Peel Oils of Different Types of *Citrus sinensis* L. and *C. aurantium* L. Growing in Egypt

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Abstract □ A comparative chromatographic study including column chromatography, TLC, and GLC was carried out on the peel essential oils (cold-pressed and steam-distilled) of different types and varieties of *Citrus sinensis* L. and *C. aurantium* L. growing in Egypt. A mineral oil capillary column was successfully employed in the GLC analysis of these oils. IR patterns of the oils were used in the differentiation between the cold-pressed and steam-distilled oils.

Keyphrases □ *Citrus sinensis, C. aurantium*—peel oil comparison □ Oils, *C. sinensis, C. aurantium*—partial component identification □ Chromatography, TLC, GLC, column—analysis □ IR spectrophotometry—identification

Since they fill an important position in the manufacture of perfumery, cosmetics, confectionery, and pharmaceutical formulations, the citrus essential oils have been the subject of numerous investigations. Mehlitz and Minas (1) used a combination of TLC and column chromatography to identify a number of oxygenated terpenes which were found in the steam-distilled oil of bitter orange peel. Karawya and Wahba (2) identified limonene, terpineol and/or linalool, geraniol and/or nerol, and methyl anthranilate in oil of sweet orange. The composition of certain citrus oils was studied by some workers (3–9) using GLC. In most of these investigations, the oils were separated into hydrocarbons and oxygenated fractions before examination with GLC. Stanley (10) employed TLC, GLC, and spectral methods for the analysis of hydrocarbons, esters, and alcohols in citrus oils. Many authors (11–14) employed IR analysis as a tool for the differentiation between synthetic and natural oils. Albaldejo (11) stated that the IR spectra of the common citrus oils lie between 2 and 16 μ.

The present work is a comparative chromatographic study using column chromatography, TLC, and GLC of the steam-distilled and cold-pressed peel oils of *Citrus sinensis* L. (sweet orange) types (Egyptian, navel, blood, and succari) and of *C. aurantium* L. (bitter orange) types (Egyptian and Spanish). IR analysis of cold-pressed and steam-distilled oils of Egyptian sweet orange was also undertaken.

EXPERIMENTAL

Materials—The cold-pressed and steam-distilled peel oils of the freshly collected mature fruits of four types of sweet orange (*C. sinensis* L.) (Egyptian, navel, succari, and blood) and two types of bitter orange (*C. aurantium* L.) (Egyptian and Spanish) growing in Egypt were used[1].

Procedures—*TLC*—The essential oils, as well as the pure constituents, were chromatographed side by side on silica gel G plates, using *n*-hexane–ethyl acetate (85:15). The chromatograms were inspected under UV light and sprayed with 1% w/v vanillin in sulfuric acid (Figs. 1 and 2). Results are shown in Table I.

Preparative TLC—The oils were chromatographed on silica gel G plates about 2 mm. thick. By this technique, most of the major constituents of the analyzed oils—*viz.*, linalool, terpineol, citral, geraniol and/or nerol, the terpene fraction, and esters were success-

Table I—Mean R_f Values of Separated Components of Cold-Pressed and Steam-Distilled Peel Oils[a]

Compound	Mean R_f	*C. sinensis* L. (Sweet Orange)								*C. aurantium* L. (Bitter Orange)			
		Egyptian		Navel		Succari		Blood		Egyptian		Spanish	
		C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.
Limonene	0.96	+	+	+	+	+	+	+	+	+	+	+	+
Linalyl acetate	0.75	+	±	+	±	+	±	+	±	+	±	+	±
Geranyl acetate	0.73												
Methyl anthranilate	0.67	+	−	+	−	+	−	+	−	+	−	+	−
Citronellal	0.68	+	+	+	+	+	+	+	+	+	−	+	−
Citral	0.51	+	±	+	±	+	±	+	±	+	±	+	±
Linalool	0.43	+	+	+	+	+	+	+	+	+	+	+	+
Terpineol	0.29	+	+	+	+	+	+	+	+	+	+	+	+
Farnesol	0.26												
Geraniol	0.24	+	+	+	+	+	+	+	+	+	+	+	+
Nerol	0.24												
Citronellol	0.25												
Unknown	0.55	+	−	+	−	+	−	+	−	+	−	+	−
Unknown	0.53	−	−	−	−	−	−	−	−	+	+	+	+
Unknown	0.46	−	+	−	+	−	+	−	+	−	−	−	−
Unknown	0.38	+	−	+	−	+	−	+	−	−	−	−	−
Unknown	0.15	−	+	−	+	−	+	−	+	−	+	−	+
Unknown	0.11	−	−	−	−	−	−	−	−	−	+	−	+
Unknown	0.08	+	+	+	+	+	+	+	+	+	−	+	−

[a] C.P. = cold pressed; S.D. = steam distilled; + = present; − = absent; and ± = located in traces.

[1] Species of oranges were identified by Dr. A. T. El Wakil, Division of Fruit Research, Horticulture Department, Ministry of Agriculture U.A.R.; voucher specimens have been deposited at this institution.

