Studies on the Essential Oils of Tobacco Leaves

Part X. Acid Fraction in the Essential Oil of the Japanese Flue Cured Tobacco Leaf Before Redrying and Aging.

By Isao ŌNISHI and Kyoji YAMASAKI

Tobacco Research Department, Central Research Institute, Japan Monopoly Corporation

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Acid fraction of the essential oil of Japanese flue cured tobacco leaf, before redrying and aging (BRA), was compared with that of the same leaf after aging (AA) as reported in previous papers^{1,2)}. The organic acids which were isolated and identified in the essential oil of BRA are similar to those of AA, except that the former contains propionic and methyl-ethylacetic acids, which were not found in the latter. While, on the other hand, total volatile acids in AA was amounted to about three times as much as that of BRA, and especially the increase of acetic and phenylacetic acids was remarkable during the process of aging.

In the previous papers we have studied the constituents of each fraction in the essential oil obtained from the Japanese flue cured tobacco leaf, after redrying and aging (AA) for in the course of about one year. The same kind of tobacco leaf, having the same grade, stalk position, growing district, before redrying and aging (BRA) was employed in this study in order to investigate changes of the chemical constituents of the essential oil during the process of aging. Besides, we expected to find whether the change of the chemical constituents in the essential oil would suggest the period suitable for the aging of tobacco leaf after it is packed in hogsheads.

From the acid fraction of the essential oil of Japanese flue cured tobacco leaf BRA, the following acids were isolated and identified; formic, acetic, propionic, α -crotonic, methyl-ethylacetic, *n*-caproic, β -methylvaleric, benzoic and phenylacetic acids.

These organic acids from the essential oil of tobacco leaf BRA are similar to those of AA, except in respect of propionic and me-

thylethylacetic acids which were newly isolated from BRA and acetic and phenylacetic acids contents increased during the process of aging.

However, it was noticed that the amount of acidic resinous constituents, which make the isolation and identification of organic acids (i. e., isooctane insoluble fraction) difficult, in the acid fraction of the essential oil BRA was smaller than that of AA. This fact, which was also observed in the case of the carbonyl fraction³⁾, would suggest that the resinous substances increased by polymerization of the dynamic substances, so called by Frankenburg⁴⁾, during the process of aging.

EXPERIMENTAL

1) **Preparation of Essential Oil:** The flue cured tobacco leaves, 1955 crop, harvested in Okayama, before redrying and aging, smoking leaf, special grade, were butted and cut by the same method as usually employed in cigarette manufacturing. As previously reported¹⁾, cut tobacco leaf, 135 kg, was steam distilled and the distillate was extracted with ether.

1) I. Ōnishi and K. Yamasaki, *This Bulletin*, **19**, 137 (1955).

2) I. Ōnishi and K. Yamasaki, *This Bulletin*, **20**, 68 (1956).

3) I. Ōnishi and M. Nagasawa, *This Bulletin*, **21**, 43 (1956).

4) W.G. Frankenburg, *Advances in Enzymology*, **10**, 371 (1950).

TABLE I
PHYSICAL AND CHEMICAL CONSTANTS OF THE
ESSENTIAL OIL OF JAPANESE FLUE CURED
TOBACCO LEAF BEFORE REDRYING AND AFTER AGING

	Before Redrying	After Aging
Yield (%)	0.133	0.215
d_{20}^{20}	0.9840	0.829
n_D^{20}	1.4798	1.4131
Acid value	76.81	—
Saponification value	119.32	—
Ester value	42.51	—

shows the yield of acid fraction, isooctane soluble and insoluble-fraction, in BRA as compared with those of AA.

3) Separation of Organic Acids: As reported previously, organic acids were separated by column chromatography employing silicic acid as an adsorbent and isooctane as an eluting solvent. The chromatograms of low- and high-boiling point fractions are represented in Figs. 1 and 2, respectively.

4) Identification of Organic Acids: Each organic acid isolated by column chromatography was derived

TABLE II
COMPARISON OF YIELD OF THE ACID FRACTION IN THE ESSENTIAL OIL OF JAPANESE
FLUE CURED TOBACCO LEAF BEFORE REDRYING AND AFTER AGING

	Before Redrying	After Aging ^a
Yield of acid fraction (mg/1 kg leaf)	141	474
Acid fraction %/Total essential oil	10.63	16.89
Isooctane soluble (mg/1 kg leaf)	51.4 (36.5%) ^b	125.0 (26.3%)
Isooctane insoluble (mg/1 kg leaf)	15.2 (10.8%)	77.4 ^c (16.3%)

a) See previous paper¹.

b) () indicates percentage for the total acid-fraction.

c) The amount of the isooctane insoluble-part of the previous paper is less than that obtained in this study, because the former was refined by extracting the ethereal-solution of the fraction with aqueous sodium bicarbonate.

d) The total sum of the isooctane soluble and insoluble part does not amount to 100%, because the acid fraction contains non-acidic compounds.

From the ethereal solution, 179.36 g of the essential oil of tobacco leaf BRA was obtained (see Table I.) and the characters of its odor were almost the same as that of the essential oil of tobacco leaf AA.

2) Preparation of Acid Fraction: As reported previously, from 179.36 g of the essential oil BRA, 19.05 g of the acid fraction which contained a considerable amount of volatile non-acidic compounds (ether, carbonyl compounds etc.) was obtained. In order to remove the volatile non-acidic compounds and collect the low-boiling point fraction, mainly consisting of acetic acid, the acid fraction was fractionally distilled at atmospheric pressure and 2.066 g of the low-boiling point fraction (b.p. 60–83°, bath temp. 100–170°) was obtained.

