Infrared Fourier Transform Spectroscopy in Flavor Analysis. III

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Some applications of interferometric infrared spectrometry employing commercial instrumentation are described. The advantages of Fourier Transform spectrometers *vis-à-vis* dispersion devices are discussed. Spectral examination of several materials of flavor importance demonstrates that highly reproducible, good-quality spectra can be obtained quickly and routinely. Minor spectral variations among a group of orange oils became clearly observable by precise ordinate scale expansion, indicating that the interferometric approach will prove useful in quality control operations.

Some time ago work was started to explore the feasibility of applying Fourier Transform spectroscopy to the analysis of flavor and fragrance materials. Infrared spectra of gas chromatography fractions were recorded (Low, 1968; Low and Freeman, 1967) with a scanning interferometer, and it was found possible to obtain spectra of microgram quantities of flavor materials (Low and Freeman, 1968). The results and methods appeared to be potentially valuable in natural products research. However, the infrared spectra which were obtained suffered from two disadvantages in that most spectra were corrected for "background" by an electronic subtraction procedure described elsewhere (Low, 1966) but were not ratioed against background, so that the band intensities were distorted; and the spectral resolution was relatively poor at 18 cm^{-1}. As new and greatly improved instrumentation has now become available, we have extended the studies and describe the measurement of infrared spectra of some pure compounds and also of a series of commercial orange oils.

INSTRUMENTATION

The instrument used was the prototype of the Digilab, Inc., Division of Block Engineering, Inc., Model FTS-14 Fourier Transform spectrometer. The FTS-14, now commercially available, is described in detail elsewhere (Dunn and Block, 1969; Low, 1970a,b). Its salient features are as follows. The instrument is a completely automated infrared Fourier Transform spectrometer incorporating a minicomputer. The latter is used not only for the data acquisition, storage, and data reduction procedures required for Fourier Transform spectroscopy, but also controls the entire instrument. The operator has access to the instrument only *via* Teletype and, as the computer monitors instrument performance and maximizes all instrument parameters, high-quality spectra are obtainable routinely by unskilled operators. The instrument is laser-controlled, leading to a wavelength accuracy 0.1 cm^{-1}. Spectral resolutions of 0.5, 1, 2, or 4 cm^{-1} are obtainable at repetitive scan times of (nominally) 8, 4, 2, and 1 sec, respectively. For the present work, a resolution of 2 cm^{-1} constant over the 3900–400 cm^{-1} range was selected. This resolution is better than that obtained by high-quality dispersion spectrometers operated in a routine fashion, and is adequate for the present purposes. The samples used were homogeneous chemicals (>95% *via* glc) and a series of commercial orange oils. Spectra were measured with the samples housed in conventional liquid cells or as thin films contained between KBr or NaCl plates.

RESULTS AND DISCUSSION

Pure Compounds. A variety of chemicals was examined under different conditions in order to obtain some representative spectra. Examples are shown in Figures 1 to 8.

The relatively short scan period of the FTS-14 (2 sec nominal at 2-cm^{-1} resolution) makes it feasible to carry out "signal averaging," *i.e.*, multiple scanning and coherent addition of signals, in order to improve the signal-to-noise (S/N) ratio. Some results are shown in Figure 1. The sample was 2,5-dimethylthiophene contained in a Wilks Minicell. The computer was given instructions (in simple code) to perform 1 scan at 2-cm^{-1} resolution in order to produce a spectrum of the sample, to ratio that spectrum against the spectrum of the source, and to plot the results ordinate scale-expanded over the 2000–600 cm^{-1} range. On command, the computer then caused the system to (a) take one scan of the sample and store the interferogram in the core memory; (b) take one scan of the source and store the interferogram; (c) perform the Fourier Transforms, with all corrections, to produce the sample and background spectra over the 3900–400 cm^{-1} range; (d) divide the sample spectrum by the background spectrum and store the ratioed spectrum covering the 3900–400 cm^{-1} range; (e) plot the ratioed, expanded spectrum over the 2000–600 cm^{-1} range (the digital plotter not only draws the spectrum but also marks the abscissa in 5-cm^{-1} intervals). A portion of the spectrum resulting from this sequence of operations is shown in Figure 1 (top trace). Spectra were then recorded, using the same sample, but with 4, 16, and 64 scans. The improvement in the S/N with increasing numbers of scans is readily apparent (S/N is proportional to the square root of the number of scans, so that an S/N improvement by a factor of 8 is obtained in going from 1 to 64 scans).

