

## Analysis of the Acid Fraction of Reunion Geranium Oil (*Pelargonium graveolens* L'Her. ex Ait)

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The free acid fraction of geranium oil Bourbon was isolated and analyzed—as such and after methylation—by gas chromatography, mass spectrometry, and infrared spectrometry. Twenty-

nine acids were identified. Most of these have not previously been reported to occur as a free acid in geranium oil.

Although investigations of acids of geranium oil have been carried out since the end of the 19th century a systematic analysis of the acid fraction of geranium oil has not been published so far. Free acids, identified by classical analytical methods, were reported by Flatau and Labbé (1898), Schimmel (1904), Glichitch and Muller (1928), and Goethals (1941a). Hydrolyzed oils were investigated by Barbier and Bouveault (1894), Schimmel (1894), Rutowski and Makarowa-Semljanskaja (1929), Naves (1934), Dodge (1941), Goethals (1941a,b), and Branigan (1944). Formic acid was reported for the first time by Béhal (1914). A crystalline sediment, which had been deposited in a zinc-lined container of geranium oil, was identified by Dodge (1922) as zinc formate.

Also chromatographic techniques, *e.g.*, paper chromatography and thin-layer chromatography, have been applied for the analysis of free and esterified acids (Alder and Schmidt, 1961; Peyron, 1962a,b; Calvarano, 1966; Gogiya and Ivanova, 1968). At the Trubek laboratories (1964) the acids (as their ethyl esters) isolated from hydrolyzed geranium oil were subjected to gas chromatographic analysis. Most of the analytical work on the acids was carried out with saponified geranium oil. This paper reports the analysis of the free acid fraction.

### EXPERIMENTAL SECTION

**Reference Substances.** Reference substances not being commercially available were synthesized. 3-Methyloctanoic acid was prepared by a free-radical reaction of *n*-pentanal to crotonic acid, followed by a Wolff-Kishner reduction of the keto acid obtained. Citronellic acid and the geranic acids were prepared by oxidation with chromic acid of citronellol, nerol, and geraniol, respectively. 3,7-Dimethyl-6-oxooctanoic acid was synthesized by oxidation of menthone with hydrogen peroxide in methanol and catalyzed by pervanadic acid. The acid and its methyl ester were purified by gas chromatography.

**Isolation of the Acids.** Ten portions of 1 kg of Reunion geranium oil were extracted at a temperature of  $-2^{\circ}$  with a 20% aqueous sodium carbonate solution ( $1 \times 200$  ml,  $2 \times 100$  ml). The extractions were completed within 10 min. The nonacid compounds were removed from the combined aqueous layers by extraction with ether ( $10 \times 50$  ml). The aqueous phase was acidified to pH 1 with 4 *N* sulfuric acid and the liberated acids were extracted with three portions of 50 ml of ether. After drying over anhydrous sodium sulfate part of the acid fraction was treated with diazomethane to methylate the acids.

**Gas Chromatography of Free Acids.** The free acids of geranium oil were analyzed on an F&M Model 810 gas chromatograph equipped with flame ionization detection. A  $\frac{1}{8}$  in. o.d. stainless steel column, 3 m in length, was packed with 20% by weight Carbowax 20M-terephthalic

acid (Varian) on 60-80 mesh Embacel support. The analysis was performed isothermally at 150 or 200° with a nitrogen carrier gas flow rate of 10 ml/min.

**Gas Chromatography of Methyl Esters.** The analysis of the methyl esters was carried out on the same gas chromatograph as mentioned above. The stationary phases (20% by weight) on 60-80 mesh Embacel support were packed in 3 m  $\times$   $\frac{1}{8}$  in. o.d. stainless steel columns. The stationary phases and the corresponding temperatures are mentioned in Table I.

**Preparative Gas Chromatography and Infrared Spectrometry.** The methyl ester mixture was separated into 13 fractions with an Autoprep Model A-700 gas chromatograph equipped with a katharometer and fitted with a 6 m  $\times$   $\frac{3}{8}$  in. o.d. stainless steel column packed with Carbowax 20M on 60-80 mesh Embacel support (20% by weight). The carrier gas (hydrogen) flow rate was 200 ml/min and the column temperature was programmed from 80 to 190° at a rate of 6°/min.

