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Qualitative Analysis of the Essential Oil of Cassia (*Cinnamomum cassia* Blume)

Roelof ter Heide

Cassia oil (*Cinnamomum cassia* Blume) has been analyzed by means of gas chromatography of fractions obtained by extraction of the oil with sodium bisulfite, sodium hydroxide, and sodium carbonate. The individual components were identified by mass, infrared, and nmr spectral methods. Thirty-five components were identified in the oil. Twenty-three of them have not previously been reported to be present in cassia oil. Except for some of the acids, all constituents found are benzene derivatives, of which 11 components are substituted in the ortho position.

Oil of cassia is the volatile oil distilled from the leaves and twigs originating from *Cinnamomum cassia* Blume, which is cultivated mainly in the southeastern part of the People's Republic of China in the provinces of Kwangsi and Kwangtung. Cassia oil is appreciated for its cinnamon-like flavor, an effect which is based on the presence of the main component of the oil, *i.e.*, cinnamaldehyde. However, it is impossible to achieve cassia flavor with cinnamaldehyde alone, so that it was interesting to know which components were present besides cinnamaldehyde. Our investigations concern the analysis of crude commercial cassia oil directly imported from the People's Republic of China. Most of the components were identified on the basis of identity of their infrared spectra, mass spectra, and retention data with those of authentic reference compounds. Only a few components of the essential oil of cassia have been reported. The presence of cinnamaldehyde and of 2-methoxycinnamaldehyde in cassia oil is already known for a long time (Bertagnini, 1853; Rochleder *et al.*, 1850, 1854). The latter compound was originally named cassia-stearoptene by Rochleder *et al.* (1850, 1854), but Bertram and Kürsten (1895) proved it to be 2-methoxycinnamaldehyde. The results of the analysis of the nonaldehydic fraction of cassia oil, in which cinnamyl acetate was found to be the major component, possibly accompanied by 3-phenylpropyl acetate, have been reported (*Ber. Schimmel*, 1889). A small amount of free cinnamic acid was also reported. Dodge (1918) and Dodge and Sherndal (1915) isolated salicylaldehyde, benzaldehyde, and 2-methoxybenzaldehyde, and further reported the presence of coumarin, cinnamic acid, benzoic acid, and salicylic acid in the alkali-soluble part of the oil.

Chowdhury and Williams (1964) described an infrared method to distinguish the leaf and twig oil from the bark oil by determination of the content of 2-methoxycinnamaldehyde. The bark oil contains less of this aldehyde.

von Schantz (1962) analyzed cassia oil by thin-layer chromatography and detected cinnamaldehyde, cinnamyl acetate, and eugenol. Paris and Godon (1963), however, did not find eugenol in their sample of cassia oil. These authors and Richter (1965) obtained a positive reaction for cinnamaldehyde by using paper and thin-layer chromatography. Betts (1965) reported the absence of eugenol in cassia bark oil. Montes (1963) applied gas chromatography for the separation of cassia oil constituents and found a number of components, including eugenol and salicylaldehyde.

The latter compound could not be traced by Wellendorf (1963).

EXPERIMENTAL

Reference Substances. Authentic samples of components were obtained from reliable commercial sources or synthesized by well established methods. They were purified by gas chromatography before use.

Operating Conditions. The gas chromatographic analysis was performed on a modified Becker instrument with a flame ionization detector. Three columns were employed. The first column (3 m × 0.25-in. o.d., stainless steel) was packed with diethyleneglycolsuccinate on 80–100 mesh acid-washed Embacel support in the weight ratio 20:80.