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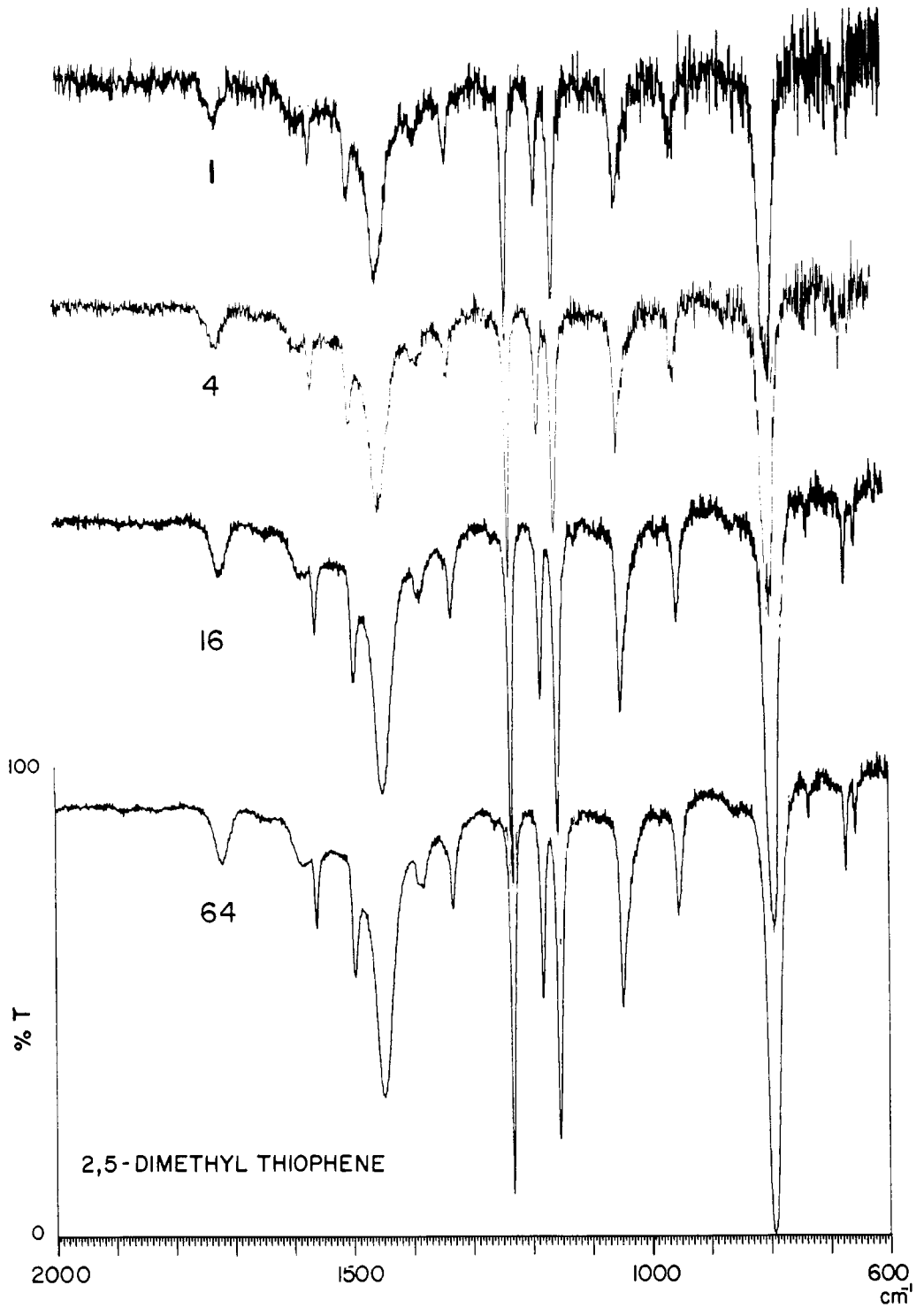

Figure 1. Effect of multiple scanning

Segments of spectra of 2,5-dimethylthiophene, recorded using the number of scans shown next to each trace. The sample was in a 0.025-mm pathlength Wilks Minicell. Each spectrum is ratioed against background and ordinate scale-expanded by approximately 30%. The ordinates of the top three traces are displaced to avoid overlapping

