Essential Oil of *Anemopsis californica*

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The constituents of the hydrodistilled essential oil from the roots and rhizomes of *Anemopsis californica* have been examined. Thymol was identified from the phenolic fraction of the oil. Gas chromatography on Hyprose SP-80 followed by collection of effluent fractions and comparison of their IR spectra with those of pure samples confirmed the presence of methyleugenol as the major constituent. A third constituent of the oil was found to be piperitone. Methyleugenol (approximately 55 percent v/v of the oil), thymol (13 percent), and piperitone (5 percent) together make up approximately 74 percent of the oil. A fourth constituent, tentatively identified as an aromatic unsaturated ether, and other constituents are being investigated.

ONLY TWO reports on the chemical constituents of the roots and rhizomes of *Anemopsis californica* (Nutt.) Hook and Arn. (Fam. *Saururaceae*, Yerba del Mansa) are available. Horton and Paul (1) first reported the presence of methyleugenol (4-allylveratrole) in the steam-distilled oil. Childs and Cole (2) later confirmed the occurrence of methyleugenol in the petrol ether extracts of the rhizomes and also reported the presence of leucoanthocyanidins and an unidentified crystalline compound in these extracts.

This paper reports the results to date of the investigation of the essential oil of *Anemopsis* (Mansa oil).

EXPERIMENTAL

Crude Drug—Hathaway Allied Products[1] supplied the whole crude drug (roots and rhizomes) in following lots: Lot RM-65-167, Lot RM-65-1029 and Lot RM-67-91.

Extraction of Essential Oil—Approximately 3 Kg. crude drug was crushed to 12–6 mm. size. The crushed drug was hydrodistilled for 30 hr. (batches of 300 Gm. drug + 1800 ml. water). The distillate was collected in a modified Clevinger's apparatus for oils lighter than water. After separation for 10 hr. the oil was removed and dried over anhydrous sodium sulfate for 30 hr. The batches were pooled for further analysis.

Thin-Layer Chromatography (TLC)—To monitor the separation steps TLC was used under the following conditions: *Equipment*—Desaga,[2] variable thickness spreader. *Carrier Plates*—glass, 8 × 8 or 2 × 8 in. *Adsorbent*—Silica Gel G (25 Gm. suspended in 50 ml. deionized water and spread over five 8 × 8 in. plates), 250 μ thick. *Activation*—dried at 110° for 2 hr. and stored over anhydrous calcium sulfate[3] before spotting. *Sample Size*—10 μl. *Solvent System*—benzene + ethylacetate, 95:5 v/v. *Development*—room temperature (22–27°), 15 cm., 35–40 min. *Spray Reagents*—(A) Anisaldehyde (anisaldehyde, 3 drops in a mixture of 10 ml. methanol and 1 ml. glacial acetic acid + 2 ml. concentrated sulfuric acid just before spraying. Sprayed plates heated at 95° for 7 min.). (B) 2:4 Dinitrophenylhydrazine (2:4 DNPH) (3). (C) Diazotized sulfanilic acid (3).

Functional Group Separation—Scheme I shows the procedure used to achieve gross separation of the constituents of Mansa oil.