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Table I. Compounds Identified in Cassia Oil

	tr/tr, rel to cinnamyl acetate (DEGS, 175°C)	tr/tr, rel to *o*-cresol OV-17 (80°–200°C)	tr/tr, methyl ester rel to methyl decylate OV-17 (80°–200°C)	Method of identification					Authentic reference	Previously reported by[a]
				glc	ms	ir	nmr	tlc		
Benzaldehyde	0.16	0.82		+	+	+			+	6, 8, 9
Salicylaldehyde	0.26			+	+	+		+	+	5, 9
2-Methoxybenzaldehyde	0.63			+		+			+	6, 9
trans-Cinnamaldehyde	0.83	2.20		+	+	+		+	+	2, 4, 7, 8, 9, 10, 11, 14, 15
trans-2-Methoxycinnamaldehyde	2.78			+		+			+	3, 4, 12, 13
2-Hydroxyacetophenone		1.52		+	+	+			+	
Methyl benzoate	0.20			+		+			+	
Phenethyl acetate	0.34			+		+			+	
3-Phenylpropyl acetate	0.50			+		+			+	9
trans-Cinnamyl acetate	1.00			+		+			+	1, 14
trans-2-Methoxycinnamyl acetate	3.18			+		+			+	
Phenethyl alcohol	0.45			+		+			+	
trans-Cinnamyl alcohol	1.44			+		+			+	
Coumarin	3.03	2.84		+	+	+			+	5
Phenol		0.76		+	+			+	+	
o-Cresol		1.00		+	+				+	
Guaiacol	0.39	1.20		+	+	+		+	+	
2-Vinylphenol		1.37			+	+	+			
4-Allylphenol (Chavicol)		1.73		+		+			+	
4-Ethylguaiacol		1.84		+	+				+	
Eugenol		2.14		+	+	+			+	9, 14
2-Methylbutyric acid			0.17	+	+				+	
3-Methylbutyric acid			0.17	+	+				+	
Hexanoic acid			0.28	+	+				+	
Heptanoic acid			0.44	+	+				+	
Octanoic acid			0.62	+	+				+	
Nonanoic acid			0.81	+	+				+	
Decanoic acid			1.00	+	+				+	
Benzoic acid			0.75	+	+			+	+	5
cis-Cinnamic acid			1.23	+	+				+	
trans-Cinnamic acid			1.42	+	+			+	+	1, 5
cis-2-Methoxycinnamic acid			1.84	+	+	+	+		+	
trans-2-Methoxycinnamic acid			2.03	+	+	+	+		+	
Dihydrocinnamic acid			1.13	+	+	+			+	
2-Methoxydihydrocinnamic acid			1.61	+	+	+	+		+	

[a] 1. *Ber. Schimmel* (1889); 2. Bertagnini (1853); 3. Bertram and Kürsten (1895); 4. Chowdhury and Williams (1964); 5. Dodge and Sherndal (1915); 6. Dodge (1918); 7. Huesler (1891); 8. Küchler *et al.* (1965); 9. Montes (1963); 10. Paris and Godon (1963); 11. Richter (1965); 12. Rochleder *et al.* (1850); 13. Rochleder *et al.* (1854); 14. von Schantz (1962); 15. Wellendorf (1963).

It was operated at a temperature of 175°C with a carrier gas (nitrogen) flow of 40 ml per min. The same column was used for preparative gas chromatography. For that purpose it was installed in the oven of an F&M Model 720 gas chromatograph with katharometer detection. The temperature was now programmed from 80 to 200°C at a rate of 2°C per min. The components were trapped in glass capillary tubes of 0.75-mm i.d. After trapping they were dissolved in carbon disulfide and transferred into an infrared microcell.

The second column (2 m × $^1/_8$-in. o.d., stainless steel) was packed with Durapak-OPN and operated at 130°C with a carrier gas (nitrogen) flow of 10 ml per min. Injector and detector temperatures were 260°C. The third column was a 3 m × $^1/_8$-in. o.d. stainless steel column packed with silicon OV-17 on acid-washed Embacel support in the weight ratio 20:80. This column, installed in a Varian Aerograph Model 1220 gas chromatograph, was coupled with a two-stage Watson-Biemann molecular separator to a single focusing 90° magnetic sector field mass spectrometer (Varian-MAT CH5, Bremen, West Germany), operating with an ionization energy of 70 eV. The temperature of the column was programmed from 80 to 200°C at a rate of 2°C per min. The helium flow rate was 40 ml per min. The infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord. Normal cells (path length 0.0091 mm) were used. In the case of trapped components the spectra were recorded in carbon tetrachloride and CS_2 solution, using microcavity cells (0.05 and 0.103 mm path length) with a 4× beam condensing unit.