These 13 fractions were further separated to obtain pure components for infrared analysis of the methyl esters. The gas chromatographic conditions were adapted to the volatility of the fractions. The components were trapped in glass capillary tubes of 0.75 mm i.d. immersed in liquid nitrogen. The spectra were recorded on a Perkin-Elmer Model 137 infracord using 0.014 mm cells or in some cases 0.05 mm microcavity cells.

**Combined Gas Chromatography-Mass Spectrometry.** The methylated acid fraction was also analyzed with a combined gas chromatograph-mass spectrometer. The gas chromatograph used was a Varian Aerograph Model 1220 with a 2.5-m stainless steel column ( $\frac{1}{8}$  in. o.d.) packed with 10% by weight Carbowax 20M on 60-80 mesh Embacel support. The furnace temperature was programmed from 50 to 200° at a rate of 1°/min.

The effluent of the column was admitted through a Watson-Biemann molecular separator to the inlet of a single focusing 90° magnetic field mass spectrometer (Varian-MAT, CH-5, Bremen, Germany). The total ionization current obtained from the ionization chamber operating at 20 eV was recorded as a gas chromatogram. From the material entering the other ionization chamber operating at 70 eV the mass spectra were obtained.

### RESULTS AND DISCUSSION

The extraction of the acids was carried out under such conditions (temperature,  $-2^{\circ}$ ; duration, 10 min) that hydrolysis of the esters of geranium oil was negligible (Timmer *et al.*, 1975). A survey of the analytical results is given in Table I. The *free acids* could be separated with Carbowax-terephthalic acid as a stationary phase. Symmetrical peaks were obtained. The identification of the free acids was based on comparison of their relative retention times with those of reference compounds. As shown in Table I 18 acids were found. Their retention times relative to *n*-valeric acid (150°) and to *n*-octanoic acid (200°) are listed.

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**Table I. Free Acids Identified in Geranium Oil<sup>a</sup>**

Acids identified in geranium oil	Means of ident. of methyl ester	Relative retention times of geranium acids <sup>b</sup>						Retention indices of geranium acids as methyl esters <sup>b</sup>		Previously reported in geranium oil <sup>h</sup>
		Free acids, CW-TPA		Methyl esters		DEGS		CW-AP-20M, L, TCEP, As	70° free acid	
		150°, c	200°, d	90°, e	150°, f	85°, f	60-200°, 2°/min, OV-17, f			
Formic acid	g									1, 2, 3, 4
Acetic acid	rrt	0.315	0.245			0.182	0.239			8, 9, 10, 11, 12, 13, 14, 15
Propionic acid	rrt	0.450	0.350			0.377				3, 4, 5
Butyric acid	ms, rrt	0.641	0.582			0.240	0.532			4
Isobutyric acid	rrt	0.500				0.427				1, 12, 13, 15, 17
Valeric acid	rrt, ri	1.000	1.000			0.369	1.000		1403	5
2-Methylbutyric acid	rrt, ri					0.662	0.673		1306	1, 9, 12, 13
Isovaleric acid	rrt, ri	0.753	0.670						1323	15, 16
2-Methylcrotonic acid (Tiglic acid)	rrt, ri					0.618	1.600		1532	6, 7
Hexanoic acid	ms, rrt, ri	1.540	2.020			0.553			1494	4
2-Ethylbutyric acid	rrt, ri					0.327	1.180		1372	
2-Methylvaleric acid	rrt, ri	1.090				1.340			1388	
3-Methylvaleric acid	ms, rrt, ri	1.300				0.433	1.370		1433	
4-Methylvaleric acid	ms, rrt, ri	1.110				0.462	1.420		1452	
Heptanoic acid	rrt, ri		0.708			0.657	0.755		1582	15
Octanoic acid	ir, ms, rrt, ri	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1413	4
6-Methylheptanoic acid	ms, rrt			0.803				0.758		15
Nonanoic acid	rrt	1.410	1.500	1.610	1.260	1.060	1.020	1.120		
3-Methyloctanoic acid	ir, ms, rrt		2.250			1.540				
Decanoic acid	ms, rrt			2.090				2.590		
8-Methylnonanoic acid	rrt								1582	1191
Citronelllic acid	ir, ms, rrt, ri	2.130				1.540			1657	7
(Z)-Geranic acid	ir, ms, rrt, ri	2.450				1.760			1716	1224
(E)-Geranic acid	ir, ms, rrt, ri	2.900				1.980				19
3,7-Dimethyl-6-oxo-octanoic acid	nmr, ir, ms, rrt					2.760				
Dodecanoic acid	rrt					2.060				15
Benzoic acid	ms, rrt					1.930				15
Phenylacetic acid	rrt	4.150	4.170			2.350				15
Salicylic acid	ms, rrt	4.490	5.090			2.410				15

