GAS-PARTITION CHROMATOGRAPHY. APPLICATIONS TO ESSENTIAL OILS AND OTHER VOLATILE MATERIALS*

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THERE HAVE BEEN a number of papers on the basic principles of gas-partition chromatography. The purpose of this paper is to discuss the application of gas chromatography to analysis and research on essential oils and aromatic chemicals as well as other volatile materials.

Figure 1 illustrates the basic principles and operation of a gas chromatograph. It shows the essential parts: a cylinder of helium with a pressure regulator and flow meter; sample introduction system, generally with a flash heater; and a column itself which is in many cases coiled or U-shaped, and is packed with a carefully chosen adsorbent material. Finally there is a detector cell, which is in two parts; the reference cell through which the helium passes first, and the actual sensing part of the cell through which the product and the helium finally pass.

Basically, there are two ways that the gas chromatograph can be used. It can be used primarily to obtain the chromatogram as a record for qualitative and/or quantitative purposes. It can also serve as a means of obtaining samples of various components of mixtures, with the chromatogram as a guide to the operation of the sample-collection system. Examples of chromatograms are discussed in conjunction with Figs. 2 and 3.

A gas chromatogram of a sample of octyl aldehyde is shown in Fig. 2. This and the other chromatograms shown later have been changed so that they read from left to right, although, normally, recording machines will record from right to left. The first pip is the air peak; then there are two very small peaks, and finally the very large octyl aldehyde peak. This, plus the shape of the major peak, is a good sign that we have quite pure octyl aldehyde. Since the octyl aldehyde peak is so smooth, any other impurity would have to be extremely close to octyl aldehyde in retention time in order for its peak to be "hidden;" the chances for this are very slim.

Figure 3 shows an example of a good separation of a mixture of com-

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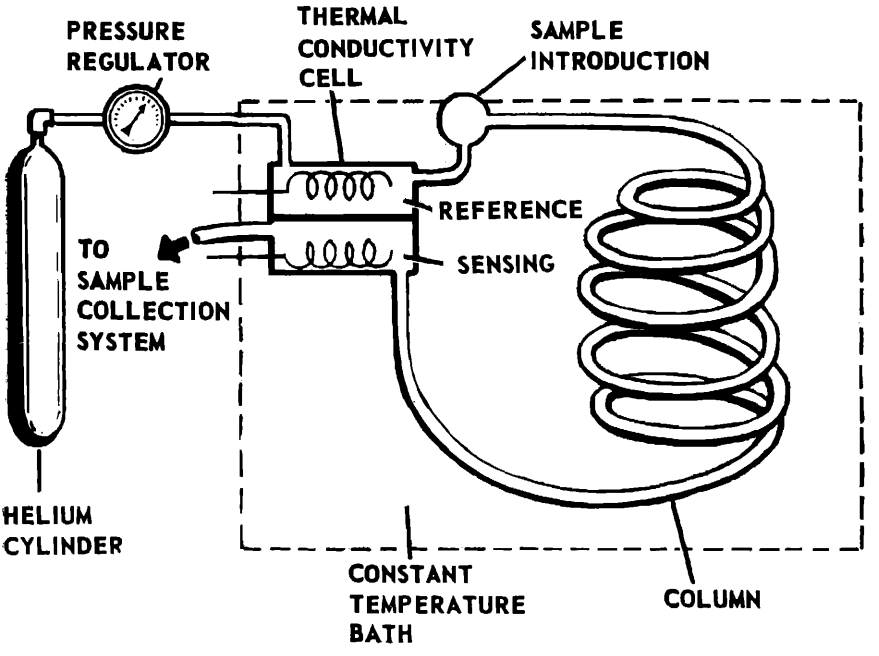

Figure 1.

pounds: methyl, ethyl and isopropyl benzoates. Their boiling points are respectively, 199°, 212° and 218°C., so that they cannot be separated nearly so cleanly by distillation. This chromatogram was run at 190°C. with a column of crushed fire brick on which was suspended Apiezon wax. The chromatogram shown in Fig. 4 was run at a higher temperature, 228°C., on the same column. The ethyl and the isopropyl peaks are pushed together and the resolution is much poorer. Operation at a much lower temperature, 167°C., is shown in Fig. 5. Here the peaks are spread out and are not nearly so amenable to quantitative interpretation; in addition, the whole process is much slower. Figure 6 shows a chromatogram using a column material at 190°C. that did not give as good a separation.