The nuclear magnetic resonance (nmr) spectra were recorded on a Varian A-60A spectrometer. The samples were dissolved in carbon tetrachloride with tetramethylsilane (TMS) as an internal standard. Peak positions are given as δ values in ppm from TMS. Thin-layer chromatography of the phenols was carried out on silica gel G plates (Merck) with chloroform, hexane–acetic acid (95:5), and hexane–pyridine (95:5) as developing solvents (Klouwen and ter Heide, 1962). They were located on the plates by spraying with 2,6-dichloroquinonechloroimid (0.1% in ethanol). The aromatic acids were separated on silica gel plates with a mixture of dibutylether and formic acid (100:1) as developing solvent and they were visualized with bromophenol blue indicator.

Carbonyl Fraction. Cassia oil (200 g) was vigorously shaken in a separating funnel at room temperature with 400 ml of a freshly prepared aqueous solution (35%) of sodium bisulfite. The aqueous layer, containing dissolved bisulfite addition products, was drawn off. The remaining semisolid

mass, containing an insoluble precipitate of bisulfite addition compounds, was stirred with 500 ml of ether to dissolve the nonreacted oil, filtered off under suction, and washed twice with 50 ml of ether. The filtrate consisted of an ether layer and an aqueous layer.

The aqueous layer was separated and added to the aqueous solution of bisulfite compounds. These combined solutions were extracted twice with 50-ml ether portions to remove residual unreacted oil. All the ether washings were combined.

The carbonyls were removed from the combined aqueous layers by regeneration with dilute sulfuric acid (10%) at pH 1 and subsequent extraction with three 25-ml volumes of ether. The ether solution of carbonyls was dried over anhydrous magnesium sulfate and concentrated to a volume of 2 ml in a rotary evaporator at 35°C. The regenerated carbonyl compounds were separated and isolated by means of preparative gas chromatography for infrared analysis. Part of the precipitate was suspended in water and regenerated in the way as described for the soluble bisulfite addition compounds.

Noncarbonyls. The ethereal solution of unreacted oil was extracted with 5 × 50 ml portions of sodium bisulfite (30% by weight) to ensure complete removal of cinnamaldehyde, washed with 50 ml of water, and dried over anhydrous magnesium sulfate. The solvent was removed by distillation in a rotary evaporator at 35°C. The residue was dried again over magnesium sulfate and distilled at a pressure of 5 mm, using a Vigreux column. Five fractions were obtained, which were separated by preparative gas chromatography. The trapped components were identified by infrared spectrometry. The phenols and acids were isolated and analyzed separately.

Phenolic Fraction. Cassia oil (500 g) was shaken with three portions of 100 ml of 1 *N* KOH at 0°C. The nonreacted oil was removed by extraction of the alkaline solution with ten 30-ml portions of ether. The phenolic compounds were regenerated by introduction of gaseous carbon dioxide until pH 10 and extracted with seven 25-ml volumes of dichloromethane. The combined extracts were dried over anhydrous magnesium sulfate and concentrated to a volume of 5 ml. This concentrate was analyzed by the combination gas chromatography–mass spectrometry. The mass spectra and retention volumes were compared with those of authentic reference substances, examined under identical conditions. Additional evidence was obtained by infrared analysis of trapped components and by thin-layer chromatography.

