# *Analysis of the Volatile Constituents of Hamanasu Absolute Oil*

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Previously[1] three of the present authors presented detailed analytical data on the volatile constituents of commercial Hamanasu flower absolute oil and predicted the presence of a minute quantity of a component which is thought to be essential for the floral note of the Hamanasu flower. This report will present some additional data and observations which have been obtained in preliminary attempts to separate this characteristic ingredient.

## Experimental

**Preliminary Separation of the Materials.** — To remove unnecessary non-volatile fractions, 100 g. of commercial Hamanasu absolute oil was steam-distilled; 65 g. of volatile oil was thereby obtained. Then, by treating this volatile oil three times with 5 g. of the Girard T reagent, 30 mg. of the carbonyl fraction was separated. As has been reported, the volatile fraction of Hamanasu absolute oil is composed mainly of phenylethyl alcohol, and it was desirable to separate the gross part of it in order to facilitate the further fractionation of minute components. For this purpose, a mixture of 62 g. of the volatile oil, 100 g. of phthalic anhydride, and 100 cc. of benzene was refluxed for 3 hr. on a water-bath. Acid phthalate and the excess phthalic anhydride were removed from the reaction mixture in the usual way, leaving 7.4 g. of unchanged oil. The same procedure was repeated to give 1.9 g. of an oil which was shown to contain only a few alcoholic compound by measuring its infrared spectrum. Alcoholic compounds regenerated from the acid phthalate by alkaline hydrolysis had a rather simple oder of phenylethyl alcohol and seemed to contain no characteristic ingredients.

**Gas Chromatography.**—Gas chromatography was carried out using a Shimadzu GC-2B type gas chromatograph: column; polyethylene glycol (25%) on celite, 3 m.; temp., 170°C; carrier gas, $N_2$.

**Liquid Chromatography.**—Mallinickrodt's silicic acid was used as the adsorbent, and a hexane solution of ether (3%, 5%, 10%, 30%), as the elute solvent.

**Paper Chromatography.** — 2,4-Dinitrophenylhydrazones were chromatographed, on a filter paper impregnated with *N,N*-dimethylformamide as the stationary phase, by developing it with cyclohexane saturated with *N,N*-dimethylformamide.

## Results and Discussion

The final non-alcoholic oil was subjected to adsorption chromatography and separated into 18 fractions. Each fraction was analyzed by means of gas chromatography and infrared spectroscopy. The compounds identified are shown in Table I.

For illustration, the full composition of the volatile fraction of Hamanasu absolute oil is presented in Table II (in which the dotted items are newly added as a result of this study), together with the composition of Bulgarian rose oil, in which the content of every component was estimated in the authors' laboratory. Carbonyl compounds that could be identified only by paper chromatography are omitted from this table, as they will be discussed in a later part of this report.

It may safely be predicted that the effect of the combination of the constituents of Hamanasu oil is essentially the same as that of Bulgarian rose oil, although the proportions of some ingredients are quite different in these two species of rose. It is curious that there seems to be no organoleptic evaluation of rose oxide in the litrature. The authors reconstituted Hamanasu oil according to their complete analytical data and observed that rose oxide has a marked effect in adding freshness to the floral note. However, some other component is still missing, the floral tone itself; for the time being, this missing component is insufficiently substituted for by ionone or irone.

The carbonyl fraction, separated by the Girard T reagent, was treated with an acidic solution of 2,4-dinitrophenylhydrazine. The products were extracted with chloroform, concentrated to a small volume, and subjected to

1) K. Nishimura, T. Sakai and Y. Hirose, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **83**, 745 (1962).

TABLE I. THE COMPOUNDS NEWLY IDENTIFIED FROM HAMANASU ABSOLUTE OIL

| Compound | Note | Fr. No. |
|---|---|---|
| Hydrocarbon | IR, solid paraffin | 1, 2 |
| Citronellyl acetate | RT,* 9.3 min<br>IR, 8.1 $\mu$ (acetate)<br>span.—citronellol | 3, 4 |
| Neryl acetate | RT, 10.5 min.<br>IR, 8.1 $\mu$ (acetate)<br>sapn.—nerol | 3 |
| Geranyl acetate | RT, 11.5 min.<br>IR, 8.1 $\mu$ (acetate)<br>sapn.—geraniol | 3, 4 |
| Rose oxide | RT, 5.2 min.<br>IR, 7.9, 8.4, 8.5, 9.1, 11.3, 11.9 $\mu$ | 4, 5 |
| Benzyl formate | RT, 14.2 min.<br>IR, 8.6 $\mu$ (formate)<br>sapn.—benzyl alcohol | 5, 6 |
| Phenylethyl acetate | RT, 13.0 min.<br>IR, 8.1 $\mu$ (acetate)<br>sapn.—phenylethyl alcohol | 7,8,9, 10, 11 |
| Eugenol | RT, 33.5 min.<br>IR, 3.0, 6.1, 7.3, 8.7, 8.9, 9.6, 10.9, 12.1, 12.5 $\mu$ | 10, 11, 12 |
| Methyleugenol | RT, 20.6 min.<br>IR, 6.6, 7.9, 8.1, 8.7, 9.7, 10.9, 11.7, 12.3, 13.0 $\mu$ | 12** |