Of course, the peaks in the various chromatograms do not *identify* the particular compounds. They can be used for this purpose only if reference chromatograms of the individual compounds obtained under the identical conditions are available for comparison, and even then considerable caution must be exercised. If unknown compounds are involved, there is obvious need for complementary analysis of fractions corresponding to each peak, e.g., by infrared spectroscopy. In any case, the type of column packing and the operating conditions must be chosen with care if best results are to be obtained.

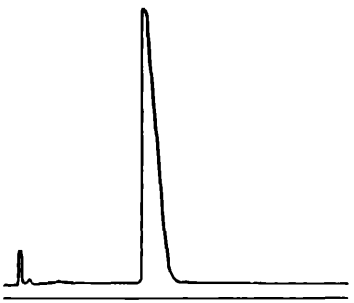

Figure 2.—Gas chromatography of octyl (C_8) aldehyde.

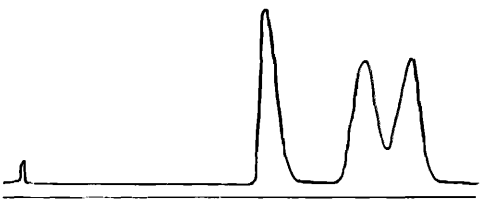

Figure 3.—Gas chromatography of 1:1:1 mixture of methyl, ethyl and isopropyl benzoates 190°C.

Analytical Uses of Gas Chromatography

One important use of gas chromatography is for the detection of impurities. Its use in detecting 1 per cent of heptaldehyde in octyl aldehyde is shown in Fig. 7. The sensitivity of the instrument and the sample size have been increased to such an extent that the octyl aldehyde peak runs off the chart. Under these conditions, the heptaldehyde peak shows up very clearly between the peaks for the very small impurities that were in the octyl aldehyde (Fig. 2) and the major peak. It is obvious that the latter materials are present in less than 1 per cent concentration since their peaks are much smaller than that of the heptaldehyde.

Figures 8–11 illustrate the use of gas chromatography for quantitative analysis of a mixture of supposedly equal amounts of C_7, C_8, C_9 and C_{10} aldehydes. This analysis could not be done by any other analytical technique with the possible exception of mass spectroscopy. The quantity of each aldehyde present is, strictly speaking, represented by the area under the corresponding peak. However, estimating the area under a peak of this sort in a rigorous way is a rather tedious and time-consuming job. For most purposes, one can use the approximation that the area under the peak is proportional to the height of the peak times the width of the peak at

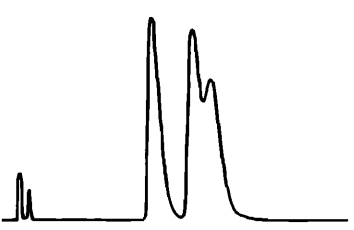

Figure 4.—Gas chromatography of 1:1:1 mixture of methyl, ethyl and isopropyl benzoates 228°C.

Figure 5.—Gas chromatography of 1:1:1 mixture of methyl, ethyl and isopropyl benzoates 167° C.

Figure 6.—Gas chromatography of methyl, ethyl and isopropyl benzoates 190°C.

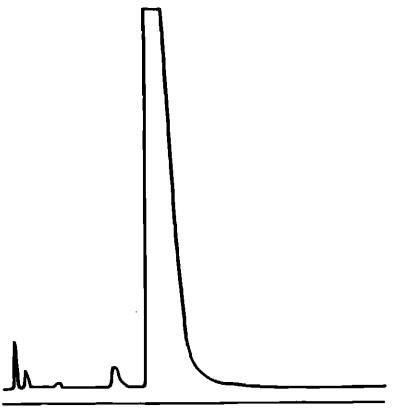

Figure 7.—Gas chromatography of octyl (C_8) aldehyde plus 1% heptyl (C_7) aldehyde.

TABLE I—QUANTITATIVE ANALYSIS OF MIXTURE OF STRAIGHT-CHAIN ALDEHYDES, C_7, C_8, C_9 AND C_{10}

λ	Found, %	Calculated, %
C_7	23.3 ± 0.45	25
C_8	28.2 ± 0.4	25
C_9	24.4 ± 0.25	25
C_{10}	24.1 ± 0.15	25

half its height. Using this method, results (Table I) accurate to within a few per cent for each aldehyde were obtained. However, the reproducibility of the method is better than this; it should be possible to get at least 1 per cent accuracy on this kind of a mixture by use of calibration chromatograms.

