Studies on the Essential Oils of Tobacco Leaves

Part VI. Phenol Fraction (II)

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Further investigation was conducted on the caustic alkali soluble compounds contained in the essential oil of Virginia tobacco leaves. Benzaldehyde, *p*-anisaldehyde, *o*-hydroxyacetophenone and eugenol were isolated and identified. Several other compounds were also isolated and investigated.

In the previous paper¹⁾, we have described isolation and identification procedures of phenol, guaiacol, salicylaldehyde, methyl salicylate, lauric acid, myristic acid and palmitic acid, also presuming the presence of several carbonyl compounds and a low boiling substance. In the course of further investigations other two phenolic compounds were detected by paper chromatography in the alkali insoluble part, which was separated when the crude phenol ethereal solution was shaken again with an aqueous solution of 3% potassium hydroxide for purification. In this paper, we have identified eugenol as one of these phenolic compounds, by fractional distillation followed by chromatography. The final identification was accomplished on melting point, and infrared spectrum of its 2, 4-dinitrophenylether. The carbonyl compounds were further separated and derived to 2, 4-dinitrophenylhydrazones. Acetaldehyde, benzaldehyde, *p*-anisaldehyde, *o*-hydroxyacetophenone as well as salicylaldehyde were isolated and identified by chromatographic adsorptions on silicic acid and celite, then, finally by their melting points, infrared spectra and elementary analyses. On the other hand, the presence of *m*-tolualdehyde was estimated. Heretofore, no

report concerning *p*-anisaldehyde, *o*-hydroxyacetophenone and *m*-tolualdehyde in the essential oil of tobacco leaves can be seen. So far as the presence of eugenol is concerned, Piatniski²⁾ has once reported on this, but his details had not been clarified. However, its presence has been clearly confirmed in this study.

EXPERIMENTAL

In order to purify an ethereal solution of crude phenol, it was shaken again with an aqueous solution of 3% potassium hydroxide, and a brown oil remained on evaporating off the ether. The residual oil solidified and separated white crystals on standing (ca. 1.2 g.). As it slightly showed a positive diazo reaction, it was dissolved in 20 ml. ether and poured into the 210×25 mm. alumina (Brockman) chromatographic column. It was eluted by ether for the first time, followed by ethyl acetate.

1. A yellow oil (20 mg.) remained on distilling off the ether effluent. Its 2, 4-dinitrophenylhydrazone is hardly soluble in hot alcohol and a black particle was obtained by recrystallization from alcohol-ethyl acetate.

2. An orange-yellow oil (10 mg.) was obtained from the ethyl acetate effluent. Two spots, R_F values: 0.35, pink and 0.55, yellow, were detected on paper chromatograms of its hydroxyazo compounds. Though these spots were not identical with the previously detected compounds¹⁾, one of them, R_F value: 0.55, was concordant with that which we detected in the volatile phenol of the "Freon" extracts of Virginia

1) I. Onishi and K. Yamamoto, This Bulletin, 19, 148 (1955).

2) W.G. Frankenburg. *Advances in Enzymology*, 6, 331 (1946).

tobacco leaves³⁾.

3. The column was separated into six portions with equal length, each portion, after acidification was extracted with ether. A yellowish-white solid substance, 1.020 g., was obtained from the upper portion. On recrystallizing from 75% alcohol, palmitic acid was identified by its melting point, 61–62°, and infrared spectrum. These compounds seemed to remain in the ether because of their incompleteness of separation. It was evident that at least two unknown phenols, and certain amounts of carbonyl compounds were presented in this fraction, so we have conducted investigation separately on carbonyl and phenol fractions.

Carbonyl Fraction: Crude phenol ether solution, which was obtained from 120 kg. Virginia tobacco leaves by the same method as described in Part III, was shaken again with an aqueous solution of 20% sodium bisulfite to separate the carbonyls. After being washed with ether, added with calculated 6 N sulfuric acid, saturated with sodium chloride, the aqueous solution was repeatedly extracted with ether. As the ethereal solution colored dark on distilling off the ether, 20 ml. alcohol was added just before the ether distilled up, this being poured into the solution consisting of 0.5 g. 2, 4-dinitrophenylhydrazine, 2 ml. concentrated sulfuric acid, 3 ml. water and 40 ml. alcohol. After thorough stirring, a separated reddish-brown precipitate of 2, 4-dinitrophenylhydrazone (I), 169 mg., was filtered off, then it was washed and dried. On the other hand, an orange-yellow precipitate of 2, 4-dinitrophenylhydrazone (II), 76 mg.,

was obtained from the filtrate.

Chromatographic Separation of (I): Forty milligrams of (I) was put into the chromatographic column by the same method as described in Part II. of this study. The same procedure was repeated four times, the results obtained are shown in Table I. The colored effluents of Bands No. 1 and 2. were collected in each flask successively while the other bands were sectioned, then extracted with ether. On distilling off the solvent, a dry precipitate remained in each flask.