* Gas chromatography, stationary phase: polyethylene glycol on celite; temp.: 180°C; carrier gas: $N_2$.

** Fractions 13 to 18, were mainly phthalic acid diester.

paper chromatography. When the same procedure was followed as with Bulgarian rose oil, it was found that the chromatogram of the carbonyl fraction of Hamanasu oil is very simillar to that of Bulgarian rose oil. These results are shown in Table III.

Every spot of each chromatogram was cut into pieces and extracted with chloroform, and the ultraviolet adsorption of each extract was measured in neutral and basic solutions (Table IV).

When the deterioration of the peak at the end of one hour was observed, all spots except spot No. 2 from Bulgarian rose oil and spots No. 1 to 4 from Hamanasu oil developed an aliphatic aldehyde character. This would account for the well-known fact that lower homologs of aliphatic aldehyde occur frequently

TABLE II. FULL COMPOSITION OF THE VOLATILE FRACTION OF HAMANASU ABSOLUTE OIL AND BULGARIAN ROSE OIL

| Compound | Hamanasu % | Bulgarian rose, % |
|---|---|---|
| Aliphatic hydrocarbon | + | 3.9 |
| *p*-Menth-1-ene | 0.1 | — |
| Myrcene | — | 0.5 |
| Rose oxide | 0.2 | 0.6 |
| Linalool | 2.9 | 3.5 |
| Citronellol | 2.5 | 38.5 |
| Geraniol | 1.5 | 9.6 |
| Nerol | 1.0 | 4.5 |
| Phenylethyl alcohol | 86.3 | 2.3 |
| Benzyl alcohol | 1.1 | ? |
| Nonyl alcohol | + | ? |
| Heptyl alcohol | + | ? |
| Hexyl alcohol | — | + |
| Phenylethyl acetate | 0.3 | ? |
| Benzyl formate | 0.1 | — |
| Citronellyl acetate | 0.2 | 4.2 |
| Geranyl acetate | 0.3 | 0.6 |
| Neryl acetate | 0.1 | + |
| Nonyl aldehyde | + | ? |
| Benzaldehyde | + | ? |
| Citral | — | 0.3 |
| $\gamma$-Nonalactone | + | ? |
| Eugenol | 0.5 | 1.5 |
| Methyleugenol | 0.3 | 4.7 |

TABLE III. THE SPOTS OF PAPER CHROMATOGRAMS

Hamanasu oil

| Spot No. | $R_f$ | Reference compound ($R_f$) |
|---|---|---|
| 1 | 0.18 | Formaldehyde (0.18) |
| 2 | 0.26 | Acetaldehyde (0.26) |
| 3 | 0.29 | Benzaldehyde (0.28) |
| 4 | 0.37 | Phenylacetaldehyde (0.35)<br>Propionaldehyde (0.38)<br>Acetone (0.36) |
| 5 | 0.50 | Ethyl methyl ketone (0.50) |
| 6 | 0.54 | Butyraldehyde (0.54) |
| 7 | 0.62 | Methyl propyl ketone (0.61) |
| 8 | 0.74 | Citral (0.74) |
| 9 | 0.91 | Methyl nonyl ketone (0.92) |

Bulgarian rose oil

| Spot No. | $R_f$ | Reference compound ($R_f$) |
|---|---|---|
| 1 | 0.18 | Formaldehyde (0.18) |
| 2 | 0.26 | Acetaldehyde (0.26) |
| 3 | 0.37 | Phenyacetaldehyde (0.35)<br>Propionaldehyde (0.38)<br>Acetone (0.36) |
| 4 | 0.54 | Butyraldehyde (0.54) |
| 5 | 0.64 | Valeraldehyde (0.63)<br>Methyl propyl ketone (0.61) |
| 6 | 0.70 | Methyl heptenone (0.71)<br>Octyl aldehyde (0.70) |
| 7 | 0.74 | Citral (0.73) |