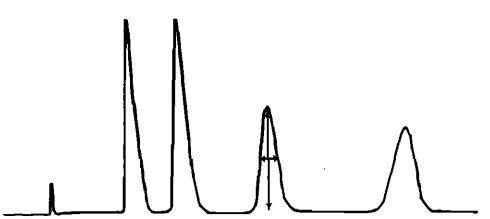

Figure 8.—Gas chromatography of 1:1:1 mixture of C_7, C_8, C_9 and C_{10} aldehydes.

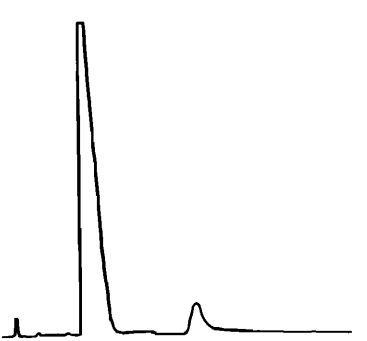

Figure 9.—Gas chromatography of heptyl (C_7) aldehyde.

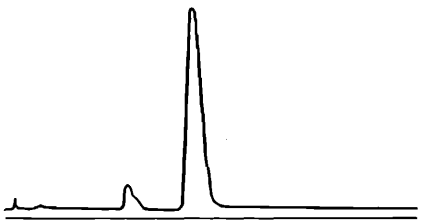

Figure 10.—Gas chromatography of nonyl (C_9) aldehyde.

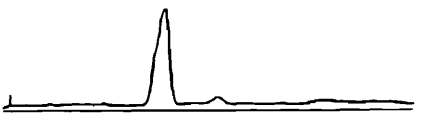

Figure 11.—Gas chromatography of decyl (C_{10}) aldehyde.

TABLE II—QUANTITATIVE ANALYSIS OF MIXTURES OF METHYL, ETHYL AND ISOPROPYL BENZOATES

	Found, %	Calculated, %	Found, %	Calculated, %	Found, %	Calculated, %
Methyl	32.6 ± 0.5	33.3	49.7 ± 0.7	50	25.1 ± 0.4	25
Ethyl	34.0 ± 0.9	33.3	25.7 ± 0.3	25	26.1 ± 0.4	25
Isopropyl	33.4 ± 0.4	33.3	24.7 ± 0.45	25	48.7 ± 0.1	50

The fact that the results in Table I were not as accurate as expected was suspicious. It was not reasonable to expect the analysis for the C_8 to be so much higher than those for C_7, C_9 and C_{10} aldehydes when all four compounds were presumably present in the same amounts. It was suspected that the C_8 was the purest of the aldehydes used in the mixture, and, therefore, that the actual C_8 content should be slightly higher than any of the others. Figure 9 shows a purity determination of the C_7 aldehyde. The impurity indicated is not, as one might suspect, the C_8 octyl aldehyde since the retention time is not quite right. The point is that the C_7 aldehyde was impure and, therefore, the low analytical figure for this aldehyde is reasonable. The same thing applied to the C_9 aldehyde (Fig. 10), which has some C_8 impurity. This would decrease the analysis for C_9 and increase it for C_8. Finally, in Fig. 11, the C_{10} aldehyde can be seen to be a complex

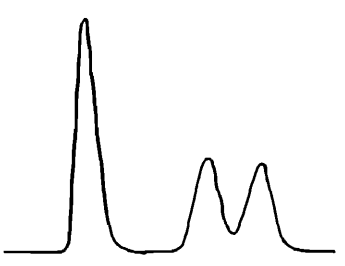

Figure 12.—Gas chromatography 2:1:1 mixture of methyl, ethyl and isopropyl benzoates.

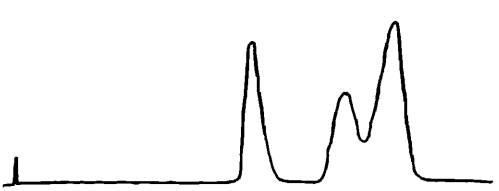

Figure 13.—Gas chromatography of 1:1:2 mixture of methyl, ethyl and isopropyl benzoates.

