hour after the addition was complete, and the yellow product was isolated and washed with water followed by ethanol. The yield was 2.3 Gm. (58.7%), and the product decomposed above 110°.

Ferrous Complex of 2-Mercaptoethylguanidine.—*S*-β-Aminoethylisothiouronium bromide hydrobromide (8.52 Gm., 0.03 mole) was dissolved in 35 ml. of water and the pH was adjusted to 8.0 using dilute ammonium hydroxide. A solution of 4.18 Gm. (0.015 mole) of ferrous sulfate heptahydrate in 15 ml. of water was added dropwise with stirring and a dark green precipitate formed immediately. After the addition was complete, the stirring was continued for 1 hour and the pH of the solution had decreased to a value of 6.6. The green product was isolated and washed with water followed by ethanol. The yield was 1.1 Gm. (17.3%) of a dark green product which started to decompose at 225°.

Anal.—Calcd. for $C_6H_{22}FeO_6S_3$: C, 16.90; H, 5.17; Fe, 13.10; N, 19.71; S, 22.56. Found: C, 17.06; H, 5.16; Fe, 11.99; N, 19.00; S, 22.24.

REFERENCES

(1) Bacq, Z. M., Herve, A., and Fischer, P., *Bull. Acad. Roy. Med. Belg.*, 18, 226(1953).
(2) Jones, M. M., *Nature*, 185, 96(1960).
(3) Kalkwarf, D. R., *Nucleonics*, 18 (5), 76(1960).
(4) Patt, H. M., *Federation Proc.*, 19, 549(1960).
(5) Baur, E., and Preis, H., *Z. Phys. Chem.*, B32, 65 (1936).
(6) Schubert, J., Abstrs. 139th Natl. Meeting, Am. Chem. Soc., March, 1961, p. 9-N.
(7) Alexander, P., Bacq, Z. M., Cousens, S. F., Fox, M., Herve, A., and Lazar, J., *Radiation Res.*, 2, 392(1955).
(8) Rixon, R. H., and Whitfield, J. F., *Intern. J. Radiation Biol.*, 3, 439(1961).
(9) Smirnova, O. I., *Radiobiologiya*, 2, 378(1962).
(10) Corradi, C., Pagletti, R., and Pozza, E., *Atti Soc. Lombarda Sci. Med. Biol.*, 13, 80(1958).
(11) Butler, J. A. V., and Robins, A. B., *Nature*, 193, 673 (1962).
(12) Knoblock, E. C., and Purdy, W. C., *J. Electroanal. Chem.*, 2, 493(1961).
(13) Foye, W. O., Marshall, J. R., and Mickles J., THIS JOURNAL, 52, 406(1963).
(14) Foye, W. O., Mickles, J., Duvall, R. N., and Marshall, J. R., *J. Med. Chem.*, 6, 509(1963).
(15) Musil, A., and Pilz, W., *Z. Anal. Chem.*, 141, 19 (1954).
(16) Greth, A., and Reese, J., German pat. 1,094,729 (1960).
(17) Foye, W. O., Duvall, R. N., and Mickles, J., THIS JOURNAL, 51, 168(1962).

Investigation of Egyptian Basil Essential Oils by Simple Chromatographic Method

By L. HOERHAMMER, A. E. HAMIDI*, and G. RICHTER

Two kinds of basil oil are produced in Egypt under the so-called white and red basil oils. The present study reveals the occurrence of terpineol, linalool, cineole, citral, eugenol, esterified terpineol, geraniol, linalool, and citronellol with acetic and formic acids (geranyl and/or citronellyl acetate, linalyl and/or terpinyl acetate and citronellyl formate) a sesquiterpene alcohol (nerolidol?) and unidentified terpenes in both types of oil. The red type contains in addition methyl chavicol and cinnamic acid ester. The white type contains methyl cinnamate and (safrol ?). A simple economic method for the oil investigation is described.

THE GENUS *Ocimum* includes, according to Hegi (1), 50–60 species in Africa, Asia, and America. Guenther (2) and Gildemeister and Hoffmann (3) state that there is a great possibility of cross pollination in the species of *O. basilicum* L., hence the occurrence of a larger number of varieties and physiological forms is possible. They claim that it seems appropriate to classify the basil oil types according to their chemical composition and geographical sources rather than ascribing them to definite plant varieties. They mention main types of *Ocimum* plants according to the chemical compound dominating in the oil.

Studies reported on the chemical composition of *O. basilicum* oils as reviewed by these authors, indicate that they contain methyl chavicol, anethole, methyl cinnamate, linalool, eugenol, cineole, camphor, terpenes, and sesquiterpenes.