Scale expansion on all or a portion of the spectrum is simple to accomplish, because the entire spectrum is stored in the computer memory. It is only necessary to instruct the computer to scale-expand and indicate the region of the spectrum which is to be plotted. An example is shown in Figure 2. The upper trace is a ratioed spectrum of ethyl octanoate. After the spectrum had been plotted, the command was given to ordinate scale-expand over the entire region; the lower plot resulted. For the upper spectrum, the highest transmittance was 52%; the lowest, 0.02%. The lower trace, plotted full-scale, is thus a twofold expansion. (The computer takes the point of highest transmittance and places it at the 100% line, places the lowest point at the 0% line, and multiplies the entire spectrum by an appropriate scaling

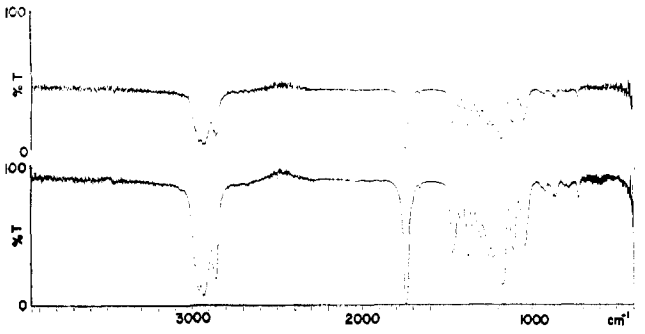

Figure 2. Spectra of ethyl octanoate

After the upper trace was recorded (16 scans, ratioed, sample in 0.025-mm Minicell), the spectrum was replotted using ordinate scale expansion

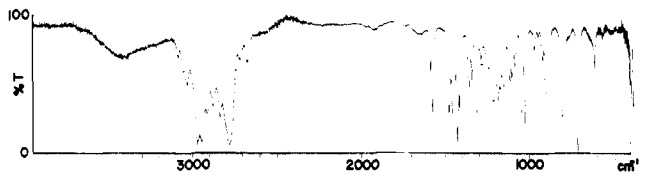

Figure 3. Spectrum of nicotine

Sample in 0.025-mm Minicell; 128 scans; ratioed; scale-expanded

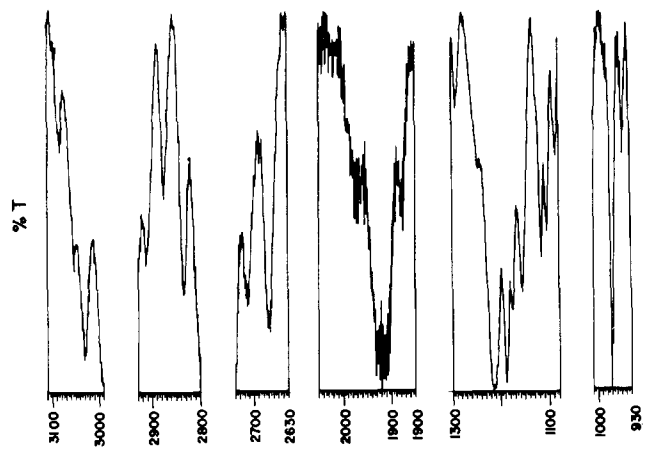

Figure 4. Nicotine—scale-expanded

The spectrum of Fig. 3 was scale-expanded over the segments shown

factor. The scaled spectrum is then plotted.) Further examples are shown in Figures 3 to 6. After the spectrum of nicotine had been recorded and plotted (Figure 3), selected portions of the spectrum were scale-expanded (Figure 4). Similarly, after the spectrum of longifolene had been obtained (lower trace, Figure 5), scale-expanded segments of the spectrum were plotted (Figure 6). Such scale expansion is of obvious utility in examining weak bands.

Maximum and minimum % transmittance values are not obtained from the plotted spectrum, but are given by the computer. When the plotting of a spectrum is complete, the computer will cause the Teletype to print out two numbers corresponding to the highest and lowest % transmittance values of the spectrum over the range plotted. Thus, for the lower plot of Figure 5, the maximum %T was 94.6, and the minimum was 35.1. Similarly, when the longifolene spectrum was expanded, the values given in Table I were obtained. For example, the transmittance was 91.6% at

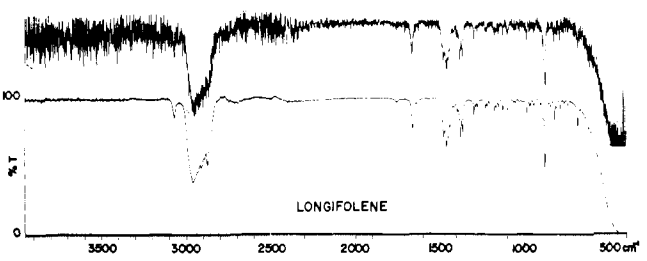

Figure 5. Spectra of longifolene

Sample between NaCl flats. Upper trace: 1 scan; lower trace: 256 scans; both spectra were ratioed but not expanded