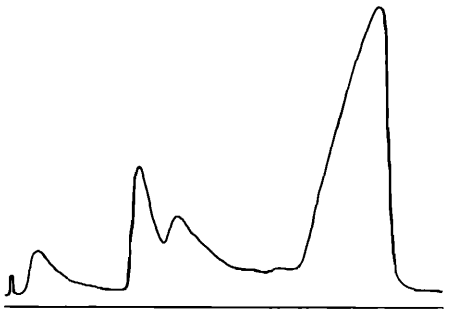

Figure 14.—Gas chromatography of rhodinol I.

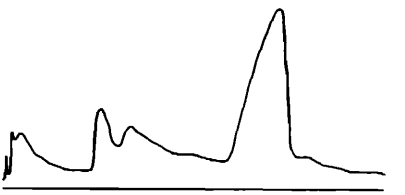

Figure 15.—Gas chromatography of rhodinol II.

mixture. The over-all conclusion was that the analysis of the mixture was reasonably accurate.

As far as quantitative analysis goes, the mixture of aldehydes was a rather easy case in that the peaks were completely resolved. In chromatographing a 1:1:1 mixture of methyl, ethyl and isopropyl esters of benzoic acid, Fig. 3 shows that the ethyl and isopropyl esters were not completely resolved from each other. This is a more critical test of the capabilities of the method. Figure 12 is a chromatogram from a 2:1:1 mixture of the benzoates and Fig. 13 is from a 1:1:2 mixture. In Table II, it can be seen that both the gross figures and the reproducibility were very good. In this case the starting materials were relatively pure. The isopropyl benzoate contained a trace of what was probably isopropyl alcohol; the others contained no obvious impurities. Here again, it would be extremely difficult to analyze these mixtures by any other method.

Differentiation of Essential Oils and Detection of Adulteration

Gas chromatography can also be very useful for analysis of complex materials whose constitution are not completely known. In these cases it

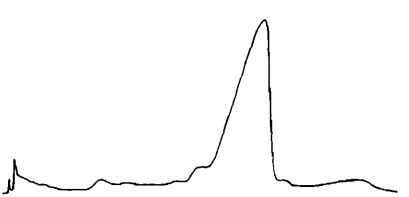

Figure 16.—Gas chromatography of citronellol I.

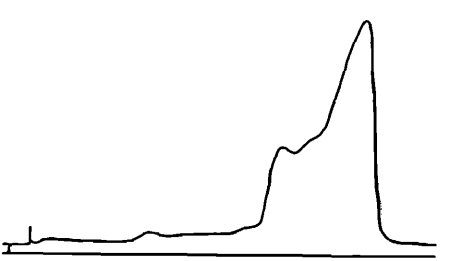

Figure 17.—Gas chromatography of citronellol II.

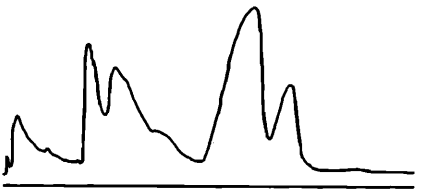

Figure 18.—Gas chromatography of Reunion geranium oil I.

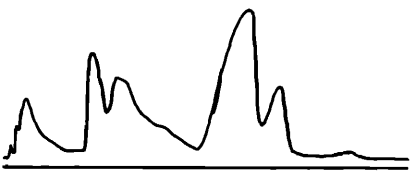

Figure 19.—Gas chromatography of Reunion geranium oil II.

may not be possible to associate each peak with a particular chemical entity, but this need not diminish the utility of the method.

Figures 14 and 15 are chromatograms of two different samples of commercial rhodinol. The peaks to the left of the main peak are apparently characteristic of rhodinol. Incidentally, there is reason to believe that some of these peaks represent thermal decomposition products rather than actual components of rhodinol. In order to study these components unchanged, it would be necessary to run the chromatography at a much lower temperature; one at which the peaks would be flattened out considerably.

Chromatograms of citronellol samples (Figs. 16 and 17) demonstrate the absence of the early rhodinol peaks. However, there is a small peak that appears as a "shoulder" on the main peak. This seems to be a distinguishing characteristic of citronellol, as related to rhodinol. These effects are quite consistent, and it appears that one can easily use them to distinguish between a citronellol and a rhodinol. In fact, one could probably detect adulteration of rhodinol with citronellol by means of the citronellol "shoulder."