Tackholm (4) describes only two indigenous *Ocimum* plants in the Egyptian flora under the names of *O. menthaefolium* Hoechst and *O. menthaefolium* var. *staminosum* Sims. Ascherson (5) mentions *O. basilicum* L. in Egypt under the cultivated plants. Basil is named "rihan" or "saatar hindy" by the natives of Egypt.

Since no previous work has been reported on the analysis of the Egyptian basil volatile oils, this study was undertaken to reveal their constituents. This study is also made to verify, as far as possible with future studies, the appropriate chemotaxonomical order of the Egyptian basil plants. Further aim of the study is to find out a simple economic method for the oil investigation which could be applied easily in agricultural experimental stations occupied with the production of essential oils, where expensive and delicate apparatus cannot always be afforded easily.

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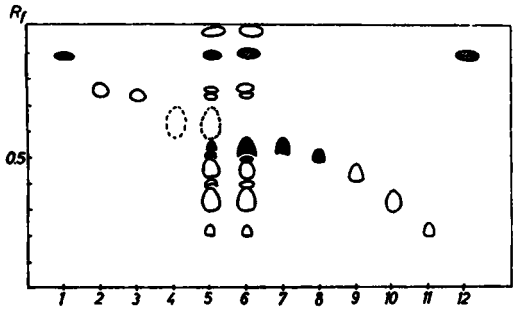

Fig. 1.—Thin-layer chromatogram of Egyptian white and red basil essential oils. Key: 1, safrol; 2, citronellyl formate; 3, geranyl acetate; 4, methyl cinnamate; 5, white basil oil; 6, red basil oil; 7, eugenol; 8, citral; 9, cineole; 10, linalool; 11, terpineol; 12, methyl chavicol.

EXPERIMENTAL

White Basil Oil

The plant from which the oil was obtained was named by the royal botanic gardens, Kew, U.K., as *O. basilicum* L. var. *basilicum*. It is an erect plant with much branching, slightly pubescent, and attaining a height of 80 cm. or more. The racemes are dense with white flowers, hence the name white basil. It grows in the whole Nile valley giving two to three cuts during the year.

Examination of the Oil.—The freshly cut herb (branches and flowering shoots) was steam distilled by the Medi inal and Aromatic Plants Research Division, Ministry of Agriculture, at Barrage north of Cairo. The oil was yellowish having a sweet odor resembling methyl cinnamate. It had the following physicochemical constants: specific gravity (20°), 0.9647; optical rotation, −4.80; refractive index (20°), 1.5078; acid number, 1.4; and ester number, 156.8.

Chromatographic Analysis

An informatory thin-layer chromatogram[1] of the oil (Fig. 1) was prepared to gain a preliminary knowledge of the possible occurrence of the oil constituents, by applying a simple method described by Hoerhammer and co-workers (6). The detection of the spots was carried out by the following reagents after Hoerhammer and co-workers (7): osmium tetraoxide, antimony trichloride, and anisaldehyde. According to the results of this chromatogram, the oil was fractionated on a column using 0.2–0.5 mm. activated silica gel as adsorbant. The column was 1-cm. i.d. and 28 cm. long. A small pledget of cotton was placed in the bottom of the column and the elution was carried out by a mixture of benzene and ethyl acetate (95:5). The fractions 1–31 of 0.5 ml. each and 32–96 of 3 ml. each were col'ected and thin-layer chromatographed (Figs. 2–4). These chromatograms show the distribution of the oil constituents isolated on the column and hav ng R_f values corresponding to authentic test references.

Verification of the Occurrence of the Oil Constituents

Terpineol.—The amount of terpineol in the oil seemed to be small. Therefore a larger column of 3-cm. i.d. and 28 cm. long was used as described above. The oil amount used was 5 ml. The first 61 fractions of 5 ml. each, fraction 62 of 30 ml., and fractions 63–64 of 200 ml. were collected. In the last fraction, which contained mainly linalool, terpineol which had an R_f value on thin-layer chromatogram as that of an authentic terpineol sample, could be detected. Since geraniol has on a thin-layer chromatogram an R_f value like terpineol, to verify the absence of geraniol in the oil, a paper chromatogram was carried out as described by Hoerhammer and co-workers (8). Pure geraniol and the obtained terpineol were tested. There were two different R_f values indicating the absence of geraniol in the oil. The occurrence of terpineol in the oil was also ascertained by obtaining its nitrosochloride derivative (9) in the form of white needle crystals which were identical to crystals obtained by the same manner from pure terpineol. We could not determine the melting point of these few crystals obtained from basil oil.