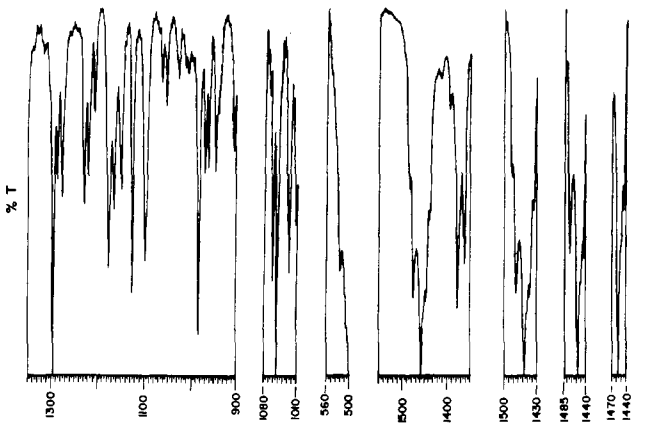

Figure 6. Longifolene—scale-expanded

The 256-scan spectrum of Fig. 5 was scale-expanded over the ranges shown

1350 cm^{-1} and 80.5% at 900 cm^{-1}, so that the difference of 11.1% was plotted full-scale, giving a ninefold expansion. As these expansions are brought about by computation, a further benefit will accrue in that the precise measurement of band intensities will be possible by writing the appropriate software for integration.

Some additional spectra of furfuryl alcohol, γ-dodecalactone, and eugenol are shown in Figure 7. Spectra of such quality can be obtained easily and routinely, at a spectral resolution of 2 cm^{-1} over the entire range. Were it not for the minor noise in the high-frequency region and below about 500 cm^{-1}, such spectra would easily exceed the criteria for Class II spectra of the Coblentz Society. (At the time these spectra were recorded, the beamsplitter had been damaged, so that the instrument response was relatively poor at the high-frequency region.) Although high-quality spectra would probably not be needed for some of the routine work of an analytical laboratory, spectra of Class II quality are

Table I. Transmittance Values for Figure 6

Range		% Transmittance		Ordinate Expansion
Max	Min	Max	Min	by Factor of
1350	900	91.6	80.5	9
1080	1010	91.6	88.7	34
600	560	82.0	67.0	6.7
1550	1350	92.6	58.1	2.9
1500	1430	90.5	58.1	3.1
1485	1440	79.8	58.1	4.6
1470	1440	88.0	73.0	6.7