Estimation of *m*-tolualdehyde: Recrystallization of the precipitate from Band No. 1 gave a red crystal, sublimizing at 193–197°. Its infrared spectrum is similar to 2, 4-dinitrophenylhydrazone of *m*-tolualdehyde, m.p. 200–201°, but not decidedly identical. Further investigation is now in progress.

Identification of benzaldehyde: An orange-yellow crystal, m.p. 240–241°, was obtained from Band No. 2. The mixed melting point with the authentic derivative of benzaldehyde, m.p. 243°, did not depress. Infrared spectra were found to agree well with each other.

Identification of *p*-anisaldehyde: A red plate crystal, m.p. 254°, was obtained from Band No. 3. by recrystallization from 95% alcohol. The melting point did not depress by mixing with the authentic derivative of *p*-anisaldehyde, m.p. 253–254°. The infrared spectra were found to agree well with each other.

Identification of salicylaldehyde and *o*-hydroxyacetophenone: Band No. 4 which developed just beneath the top band, was separated into two bands by repeated chromatography employing 0–10% ethyl acetate in petroleum benzene. Although the separation was not complete, finally, the mainly colored portions were extruded, purified by the same chromatographic procedure and recrystallized from the alcohol-ethyl acetate mixture. A fine red needle crystal, m.p. 252°, was obtained from the upper band. The mixed melting point with the authentic derivative of salicylaldehyde did not depress, both infrared spectra were found to be identical with each other. From the lower band, a fine orange needle crystal, m.p. 210°, was obtained. *Anal.* Found: C, 53.24; H, 3.84; N, 17.65, Calcd. for C₁₄H₁₂O₅N₄: C, 53.15; H, 3.76; N, 17.72. The infrared spectrum of this crystal was identical with 2, 4-dinitrophenylhydrazone of the authentic *o*-hydroxyacetophenone⁴⁾.

Estimation of α -pyrrol methyl ketone: The top band did not descend entirely and was hardly soluble in hot alcohol. It was therefore extracted with ether and a black particle was obtained after removing the

TABLE I
CHROMATOGRAPHIC SEPARATION OF THE MIXTURE
OF 2, 4-DINITROPHENYLHYDRAZONES (I) FROM THE
CARBONYL FRACTION (sample 169 mg.)

Band No.	Yield (mg.)	Compound	Color
5	115	(α -Pyrrol methyl ketone)	Reddish-black
	28	Salicylaldehyde	Red
4	12	<i>o</i> -Hydroxyacetophenone	Orange
3	1	<i>p</i> -Anisaldehyde	Red
2	5	Benzaldehyde	Orange-yellow
1	4	(<i>m</i> -Tolualdehyde)	Red

Band number in order of elution.

Adsorbent: Silicic acid + Celite (2:1).

Column: 200 × 36 mm.

Developing solvent: 1–10% Ether in petroleum benzene (b.p. 40–80°),

3) I. Onishi and K. Yamamoto, *Scientific Papers of Central Research Institute, Japan Monopoly Corporation*, No. 90, 39 (1954).

4) K. Fries and W. Phaffendorf, *Ber.*, 43, 215 (1910).

solvent and recrystallizing from alcohol and ethyl acetate. Although we could not determine the melting point, this substance is similar to that of the component isolated from Fraction 4, Table I., in the previous paper¹⁾, the infrared spectrum was identical with 2, 4-dinitrophenylhydrazone of α -pyrryl methyl ketone, found in the neutral fraction of this study.

Chromatographic Separation of (II): The results are summarized in Table II.

TABLE II

CHROMATOGRAPHIC SEPARATION OF THE MIXTURE OF 2, 4-DINITROPHENYLHYDRAZONES (II) FROM THE CARBONYL FRACTION (sample 76 mg.)

Band No.	Yield (mg.)	Compound	Color
4	25	Unknown	Reddish-black
3	42	Acetaldehyde	Orange
2	less than 1	Unknown	Yellow
1	3	Unknown	Orange

Foot notes are the same as in Table I.

Band No. 1: A fine orange needle crystal obtained from Band No. 1. was recrystallized twice from 94% alcohol, m.p. 134°. We cannot find any identical 2, 4-dinitrophenylhydrazone with this in the literature, so further investigation is now in progress.

Band No. 2: We could not measure neither the melting point nor the infrared spectrum because the amount was too small.

Identification of acetaldehyde: From Band No. 3, an orange needle crystal was obtained, recrystallized from 90% alcohol, m.p. 162°. The mixed melting point with the authentic acetaldehyde 2, 4-dinitrophenylhydrazone, m.p. 164°, showed no depression and the infrared spectra of both derivatives agreed well with each other.