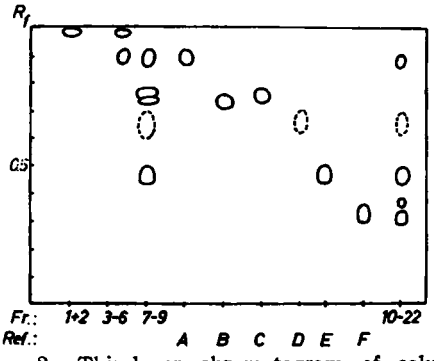

Fig. 2.—Thin-layer chromatogram of column fractionated Egyptian white basil essential oil. Key: Fr., fractions, Ref., test references; A, safrol; B, geranyl acetate; C, citronellyl formate; D, methyl cinnamate; E, cineole; F, linalool.

Linalool.—The fractions 59–66 (Fig. 4) were combined and the solvent evaporated under reduced pressure. An authentic sample of linalool produced the same R_f value as the isolated linalool on the thin-layer chromatogram. The oxidation of the collected fractions with Beckman oxidation reagent (10) at 16° into citral and examining the oxidation product by thin-layer chromatography, resulted in an R_f value and spot color the same as pure linalool treated the same way. The occurrence of linalool was also proved by treating these fractions with 2,4-dinitrophenylhydrazine according to Vogel (11). For comparison, pure linalool was treated the same.

Cineole and Cinnamic Acid Ester.—The fractions 23–29 (Fig. 3) were combined and the solvent evaporated as usual. The oily residue was fractionated on a column as described previously for the oil fractionation. Chloroform was used as eluent. Forty-four fractions of 3 ml. each were collected. The fractions 31–33 on standing for a few hours produced white needle crystals of pleasant odor. These crystals had on thin-layer chromatogram an R_f value and melting point (36.5°) identical to an authentic sample of methyl cinnamate. The oily residue was treated with 2 *N* NaOH for 1 hr. A eucalyptus odor was noticed indicating the presence of cineole. Its occurrence was also proved by means of paper chromatography against pure cineole (7).

The aqueous saponifiable solution was rendered

[1] All thin-layer chromatograms were eluted by a mixture of benzene and ethyl acetate (95:5 v/v) unless otherwise stated, and silica gel G Merck was used as adsorbant.

acidic by means of sulfuric acid. A white precipitate was formed which, after recrystallization, had the odor and melting point (135°) of pure cinnamic acid (see under *The Esters*).

Citral.—This compound was detected by thin-layer chromatography against pure citral. The reproduction of the spots was achieved by means of 2,4-dinitrophenylhydrazine (concentrated solution in 2 *N* HCl). Its occurrence was also proved as follows: 0.5 ml. of the oil was treated with 2,4-dinitrophenylhydrazine to obtain the citral hydrazones, whose crystallization was not possible. The solution was then shaken with petroleum ether (b.p. 30–68°). The petroleum ether extract was dried over anhydrous sodium sulfate and thin-layer chromatographed against the hydrazones of authentic citral. A mixture of benzine (b.p. 50–70°) and ethyl acetate (95:5 v/v) was used for elution. The R_f values and spots color were identical. This system was found applicable to separate the two types of citral hydrazones.

The Esters and Their Corresponding Acids and Alcohols.—The fractions 7–9 (Fig. 2) were combined, reduced to a small volume, and column-fractionated as outlined above. For elution, a mixture of benzene and *n*-heptane (19:1 v/v) was used. Fifty-four fractions of 1 ml. each were collected and thin-layer chromatographed. There were at least three spots indicating the presence of three ester types and a fourth spot with a higher R_f value (Fig. 2). The presence of the corresponding acids was verified through their hydroxamic acids by treating 0.5 ml. of the oil with hydroxylamine as described by Hais (12). Authentic samples of formic, acetic, and cinnamic acids were treated in the same way. These hydroxamic acids were paper chromatographed applying a system described by Hais (13) and using filter paper S.S. 2043b,gl., washed. For the reproduction of the spots, an

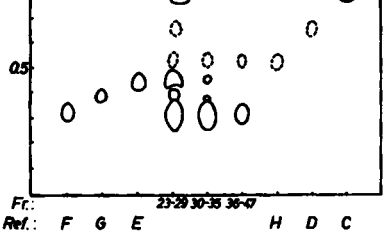

Fig. 3.—Thin-layer chromatogram of column fractionated Egyptian white basil essential oil. Key: Fr., fractions; Ref., test references; F, linalool; G, geraniol; E, cineole; H, citral; D, methyl cinnamate; C, citronellyl formate.

ethanolic solution of 0.1% ferric chloride was used. The spots were identical both in R_f values and color indicating the presence of the three acids respectively.