A negative test with 2:4 DNPH on TLC indicated initially that the oil was devoid of carbonyls. However, Stahl (4) has pointed out that ketones such as menthone, piperitone, and fenchone should be present in larger amounts to yield a positive color reaction with the reagent. If such ketones were present in Mansa oil in low concentrations, the negative test could be expected. In order to further test for the presence of carbonyls a procedure reviewed by Parsons (5) was adopted as follows. Analytical grade diatomaceous earth,[4] 10 Gm. was triturated with a solution of 500 mg. of 2:4 DNPH in 6 ml. phosphoric acid (d = 1.75) and 4 ml. of deionized water. The impregnated diatomaceous earth was then packed in a glass column (2.1 cm. i. d.) filled with cyclohexane. The cyclohexane was run off the column and the diatomaceous earth washed with benzene to remove excess 2:4 DNPH. A solution of 0.5 ml. oil in 0.5 ml. cyclohexane was then applied to the column and the column eluted with cyclohexane until the eluate gave no color with vanillin-sulfuric acid reagent (3). The column was now eluted with benzene. The benzene effluent was deep orange-red; a characteristic color of 2:4-dinitrophenylhydrazones. It was therefore apparent that small amounts of carbonyl compounds were present in Mansa oil and that these could not be grossly separated by the usual 2:4 DNPH treatment of the oil. A method similar to that of Teitelbaum (6) was therefore used to obtain the carbonyl fraction from the phenol-free oil (Scheme I). A mixture of 5 Gm. phenol-free oil, 5 Gm. Girard "T"[5] reagent, and 0.1 Gm. of a cation exchanger[6] were refluxed in a 50-ml. round-bottom flask with 10 ml. absolute ethanol for 1.5 hr., during which period Girard "T" dissolved. The hot solution was then stirred into 40 ml. ice water and allowed to stand for 0.5 hr. The aqueous mixture was extracted with ether (15 ml. × 3) and the combined ether extracts were dried over Na_2SO_4 anhyd. Removal of ether *in vacuo* gave the noncarbonyl fraction (4.15 Gm.). The aqueous phase was acidified by 10 ml. concentrated HCl and kept overnight. The oily carbonyl fraction which separated was extracted with ether (20 ml. × 3), the combined ether extracts dried over Na_2SO_4 anhyd., and the ether removed *in vacuo*

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[1] Hathaway Allied Products, Los Angeles, Calif.

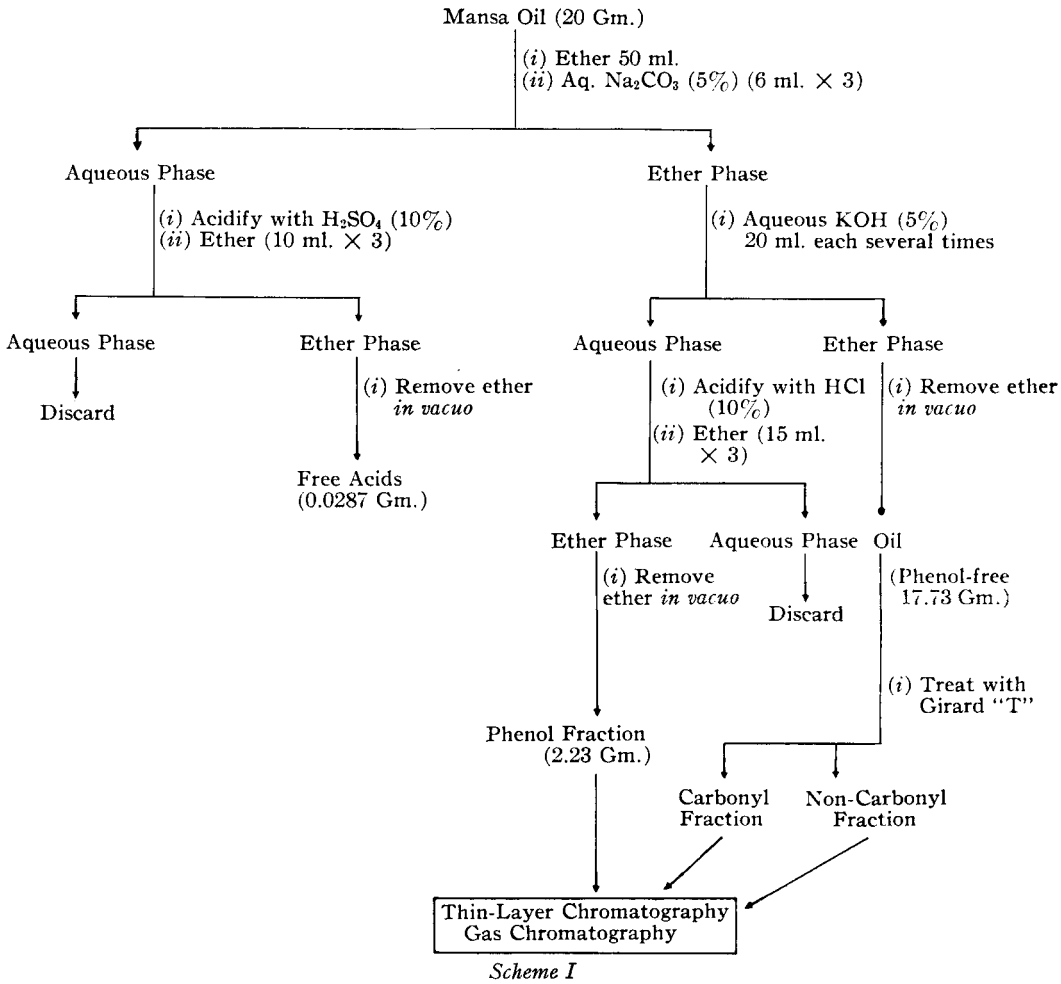
[2] Brinkmann Instruments Inc., Westbury, N. Y.