The distilling residue was dissolved in ether, and the ethereal solution was extracted with saturated aqueous sodium bicarbonate solution. This aqueous solution was acidified with sulfuric acid, then saturated with sodium chloride and extracted with ether. After removal of ether, 6.918 g of high-boiling point fraction was obtained. This fraction was extracted two or three times with hot isooctane and 2.047 g of the isooctane soluble-fraction was obtained. Table II.

to its *p*-bromphenacyl ester and identified by its melting point, infrared spectrum and elementary analysis.

FIG. 1. Chromatographic Separation of Low-boiling Point Fraction.

Developers used were as follows: Isooctane 500 ml, Isooctane-Ether (10% v/v) 100 ml, Ether 200 ml. 13 g. of Silicic acid was used as packing material.

FIG. 2. Chromatographic Separation of High-boiling Point Fraction.

Developers used were as follows: Isooctane 800 ml, Iso-octane-Ether (10% v/v) 100 ml, Ether 200 ml.

with each other.

RESULTS AND DISCUSSION

Table III. shows the content of each organic acid isolated and identified from the essential oil before redrying and aging, compared with that of after aging. There was no difference in the kind of organic acid between BRA and AA, except that methylethylacetic and propionic acids were newly isolated from BRA.

However, Table II. shows the increase of total volatile organic acid from 141 mg BRA to 474 mg AA, per 1 kg of tobacco leaf. The content of low-molecular fatty acid, especially acetic acid in the essential oil of

TABLE III
COMPARISON OF THE ACID FRACTION IN THE ESSENTIAL OIL OF JAPANESE FLUE CURED TOBACCO LEAF BEFORE REDRYING AND AFTER AGING

	Before Redrying		After Aging	
	mg/10 kg leaf	% for total acid	mg/10 kg leaf	% for total acid
Formic acid	—	—	trace	—
Acetic acid	98	33.9	268	49.2
Propionic acid	9	2.9	—	—
Isobutyric acid	—	—	8	1.5
α-Crotonic acid	22	7.6	40	7.3
Methylethylacetic acid	10	3.5	—	—
n-Caproic acid	15	5.3	19	3.5
β-Methylvaleric acid	82	28.3	106	19.4
Benzoic acid	32	11.2	34	6.2
Phenylacetic acid	21	7.3	70	12.9

5) Identification of Methylethylacetic Acid: *p*-Bromphenacyl ester of the acid corresponding to the first peak in Fig. 1 (m.p. 54–5°), showed no depression of melting point upon admixture with *p*-bromphenacyl ester of authentic methylethylacetic acid (m.p. 55–6°), and infrared spectra of both derivatives agreed well with each other.

Anal. Found: C, 51.88; H, 4.67. Calcd. for C₁₃H₁₅BrO₃ (as the methylethylacetic acid derivative): C, 52.19; H, 5.05.

6) Identification of Propionic Acid: *p*-Bromphenacyl ester of the acid corresponding to the second peak in Fig. 1 (m.p. 58–60°) showed no depression of melting point, upon admixture with *p*-bromphenacyl ester of authentic propionic acid (m.p. 58–9°) and infrared spectra of both derivatives agreed completely

tobacco leaf AA was about 2.5 times as much as that of BRA and an increase of phenylacetic acid by 3 times was observed during the process of aging, but the question of the formation of these acids has not been clarified in detail.

Phenylacetic acid is distributed in many plants, for example, in Japanese peppermint oil, being found as an ester with β,γ-hexenol⁵⁾. In the process of biological degradation of phenylalanine by microorganism^{6,7)}, phenyl-

5) N. Hirao, "Nihon-Seiyu-Kagaku", Sasaki-rosho Pub. Co., Tokyo, 1939, p. 99.

6) K. Felix, K. Zorn und H. Dirr-Kaltenbach, *Z. physiol. Chem.*, **247**, 141 (1937).

7) T. Uemura, *J. Agr. Chem. Soc. Japan*, **13**, 1146 (1937).

acetic acid is produced through phenylpyruvic acid and Polak⁸⁾ reports, that this process would be observed in the biogenesis of phenylacetic acid in plants. Although phenylalanine has been found in the tobacco leaf after flue-curing⁹⁾, it is not experimentally proved whether phenylacetic acid is produced from the phenylalanine contained in the tobacco leaf.

α -Crotonic acid was not generally found in plants except of its presence in *Ramalina reticulata*¹⁰⁾ and animal fat¹¹⁾, but it is considered that this acid might be produced from the fatty acid-cycle of Lynen¹²⁾, which

plays an important role in the biogenesis of fatty acids.

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8) E.H. Polak, *Perfum. Ess. Oil Record*, **46**, 369, 401 (1955); **47**, 6 (1956).

9) S. Ranjan and M.M. Laloraya, *Nature*, **177**, 235 (1956).

10) J.B. Stark, E.D. Walter and H.S. Owens, *J. Am. Chem. Soc.*, **72**, 1819 (1950).

11) F.L. Bensch and E. Ulysoy, *Arch. Biochem.*, **11**, 489 (1946).

12) F. Lynen, *Angew. Chem.*, **67**, 463 (1955).