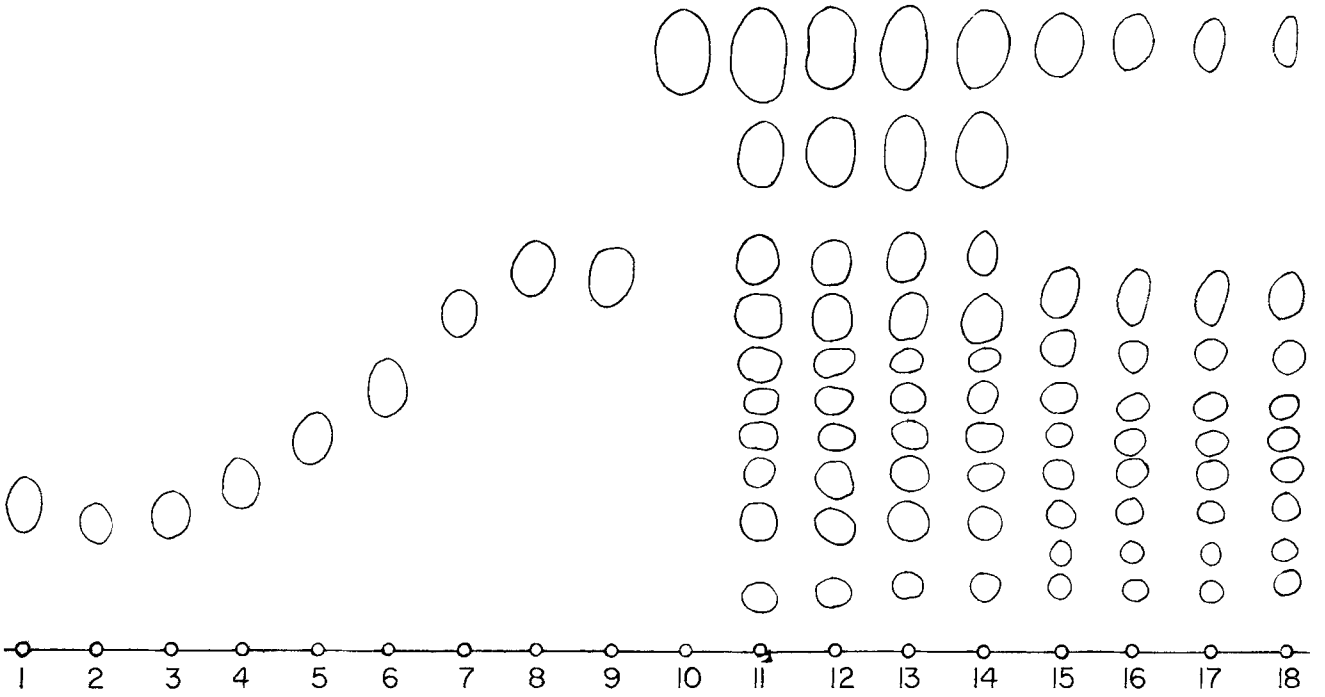

Figure 1—*TLC of the cold-pressed and steam-distilled peel oils of different types of* C. sinensis *L. The solvent used was* n*-hexane–ethyl acetate (85:15). Key: 1, citronellol; 2, nerol; 3, geraniol; 4, terpineol; 5, linalool; 6, citral; 7, citronellal; 8, geranyl acetate; 9, linalyl acetate; 10, limonene; 11, Egyptian orange oil (cold-pressed); 12, navel orange oil (cold-pressed); 13, blood orange oil (cold-pressed); 14, succari orange oil (cold-pressed); 15, Egyptian orange oil (steam-distilled); 16, navel orange oil (steam-distilled); 17, blood orange oil (steam-distilled); and 18, succari orange oil (steam-distilled).*

