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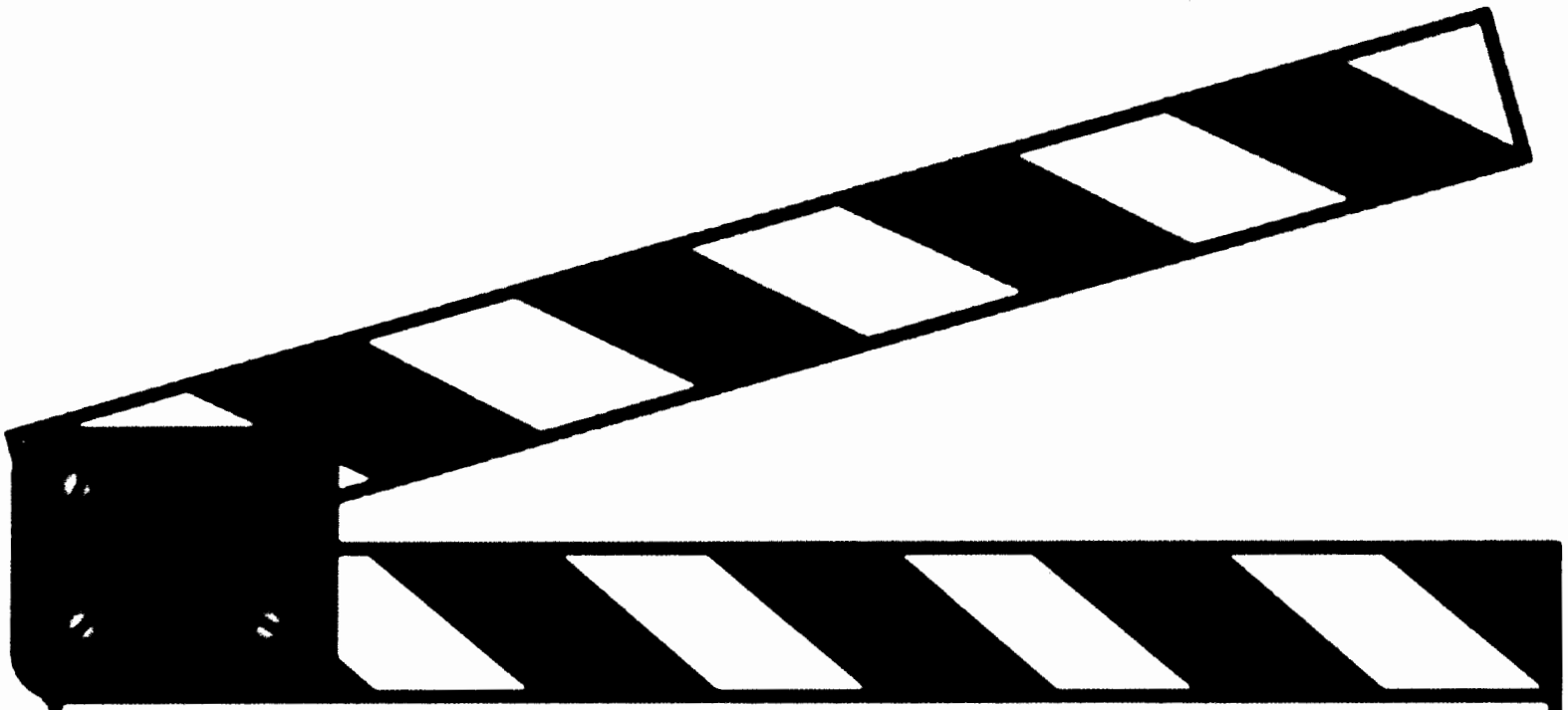
An investigation of the analysis of turpentine by gas chromatography

John R. Wagner



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AN INVESTIGATION OF THE ANALYSIS OF TURPENTINE BY GAS CHROMATOGRAPHY

by

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INTRODUCTION

Turpentine is that fraction of the essential oil from pine sources boiling approximately between one hundred-fifty and one hundred-seventy degrees centigrade. Turpentines are classified into four types according to the source and method of production.

Gum Spirits of Turpentine is obtained by distillation of the oleoresin from living trees.

Steam Distilled Wood Turpentine is obtained by the distillation of wood itself, or its extract.

Destructively Distilled Wood Turpentine is produced by the carbonization of resinous wood.

Sulfate Wood Turpentine is distilled from the oil obtained as a by-product of the sulfate pulp process.

These four types of turpentine were recognized under the Federal Naval Stores Act of 1923.[24] Another type of turpentine, not officially recognized, is derived from the sulfite wood pulping process. The quality of turpentine is controlled by Federal,[24] State,[34] and ASTM[1, 2] standards. Twenty-one States as well as several municipalities have laws relating to turpentine.

The specifications for turpentine described in these various laws and regulations are based on physical tests, such as color, distillation range, refractive index, odor, acid and soponification numbers.

The improvements in turpentine refining processes have made the physical differences between types of turpentine quite small, which has lead to attempts to characterize these types by chemical means. The tests which have been developed are based on minor constituents present in one type and not the other, rather than the composition of the turpentine as a whole.