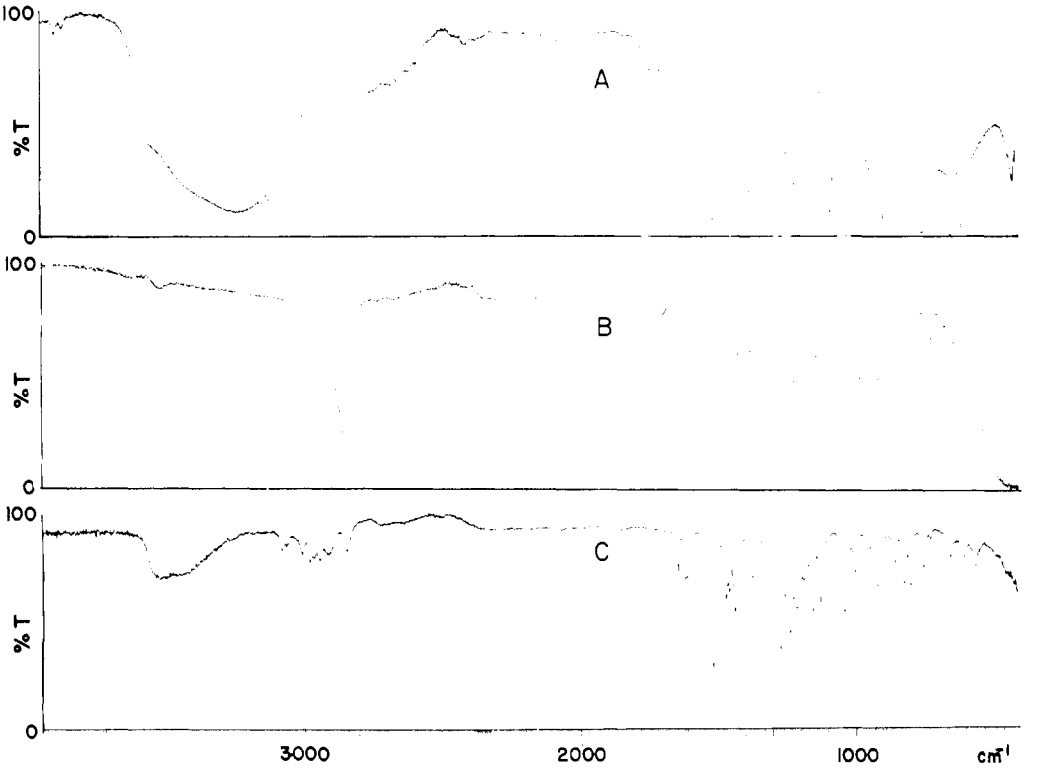

Figure 7. Representative spectra

A: furfuryl alcohol; B: dodecalactone; C: eugenol. Each spectrum was obtained with 256 scans; 2-cm^{-1} resolution over the entire range; ratioed but not scale-expanded

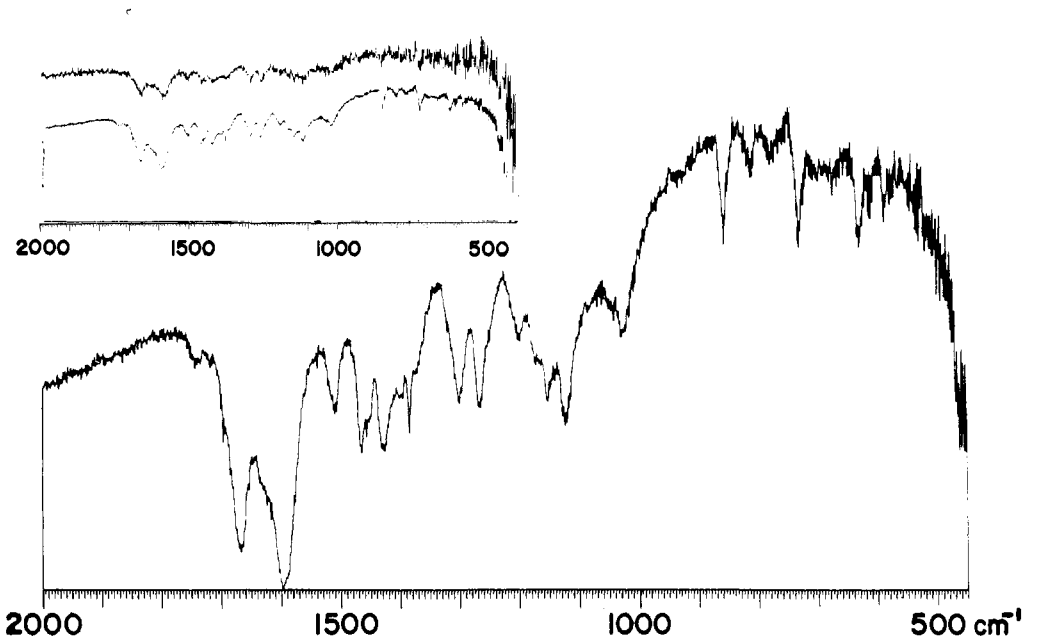

Figure 8. Spectra of microsample of vanillin

1-2 μg vanillin, in KrB pellet, used with 4X Perkin-Elmer beam condenser. Insert; top trace, 1 scan; bottom trace, 256 scans. The 256-scan spectrum was then scale-expanded and is shown as the lowest trace; 2-cm^{-1} resolution

now so easy to obtain that their use would probably become routine. Certainly, spectra such as these, having good resolution and high wavenumber precision over the entire spectral range, will be useful in setting up spectral files and computerized search-match-retrieval programs.

Time did not permit us to study the problem of instrument sensitivity extensively, but it was possible to run a 2-μg sample of vanillin in KBr using a 4X Perkin-Elmer beam condenser. Some spectra are shown in Figure 8. The insert of Figure 8 shows the results obtained with 1 scan (top trace) and 256 scans (lower trace). Note that even with 1 scan the pertinent spectral features could be discerned. The lower trace of the