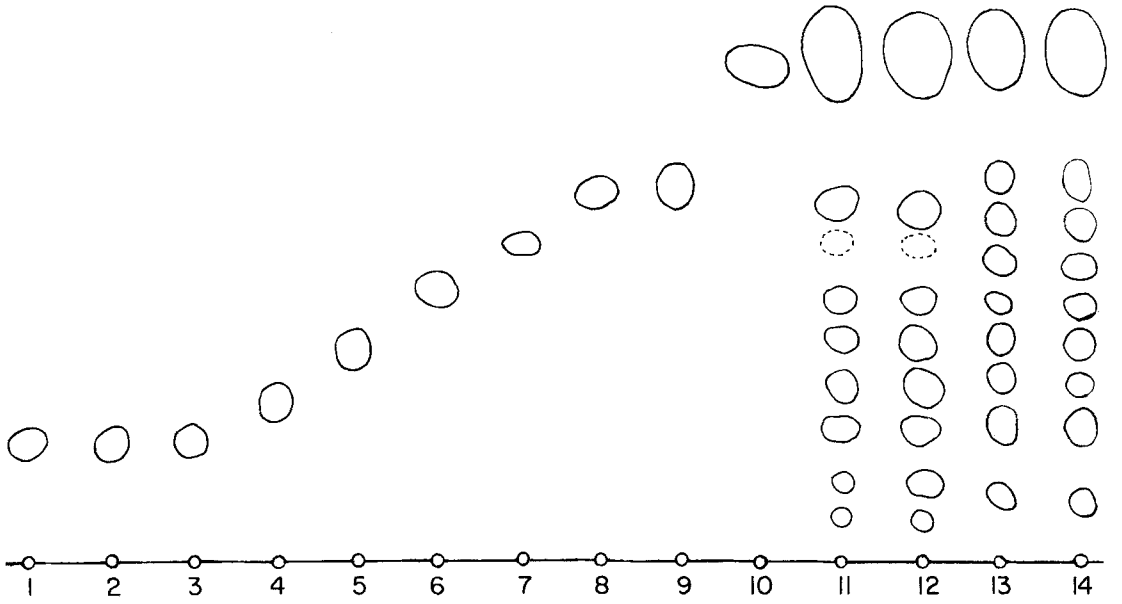

Figure 2—*TLC of the cold-pressed and steam-distilled peel oils of different types of* C. aurantium *L. The solvent used was* n*-hexane–ethyl acetate (85:15). Key: 1, citronellol; 2, nerol; 3, geraniol; 4, terpineol; 5, linalool; 6, citral; 7, citranellal; 8, geranyl acetate; 9, linalyl acetate; 10, limonene; 11, Egyptian bitter orange oil (steam-distilled); 12, Spanish bitter orange oil (steam-distilled); 13, Egyptian bitter orange oil (cold-pressed); and 14, Spanish bitter orange oil (cold-pressed).*

fully separated. These isolates could be used as reference materials for the GLC analyses of the investigated oils.

Column Chromatography—The oil of Egyptian bitter orange, containing most of the constituents of the investigated oils, was chosen for column chromatographic analysis. About 0.3 ml. of the oil was chromatographed on a 10-g. column of silica gel[2] (1-cm. diameter), using successively as eluents: petroleum ether (30–50°); petroleum ether containing 1, 2, and 5% ether; benzene; ether; and ethanol. Complete elution of any constituent was determined by applying a few drops of the effluent directly on silica gel G strips, evaporating the solvent, and spraying with the color reagent. The absence of any color indicated almost complete elution with the solvent. All fractions were then examined by TLC, using a series of microscope slides coated with silica gel G. The fractions were regrouped on the basis of identical R_f values, size, and color of spots, as well as their color under UV light. Results are shown in Table II.

GLC—GLC analyses were performed on an F&M model 500 equipped with flame-ionization detector apparatus (model 1609).

[2] British Drug House.

Table II—Column Chromatogram of the Cold-Pressed Peel Oil of *C. aurantium* L. (Egyptian Bitter Orange)

Number	Eluent	Fraction Number	Vol., ml.	R_f	Color with Spray Reagent	Authentic Sample
1	Petroleum ether	1 + 2	20	—	—	—
		3 to 11	140	0.96	Orange to green	Limonene
		12	10	—	—	—
2	(*a*) 1% Ether in petroleum ether	1 to 6	50	—	—	—
	(*b*) 2% Ether in petroleum ether	1 to 6	50	—	—	—
	(*c*) 5% Ether in petroleum ether	1	5	—	—	—
		2 to 4	60	1. 0.73	Purple to violet	Linalyl and geranyl acetate
				2. 0.40	Violet	Unknown
				3. 0.43	Orange to violet	Linalool
				4. 0.24	Orange to violet	Geraniol and/or nerol and/or citronellol
		5	5	5. —	—	—
3	Benzene	1	5	—	—	—
		2 to 4	30	0.72	Yellow	Unknown
		5 to 10	60	0.29	Orange to green	Terpineol
		11 to 13	15	—	—	—
4	Ether	1	10	—	—	—
		2 to 7	60	1. 0.51	Brown	Citral
				2. 0.08	Faint brown	Unknown
				3. 0.24	Fluorescent under UV	Unknown
				4. 0.19		Unknown
				5. 0.16		Unknown
				6. 0.12		Unknown
5	Ethanol	1	10	—	—	—
		2 to 4	50	Yellow band eluted and gave four unknown spots on TLC		

Mineral oil capillary column, 9144.0 cm. (300 ft.) in length, stainless steel, 0.025-cm. (0.01-in.) internal diameter, was used with the following operating conditions: injection port temperature, 275°; block temperature, 175°; column temperature, 90° (kept isothermal during the whole resolution); attenuation, 8; range, 10; chart speed, 30.48 cm. (12 in.)/hr.; carrier gas, nitrogen; inlet pressure, 7.2–7.5 kg.; and sample size, 0.5–0.8 μl.

The identification of the individual peaks of the chromatogram was achieved by comparing their relative retention distances with those of the different authentic samples under the same conditions. The relative retention distance of each peak was determined with respect to the peak corresponding to terpinene (No. 18), which occurred in all chromatograms of investigated samples and exhibited a sharp and well-defined peak.

The relative retention distances of the peaks in the sequence of their emergence, along with the weight percentages of the corresponding constituents, are listed in Tables III and IV. The numbers in the first column of the table refer to the numbers of the peaks in the order of their increasing relative retention distances.

