

Presence of Three New Ingredients in Spearmint Oil

By THOMAS F. BURKS* and GUNNAR GJERSTAD

By use of gas chromatography, spearmint oil N. F. was separated into 19 fractions. Co-chromatography with known reference substances conclusively demonstrated the presence of cineole, α -pinene, and linalool in the oil.

DUE TO THEIR chemical heterogeneity, volatile oils have so far defied a complete and accurate analysis. Until recently, the only available methods for proximate analyses of these oils were fractional distillation and derivative formation of variable accuracy. The relatively recent development of gas chromatography as an analytical

method for volatile oils appeared to us to offer great advantages over conventional methods.

Carvone has for some time been recognized as the main ingredient of spearmint oil, the official purity rubric specifying a minimum concentration of 55% (1). Among the other known ingredients, the exact percentages of which have not been conclusively established, are pulegone (2), *d*-3-octanol (3), limonene, phellandrene, acetic and valeric acids esterified with dihydrocumyl alcohol, and dihydrocarveol esterified with acetic acid (4). In most instances the literature gives no particular data on conformational structural formulas. The presence of pinene, linalool, and cineole has been indicated in various oils of related species from certain foreign habitats; these reports have been questioned by other workers, and we can find no substantiation hereof in American oils (4, 5).

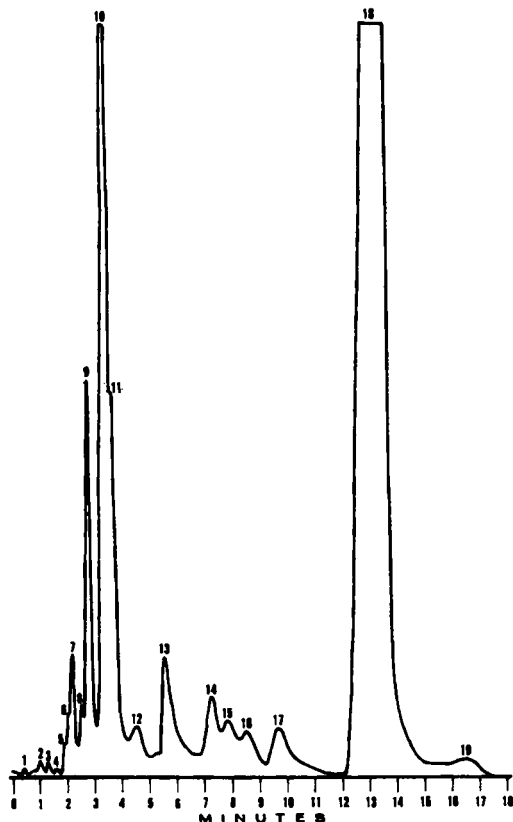


Fig. 1.—Gas chromatogram of spearmint oil N. F. on a 7.5 ft. stainless steel column of 1/4-in. diameter; Silicone Dow 710 on firebrick; helium flow 50 ml./minute; sensitivity attenuation 2 X. Total time required for separation into 19 fractions was 18 minutes. This recording was obtained at 160°C. However, lower temperatures gave the same number of peaks.

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This paper represents a part of a more comprehensive study of plant tranquilizers to be published elsewhere.

* Recipient of the Kilmer Prize for 1962 and the 1961 Edwin L. Newcomb Memorial Award.

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After the completion of this paper, it was noted that J. J. Broderick demonstrated the presence of cineole in spearmint oil. (Burchfield, H. P., and Storrs, E. E., "Biochemical Applications of Gas Chromatography," Academic Press, Inc., New York, N. Y., 1962, pp. 463-465.)

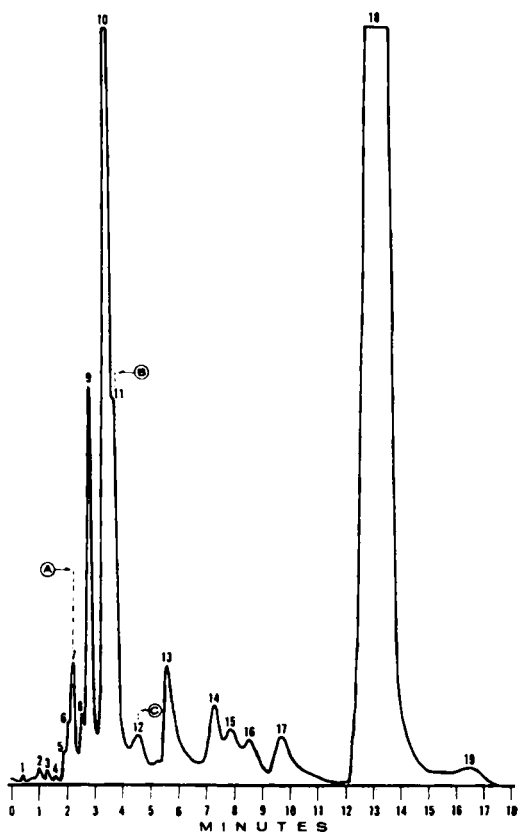


Fig. 2.—A composite graph of the effects of individual chromatograms from co-chromatography. The broken lines indicate the heights of the peaks after the reference standards were added. Key: A, pinene; B, cineole; and C, linalool.

