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SHORT COMMUNICATION

ANALYSIS OF COLD-PRESSED ORANGE OIL PARAFFIN WAXES

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Abstract—The paraffins in cold-pressed Valencia orange oil were isolated by column chromatography, separated by gas chromatography, and identified by mass spectroscopy. Normal and branched chain paraffins from C_{21} to C_{29} were found. The normal and the branched C_{23} and C_{25} predominate; n-C_{23} is the major component. The preponderance of the low carbon number paraffins and, in particular the isoparaffins are characteristic to the orange in the plant kingdom. These isoparaffins are identified for the first time in natural products.

INTRODUCTION

DURING an investigation of the hydrocarbon composition of cold-pressed orange oil, the terpene and sesquiterpene constituents were isolated and identified.[1,2] Since the literature contains no information on the composition of the hydrocarbon waxes in citrus oils, this work was undertaken to extend the analysis to include the paraffin waxes.

With the advent of gas chromatography and mass spectroscopy the analysis of these paraffins in natural products is receiving much more attention. Using mass spectroscopy alone, Waldron *et al.*[3] identified the *n*-paraffins in rose petal and tobacco leaf wax among others. Although they recognized the presence of isoparaffins in these waxes they did not identify them. Later, Eglinton *et al.*[4] extended this work to include the wax coating of plant leaves. More recently Oró *et al.*,[5] using the gas chromatographic–mass spectrometric technique recently devised by Ryhage,[6] identified the paraffin hydrocarbons in pasture plants, while Mold *et al.*[7] using the same technique identified the isoparaffins in tobacco.

In the present study the normal and isoparaffins in cold-pressed orange oil were isolated by column chromatography, separated by gas chromatography, and identified by mass spectroscopy, the latter based on analogous reference spectra published by the American Petroleum Institute. In this way seventeen paraffinic hydrocarbons were identified in orange oil, as shown in Fig. 1, ranging from C_{21} to C_{29}. From this gas chromatographic curve it can be seen that the paraffins having an odd number of carbon atoms are more abundant than the alkanes with an even number of carbon atoms. This has generally been

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[1] G. L. K. HUNTER and W. B. BROGDEN, JR., *J. Food Sci.* **30**, 1 (1965).

[2] G. L. K. HUNTER and W. B. BROGDEN, JR., *J. Food Sci.* **30**, 383 (1965).

[3] J. D. WALDRON, D. S. GOWERS, A. C. CHIBNALL and S. H. PIPER, *Biochem. J.* **78**, 435 (1961).

[4] G. EGLINTON, A. G. GONZOLEZ, R. J. HAMILTON and R. A. RAPHAEL, *Phytochem.* **1**, 89 (1962).

[5] J. ORÓ, D. W. NOONER and S. A. WIKSTRÖM, *Science* **147**, 870 (1965).

[6] R. RYHAGE, *Anal. Chem.* **36**, 759 (1964).

[7] J. D. MOLD, R. K. STEVENS, R. E. MEANS and J. M. RUTH, *Biochem. J.* **2**, 605 (1963).

found to be true in the distribution of these high molecular weight alkanes in soils, sediments, and plants.[3–5, 8–9] These same workers have also shown that *n*-paraffins, which are found widely distributed in the plant kingdom, range from C_{25} to C_{35} with C_{25},[8] C_{29},[9] C_{31}[7] and C_{33}[4, 5] paraffins being the most abundant. Because C_{23} is the most abundant normal hydrocarbon in orange oil, this oil appears to be an exception. In addition, the branched chain paraffins, such as 2-methyl-$C_{22}H_{45}$, 3-methyl-$C_{23}H_{47}$, and 2-methyl-$C_{24}H_{49}$, also appear in high concentrations and, from all indications of what has been published, this too, is unusual.

It should be noted that the various paraffins in orange oil occur in a rhythmic sequence of structural analogs and isomers. The sequence alternates between the 2-methyl-paraffin occurring with its normal isomer and the 3-methyl-paraffin accompanied by its normal isomer, the former having an odd number of carbon atoms, the latter an even number. The one exception in this sequence is the appearance of 2-methyl-$C_{21}H_{43}$ with n-$C_{22}H_{46}$. In every case, however, the amount of 2-methyl-paraffin is smaller and the amount of 3-methyl-paraffin is larger than their respective normal isomers.

In summary, seventeen paraffins were identified in cold-pressed Valencia orange oil. The C_{23} and C_{25} normal and isoalkanes appear in greatest abundance which appears to be unique to the orange. The branched chain isomeric paraffins have been identified and shown to occur in an alternating sequence.