Figures 3 and 4 show the gas chromatograms of the cold-pressed

Table III—Relative Percentage (w/w) of Terpene Hydrocarbons of Citrus Peel Oils[a]

Peak Number	Compound	d_{Rrel}	*C. sinensis* L. (Sweet Orange)								*C. aurantium* L. (Bitter Orange)			
			Egyptian		Navel		Succari		Blood		Egyptian		Spanish	
			C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.
1	Unknown	0.12	6.4	6.6	7.1	7.4	15.7	14.2	6.6	9.0	4.3	13.5	9.4	13.5
2	Unknown	0.18	2.8	1.6	4.00	4.0	6.9	5.9	4.1	8.2	3.4	7.1	5.1	7.2
2′	Unknown	0.24	—	—	—	—	—	0.9	—	—	—	—	—	—
3	Unknown	0.25	1.1	0.6	2.2	Traces	3.1	2.4	1.2	0.9	1.3	1.4	1.9	1.2
3′	Unknown	0.26	—	—	—	—	—	0.5	—	—	—	—	—	—
4	Unknown	0.32	—	0.4	Traces	—	1.0	1.4	0.4	0.5	—	1.6	—	1.5
5	α-Pinene	0.37	1.5	1.1	1.3	1.5	1.8	1.8	1.6	0.9	3.4	1.6	1.3	1.4
6	Sabinene	0.43	0.3	0.5	—	—	1.4	1.0	0.6	—	—	1.0	—	1.0
7	β-Pinene	0.47	5.3	1.2	5.1	2.6	3.2	2.2	3.9	2.8	8.8	4.0	3.8	3.8
8	Camphene	0.53	0.3	1.6	0.6	0.6	0.7	0.7	0.7	2.6	0.8	—	0.6	—
9	Unknown	0.55	—	—	—	—	—	—	—	0.2	—	—	—	—
10	Myrcene	0.61	0.5	1.1	2.7	1.1	1.7	Traces	2.6	1.3	0.7	2.5	0.9	2.3
12	Limonene	0.72	55.5	31.9	47.7	30.7	30.7	21.5	49.7	46.1	47.5	23.0	38.3	23.0
13	β-Phellandrene	0.83	—	—	—	—	—	0.6	Traces	2.1	1.7	2.8	Traces	1.6
14	Ocimene	0.85	0.5	2.5	Traces	2.5	Traces	0.4	2.4	5.8	1.1	—	2.6	—
15	*p*-Cymene	0.88	1.1	2.3	2.7	2.3	2.9	1.2	1.3	1.7	1.5	1.6	2.0	1.6
16	Unknown	0.90	0.3	1.7	1.2	1.7	1.3	2.4	0.5	Traces	1.1	2.3	1.0	2.2
17	Unknown	0.95	—	—	1.1	—	1.1	—	1.1	—	—	2.0	—	2.0
18	Terpinene	1.00	2.2	10.4	2.3	10.6	2.3	3.6	3.2	6.5	4.2	5.4	3.00	5.4
19	Unknown	1.04	2.8	5.3	2.7	5.3	5.8	3.2	2.7	0.9	0.1	1.8	4.2	1.8
19′	Unknown	1.06	—	—	—	—	—	1.0	—	—	—	—	—	—
20	Unknown	1.08	2.0	3.2	1.8	3.2	2.9	6.3	1.7	1.1	1.0	4.3	2.5	4.2
20′	Unknown	1.09	—	—	—	—	—	—	—	—	0.2	—	—	—
	Total Terpene Hydrocarbons		77.7	74.0	82.1	74.5	81.5	71.4	84.3	90.5	81.0	76.5	76.9	73.7

[a] d_{Rrel} = relative retention distance; C.P. = cold pressed; and S.D. = steam distilled.

Table IV—Relative Percentage (w/w) of Oxygenated Hydrocarbons of Citrus Peel Oils[a]