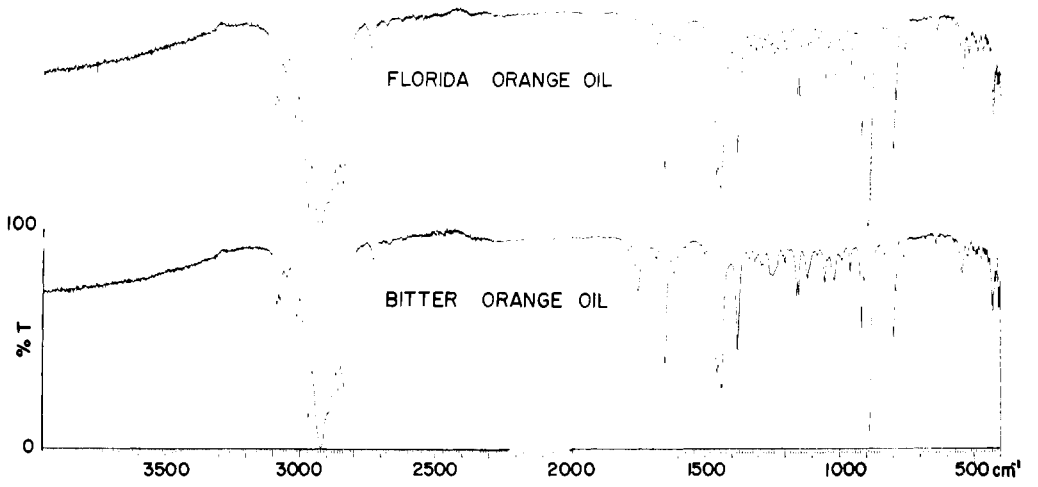

Figure 9. Spectra of orange oils

Sample between KBr plates; 256 scans; spectra were ratioed, scale-expanded; 2-cm^{-1} resolution

insert was then scale-expanded over the range shown: the spectral detail stands out quite clearly.

It is apparent from the various figures that high-quality infrared spectra can be readily obtained. If the sample is of a "reasonable" size, *i.e.*, of the order of a few hundred micrograms, traces showing the spectral structure at a usable S/N can be obtained with just a few scans, as shown by the spectra of Figures 1 and 5. It is important to note that such spectra resulting from short scan periods retain the 2-cm^{-1} resolution and are undistorted, in distinct contrast to the results obtained with dispersion spectrometers in which a shortened scan period leads to a decrease in resolution and can cause shifts in band positions and changes in band shapes. Spectra obtained with a small number of scans could consequently be used as "survey" spectra to examine large numbers of samples quickly and economically, quantitatively and qualitatively, *e.g.*, batteries of samples arising from distillations or plant-breeding experiments, or a reaction could be continuously monitored.

The short times required to produce spectra are also beneficial. However, the time period can be divided into three parts: the scan period, the computation period, and the plotting period. The time required for a single scan, for operation in the 2-cm^{-1} resolution mode, is 2 sec. For a ratioed spectrum, two such scans would be required, *i.e.*, a total of 4 sec. The computation period is 30 sec, irrespective of the number of scans. The periods, without plotting times, required for 1-, 4-, 16-, and 64-scan spectra would consequently be 34, 46, 94, and 286 sec, respectively; or, about $^1/_2$ to 5 min would be needed, plus plotting times. The latter will vary from several seconds to a few minutes according to the length of the plot, *i.e.*, the spectral range covered, and also according to the S/N of the spectrum. Consequently the specific time required for the total recording period (scan plus computation plus plotting) cannot be stated. Also, with a constant spectral range, one may arrive at the paradoxical situation where the total recording time *increases* if the scan period is shortened—it may take longer to record a 1-scan spectrum than a 16-scan spectrum, for example, because the 1-scan spectrum is four times "noisier" than the 16-scan spectrum, and the noisier spectrum would require a longer plotting time. In general, spectra of acceptable or good quality can be produced in 5 to 10 min, *e.g.*, the 64-scan spectrum of Figure 1 or the 128-scan spectrum of nicotine of

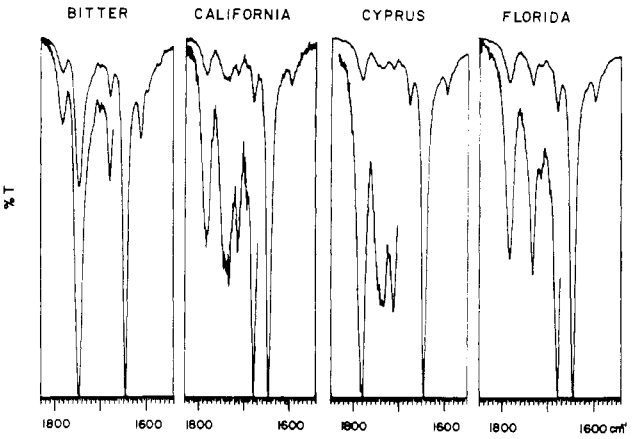

Figure 10. Expanded spectra of orange oils

Segments of spectra such as those of Fig. 9 were ordinate scale-expanded. The lower traces in the 1830–1680 cm^{-1} region are scale-expanded segments of the upper traces

Figure 3, while research-grade spectra can be obtained in about 20 min.