<sup>a</sup> Abbreviations used in the table are: rrt, relative retention time; ri, retention index; ms, mass methyl octanoate. <sup>b</sup> Determined according to Warner *et al.* (1955). <sup>c</sup> Literature references are: (1) spectrum; ir, infrared spectrum; nmr, nuclear magnetic resonance; CW-TPA, Carbowax 20M-Goethals (1941a); (2) Dodge (1922); (3) Catvarano (1966); (4) Gogya and Ivanova (1968); (5) Flatau terephthalic acid; CW-20M, Carbowax 20M; DEGS, diethylene glycol succinate; OV-17, silicone and Labbé (1898); (6) Schimmel (1904); (7) Gilchich and Muller (1928); (8) Goethals (1941b); OV-17; TCEP, 1,2,3-tris(2-cyanoethoxy)propane; AP-L, Apiezon-L. <sup>b</sup> The relative retention times (9) Branigan (1944); (10) Béhal (1914); (11) Dodge (1941); (12) Alder and Schmidt (1961); (13) and retention indices of the free and methylated geranium acids mentioned in this table agreed with Peyron (1962a); (14) Peyron (1962b); (15) Trubek Laboratories (1964); (16) Barbier and Bouveault those of authentic reference compounds. <sup>c</sup> Retention time relative to valeric acid. <sup>d</sup> Retention time (1894); (17) Rutowski and Makarowa-Semijanskaja (1929); (18) Schimmel (1894); (19) Naves relative to octanoic acid. <sup>e</sup> Retention time relative to methyl valerate. <sup>f</sup> Retention time relative to (1934).

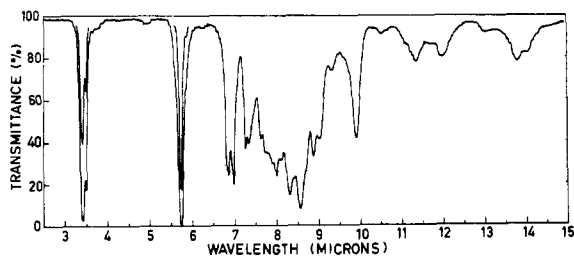


Figure 1. Infrared spectrum of methyl 3-methyloctanoate; neat, 0.014 mm; dilution (CCl<sub>4</sub>), 0.5 mm.

Formic acid could not be detected by gas chromatography because of a lack of response in the flame ionization detector. Moreover the peak of methyl formate obtained after methylation of the acid fraction coincided with the peak of ether used as a solvent. Therefore, formic acid was determined in the oil according to Warner and Raptis (1955). Their method is based on the azeotropic distillation of formic acid with chloroform.

The methylated acids were analyzed by gas chromatography on six different stationary phases. The retention times of the methyl esters relative to methyl valerate or to methyl octanoate were determined with Carbowax 20M-terephthalic acid, diethylene glycol succinate, Silicone OV-17, and Carbowax 20M (40 or 85°). They are listed in Table I. With Carbowax 20M (150°), Apiezon L, and 1,2,3-tris(2-cyanoethoxy)propane as stationary phases the retention indices according to Kovats using *n*-alkanes as reference substances were determined. These indices are also mentioned in the table. Most of the acids—as their methyl esters—could be separated on a diethylene glycol succinate column at programmed temperature.

The retention values of the esters agreed with those of authentic reference compounds. Application of gas chromatography-mass spectrometry resulted in the identification of 14 methyl esters. Six of these could be collected in sufficient amounts for an infrared recording. Only in the case of dodecanoic acid was identification based on glc retention time on one liquid phase without further confirmation by infrared and/or mass spectrometry. The mass spectra of most of the compounds identified were readily available in the literature. Methyl octanoate could be trapped for infrared analysis. Its spectrum proved to be identical with that published by van Wijk (1966).

The presence of 3-methyloctanoic acid was concluded from the mass spectrum of the isolated methyl ester: *m/e* (rel intensity) 172 M (1), 141 (5), 101 (29), 74 (100), 69 (9), 59 (8), 57 (14), 55 (12), 43 (21), 41 (18), and 39 (7). The infrared spectrum of this substance is shown in Figure 1. Furthermore, the structure was proved by synthesis (see Experimental Section).