EXPERIMENTAL

The hydrocarbon waxes were isolated for analysis from 2·2 l. of cold-pressed Valencia orange oil. The more volatile constituents were removed on a Swissco Rota-Vac distillation apparatus,* which was operated at 80° and 1·0 mm Hg, to yield a residue of 90 ml. The hydrocarbons, in a 30 ml aliquot of the residue, were separated from the oxygen containing materials by column chromatography using 300 g of Woelm basic alumina and 300 ml of *n*-hexane. The residue, following removal of the solvent, was placed on an identical but dry basic alumina column to remove the major portion of unsaturated hydrocarbons by collecting only the first 50 ml of *n*-hexane eluate. The remaining traces of olefinic material were removed from this residue on a $1{\cdot}25 \times 45$ cm column containing Woelm alumina impregnated with 10% $AgNO_3$ on a weight basis. The paraffins, which eluted in the first 50 ml portion with *n*-hexane amounted to 0·1 g or 150 ppm in the original oil.

The paraffin waxes were separated by gas chromatography on a 65 mm × 2 m column containing 10% Carbowax 30 M on 60–80 mesh acid-washed firebrick. The column was temperature programmed from 180° to 224° at 1° per min with the helium flow rate set at 30 ml per min. The chromatogram shown in Fig. 1 was obtained using 10 μl of the sample which had been diluted with 2 vols. of *n*-hexane.

Each hydrocarbon was collected in a short length of glass capillary as it eluted from the gas chromatographic column, and by means of the solid inlet probe, analyzed in a Bendix Time-of-Flight mass spectrometer. The mass spectrometer was operated in the continuous mode at 70 eV with the source temperature maintained at 50°. The identification of each constituent was made by comparison of its mass spectrum with spectra of analogous compounds published by the American Petroleum Institute. In each case the spectrum obtained

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* The mention of brand names is for identification of type of equipment used and does not imply recommendation by the U.S. Department of Agriculture.

[8] R. O. WEENINK, *Biochem. J.* **82**, 523 (1962).

[9] A. KUKSIS, *Biochem. J.* **3**, 1086 (1964).

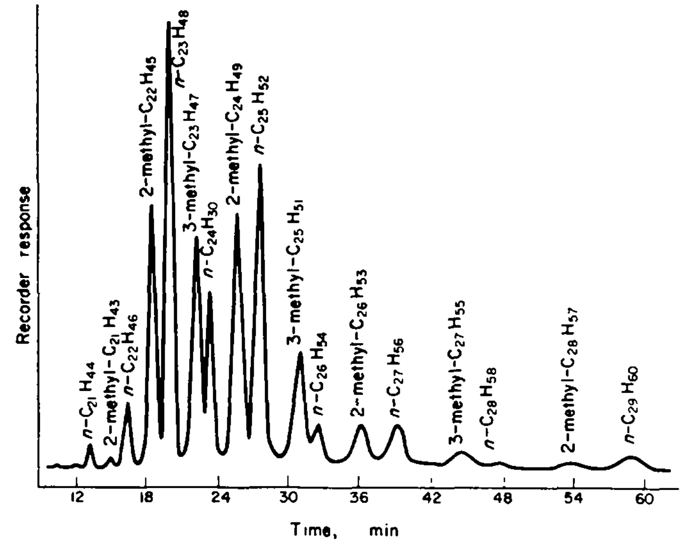

FIG. 1. GAS CHROMATOGRAM OF A 4 μl SAMPLE OF PARAFFINIC HYDROCARBONS IN COLD-PRESSED VALENCIA ORANGE OIL.

from the mass spectrometer showed a higher molecular ion peak. This is best explained by differences in the source temperature and other instrumental parameters.

Figure 2 shows a partial mass spectrum of a normal, a 2-methyl, and a 3-methyl alkane which were obtained during the orange oil analysis. The 2-methyl alkanes are characterized by the loss of an isopropyl group (M-43) and the 3-methyl alkanes by a loss of an ethyl group (M-29).

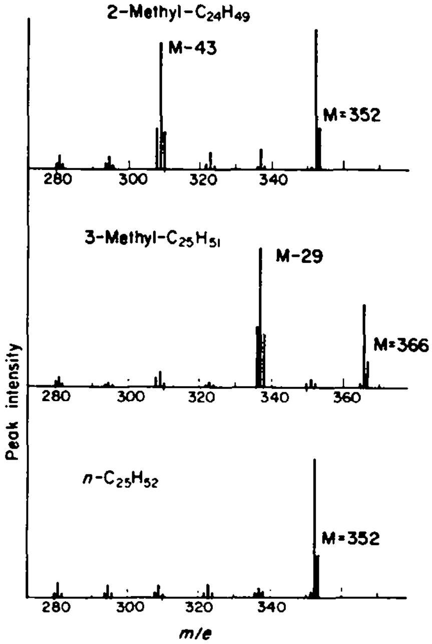

FIG. 2. TYPICAL MASS SPECTRA OF THREE DIFFERENT PARAFFINIC HYDROCARBON TYPES IN ORANGE OIL.