Peak Number	Compound	d_{Rrel}	*C. sinensis* L. (Sweet Orange)								*C. aurantium* L. (Bitter Orange)			
			Egyptian		Navel		Succari		Blood		Egyptian		Spanish	
			C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.	C.P.	S.D.
21	Unknown	1.15	2.0	0.5	0.6	0.5	0.5	Traces	0.6	0.7	0.6	—	0.6	—
22	Terpineol	1.18	0.6	0.7	1.0	0.7	1.0	1.4	1.0	1.1	1.1	1.6	1.4	1.6
23	Unknown	1.23	2.0	0.6	—	0.6	—	1.1	—	—	—	1.6	—	1.6
24	Citronellal	1.26	0.7	0.5	0.5	0.5	0.6	1.0	0.4	0.1	1.1	0.7	0.3	0.8
25	Unknown	1.3	—	—	2.5	Traces	1.1	Traces	—	—	0.1	0.6	0.5	0.6
26	Linalool	1.33	2.1	5.2	1.2	3.2	4.0	7.2	2.1	2.8	1.9	3.0	4.7	3.1
27	Linalyl acetate	1.37	2.2	1.4	2.5	1.5	2.6	2.1	2.0	1.2	1.9	1.0	1.5	1.0
27′	Unknown	1.43	—	—	—	—	—	—	—	—	1.3	—	—	—
28	α-Citral	1.51	1.1	1.1	1.1	0.9	1.0	1.0	1.0	0.1	0.5	1.0	0.9	1.0
29	Citronellol	1.64	1.0	—	—	—	—	—	0.7	—	0.4	—	—	—
29′	β-Citral	1.76	—	—	—	1.1	—	Traces	—	Traces	—	0.3	4.2	0.2
30	Nerol, and farnesol, and unknown	1.82	7.1	5.5	0.5	5.6	6.4	2.8	7.8	2.3	5.2	5.0	3.7	5.1
31	Geraniol	1.89	0.8	1.1	1.4	1.1	1.1	4.9	1.1	0.2	0.7	3.0	2.0	3.0
32	Geranyl acetate	2.05	1.0	6.5	2.1	5.5	0.4	2.2	0.7	0.2	0.7	3.7	1.4	3.7
32′	Unknown	2.14	—	4.4	—	4.4	—	—	—	—	—	—	—	—
33	Methyl anthranilate	2.4	0.5	0.1	0.4	0.1	0.4	0.4	Traces	Traces	Traces	1.0	0.3	1.0
34	Unknown	2.5	—	—	—	Traces	—	—	—	—	—	0.3	—	0.6
35	Unknown	2.6	—	—	—	—	—	—	—	—	—	0.3	—	0.3
	Total Oxygenated Hydrocarbons		21.1	27.6	13.7	25.7	19.0	24.10	16.3	8.7	15.5	23.1	21.5	23.6

[a] d_{Rrel} = relative retention distance; C.P. = cold pressed; and S.D. = steam distilled.

and steam-distilled peel oils of the Egyptian bitter orange, respectively.

IR Analysis—To study the potentialities of IR spectroscopy in the qualitative comparison between cold-pressed and steam-distilled peel oils of different oranges, those of Egyptian orange were studied as an example. The spectra of both the cold-pressed and steam-distilled peel oils of the Egyptian orange were made with a Unicam SP 200 spectrophotometer, using potassium bromide disks smeared lightly with the investigated oil. The charts are shown in Figs. 5 and 6.

RESULTS AND DISCUSSION

TLC chromatograms (Figs. 1 and 2) of the citrus peel oils investigated were well developed by *n*-hexane–ethyl acetate (85:15). Constituents corresponding to the major spots could be isolated by preparative TLC and analyzed by GLC. It was possible to chromatograph 5 ml. of the oil on 10 plates at one time. The terpenes were successfully separated from the oxygenated hydrocarbons by column chromatography.

In a previous communication (15), it was reported that mineral oil capillary columns were very efficient in the separation of the constituents of the volatile oils of some *Labiatae* plants. This process has been successfully applied to the citrus peel oils under investigation. The following constituents were determined qualitatively and quantitatively.

Hydrocarbons—The terpene hydrocarbons gave one spot on TLC and emerged together in the front zone from the column with petroleum ether. However, the GLC study of the terpene fraction, isolated by TLC and by silica gel column, showed the following. α-Pinene, β-pinene, limonene, and terpinene were identified in all the oils under investigation. The occurrence of the other constituents in the investigated oils is shown in Tables II and III.

Limonene normally accounted for the largest content of any constituent of citrus oils being present in higher concentration in the cold-pressed (30.7–55.5%) than in the steam-distilled peel oils (21.5–46.1). α-Pinene occurred in low concentrations in the investigated oils, ranging from 0.9 to 3.4%. β-Pinene, however, was more abundant than α-pinene; its concentration ranged from 1.12 to 8.8% in the examined oils. The cold-pressed oil of the Egyptian bitter orange showed the highest concentration of α-pinene and β-pinene, 3.4 and 8.8%, respectively.

Sabinene content reached up to 1.4% in cold-pressed oil of succari orange. Camphene occurred in low concentrations, ranging from 0.3 to 2.5% in the investigated oils. Myrcene and β-phellandrene were identified (up to 2.6 and 2.8%, respectively) in all the oils. Ocimene and *p*-cymene were present in low to moderate concentrations, up to 5.8 and 2.9%, respectively. Terpinene occurred in higher concentrations in the steam-distilled than in the cold-pressed oils, being 3.6–10.6% in the former oils and 2.2–4.2% in the latter oils.

Thirteen additional unknown peaks were located in the chromatograms of the investigated oils, as presented in Table III.