A portion of these concentrated fractions was thin-layer chromatographed against the available authentic samples of geranyl, citronellyl, terpinyl, and linalyl acetates, citronellyl formate, and methyl cinnamate. Miller and Kirchner (14) described several systems for the separation of terpene alcohols and esters on silicic acid glass plates. Our trials with most of these systems using silica gel were unsuccessful. Therefore a mixture of *n*-heptane, *n*-hexane, and diisobutylketone (1:1:5) was used for elution. The spots were developed by spraying with antimony trichloride. There were (*a*) a lower spot corresponding to methyl cinnamate, (*b*) above it, another spot corresponding to geranyl and/or citronellyl acetate, (*c*) a third higher spot corresponding to linaryl and/or terpinyl acetate, and (*d*) a fourth farther higher spot corresponding to citronellyl formate. It was not possible by this system to differentiate between geranyl and citronellyl acetate on one hand, and linalyl and terpinyl acetate on the other hand. The verification of the corresponding alcohols was carried out as follows. The ester fractions were saponified as usual. The liberated alcohols were paper chromatographed (8) against authentic samples of geraniol, terpineol, citronellol, and linalool. The obtained R_f values were identical, respectively. It is noteworthy to state that the reproduction of a methyl cinnamate spot can also be achieved by spraying the plates with 2,4-dinitrophenylhydrazine, where a stable yellow color is produced. Methyl chavicol could not be detected in these fractions.

Eugenol.—The isolation of this compound on the column was unsuccessful. It was obtained by shaking 0.5 ml. of the oil with 3% aqueous solution of sodium hydroxide, extracting the phenolic substances with ether, drying the ether extract over anhydrous sodium sulfate and thin-layer chromatographed against authentic eugenol. The spots reproduced by anisaldehyde reagent were identical in their R_f values and color.

Safrol.—In the ester fractions 7–9 (Fig. 2) a fourth compound with higher R_f value could be observed; presumably a phenol ether. It had the same R_f value on the thin-layer chromatogram and spot color when sprayed with anisaldehyde and exposed to osmium tetraoxide as an authentic sample of safrol.

Sesquiterpene Alcohol (Nerolidol?).—The fractions 23–29 (Fig. 3) contained a compound located on the thin-layer chromatogram between linalool and cineole. It had an R_f value as an authentic sample of nerolidol. When exposed to osmium tetraoxide it showed an unsaturated property presumably a sesquiterpene alcohol.

Terpenes.—The fractions 1–6 (Fig. 2) contained the terpenes. These were not investigated further because of lack of test references.

Red Basil Oil

The plant from which the oil was produced was named by the royal botanic gardens, Kew, U.K., as *O. basilicum* L. var. *purpurascens* Benth The morphological feature of the plant does not differ greatly from the white type except that the flowers are violet to red, hence the name red basil.

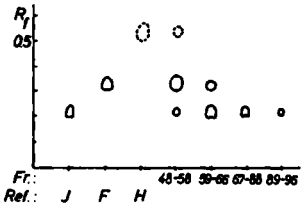

Fig. 4.—Thin-layer chromatogram of column fractionated Egyptian white basil essential oil. Key: Fr., fractions; Ref., test references; J, terpineol; F, linalool; H, citral.

Examination of the Oil.—The red basil oil which was produced by the same method as the white basil oil, had a strong spicy odor and its color was reddish. It had the following physicochemical constants: specific gravity (20°), 0.9111; optical rotation, −10.75°; refractive index (20°), 1.4830; acid number, 6.5; and ester number, 14.5.

Chromatographic Analysis

The oil was investigated the same way as the white basil oil. It had the same chemical composition (Fig. 1) except for the following:

Methyl Chavicol.—This could be detected by thin-layer chromatography against authentic methyl chavicol.

Cinnamic Acid Ester.—Cinnamic acid could be detected by its hydroxamic acid as described in the white basil oil investigation. The isolation of the ester on the column or on the chromatoplates was unsuccessful.

Safrol.—Could not be detected.

Eugenol.—Could be easily isolated on the column.