Acid Fraction. Cassia oil (500 g) was extracted successively with one 100-ml and two 50-ml volumes of sodium carbonate solution (20% by weight) at a temperature of −2°C. The aqueous solutions were combined, extracted with ether (10 × 25 ml) to remove nonreacted oil, acidified to pH 1 with dilute sulfuric acid (4 *N*), and extracted with ether (3 × 25 ml). Part of the combined ether extracts was analyzed by thin-layer chromatography.

The greater part was dried over anhydrous magnesium sulfate and methylated with diazomethane. The ethereal solution of methyl esters was concentrated by distillation at atmospheric pressure using a Vigreux column until a residue of about 5 ml remained. The analysis was performed by the combination glc–ms. The mass spectra and retention volumes were compared with those of authentic reference compounds.

RESULTS AND DISCUSSION

From cassia oil the carbonyls, phenols, and acids were isolated by reactions with $NaHSO_3$, KOH, and Na_2CO_3. It is obvious that, in principle, compounds with more than one functional group will occur in different fractions. Sometimes they can more easily be detected in the one than in the other fraction, depending on the completeness of the reactions and the relative concentrations. Treatment with $NaHSO_3$ gave soluble and insoluble addition products with carbonyls. The unreacted oil containing predominantly the noncarbonyls has also been analyzed.

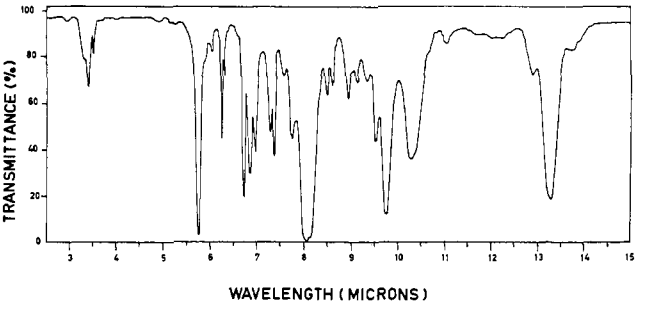

Figure 1. Infrared spectrum of synthetic *trans*-2-methoxycinnamyl acetate

The compounds found in the various fractions of cassia oil and the methods of identification are listed in Table I. The table includes a list of glc retention times on two different liquid phases and relative to three different internal standard compounds, *i.e.*, cinnamyl acetate, *o*-cresol, and methyl decylate. Mass spectra, infrared spectra and nmr spectra of most of the listed compounds were readily available in the literature, except those of 2-methoxycinnamyl acetate, 2-vinyl phenol, the methyl esters of *cis*- and *trans*-2-methoxycinnamic acid, and the methyl ester of 2-methoxydihydrocinnamic acid. The infrared spectra of these compounds are included in the paper. Other spectral data of these compounds are discussed below.

Infrared analysis of the regenerated bisulfite addition products of the carbonyls revealed the presence of benzaldehyde, 2-methoxybenzaldehyde, *trans*-cinnamaldehyde, and *trans*-2-methoxycinnamaldehyde. Only a small proportion of the total cinnamaldehyde was found in this fraction; most of this substance, together with fairly large amounts of 2-methoxycinnamaldehyde, was contained in the insoluble bisulfite precipitate. The noncarbonyl fraction consisted mainly of *trans*-cinnamyl acetate, which is in accordance with other observations (*Ber. Schimmel*, 1889). By comparison of the infrared spectra of trapped components with those of authentic reference substances, the following constituents were identified: *trans*-cinnamyl alcohol, phenethyl alcohol, *trans*-cinnamyl acetate, phenethyl acetate, 3-phenylpropyl acetate, methyl benzoate, and *trans*-2-methoxycinnamyl acetate. The latter compound was synthesized. Structural evidence was obtained by the following spectral data: nmr spectrum (δ values in ppm from TMS) 1.98 (3 H, s), 3.77 (3 H, s), 4.63 (2 H, dd, J = 6.5 and 1 Hz), 6.20 (1 H, dt, J_d = 16 Hz, J_t = 6.5 Hz), 6.6–7.5 (5 H); mass spectrum 206 (M^+) (87%), 164 (87%), 163 (97%), 135 (100%), 131 (93%), 108 (65%), 103 (59%), 91 (64%), 77 (53%), 43 (52%). The infrared spectrum of the synthesized product is given in Figure 1. The ir spectrum of the trapped cassia oil component was consistent.