The first part of the chromatogram of a Reunion geranium oil is shown in Fig. 18. This pattern is characteristic of geranium oils in general although the ratios of the areas under the peak will vary from one sample to another. A chromatogram of another Reunion geranium oil is shown in Fig. 19, and it demonstrates different main peak ratios. It was hoped at first that there might be some consistent difference in the ratios of these major peaks that would enable one to distinguish Reunion geranium oil from Algerian geranium oil. However, as is shown in Figs. 20 and 21, the Algerian oil looks quite similar to the Reunion oil in the first part of the chromatogram.

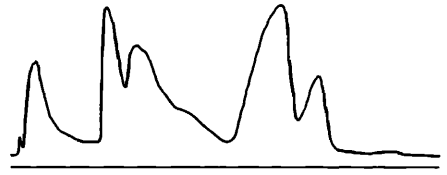

Figure 20.—Gas chromatography of Algerian geranium oil I.

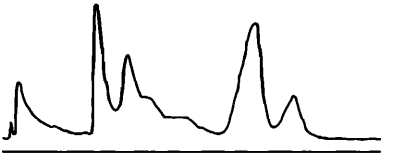

Figure 21.—Gas chromatography of Algerian geranium oil II.

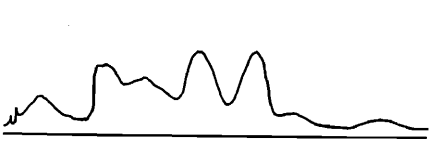

Figure 22.—Gas chromatography of synthetic geranium oil I.

Figure 23.—Gas chromatography of synthetic geranium oil II.

Figures 22 and 23 show two typical synthetic geranium oils, and it is obvious that these peaks are entirely different from those of the natural products. The third synthetic oil (Fig. 24) gives a chromatogram closest to that of a natural geranium oil, yet it can be distinguished from the natural product. Thus, the pattern of peaks, even though they vary somewhat in ratio for similar samples, serve quite well to distinguish true geranium oils from synthetic geranium oils. The chromatogram of Turkish geranium oil (Fig. 25) shows it is not really a geranium oil at all but an entirely different product. This illustrates the ease with which gas chromatography distinguishes between different kinds of essential oils.

Far out in the chromatogram of the Reunion geranium oils (Fig. 26) there is a single peak that appears consistently but never appears for Algerian geranium oils. This difference has proved to be consistent with four samples of Reunion oil and four samples of Algerian oil, and thus might be the basis for a routine differentiation procedure.

An area of great potential practical use for gas chromatography is in the detection of adulteration of essential oils. Not enough work has been done to be certain that adulteration can be detected in any given oil. However, the possibilities in connection with rhodinol-citronellol were mentioned above. Also two lavender oils were studied from this standpoint; one was presumably pure lavender oil and the other was carefully sophisticated (Fig. 27). There are definite differences between the adulterated and the pure lavender oils. However, there is no question, as was shown in the case of the geranium oils, that natural differences are to be expected between one and another batch of a pure essential oil. One has to find whether the chromatographic differences between an adulterated oil and a pure oil are

Figure 24.—Gas chromatography of synthetic geranium oil III.

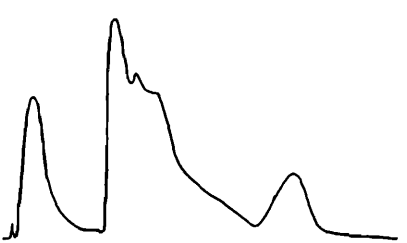

Figure 25.—Gas chromatography of "Turkish geranium" oil.

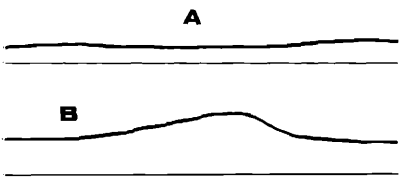

Figure 26.—"Tail end" of gas chromatography of Algerian (*A*) and Reunion (*B*) geranium oils.

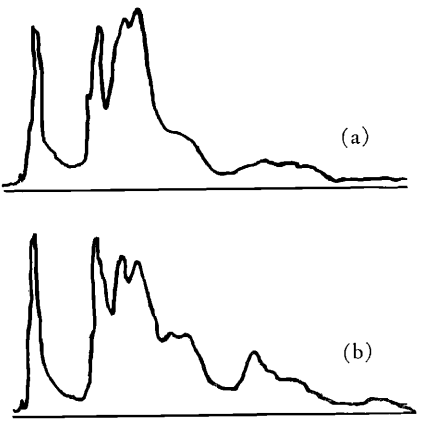

Figure 27.—Gas chromatography of; (a) pure lavender oil, (b) sophisticated lavender oil.

outside the range of differences to be expected from one batch of pure oil to another.