The relatively fast rate at which usable spectra can be generated will lead to significant economic advantages for quality-control and other situations where numerous samples are examined routinely. It is again difficult to be specific because there are numerous variables to be considered, such as instrument cost and capital-equipment depreciation rate, technician's salary, overhead, etc. However, the cost per FTS-14 spectrum is probably $^1/_5$th to $^1/_{10}$th the cost of spectra recorded with research-grade dispersion spectrometers.

Orange Oils. It is well known that minor variations in the compositions of natural products can often be readily detected by glc methods and, although extremely complex chromatograms may contain many unknown peaks, the entire glc pattern can be useful in characterizing materials. For example, there are consistent correlations between glc data and varietal differences for a range of foodstuffs (Jennings *et al.*, 1960; McCarthy *et al.*, 1963; Mackay *et al.*, 1961; Wolford *et al.*, 1965), as well as quantitative differences in the glc patterns of tobaccos (Smith *et al.*, 1963), of potatoes of different varieties and geographical origin (Self, 1963), of spearmint oil (Smith *et al.*, 1963) and peppermint oil (Hawkes

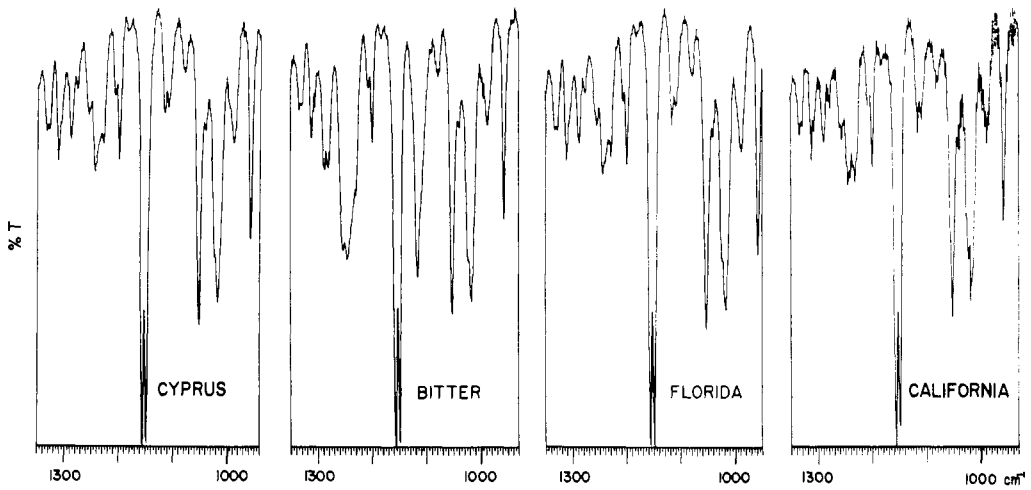

Figure 11. Expanded spectra of orange oils

Segments of spectra such as those of Fig. 9 were ordinate scale-expanded

and Wheaton, 1967), and the unequivocal differentiation between *Coffea arabica* and *Coffea robusta* on the basis of glc profiles has been described (Biggers *et al.*, 1969).

Minor composition variations should also appear in the infrared spectral profiles of natural products, in analogy to variations in glc profiles. In contrast to the glc work, however, small changes in the concentrations of minor constituents of a complex sample would be difficult to detect by conventional infrared methods because of the frequent overlapping of infrared bands and lower sensitivity. In order to detect small intensity changes in the infrared pattern, the entire infrared spectrum would have to be measured very precisely. Unfortunately, attempts to look for minute differences by recording infrared spectra with dispersion instruments using ordinate scale expansion have not been fruitful because of difficulties arising from decreased resolution, lowered S/N, longer recording time, and instrument instability.

On the other hand, information related to the flavor quality of, say, an essential oil might be gained from its complex infrared pattern, generated by Fourier Transform spectroscopy. The fact that data acquisition using Fourier Transform spectroscopy requires much less time than glc methods makes the infrared approach an attractive one, and we describe our initial tests to evaluate its feasibility.