From the previous data, it is clear that the terpene hydrocarbons constitute the higher proportion of the total makeup of the investigated peel oils, being slightly higher in the cold-pressed oils (77.5–85.6%) than in the steam-distilled oils (72.0–75.6%). The hydrocarbon content of the cold-pressed oil of blood orange (85.6%)

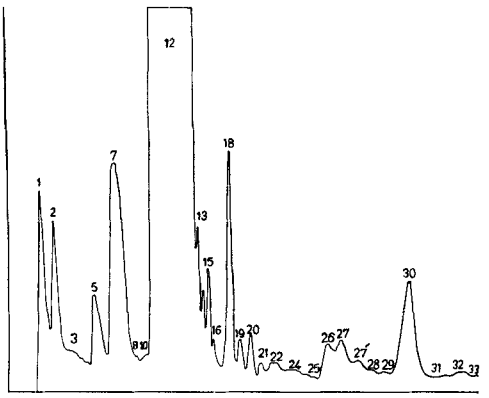

Figure 3—*GLC of the cold-pressed peel oil of Egyptian bitter orange.*

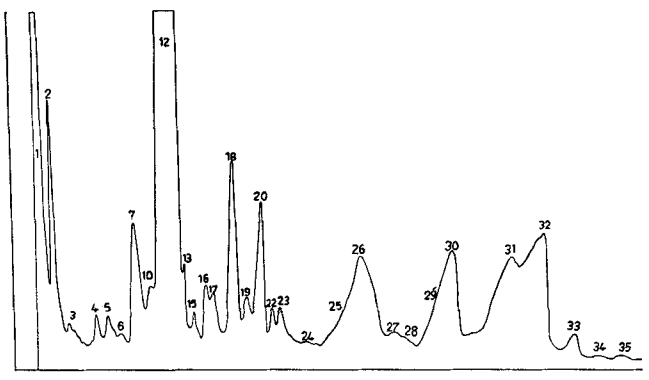

Figure 4—*GLC of the steam-distilled peel oil of Egyptian bitter orange.*

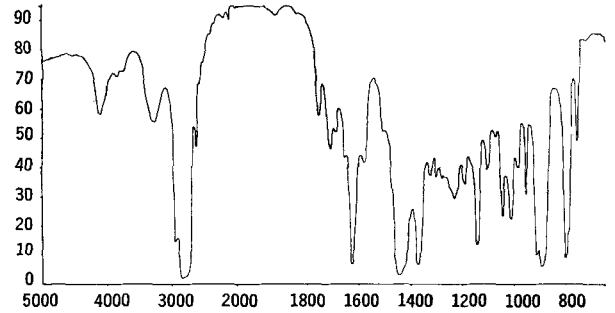

Figure 5—*IR spectrum of cold-pressed peel oil of Egyptian sweet orange.*

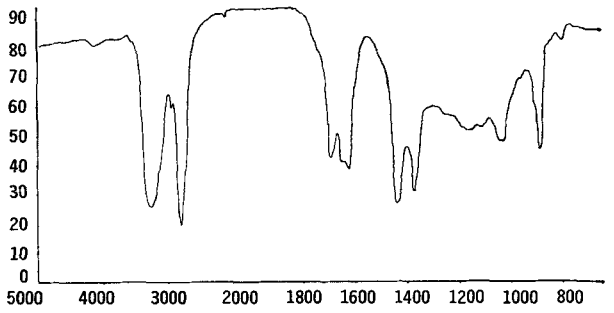

Figure 6—*IR spectrum of steam-distilled peel oil of Egyptian sweet orange.*

is exceptionally lower than that of the respective steam-distilled oil (90.4%).

Oxygenated Hydrocarbons—The oxygenated hydrocarbons were resolved into many spots by TLC, emerged into different zones by various eluents on a silica gel column, and gave numerous peaks by GLC. The following constituents were identified when compared with the respective authentic samples.

Linalool, terpineol, geraniol and/or nerol and/or citronellol and/or farnesol, citronellal, citral, linalyl acetate, and geranyl acetate were of common occurrence in the investigated oils. Methyl anthranilate, although detected in all the oils by GLC, could not be located in the TLC chromatograms of the steam-distilled oils (blue fluorescence under UV). This indicates that the steam-distilled oils may contain a substance that separates in the same zone as methyl anthranilate, quenching its fluorescence.

Linalool was located as one spot by TLC; it was eluted from the column together with other alcohols such as geraniol, nerol, citronellol, farnesol, and certain esters by 5% ether in petroleum ether. However, it gave a well-defined peak by GLC. Linalool content ranged from 2.8 to 7.2% in the steam-distilled oils and from 1.2 to 4.7% in the cold-pressed oils. Terpineol and geraniol were of low concentration in the investigated oils, ranging from 0.6 to 1.4% and from 0.2 to 4.9%, respectively. Terpineol gave one spot on TLC and was eluted from the column by benzene.

Citronellol was identified in the cold-pressed oils of Egyptian orange (1.0%), blood orange (0.7%), and Egyptian bitter orange (0.4%). Nerol and farnesol, together with other unidentified components, emerged as a broad peak, ranging from 0.5 to 7.8%. Citronellal gave almost the same R_f as esters by TLC, yet it could be identified in the investigated oils by GLC. It ranged from traces to 1.1%. Citral gave one spot on TLC and emerged from the column together with other unidentified components on ether elution. By GLC, authentic citral showed two peaks of different retention distances (1.5 and 1.8), which are expected to correspond to α- and β-citral, respectively. The first peak was identified in all the investigated oils, ranging from trace to 1.1%; the second peak appeared as a shoulder in steam-distilled oils of navel orange, succari orange, blood orange, and both kinds of bitter orange and in a comparatively high concentration (4.2%) in cold-pressed oil of Spanish bitter orange.

