Analysis of Thymol and Eugenol Employing Ion Exchange Resins

By FLOYD A. WAGNER and MURIEL C. VINCENT

A quantitative analysis for thymol, eugenol, and clove oil has been developed by means of a spectrophotometric-anion exchange procedure. Suitable reagents which will produce colored compounds with small amounts of the phenolic substances have been selected. A comparison with the official assay for clove oil has been made. The ion exchange method indicated a full recovery of the phenols present in the oil and could be conducted in a much shorter period of time than the official method.

THE QUANTITATIVE determinations for volatile oils and their constituents are numerous, and in the case of the oils the assays frequently depend on a particular group of chemical substances rather than a specific compound. In this respect, then, it would be quite desirable to have a procedure capable of rapidly, accurately, and conveniently assaying an aqueous or nonaqueous mixture of such products for the major constituent only.

The presently accepted assay procedure for thyme oil (1) and clove oil (2) for their respective principal constituents, thymol and eugenol, have several disadvantages. These are primarily the nonspecificity of the alkali hydroxide to eugenol and thymol because of its reaction to phenolic compounds in general, inexact measurements of volumes, and inaccurate readings of the meniscus (3).

It was the purpose of this study to develop a method which would rapidly and accurately remove eugenol or thymol from a solution by means of an ion exchange resin.

The application of chromatographic techniques in the analysis of volatile oils has been somewhat limited. However, gas chromatographic analysis has been employed by Naves (4) for thyme oils and by Naves and Odermatt (5) and Stahl and Trennheuser (6) for eugenol. Bielenberg and Fischer (7) have reported the adsorption of thymol and other monohydroxy benzenes on alumina columns. Frydman (8) and coworkers used silicic acid-starch paste-covered glass plates to achieve the separation of thymol and other compounds of volatile oils.

EXPERIMENTAL

Experiments were first undertaken to find reagents which would produce soluble colored compounds with various known concentrations of thymol and eugenol. These solutions were observed at different wavelengths of light using a spectrophotometer to obtain the characteristic curves, and standard curves were then established. Subsequently, samples of thymol, eugenol, and clove oil were passed through a weakly basic ion exchange resin, and the eluates were treated with the reagent and observed at the required wavelength.

Reference to the standard curve showed that quantitative recoveries were obtained.

Color Reagents and Standard Curves.—Alcoholic thymol solutions of 0.1 *M*, 0.01 *M*, and 0.001 *M* concentrations were treated in the following manner with Hammarsten-Rolbert reagent (9). One milliliter of solution to be tested was placed in a cell and 5 ml. each of glacial acetic acid and concentrated sulfuric acid were added to produce the characteristic red-violet color. The solutions were allowed to stand 5 minutes to permit maximum color development before viewing in the Colemen spectrophotometer between wavelengths of 400 and 650 mμ (Fig. 1). The standard curve was prepared from the data, using transmittance at 520 mμ (Fig. 2).

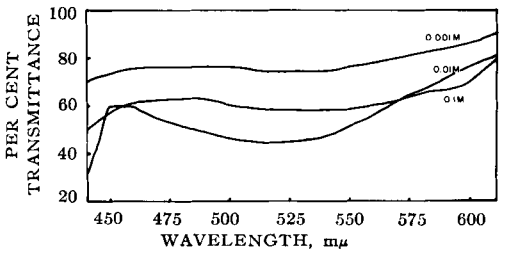

Fig. 1.—Concentration-transmittance curves for thymol.

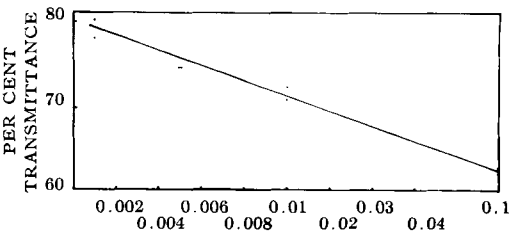

Fig. 2.—Standard curve for thymol, 520 mμ.