Study of Essential Oil Composition by Isolation of Compounds

Another area of great potential utility for gas chromatography is in the investigation of the compositions of essential oils. Here it must be stressed that gas chromatography itself is not the complete solution to the problem of separating all the components of such complex mixtures. It is almost always necessary to use an approach based on a number of different separation techniques applied in proper sequence. This is especially true in dealing with the minor components. For such purposes, gas chromatography can be regarded as a novel and extremely efficient distillation technique which routinely has a separation efficiency equivalent to 1000 to 2000 theoretical plates. Conventional distillation cannot begin to approach this efficiency. In addition, azeotropes do not form under the conditions of gas chromatography, and small amounts of material can be handled with ease.

There are distinct differences in operation of a gas chromatograph when used as a separation tool, as compared with its use as an analytical or characterization tool. When used for analysis or characterization, it does not really matter what the chromatographic peaks represent; reproducibility of the peaks is the main concern, whether they represent actual components of the original mixture or their decomposition products. However, if the aim is to identify the actual components, one must be certain that it is they that are being recovered, not their decomposition products.

There are several ways by which one can detect a decomposition problem in such work (frequently caused by too high a column temperature). One

can sometimes tell from the odor of a recovered fraction whether it is a decomposition product. If the fraction contains an olefin, occurrence of dehydration of an alcohol during the chromatography may be indicated. Infrared spectroscopy can be very helpful in this respect. For example, if two or three absorption peaks are present in the infrared spectrum of the isolate that are also present in the spectrum of the original mixture, it is a good indication that the isolate was present as such in the original mixture.

Questions about differences in the structure and/or composition of rhodinol and citronellol have been puzzling essential oil and terpene chemists literally for decades. The two materials have markedly different odors, which is the reason rhodinol costs eight or ten times as much as citronellol. Some investigators have ascribed the difference in odor solely to an isomeric difference between the major components; citronellol possesses the isopropenyl structure, while the major component of rhodinol has been thought by some to be an isomer of citronellol with an isopropylidene structure. Other investigators have maintained that the difference in odor is due primarily to desirable "impurities" or minor components of rhodinol that are not present in citronellol. Some earlier work (without benefit of gas chromatography) had led us to agree with the latter explanation, but unequivocal evidence was still lacking.

Figure 28 shows a chromatogram of one of the rhodinol samples obtained for this work. This sample was unusual in that it did not give a sharp peak but a rounded one as well as some unexpected peaks earlier in the

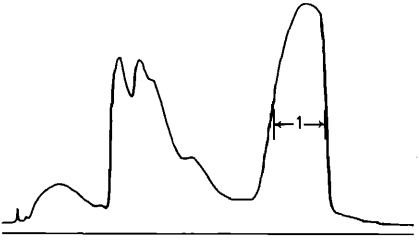

Figure 28.—Isolative gas chromatography of "abnormal" rhodinol.

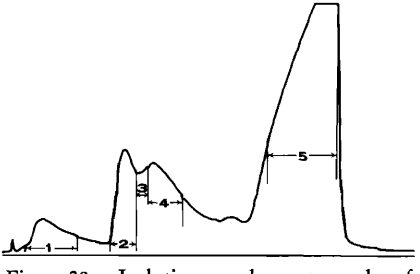

Figure 29.—Isolative gas chromatography of rhodinol I

chromatogram. The main fraction was collected, and its infrared spectrum was that of a reasonably pure sample of a member of the menthone series. Obviously, the original sample was spurious. This illustrates the fact that very often one can use a *combination* of gas chromatography and infrared

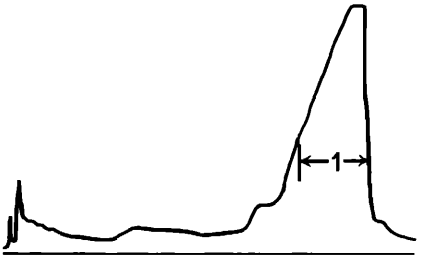

Figure 30.—Isolative gas chromatography of citronellol I.

spectroscopy as an analytical tool. The two techniques are really complementary to one another, and by isolating the fractions by gas chromatography and studying them by infrared spectroscopy, one has a pair of methods for isolation and identification that work together remarkably well.