The presence of phenylpropyl acetate was already presumed in 1889 (*Ber. Schimmel*, 1889). Montes (1963) found it by glc analysis. Some earlier investigators (Dodge, 1918; Montes, 1963) failed to prove the presence of cinnamyl alcohol. The main constituent in the phenolic fraction was

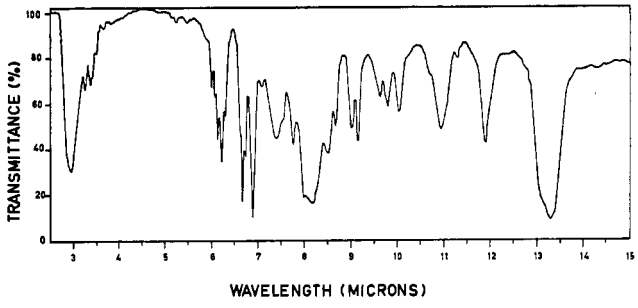

Figure 2. Infrared spectrum of 2-vinylphenol isolated from the phenolic fraction of cassia oil

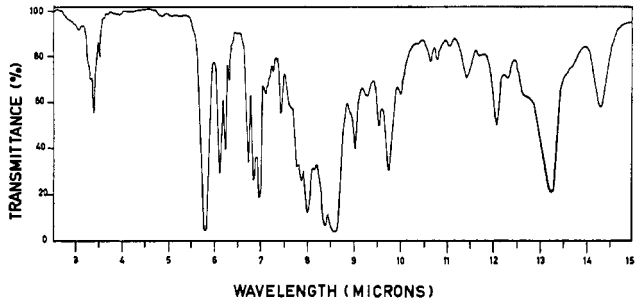

Figure 3. Infrared spectrum of the methyl ester of *cis*-2-methoxycinnamic acid isolated from the methylated acid fraction of cassia oil

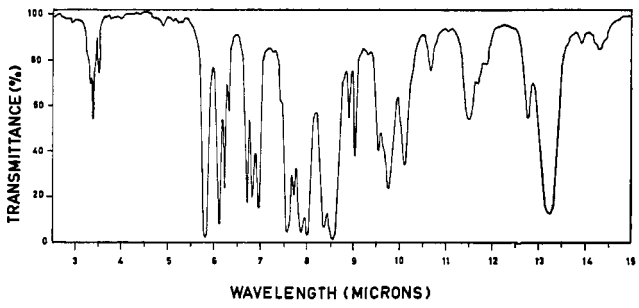

Figure 4. Infrared spectrum of the methyl ester of *trans*-2-methoxycinnamic acid isolated from the methylated acid fraction of cassia oil

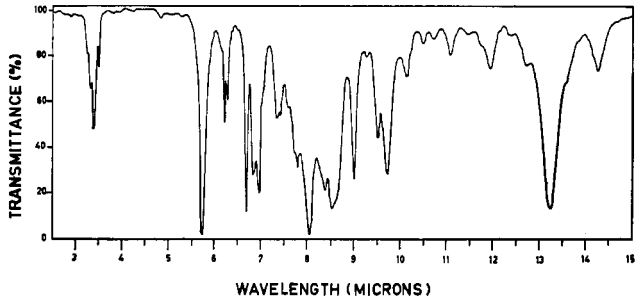

Figure 5. Infrared spectrum of the methyl ester of 2-methoxydihydrocinnamic acid isolated from the methylated acid fraction of cassia oil