EXPERIMENTAL

Two-microliter samples of spearmint oil N. F.¹ were chromatographed in an Aerograph model A-90-P gas chromatograph equipped with a thermal conductivity detector and a Honeywell-Brown

¹ Obtained from Magnus, Mabee & Reynard, Inc., New York, N. Y. No data on its provenience are available.

Elektronik recorder of 1-mv. sensitivity. Silicone Dow 710 and diethyleneglycol succinate, both adsorbed on firebrick, were the most suitable stationary phases; the other experimental conditions are listed under Fig. 1.

Inspection of Fig. 1 reveals that the oil may be separated into a total of 19 fractions, eight representing minor and 11 major components. Due to the lack of authentic blank substances, all of these ingredients have yet to be positively identified, but work is in progress. Carvone produced the most prominent peak (No. 18) which was readily identified by use of added reference standard.

Comparison of retention volumes and co-chromatography with added pure reference standard under identical experimental conditions were employed in the identification of three compounds in the oil, the presence of which had previously been disputed. Authenticated reference substances, pure and in 2% mixtures in natural oil, were gas chromatographed individually and in various combinations. The results are presented in Fig. 2.

The addition of 2% linalool to the oil produced an

increase in the size of peak 12 at a retention time of 4.3 minutes, which is identical to that of pure linalool under the stated conditions. Added cineole similarly effected an increase in peak 11, which appeared only as a "shoulder" on peak 10 in the natural oil, and clearly separated it from peak 10. The retention time of 3.7 minutes for peak 11 corresponds to that of pure cineole. Pinene, with a retention time of 2.2 minutes, caused a marked increase in peak 7.

Gas chromatography was found to be a highly versatile and valuable method for the complete analysis of complex naturally occurring volatile substances.

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Separation and Investigation of a Stable Solid Free Radical of Chlorpromazine

By F. H. MERKLE, C. A. DISCHER, and A. FELMEISTER*

A solid stable free radical of chlorpromazine was prepared and characterized. The free radical nature of this compound was verified by electron spin resonance and ultraviolet spectral studies. The molecular weight and melting point of the free radical were determined.

ALTHOUGH the pharmacological importance of chlorpromazine is well established, its metabolic fate is not fully known (1). While studies of the oxidation of chlorpromazine have been reported (2, 3) and the existence of a free radical intermediate proposed (4, 5), little is known regarding the nature of this radical in the solid state (6, 7). The preparation and characterization of this free radical intermediate was undertaken as a preliminary step to the study of its role in the electrochemical and photo-oxidation mechanisms of chlorpromazine.

Preparation of Free Radical Intermediate

A procedure based on that outlined by Billon (8) for the preparation of a phenazothionium salt of phenothiazine was used in the preparation and separation of solid free radical. Approximately 0.5 Gm. of chlorpromazine 5-oxide hydrochloride¹ was dissolved in 10 ml. of 70% perchloric acid. The resulting dark red solution was agitated for 10 to 15 minutes and diluted first with an equal volume of acetone and then ether. Upon cooling to about -5° a strongly red-purple, fine crystalline solid separated. This solid material will be referred to in future discussions as Compound R. The solid softened over the range of 185-195 $^{\circ}$, before melting.

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* Present address: College of Pharmacy, Columbia University, New York, N. Y.

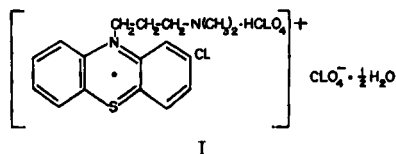
¹ Chlorpromazine hydrochloride and chlorpromazine 5-oxide were supplied by Smith Kline and French Laboratories, Philadelphia, Pa.

Characterization of Compound R

Schieser and Tuck (9) prepared a semiquinone free radical of chlorpromazine by dissolving chlorpromazine in concentrated sulfuric acid; electron spin resonance studies were used to confirm the presence of the free radical in the reaction mixture. Visible and ultraviolet spectra of the radical, prepared by the method of these workers, were obtained on a Beckman DK-2 ratio-recording spectrophotometer. These spectra were identical to those of Compound R, Fig. 1.

Electron spin resonance spectra indicate that Compound R is strongly paramagnetic.²

Elemental analysis of the free radical, Compound R, indicates that it probably exists as the hemihydrate of the diperchlorate salt. Structure I is



proposed. Calculated theoretical percentages of the elements in the proposed structure, based on a molecular weight of 527.5, are in good agreement with the analytical results, Table I.

² Electron spin resonance measurements were performed by Mrs. N. Steinberger, Columbia University, Department of Chemistry. The authors wish to thank Dr. G. Fraenkel and his staff for the use of their laboratory facilities in making these measurements.