TABLE IV. UV ABSORPTION SPECTRA OF EACH SPOT OF PAPER CHROMATOGRAM

Hamanasu oil

| Spot No. | Neutral solution $\lambda_{max}$ | Alkaline solution (0.25 N NaOH in EtOH) $\lambda_{max}$ | Optical density ($E$) $t=$ 0 min. | $t=$ 60 min. | $E_{t=60}/E_{t=0}$ |
|---|---|---|---|---|---|
| | mμ | mμ | | | % |
| 1 | 367 | 440 | 0.40 | 0.20 | 50.0 |
| | | 530 | 0.26 | 0.14 | 54.0 |
| 2 | 366 | 444 | 0.65 | 0.59 | 90.8 |
| | | 535 | 0.30 | 0.25 | 83.3 |
| 3 | 368 | 437 | 0.80 | 0.70 | 87.5 |
| | | 540 | 0.48 | 0.30 | 62.5 |
| 4 | 368 | 440 | 0.70 | 0.48 | 68.6 |
| | | 535 | 0.48 | 0.30 | 62.5 |
| 5 | 367 | 438 | 0.65 | 0.64 | 98.4 |
| | | 545 | 0.40 | 0.40 | 100 |
| 6 | 368 | 440 | 0.20 | 0.20 | 100 |
| | | 540 | 0.11 | 0.11 | 100 |
| 7 | 368 | 440 | 0.20 | 0.19 | 95.0 |
| | | — | — | — | — |
| 8 | 368 | 442 | 0.29 | 0.29 | 100 |
| | | 530 | 0.17 | 0.17 | 100 |
| 9 | 367 | 438 | 0.57 | 0.56 | 98.2 |
| | | 540 | 0.26 | 0.26 | 100 |

Bulgarian rose oil

| Spot No. | Neutral solution $\lambda_{max}$ | Alkaline solution (0.25 N NaOH in EtOH) $\lambda_{max}$ | Optical density ($E$) $t=$ 0 min. | $t=$ 60 min. | $E_{t=60}/E_{t=0}$ |
|---|---|---|---|---|---|
| | mμ | mμ | | | % |
| 1 | 356 | 435 | 0.25 | 0.16 | 64.2 |
| | | 525 | 0.10 | 0.03 | 30.0 |
| 2 | 364 | 435 | 0.19 | 0.19 | 100 |
| | | 525 | 0.08 | 0.08 | 100 |
| 3 | 365 | 435 | 0.49 | 0.47 | 96.3 |
| | | 520 | 0.29 | 0.28 | 98.1 |
| 4 | 365 | 440 | 0.13 | 0.09 | 68.4 |
| | | 530 | 0.05 | 0.03 | 60.0 |
| 5 | 364 | 437 | 0.38 | 0.25 | 66.8 |
| | | 530 | 0.21 | 0.11 | 52.8 |
| 6 | 360 | 437 | 0.80 | 0.53 | 65.8 |
| | | 530 | 0.48 | 0.25 | 52.6 |
| 7 | 360 | 437 | 0.63 | 0.42 | 67.7 |
| | | 540 | 0.35 | 0.19 | 53.3 |

in natural products as common ingredients and/or as degradation products in the course of processing.

The aliphatic aldehyde character was not shown by spot No. 2 from Bulgarian rose oil and spot No. 6 from Hamanasu oil, however, in spite of the fact that the $R_f$ values of these spots correspond to those of acetaldehyde and butyraldehyde respectively. Neither spot corresponds to any simple ketones or aromatic aldehydes. After all, these rose oils probably contain some unknown compounds of an $\alpha,\beta$-unsaturated aldehyde or polyfunctional ketone character.

In 1958, Panayotov and Ivanov[2] applied the paper chromatographic technique to the carbonyl fraction isolated by the extraction of Bulgarian rose oil with a sodium bisulfite solution; they thus identified eight components, including cinnamaldehyde, salicylic aldehyde and carvone, by merely referring to the $R_f$ value of each spot. However, it was difficult for the authors of this paper to derive any positive evidence of the presence of those carbonyl compounds in either Bulgarian rose oil or Hamanasu oil, even by taking both liquid and paper chromatographic data into account.

## Summary

The separation of the carbonyl fraction and the non-alcoholic fraction, by the Girard T reagent and phthalic anhydride respectively, from the principal constituent, phenylethyl alcohol, of Hamanasu flower absolute oil, and the successive analyses of each fraction have been carried out in order to elucidate the essential olfactory ingredient of the rose oil. The attempt resulted in adding some new components to those listed in the previous report: rose oxide, citronellyl acetate, geranyl acetate and methyleugenol.

The variety of the components has been proven to be essentially the same as in Bulgarian rose oil, although the proportion differs in some components.

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2) I. Panayotov and D. Ivanov, *Perf. Essent. Oil Record*, **49**, 231 (1958).