salicylaldehyde. Its presence in cassia oil has been reported (Dodge and Sherndal, 1915; Dodge, 1918; Montes, 1963). Wellendorf (1963), however, could not detect it in his gas chromatogram. In this fraction another 2-substituted aromatic carbonyl compound was identified, namely, 2-hydroxyacetophenone, which has not been found previously in cassia oil. The presence of eugenol has sometimes been denied in the literature (Paris and Godon, 1963; Betts, 1965). In our sample of cassia oil, eugenol was positively identified by mass spectral and infrared analysis. In addition the retention times of the trapped component on three different column liquids were identical with those of an authentic reference sample. Other phenols were found for the first time in cassia oil, *viz.* phenol, *o*-cresol, guaiacol, 4-allylphenol (chavicol), 4-ethylguaiacol, and 2-vinylphenol. The latter compound was not available as a reference. The infrared spectrum of the trapped component is shown in Figure 2. Its mass spectral data were 120 (M^+) (83%), 119 (25%), 92 (23%), 91 (100%), 65 (28%), 63 (17%), 51 (18%). The nmr spectrum was of poor quality because of the small sample available, but supported the structure of 2-vinylphenol. Salicylaldehyde, phenol, and guaiacol were also detected by thin-layer chromatography, by comparison with authentic reference compounds.

Fourteen acids were identified as their methyl esters. Twelve were found in cassia oil for the first time. The acid fraction consisted mainly (about 70%) of *trans*-cinnamic acid. The cis isomer was present in much smaller amounts. Cinnamic acid was already reported as a constituent of cassia oil (*Ber. Schimmel*, 1889). Other major components included *trans*-2-methoxycinnamic acid, dihydrocinnamic acid, and benzoic acid. The latter compound was also found by Dodge and Sherndal (1915). The same investigators mentioned the presence of salicylic acid in cassia oil. We found a small peak with the same retention time as methylsalicylate but its mass spectrum was not conclusive. The infrared spectra of the isolated methyl esters of *cis*- and *trans*-2-methoxycinnamic acid are shown in Figures 3 and 4, respectively. Other spectral data of these components were as follows. *cis*-2-Methoxycinnamic acid methyl ester: mass spectrum 192 (M^+) (24%), 161 (100%), 131 (18%), 118 (29%), 105 (27%), 91 (24%), 89 (18%), 77 (30%), 63 (16%), 51 (23%). The nmr spectrum was of poor quality but supported the structure. *trans*-2-Methoxycinnamic acid methyl ester: mass spectrum 192 (M^+) (35%), 161 (100%), 131 (14%), 118 (23%), 105 (23%), 91 (12%), 89 (14%), 77 (17%), 63 (10%), 51 (10%); nmr spectrum δ (CCl_4) 3.72 (3 H, s), 3.87 (3 H, s) 6.38 (1 H, d, J = 16 Hz), 6.7–7.6 (4 H), 7.90 (1 H, d, J = 16 Hz). The infrared spectrum of the methyl ester of 2-methoxydihydrocinnamic acid isolated by glc is given in Figure 5. The mass spectrum was 194 (M^+) (29%), 134 (50%), 121 (79%), 119 (31%), 91 (100%), 77 (30%), 65 (29%), 51 (30%); nmr signals δ (CCl_4) occurred at 2.2–3.1 (4 H, AA'BB' system), 3.58 (3 H, s), 3.81 (3 H, s), 6.5–7.5 (4 H). The methyl esters of 2-methylbutyric acid and 3-methylbutyric acid were separated on a column filled with Durapak-OPN (see experimental). The peak resolution was 1.46. Both acids were detected for the first time in cassia oil.

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Analysis of Volatile Constituents from Meyer Lemon Oil

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Thirty-one compounds were isolated and identified as Meyer lemon oil volatile constituents. Vacuum distillation, column chromatography, and gas chromatography were used for separation and purification and compound identifications were made by infrared spectroscopy and mass spectrometry. Found were seven terpene hydrocarbons, seven sesquiterpene hydrocarbons, nine alcohols, seven carbonyl compounds, and the fungicide, *o*-phenylphenol. The taste threshold was determined for thymol, which was the major oxygenated constituent.