Orange oils were chosen for the exploratory studies, as it was believed that this type of oil would prove to be a severe test of the ability of infrared Fourier Transform spectroscopy to detect minor spectral differences. Orange oils contain about 95% (glc) of the terpene hydrocarbon limonene, the remaining few percent comprising more than 100 constituents. Consequently, the infrared spectra of orange oils closely resemble those of pure limonene, and differences among orange oils would be evidenced by the appearance of new bands of very low intensity and by small and subtle variations in the shapes and intensities of weak absorptions.

Samples of Italian Bitter, California Valencia, Cyprus, and Florida Valencia orange oils were examined. The components present in amounts of 0.1% and higher are given in Table II. Those values were obtained by glc computerized area determinations and identities were ascertained by glc-MS. It can be seen that there are only small differences among the oils.

Examples of spectra of the orange oils recorded at 2-cm^{-1} resolution over the entire 3850–400 cm^{-1} region are shown in

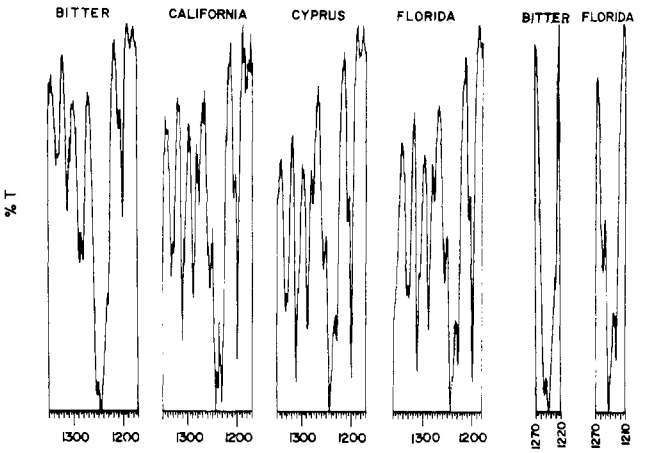

Figure 12. Expanded spectra of orange oils

Segments of spectra such as those of Fig. 9 were ordinate scale-expanded

Figure 9. The spectra differ from that of limonene and from each other only in small details. These variations in spectral structure become more evident when the bands are scale-expanded. Ordinate scale expansions were carried out with no accompanying band distortions or resolution loss, as described earlier. Some examples are shown in Figures 10, 11, and 12.

Significant, reproducible spectral differences are apparent in all instances. These should not necessarily be regarded as a measure of compositional variations attributable to geographic origin of the oils. The high degree of sophistication

Table II. Glc-MS Analysis of Orange Oils

Component	Italian Bitter	Florida Valencia	California Valencia	Cyprus
α-Pinene	0.5	0.4	0.4	0.4
Salunene	1.2	0.2	0.5	0.5
Myrcene	1.3	1.4	1.5	1.3
Limonene	95.5	96.5	97	96
cis-3-Hexenol	0.1	0.1	0.1	...
Decanol	0.1	0.1	...	0.1
Linalool	0.4	0.25	0.1	0.2

enerally encountered in orange oils in addition to their states of oxidation, past history, etc., militates against such an assumption. Indeed, the flavor and spectral fluctuations in oils derived from a specific region may be as great as those of oils coming from various parts of the world. The spectra do show, however, the merit of the proposed approach using infrared spectra obtained by Fourier Transform spectroscopy.

Further work is indicated on relating infrared profiles to sample varietal differences or geographic origin. With extremely complex samples, recording infrared spectra using Fourier Transform methods is similar to the infrared "fingerprinting" carried out by many industrial laboratories. However, the fingerprinting *via* Fourier Transform spectroscopy is capable of higher sophistication than that performed by conventional dispersion instrumentation. The high S/N capabilities of the Fourier Transform spectrometer have already been mentioned—these can be considered in terms of "sensitivity," speed, or economics—and lead one to propose that it will be possible to carry out quality-control work on natural products quite rapidly using infrared spectra, because it is possible to examine extremely complex samples in just a few minutes. Also, the digital nature of the spectral data and the data-handling procedures lead to flexibility (the storage of spectra, spectra files, and computerized search-match-retrieval procedures has been mentioned earlier). For example, by writing the appropriate software, it will be possible to simplify a spectral profile by subtracting the spectrum of a major component, thereby enabling the experimenter to observe smaller differences between samples. Similarly, computerized band intensity measurements and computerized fingerprint matching are feasible. Work on such topics is under way.

ACKNOWLEDGMENT

The authors are indebted to Anne Sanderson, International Flavors & Fragrances, for her role in recording and interpreting the glc-MS data.

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Received for review March 31, 1970. Accepted May 22, 1970. Partial support from the National Center for Air Pollution Control is gratefully acknowledged.