Calcutta, and this indeed, was borne out by the trials with DB 1, 2 and 5 columns. Of the molecules identified by GCMS analysis (Table 1), only benzaldehyde and dimethyl disulfide are well-known. Others were not available in the market, and inquiry at Alfred Bader library, Aldrich, also failed to elicit any further information. Dimethyl disulfide was also difficult to procure in Calcutta, and this indeed, was borne out by the trials with DB 1, 2 and 5 columns.

The compound, is a possible Strecker degradation product of cooking rice, an important aroma component of cooking rice, Chem Ind, (1982) 958.

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The presence of these mammalian pheromones in boiled/fried mung bean is apparently co-incident products of the high-temperature Maillard reaction. The presence of a mammalian pheromone in unboiled vegetables has earlier been reported.

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As for 2-phenylmethylaminoethanol, mw 151. (Table 1) the m/e is 120 because the terminal-CH₂OH (mw = 31) immediately breaks away. The compound may be helpful against bacterial and fungal attacks. It is customary to store certain mung beans after frying. Dehydration coupled with 2-phenylmethyl-

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References