Band No. 4: The top band was similar to Band No. 5. in Table I.

Phenol Fraction: After removal of the carbonyl compounds, crude phenol, 19.6251 g., was put into the fractional distillation under a nitrogen stream. The results are shown in Table III. Fraction 1. was almost ether. Fraction 2. was also almost ether, but it seemed to contain some other compounds. Two peculiar infrared absorption bands, 1730 and 1230 cm⁻¹, which were absent in the authentic ether, were found in this fraction. As these bands indicated the presence

of C=O linkage in the form of $\text{RC} \begin{smallmatrix} \text{O} \\ \diagup \\ \text{OR} \end{smallmatrix}$, and especially from the latter band attributed to acetate, the

TABLE III
SEPARATION OF THE CRUDE PHENOLS
BY FRACTIONAL DISTILLATION
(sample 19.6251 g.)

Fraction	Pr.	b.p. (°C)	Bath T. (°C)	Yield (g.)
1		(~ 35)	83	1.2032
2		(~ 45)	125-145	0.8650
3	6 mm. (Hg.)	(~ 57)	180-200	0.1265
4	"	(~160)	236	1.2016
Residue				13.3201

presence of a low molecular ester was presumed, but we could not identify any component after saponification because of the lack of sufficient amount for further investigation.

Detection of phenol and guaiacol: Two spots were detected on the paper chromatogram of Fraction 3. They were identical with phenol and guaiacol both in their R_F values and colors, according to our previous paper¹⁾.

Identification of eugenol: Fraction 4, a mixture of phenolic compounds and high molecular fatty acids, was put into the liquid chromatography as follows: the mixture was dissolved in 20 ml. of ether then poured into the chromatographic column of silicic acid (230×30 mm.). On eluting with petroleum benzine, b.p. 30-65°, a fatty acid, 1.006 g., appeared in the first effluent. Thirty-one milligram of orange-yellow oil was obtained from the ethyl acetate effluent. One spot, which corresponds to R_F value: 0.35, pink, described above, was detected on the paper chromatogram. This oil was derived to 2, 4-dinitrophenylether according to the method of Bost and et al.⁵⁾. A yellow plate precipitate, 7 mg., was obtained by final recrystallization from alcohol. The mixed melting point of this derivative, m.p. 112-113°, with the authentic derivative of eugenol, m.p. 114°, did not depress, and the infrared spectrum was identical with that of the authentic specimen. The fact that eugenol was not identified from the corresponding fraction in the previous experiment might be attributable to the quantitative loss by purification and the mixture containing a large amount of fatty acids. In such a case it is hard to detect the phenols by any method. Another phenol corresponding to R_F value: 0.55, was not identified in this investigation.

5) R.W. Bost and F. Nicholson, *J. Am. Chem. Soc.*, **57**, 2368 (1935).

DISCUSSION

From the phenolic fraction of Virginia tobacco essential oil the following substances were isolated and identified: phenol, guaiacol, eugenol, *p*-anisaldehyde, *o*-hydroxyacetophenone, benzaldehyde, acetaldehyde, salicylaldehyde and palmitic acid. The presence of α -pyrrol methyl ketone was also proved in this fraction. Another compound such as *m*-tolualdehyde was isolated but could not be decidedly identified.

Among these compounds, phenol, guaiacol, salicylaldehyde and palmitic acid have already been reported in the previous paper¹⁾. Acetaldehyde and benzaldehyde were also reported in this study⁶⁾. Heretofore, we have no report indicating the presence of *p*-anisaldehyde, *o*-hydroxyacetophenone, *m*-tolualdehyde and α -pyrrol methyl ketone in tobacco essential oils. Eugenol has been reported by other authors²⁾, but the method of identification was not so distinct such as in this study.

It is an interesting fact that α -pyrrol methyl ketone, which was found in the neutral fraction described in the previous paper⁷⁾, was also found in this phenol fraction. As mentioned above, many carbonyls and some acidic compounds were isolated besides the

true phenolic compounds from this fraction. The isolation of phenols seems to be difficult, and the solubility in aqueous and ethereal solutions has influence on the partition of these chemically inactive compounds, especially when the amount is very small.

Although we could not determine accurate quantity, the amount of palmitic acid far exceeds the others. It is well known that high molecular fatty acids have the property making tobacco smoke mild. On the other hand, undoubtedly, each of the other substances mentioned above also have their characteristic intense odor. So, it is clear that they have influence upon the aromas and tastes of tobacco smoke, either individually or together, though the amounts are small.

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6) I. Ōnishi and M. Nagasawa, This Bulletin, **19**, 143 (1955).

7) I. Ōnishi, H. Tomita and T. Fukuzumi, This Bulletin, **20**, 67, (1956).