[3] Drierite, W. A. Hammond, Drierite Co., Xenia, Ohio.

[4] Celite 545, Johns-Manville Corp., New York, N. Y.

[5] Eastman Organic Chemicals.

[6] Dowex 50WX-8, Dow Chemical Co., Midland, Mich.



Scheme I

(0.85 Gm.). TLC of this carbonyl fraction now gave a 2:4 DNPH positive spot (Fig. 1B).

Gas Chromatography (GC) Apparatus—Autoprep[7] model A-700 with thermal conductivity detector. *Column*—Aluminum, 1/4 in. o.d., 20 ft. *Packing*—Hyprose SP-80,[7] 20% w/w on 60–80 mesh Chromosorb W (Hyprose SP-80, 20 Gm., in hot chloroform solution was deposited on 100 Gm. Chromosorb W with constant stirring. The solvent was then removed in a rotary evaporator under water-aspirator suction. The packed column was preconditioned overnight at 190° under a slow stream of nitrogen). *Carrier Gas*—Helium, 75 ml./min. at 50 p.s.i. inlet pressure. *Injection Port Temperature*—200°. *Column Temperature*—180°. *Detector Temperature*—250°. *Recorder Span*—1 mv. *Chart Speed*—1 in. 5 min. The effluent fractions were collected directly on KBr pellets placed in a KBr bottle[7] or by passing through a trap containing spectral grade carbon tetrachloride. The IR spectra were obtained with a Perkin-Elmer model 337 IR spectrophotometer.

RESULTS AND DISCUSSION

Approximately 250 Gm. of a dark green oil was collected. The average yield from several batches was 6.5% v/w of crude drug. Finer grinding of the crude drug increased the yield to approximately 7.5% however under these conditions charring of the fine powder occurred. The oil distilling in the initial stages was pale yellow gradually changing to dark green. The pooled oil had the following physical constants: d_{25}^{25} 0.9980; n_D^{25} 1.5195, and $[\alpha]_D^{25}$ less than −3.5° (dark color interfered).

The three spray reagents for TLC were selected so as to detect most of the common constituents of essential oils (3). Anisaldehyde gives colored spots with terpenes, phenols, steroids, and sugars and 10 well-marked spots were obtained with Mansa oil (Fig. 1A). The carbonyl compounds are detected, more or less specifically, with 2:4 DNPH. This reagent failed to give any colored spot with the oil. Diazotized sulfanilic acid gave only one yellow spot indicating the presence of one phenol (Fig. 1C).

Repeated extractions with aqueous KOH removed the phenol fraction from the oil. Addition of alkali to the oil changed the color of the oil to deep red as long as phenols were present. TLC of the dark brown oily phenol fraction (Scheme I) gave only one yellow spot with reagent (C) (Fig. 1D). The odor of the fraction was similar to that of thyme oil. Distillation of this fraction at 230–238° gave a clear heavy liquid which solidified on keeping at 4° for 4 hr. The oily film adhering to

[7] Wilkens Instrument and Research Inc., Walnut Creek, Calif.