CONCLUSION AND DISCUSSION

The investigation of the two Egyptian basil oils undertaken in this study, revealed the occurrence of terpineol, linalool, cineole, eugenol, esterified geraniol, citronellol, linalool, and terpineol with acetic and formic acids (geranyl and/or citronellyl acetate, linalyl and/or terpinyl acetate, citronellyl formate), and a sesquiterpene alcohol (nerolidol?) in both types of oils. In addition, the white type contained methyl cinnamate and (safrol?). The red type contained methyl chavicol and traces of cinnamic acid ester. The presence of terpineol, citral, and safrol has not been reported before in the *O. basilicum* oils. Therefore the basil oil types outlined by Guenther (2) and Gildemeister and Hoffmann (3) cannot be applied in arranging these two oil types in any of the reported types. This viewpoint is supported by the occurrence of citral in other *Ocimum* species, *e.g.*, *O. canum* Sims (2, 3), *O. gratissimum* L., and *O. menthaefolium* Hochst. (3). The occurrence of constituents in the Egyptian basil oils, which have not been reported previously, and their differences from basil oil samples of European origin analyzed at the same time, lead to the possibility of arranging these two Egyptian basils in separate chemical or physiological races [Dillemann (15), Rowson (16), Huerlimann (17)], the category to include those plants indigenous to and/or cultivated in Egypt. Further study of the oils of these plants in different periods of plant growth is recommended to ascertain the occurrence and percentage of their chemical constituents which may change according to climatic, edaphic, and genetic factors. These qualitative and quantitative studies are being carried out.

REFERENCES

(1) Hegi, G., "Illustrierte Flora von Mittelleuropa," Vol. 5/4, 2nd ed., J. F. Lehmann, München, Germany, 1939, p. 2267.
(2) Guenther, E., "The Essential Oils," Vol. 3, 3rd ed., D. Van Nostrand Co., Inc., Princeton, N. J., 1958, pp. 399–433.
(3) Gildemeister, E., and Hoffmann, F., "Die Aetherischen Oele," Vol. 7, 5th ed., Akademische Verlag, Berlin, Germany, 1961, pp. 478–516.
(4) Tackholm, V., "The Student's Flora of Egypt," The Anglo-Egyptian Bookshop, Cairo, Egypt, 1956, p. 140.
(5) Ascherson, F., and Schweinfurth, G., "Illustration de la flore d'Egypte," Le Claire, 1887, p. 120.
(6) Hoerhammer, L., and Wagner, H., *Deut. Apotheker-Ztg.*, **101/26**, 779(1961).
(7) Hoerhammer, L., Richter, G., and Wagner, H., *J. Chromatog.*, **10**, 108(1963).
(8) Hoerhammer, L., private communication, Institut für Pharmazeutische Arzneimittellehre der Universität München.
(9) Guenther, E., "The Essential Oils," Vol. 2, 2nd ed., D. Van Nostrand Co., Inc., Princeton, N. J., 1958, p. 775.
(10) *Ibid.*, p. 275.
(11) Vogel, A., "A Textbook of Practical Organic Chemistry," 3rd ed., Longmans, Green, London, 1959, p. 344.
(12) Hais, M., and Macek, K., "Handbuch der Papierchromatographie," Vol. 1, VEB Gustav Fischer Verlag, Jena, Germany, 1958, p. 770.
(13) *Ibid.*, p. 214.
(14) Miller, J. M., and Kirchner, J. G., *Anal. Chem.*, **25**, 1107(1953).
(15) Dillemann, G., *Planta Med.*, **8**, 263(1960).
(16) Rowson, J. M., *ibid.*, p. 243.
(17) Huerlimann, H., *ibid.*, p. 216.

Synthesis and Antitubercular Activity of Isonicotinoyl and Cyanoacetyl Hydrazones

By DIPTISH CHAKRAVARTY, ARUN BOSE, and SAMIR BOSE

Seven isonicotinoyl hydrazones and four cyanoacetyl hydrazones were synthesized. Their antitubercular activities *in vitro* and *in vivo* were evaluated.

THE ANTIMICROBIAL activity of isoniazid against tubercle bacilli depends on the amount of free and unaltered isoniazid (1). The observations of Fox and Gibas (2) show that one or both of the hydrogen atoms attached to nitrogen in the hydrazine moiety of isoniazid may be replaced by a variety of groups with little loss of activity.

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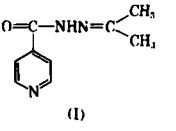
Fox and Gibas (3) have shown that isonicotiny hydrazine when reacted with acetone gives 1-isonicotinyl-2-iso-propylidene hydrazine (I)



(I)

which proved to be very active against tubercle bacilli. These authors (3) have investigated systematically alkylidene derivatives of isonicotinyl hydrazone with the twofold view of dis-