The gas chromatograms of authentic rhodinol and citronellol were shown in Figs. 16–19. For the purposes of this work, several fractions from the respective chromatographies of rhodinol and of citronellol were collected as illustrated in Figs. 29 and 30. These fractions were examined by infrared spectroscopy. Fractions 1–4 of rhodinol were found to consist mostly of hydrocarbons. These were probably decomposition products and were not investigated further.

Figure 31 shows the infrared spectra of the fractions corresponding to the major peaks from the chromatographies of the authentic rhodinol and citronellol. They are very similar but not quite identical; the isolates are not completely pure. However, the major infrared peaks are identical, and, most significantly, the ratios of the peaks for the isopropylidene (12.0μ) and isopropenyl (11.25μ) groups are very close in the two curves; that is, 0.57 and 0.49. It is not possible at this point to make conclusive statements to the effect that the C_{10} terpene alcohol is the same in both rhodinol and citronellol. The samples are not quite pure enough to be able to say that. There is a small amount of carbonyl absorption at about 5.85μ in the alcohol obtained from citronellol. However, it seems reasonably certain that the highly purified major components of rhodinol and citronellol will eventually prove to be practically identical. The important conclusion at this point is that the difference in odor between rhodinol and citronellol is not *only* due to the difference in the ratios of the double-bond isomers. The gas chromatogram of rhodinol shows so many other constituents that

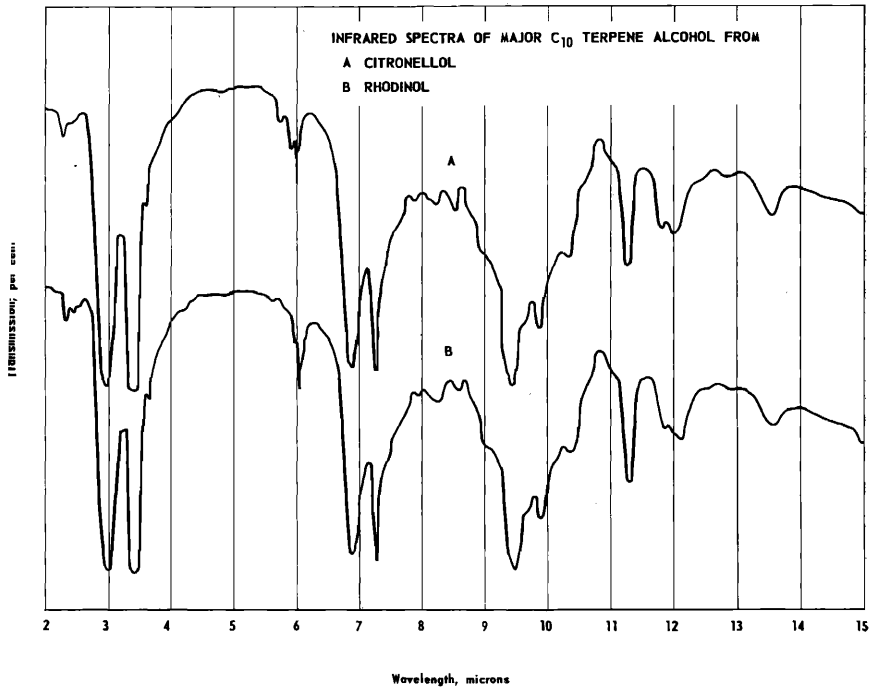

Figure 31.

the difference in odor between rhodinol and citronellol cannot be ascribed merely to a difference in isomers.

This discussion brings up the question of how pure aromatic chemicals should be. Gas chromatography shows every sign that it could be used for commercial purification of some materials. The technique apparently can be scaled up very readily and can be put on an automatic cycling basis. It may develop that any suitable chemical that costs over $5 or $10 a pound, and perhaps even considerably less, can be commercially processed by gas chromatography.

All the essential oil catalogs offer "pure" rhodinol, or "pure" geraniol or "pure" linalool, but it is doubtful that the chemically pure products are desired. For example, the indications are that rhodinol is not as pure as citronellol and yet the price of the former is higher than that of the latter. What the purchaser is probably doing in the case of rhodinol is buying some extremely expensive and desirable impurities, and since the impurities are present in low concentration they may be considered extremely expensive. In terms of producing pure aromatic chemicals by means of gas chromatography, one must be quite certain how pure one's customers want these materials; what are probably desired in most cases are products with controlled and uniform impurities.