Three terpenic acids were identified as their methyl esters, *viz.* citronellic acid and (*Z*)- and (*E*)-geranic acid. The mass spectral data of the isolated methyl citronellate were: *m/e* 184 (M) (7), 152 (34), 110 (43), 109 (22), 95 (35), 94 (18), 82 (21), 69 (93), 59 (18), and 41 (100). The infrared spectrum of the same compound is given in Figure 2. Some published ir data (Howard and Stevens, 1960; von Rudloff, 1963) were in agreement.

The mass spectra of the isomeric methyl geranates were identical and agreed with the spectrum published by Thomas *et al.* (1969). The infrared spectra of the isolated methyl esters of the geranic acids were also identical with literature spectra (Stadler and Oberhänsli, 1959).

The structure of 3,7-dimethyl-6-oxooctanoic acid was elucidated by ir, mass spectral and nmr analyses and confirmed by synthesis (see Experimental Section). The infrared spectrum of the isolated acid (as methyl ester) is shown in Figure 3. Other spectral data were: nmr ( $\delta$  values; from Me<sub>4</sub>Si; 60 MHz in CCl<sub>4</sub>) 0.95 (3 H, d, *J* = ~

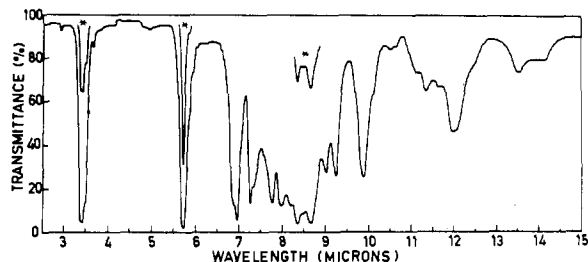


Figure 2. Infrared spectrum of methyl citronellate; neat, 0.014 mm; dilution (CCl<sub>4</sub>), 0.5 mm.

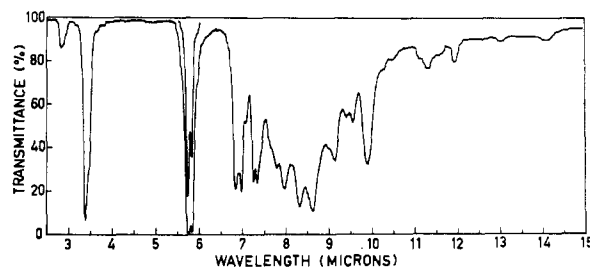


Figure 3. Infrared spectrum of methyl 3,7-dimethyl-6-oxooctanoate; neat, 0.014 mm; dilution (CCl<sub>4</sub>), 0.5 mm.

6 Hz), 1.07 (6 H, d, *J* = 7 Hz), 2.40 (1 H, septet, *J* = 7 Hz), 3.62 (1 H, s); mass spectrum *m/e* 200 (M) (5), 169 (5), 168 (5), 157 (33), 129 (9), 125 (93), 115 (17), 105 (8), 97 (32), 86 (22), 69 (57), 59 (25), and 43 (100).

Mass spectral analysis revealed the presence of at least 11 methyl esters of which the structure could not be elucidated. They are believed to be unsaturated and branched esters of C<sub>8</sub>, C<sub>8</sub>, C<sub>9</sub>, and C<sub>10</sub> acids. Table I shows that the saturated carboxylic acids with an unbranched chain up to and including decanoic acid and also dodecanoic acid were present in geranium oil. Noteworthy is the presence of a great number of acids bearing a methyl substituent. All iso acids listed were newly found in geranium oil. Only the occurrence of isobutyric acid and isovaleric acid in saponified geranium oil was reported (Barbier and Bouveault, 1894; Trubek, 1964). Of the acids with a methyl group in the 3 position 3-methyloctanoic acid has never been reported as a constituent of essential oils. There was also an indication (from the mass spectrum) for the presence of 3-methylnonanoic acid. Its structure could not be definitely established because of a lack of a reference sample.

The presence of citronellic acid, reported by Glichitch and Muller (1928), in a mawah geranium oil was confirmed. The geranic acids (predominantly the *E* isomer) were detected for the first time as free acids in geranium oil. Noteworthy is the identification of 3,7-dimethyl-6-oxooctanoic acid. This compound has never been reported as a constituent of essential oils.