Linalyl acetate occurred in the cold-pressed oils in a comparatively moderate concentration, ranging from 1.5 to 2.6% and from 2.3 to 2.1% in the respective steam-distilled oils. Geranyl acetate was of a lower concentration than linalyl acetate. In the case of the steam-distilled oils of Egyptian and navel orange, the peak corresponding to geranyl acetate was flat due to the probable presence of other components of the same retention distance; its concentration was 6.5% in both oils. Methyl anthranilate concentration in the cold-pressed oils was usually higher than in the steam-distilled oils and ranged from a trace to 0.5%.

Seven unknown peaks were also located in the chromatograms of one or more of the investigated oils, as shown in Table IV. The total oxygenated constituents ranged from 14.4 to 19.2% in the cold-pressed oils and from 9.5 to 25.6% in the steam-distilled oils.

With respect to the IR spectroscopy, the spectra of the cold-pressed oil (Fig. 5) and steam-distilled oil (Fig. 6) of Egyptian orange peel show common bands and differ in others with respect to their position and comparative intensity. The broad band at about 3430 cm.$^{-1}$ measures the free alcohols, *e.g.*, citronellol, geraniol, linalool, and some other low boiling alcohols. This band is more intense in the steam-distilled than in the cold-pressed oil due to the probable hydrolysis of the esters during the distillation process resulting in a higher alcohol content. The unresolved bands at about 2960, 2930, and 2860 cm.$^{-1}$ are due to the C—H stretching frequencies of the CH_3 and CH_2 groups and are a measure of the aliphatic constituents in the oil. They are more pronounced in the cold-pressed than in the steam-distilled oil. Acetic esters appear clearly at about 1740 cm.$^{-1}$ in the cold-pressed oil and are almost absent in the steam-distilled oil. The small band at 1665 cm.$^{-1}$ is the C=C stretching frequency of the isopropylidene group, common to the constituents of both oils. There is a small band at about 1680 cm.$^{-1}$ which corresponds to citral and appears for both steam-distilled and cold-pressed oils. Citronellol and geraniol show absorptions at 1059 and 1003 cm.$^{-1}$, respectively, and are more distinct in the cold-pressed oil than in the respective steam-distilled oil. A common band at 890 cm.$^{-1}$, somewhat larger in the cold-pressed oil than in the steam-distilled oil, is due to the isopropenyl band of the sesquiterpenes.

On examining the IR patterns of the investigated cold-pressed and steam-distilled peel oils of Egyptian orange, it is evident that the cold-pressed oil spectra show numerous bands which are weak or completely absent in the spectra of the steam-distilled oil of the same species. Bands at about 845–920, 1300–1500, and 3900–4400 cm.$^{-1}$ are much stronger in the case of the cold-pressed oil. Bands at about 740–765, 800, and 940–970 cm.$^{-1}$, although strongly exhibited in the spectra of the cold-pressed oil, are nearly absent in those of the steam-distilled oil.

REFERENCES

(1) A. Mehlitz and T. Minas, *Riechst. Aromen Koerperpflegem.*, **15**, 204 (1965); through *Chem. Abstr.*, **63**, 11243(1965).

(2) M. S. Karawya and S. K. Wahba, *Egypt. Pharm. Bull.*, **44**, 31(1962).

(3) C. A. Slater, *J. Sci. Food Agr.*, **12**, 257(1961).

(4) A. Liberti and C. P. Cartoni, *J. Chromatogr.*, **3**, 121(1960).

(5) R. A. Bernhard, *ibid.*, **3**, 476(1960).

(6) J. R. Clark and R. A. Bernhard, *Food Res.*, **25**, 389(1960).

(7) J. T. Kung, P. Bambara, and F. Perkins, Jr., *Amer. Perfum. Cosmet.*, **4**, 37(1967).

(8) W. D. Macleod, Jr., and N. M. Buigues, *J. Food Sci.*, **31**, 588(1966).

(9) W. L. Stanley, M. R. Ikedd, and S. Look, *Food Technol.*, **15**, 381(1961).

(10) W. L. Stanley, *ibid.*, **4**, 9(1962).

(11) F. N. Albaldejo, *Quim. Ind.*, **9**, 199(1962).

(12) F. A. Rotondaro, *J. Ass. Offic. Agr. Chem.*, **47**, 611(1964).

(13) H. V. Morales, *An. Fac. Quim. Farm. Univ. Chile*, **16**, 182(1964).

(14) M. F. Carroll and W. J. Price, *Perfum. Essent. Oil Rec.*, **55**, 114(1964).

(15) M. S. Karawya, S. I. Balbaa, and M. S. Hifnawy, *Amer. Perfum. Cosmet.*, **85** (8), 23(1970).