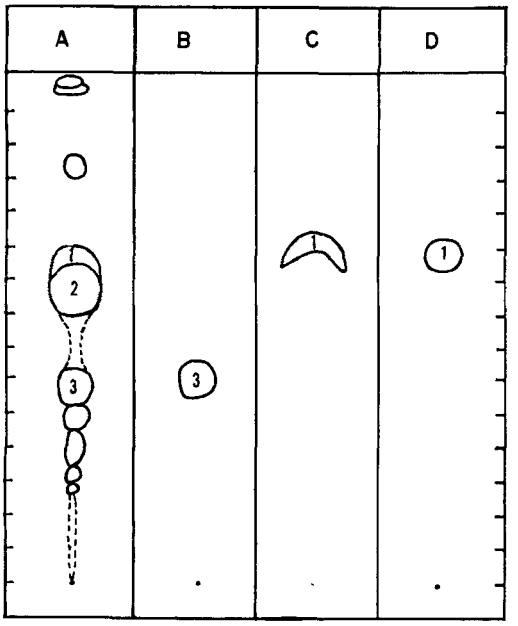


Fig. 1—Thin-layer chromatograms of hydrodistilled oil of Anemopsis californica. *Key: A, anisaldehyde; B, 2:4 DNPH, C, diazotized sulfanilic acid; carbonyl fraction of the oil; D, diazotized sulfanilic acid phenol fraction of the oil; 1, thymol; 2, methyleugenol; 3, piperitone.*

the solid was washed with chilled hexane and the solid recrystallized from boiling hexane (b.p. 68.7°). The purified solid was identified as thymol: m.p. 48–49° (49–50°) uncorrected; Positive thymolphthalein test; *N*-naphthylurethane—m.p. 106–107° (107–108°); α-naphthylurethane—m.p. 160° (161°); identical IR spectra, retention time (RT) on Hyprose SP-80 and R_f on TLC as that of pure thymol (reagent, Merck) (values in parentheses for thymol reagent, Merck).

The gas chromatogram of Mansa oil showed 20 peaks (Fig. 2). Four compounds (Peaks 1,2,3,12) were present in appreciable amounts and the authors concentrated on their identification. Tests with known samples[8] of common essential oil constituents had shown that, under these conditions, terpenes eluted out in the early region (0–5 min.), oxygenated compounds in the middle region (6–15 min.), and phenols and derivatives in the late region (16–40 min.) (Fig. 2). Injection of pure compounds showed that Peaks 1 and 2 (Fig. 2) were thymol and methyleugenol, respectively. The fractions from these peaks collected directly on KBr pellets gave IR spectra identical with those of the pure compounds. The GC of the carbonyl fraction (Scheme I) showed it to be enriched in the third major compound (Fig. 2, Peak 3). Successive injections of 5 μl. of the

[8] Supplied by Fritzsche Brothers Inc., New York, N. Y.

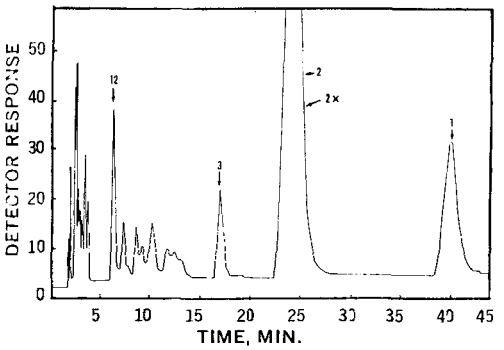


Fig. 2—Gas chromatogram of hydrodistilled oil o Anemopsis californica *(5 μl). Hyprose SP-80 stationary phase.*

carbonyl fraction were therefore used to collect sufficient quantity of Peak 3 in spectral grade carbon tetrachloride for IR analysis. The IR spectrum of the compound was identical with that of pure piperitone collected under identical conditions. The fourth compound (Fig. 2, Peak 12) collected in carbon tetrachloride gave an IR spectrum indicative of an aromatic ether (strong absorption at 1600–1500 $cm.^{-1}$ and at 1262 $cm.^{-1}$) with probably an isolated unsaturation (weak absorption at 1620 $cm.^{-1}$) and which is not monosubstituted (absence of absorption near 700 $cm.^{-1}$).

With pure methyleugenol and thymol as internal standards in GC the three identified compounds were found to be present in following amounts in the oil (% v/v); methyleugenol (55.30), thymol (13.57), and piperitone (5.19).

The aromatic ether and remaining 16 peaks constitute 25.94% of Mansa oil. In order to obtain sufficient quantities of these for identification, fractionation of the oil and subsequent GC on various stationary phases is under progress.

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(4) *Ibid.*, p. 193.
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(6) Teitelbaum, C. L., *J. Org. Chem.*, **23**, 646(1958).

Keyphrases

Anemopsis californica—essential oil
Mansa oil—*Anemopsis californica*
Methyleugenol, thymol, piperitone—isolated, identified
Column chromatography—separation
Cation-exchange resin—separation
TLC—separation monitoring
GLC—analysis
IR spectrophotometry—structure