Our sample of geranium oil contains benzoic acid, salicylic acid, and phenylacetic acid in the free state. Workers of the Trubek laboratories (1964) noted the occurrence of these acids in a saponified geranium oil. The presence of anisic acid reported by Goethals (1941b) neither has been confirmed by other authors, nor did we detect it in the free acid fraction. In conclusion, 29 acids were shown to be present in geranium oil of which 21 have not been reported previously as free acids of this oil.

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## Chemicals Related to the Gypsy Moth Sex Pheromone as Attractants and as Synergists or Inhibitors of the Pheromone

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Seven compounds and 11 isomeric mixtures related to disparlure, the sex pheromone of the gypsy moth [*Porthetria dispar* (L.)], were synthesized and tested in the field both alone and in combination with disparlure to determine their activity as attractants and as synergists or inhibi-

tors of the attraction of disparlure. Structural modifications of disparlure were mainly of its epoxy group. Many of the chemicals were attractive to the male moths, but none were as effective as disparlure. None of the compounds had any significant synergistic effect.

Eighteen compounds related to disparlure (*cis*-7,8-epoxy-2-methyloctadecane (3)) the sex attractant pheromone of the gypsy moth, *Porthetria dispar* (L.) (Bierl *et al.*, 1970, 1972), were synthesized to study the effect of structural changes in the molecule on the response of the insect. The compounds were tested for activity as attractants and as synergists or inhibitors of the attraction of disparlure. Some of the compounds may be metabolites of disparlure and so are of special interest.

Most of the syntheses involved reactions of disparlure and (*Z*)-2-methyl-7-octadecene with various reagents, in which the epoxy group was replaced by another group or groups. For example, the oxygen atom in disparlure was replaced by methylene and imino groups and sulfur. In this respect, the chemicals differed from the disparlure-related compounds synthesized and tested previously (Sarmiento *et al.*, 1972), since they were mostly positional isomers or homologs of disparlure. The flow sheet (Chart I) summarizes the syntheses of the 7 compounds and 11 isomeric mixtures that were prepared.

### MATERIALS AND METHODS

**Chemicals and Adsorbents.** Solvents and chemicals were reagent grade. Chromatographic adsorbents were silica gel (J. T. Baker Co., no. 3405, used as received), Florisil (60-100 mesh, Fisher Scientific Co. F-100, activated

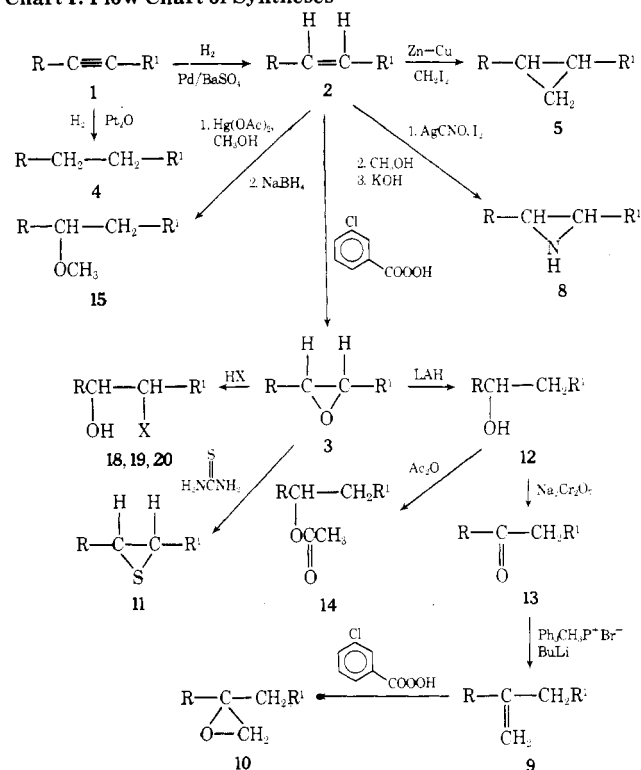
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Chart I. Flow Chart of Syntheses<sup>a</sup>



<sup>a</sup> R and R<sup>1</sup> are 5-methylhexyl and decyl or vice versa; X is Cl, Br, or I.

overnight at 110° before use), and Adsorbosil-CABN (100-140 mesh, 25% silver nitrate on silica gel).

For thin-layer chromatography (tlc), Brinkmann plates (5 × 20 cm, precoated 0.25-mm thick silica gel F254) were