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Synthesis and *In Vitro* Antimicrobial Evaluation of Hydrazones of 1-Phenyl-, 1-Benzyl-, and 1-Benzhydryl-4-aminopiperazines

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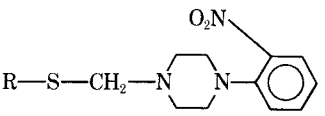
Abstract □ Thirty new hydrazones of 1-phenyl-, 1-benzyl-, and 1-benzhydryl-4-aminopiperazines were prepared and tested for *in vitro* antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Mycobacterium phlei*, and for antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans*. Activities were detected in seven compounds. A bactericidal action against *B. subtilis* and *Cl. perfringens* was noted in 1-phenyl-4-(5-nitro-2-furfurylideneamino)piperazine. 1-Benzyl-4-(5-nitro-2-furfurylideneamino)piperazine and 1-benzhydryl-4-(5-nitro-2-furfurylideneamino)piperazine were found to suppress the growth of *B. subtilis*. 1-Benzhydryl-4-isonicotinylideneaminopiperazine showed a broad spectrum of activity, inhibiting the growth of *M. phlei*, *S. aureus*, *B. subtilis*, and *S. cerevisiae*. 1-Benzhydryl-4-nicotinylideneaminopiperazine had a bacteriostatic effect on *M. phlei*, while 1-benzyl-4-[4-(4-methoxyphenyl)-3-butene-2-ylideneamino]piperazine exhibited a bactericidal action against the same bacteria. A bacteriostatic effect on *S. aureus* was observed in 1-benzyl-4-(5-nitro-2-hydroxybenzylideneamino)piperazine. None of the hydrazones showed antimicrobial activity against *E. coli*, *P. aeruginosa*, and *C. albicans*.

Keyphrases □ Hydrazones of 1-phenyl-, 1-benzyl-, and 1-benzhydryl-4-aminopiperazines—synthesis □ Antimicrobial activity—hydrazones □ Structure-activity relationships—hydrazones □ IR spectrophotometry—structure

Numerous piperazine derivatives have been reported in the literature to have antibacterial activity. For example, Nakanishi and Muro (1) noted that the unsymmetrically 1,4-disubstituted piperazines (I) were



R = 4-ClC_6H_4, CH_3CH_2, $C_6H_5CH_2$, 4-$CH_3OC_6H_4$, or 4-$CH_3C_6H_4$

I

useful bactericides and fungicides. Pedrazzoli and Dall'-Asta (2) synthesized 1-[2-(2,4-dichlorobenzoyloxy)-2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine and a number of other related 1-phenethyl-4-methylpiperazine derivatives, and they reported that these compounds exhibited both trichomonicide and amebicide activities. Hydrazones containing the piperazine nucleus have recently been examined as potential antimicrobial agents. Kozhukharov and Kharizanova (3) reported that the diisonicotinyl hydrazone of 1,4-bis(benzoylethyl)piperazine showed tuberculostatic activity. Recent work by Prescott *et al.* (4) indicated that the bishydrazones obtained by reacting 1,4-diaminopiperazine with salicylaldehyde, 5-nitrosalicylaldehyde, and 3,4-dichlorobenzaldehyde showed a high *in vitro* antibacterial activity against *Staphylococcus aureus*.

As part of a continuous investigation on piperazine compounds with potential medicinal uses, series of hydrazones of 1-phenyl-, 1-benzyl-, and 1-benzhydryl-4-aminopiperazines were prepared and their *in vitro* antibacterial and antifungal activities were determined. Eight aldehydes, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-diethylaminobenzaldehyde, 5-nitro-2-hydroxybenzaldehyde, 3,4-dichlorobenzaldehyde, 5-nitro-2-furfural, isonicotinaldehyde, and nicotinaldehyde, and three ketones, 4-methoxypropiophenone, 4-(2-hydroxyphenyl)-3-buten-2-one, and 4-(4-methoxyphenyl)-3-buten-2-one, were used in the formation of the hydrazones.

EXPERIMENTAL[1]

Chemical Synthesis—All melting points were determined using a Thomas-Hoover Unimelt apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer model 237B spectrophotometer and were in agreement with the assigned structures.

With the exception of 5-nitro-2-furfural, all aldehydes and ketones used in this work were obtained from commercial sources. 5-Nitro-2-furfural was prepared according to the procedure reported by Kochergin and Karpov (5).

The preparation of the title compounds consisted of monosubstitution of piperazine, nitrosation of the monosubstituted piperazines, reduction of the nitroso group, and condensation of the aldehydes or ketones with the 1-substituted-4-aminopiperazines.

1-Benzylpiperazine and 1-benzhydrylpiperazine were prepared according to the method of Kitchen and Pollard (6). 1-Phenylpiperazine was obtained commercially.

Nitrosation of 1-phenyl-, 1-benzyl-, and 1-benzhydrylpiperazines and subsequent reduction to 4-aminopiperazines were carried out by the methods described in the literature (7–10).

Formation of Hydrazones—A solution of the 1-substituted-4-aminopiperazine (0.0125 mole) in ethanol was treated with the appropriate aldehyde or ketone (0.0125 mole) dissolved in the same solvent. If no hydrazone was formed immediately, the reaction

[1] Elemental analyses were performed by Strauss Microanalytical Laboratory, Oxford, England.