Expansion of Florida's lemon crop from 610,000 boxes in 1967 to 1,510,000 boxes 2 years later indicates a rapidly developing lemon industry in Florida (Florida Agricultural Statistics Citrus Summary, 1970). The Meyer lemon, which grows well in Florida, is considered by Swingle (1967) as probably a hybrid of lemon and some other species of citrus and is commonly designated as [*Citrus limon* × *C. sinensis*]. The juice is sufficiently similar to true lemon so that it is used for making both lemon concentrate and lemonade (Wenzel *et al.*, 1958). The oil has an unusual flavor and aroma and information on oil composition would be of value to the citrus industry.

The only previous studies on Meyer lemons compared juice yield and certain physical constants of 42 lemon varieties, but no individual components of the oils were identified (Kesterson and Hendrickson, 1958; Wenzel *et al.*, 1958). Wenzel *et al.* (1958) prepared and examined frozen concentrates from the 42 varieties and showed Meyer lemons to have the highest juice yield per box. However, they also noted that Meyer lemon peel oil causes an off-flavor in both the juice and concentrate. Kesterson and Hendrickson (1958) showed Meyer lemon oil to have the lowest aldehyde content of the 42 varieties studied.

The unique physical characteristics of Meyer lemon oil and the thymol-like aroma noted when handling whole fruit of this variety prompted an analytical study of its composition. The present paper describes the isolation and identification of volatile constituents from cold-pressed Meyer lemon oil and determination of the taste threshold of thymol, the major oxygenated component.

EXPERIMENTAL

Cold-pressed Meyer lemon oil (43 g) was obtained using an FMC in-line extractor from five boxes of fresh Meyer lemons picked on March 29, 1971.

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Gross Separation Procedures. DISTILLATION. This oil was distilled at a bath temperature of 34°C and 0.5 mm in a rotary evaporator until most of the terpene hydrocarbons (99% limonene) were removed (39.5 g). The liquid nitrogen trap located between the receiver and the vacuum pump contained 0.9 g of liquid and the nondistilled residue weighed 2.1 g.

Liquid Adsorption Chromatography. The 2.1 g of residue was further separated into three fractions on a 1-in. × 17-in. ice water-jacketed (9°C) column containing 60–100 mesh Florisil (Fisher Scientific Co.). The fractions were eluted successively with 400 ml of hexane to remove the hydrocarbons, 400 ml of a 50/50 hexane–ethyl ether solution to concentrate the carbonyl-containing compounds, and 300 ml of absolute ethanol to strip the column of any remaining compounds. The weight of material in each fraction upon removal of the solvent was as follows: hexane, 0.5 g; hexane–ether solution, 1.3 g; and ethanol, 0.2 g.

Glc Procedures. Analyses of these fractions as well as material collected in the liquid nitrogen trap and the distillate from the rotary evaporator were carried out on an F&M Model 810 gas chromatograph equipped with dual thermal conductivity detectors using either a $^1/_4$-in. × 20-ft column packed with 20% Carbowax 20M on 60–80 mesh Gas Chrom P or a $^1/_4$-in. × 20-ft column packed with 10% of the nonpolar liquid phase UCW-98 (Applied Science Laboratories, Inc., State College, Pa.) on 60–80 mesh Gas Chrom P (Applied Science Laboratories, Inc.). For all runs the injection temperature was 250°C, the detector temperature was 280°C, the detector milliamperage was 150, and the carrier gas was helium.

Mass and Infrared Spectral Methods. Fractions were either collected in short capillary tubes for infrared and mass spectral analysis or were run into the mass spectrometer directly from gas chromatography. Mass spectra were obtained with either the Bendix Model 3012 (TOF) or the CEC Model 21–490 mass spectrometer, and infrared spectra were obtained with a Perkin-Elmer Model 137 spectrophotometer.

Spectra for each compound identified were compared to