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TABLE I.—ANALYSIS OF PHENOLS AND PHENOL-CONTAINING OILS

Phenol or Volatile Oil	*T*, %	Concentration, *M*	Phenols,[a] %	Phenols Recovered,[b] %
Thymol	73.8	0.0067	.....	100.0 ± 2.99 (11)[c]
Eugenol	23.1	0.0251	.....	100.0 ± 0.80 (3)
Clove oil	21.8	0.0289	87.9 ± 2.0 (3)	98.8 ± 2.10 (3)

[a] Calculated by official assay. [b] Analyzed by ion exchange-spectrophotometric method. [c] Number of determinations.

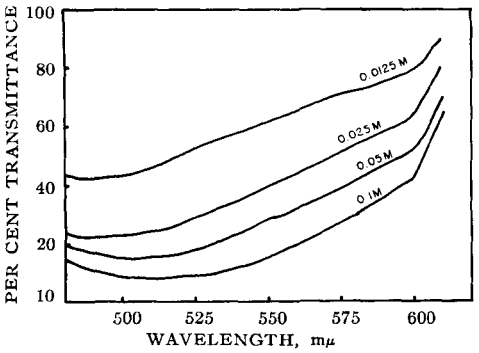

Fig. 3.—Concentration-transmittance curves for eugenol.

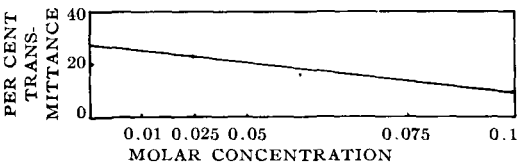

Fig. 4.—Standard curve for eugenol, 500 mμ.

Solutions of eugenol in dimethylformamide were prepared in concentrations of 0.1 *M*, 0.05 *M*, 0.025 *M*, and 0.0125 *M* and treated with Ehrlich's diazo reagent (10). In performing the test, 3 ml. of sulfanilic acid solution was added to 3 ml. of sodium nitrite solution previously placed in a cell; to this solution was added 0.1 ml. of eugenol sample and finally 3 ml. of sodium bicarbonate solution. The resulting yellow-brown solution was observed in the spectrophotometer after 15 minutes and the transmittance of light recorded between 400 and 650 mμ (Fig. 3). The standard curve was based on the transmittance of light at 500 mμ (Fig. 4).

Ion Exchange Procedure.—The weakly basic anion exchange resin, Amberlite IR-45, was employed. Ten-gram samples of the air dried resin were moistened with water, transferred to columns (22 × 3 cm.), and regenerated with 50 ml. of 4% sodium carbonate solution. After regeneration and rinsing of the columns, the distilled water was removed from the columns by washing with 30 ml. of alcohol or dimethylformamide for thymol and eugenol, respectively.

The sample to be analyzed was then added to the columns by means of a graduated 1-ml. pipet to provide 50 to 100 mg. of thymol or eugenol. All columns were washed with alcohol or dimethylformamide at a rate of 1 ml. per minute until 100 ml. of eluate was collected in a volumetric flask. After thorough mixing of the eluate, the color developing agent was added as previously described. Blank determinations were made with all samples.

Table I shows the data obtained when the phenols and clove oil were analyzed in this manner. Comparative official assays were performed on clove oil.

SUMMARY

A quantitative analysis for thymol, eugenol, and clove oil has been developed by means of an anion exchange-spectrophotometric procedure.

Suitable reagents which will produce colored compounds with small amounts of the phenolic substances have been selected. Ehrlich's reagent, which produces an azo dye with eugenol, is very sensitive and will give the characteristic yellow-brown color with eugenol in quantities as minute as 0.2 mcg. The Hammarsten-Rolbert reagent will give the characteristic red-violet color of thymosulfonic acid with amounts as low as 10 mcg. of thymol.

A comparison with the official assay for clove oil has been made. The method indicated a full recovery of the phenols present in the oil and could be conducted in approximately 3.5 hours, which is considerably less than the 18 hours required for the official assay.

Attempts to perform a similar assay on thyme oil gave consistently high results, which indicated that the reagent evidently reacted with other components of the oil. Thus, more specific color reagents for thymol will have to be developed or more selective ion exchange resins employed. Such improvements are presently being investigated.

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