The development of gas chromatography[19] and the publication of data on terpene analysis by this method has lead to this attempt to analyze turpentine by gas chromatography. The work has been limited to Gum Spirits of Turpentine and Steam Distilled Wood Turpentine since these are of chief importance and are the only types commonly retailed in small packages.

There have been three objectives:

1. To develop a method based on gas chromatography for distinguishing between Steam Distilled Wood Turpentine and Gum Spirits of Turpentine, and detection of mixtures and adulteration.
2. To identify as many components of the two turpentines as possible, using the available data and equipment.
3. To develop a rapid means of proximate analysis.

HISTORICAL

Turpentine is produced in greater quantity than any other essential oil,[18] and has been produced for over twenty centuries. The chemical and analytical investigation of the composition of the commercial product has never been undertaken routinely.

Until comparatively recent times distillation methods were the only ones available for the separation of the components of turpentine. The fractions of such distillations were subjected to classical chemical identifications and physical tests.

There are about forty-eight species of the genus pinus which are recognized in the United States,[23] and of these only two are used in the production of Gum Turpentine, Pinus palustris (longleaf) and Pinus caribaea (slash pine).[33] The composition of Gum Turpentine from these two sources has been investigated by Palkin[27] by fractional distillation and evaluation of its pinene content by optical means. His results are given below. (Table)

The work by Palkin was based on work done some years earlier by Darmois who had experimented with the fractionation of both French and American turpentines. Darmois[10, 11] showed that by effective fractional distillation, turpentine was largely separated into alpha and beta pinene with a small fraction present at the "head" and "tail" of the distillation. It was further shown that the Biot relationship could be applied to the fractions

TABLE I

COMPOSITION OF GUM TURPENTINE

Source and sample No.	Rotation (10 Cm. Tube) α_j=578mμ	Rotation (10 Cm. Tube) α_v=546mμ	Refractive Index ($n_D^{25°}$)	Density ($d^{15.6°}$)	Composition: Pinenes α	Composition: Pinenes β	Composition: Tailings
Commercial from mixed fields of slash and longleaf pine							
G-7	-2.4	-2.2	1.4697	0.8707	63.0	31.5	5.6
G-15	-9.2	-9.98	1.4692	0.8662	61.9	32.6	5.3
G-16	+1.9	+2.5	1.4680	0.8664	62.2	28.8	6.1
Samples from Authentic Sources:			1.4698	0.8667			
Slash pine 3, old	-20.50	-22.56	1.4663	0.8624	58.0	35.6	6.4
Longleaf 1, fresh	+14.04	+16.27	1.691	0.8680	68.4	28.6	3.01
Longleaf pine 2, fresh	+12.29	+14.34	1.4692	0.8694	62.5	33.3	4.2
Gum dip 8, slash	-22.58	-24.9	1.4694	0.8657	61.0	33.7	5.3
Gum dip 9, longleaf	+11.0	12.86	1.4690	0.667	62.5	34.4	3.1
Scrape 10, slash	-25.05	-27.8	1.4700	0.8704	58.8	33.6	7.6
Scrape 11, longleaf	+7.35	+8.85	1.4681	0.8675	67.0	29.2	3.8

obtained,[12] and their relative proportions determined. These procedures require fifteen kilogram samples, quite a bit of complex distillation apparatus, and a great deal of time.

Palkin and Chadwick later[28] examined Gum Turpentine and found that the "tailings" contained major portions of Dipentene and Methyl Chavicol and smaller portions of Terpinolene, Bornyl Acetate, and Pinocarveol, along with a number of hydrocarbons, alcohols, ethers, and esters which they were unable to identify. They found that fenchyl alcohol which was known to be present in steam distilled wood turpentine was not present in any detectable quantity.

The hydrocarbon content of Gum Turpentine has recently been reported in an unpublished preliminary report by Fisher, et al.,[14] using distillation and infra-red methods. Two four liter samples of Gum Turpentine of a known source were distilled using distillation columns rated at one hundred theoretical plates and by operating at a reflux ratio of one hundred to one when distilling all but the major components. Except for pure alpha and beta pinene fractions, all samples were analyzed by means of their infra-red spectra. Using this data and the weights of the fractions, the following composition was found: 1) A trace of unidentified hydrocarbon, probably fenchenes, 2) alpha Pinene, 56%, 3) Camphene, 1%, 4) Myrcene, 0.5%, 5) alpha Phellandrene, about 1%, 6) beta Phellandrene, 2.4%, other monocyclics including Limonene, Terpinolene, 1.3%, residue, 4.2%. Even with the highly efficient separation used, Camphene was not cleanly separated from alpha Pinene and beta

Phellandrene was contaminated with limonene. Alpha Phellandrene may have been produced from beta Phellandrene during the distillation.

Infra-red analysis of this same turpentine indicated 58% of alpha Pinene, based on its band at 12.75 millimicrons, 31% of beta Pinene on the basis of the band at 11.75, and 4% beta Phellandrene on the basis of the 14.75 band.

Since the results of the infra-red analysis was satisfactory, the infra-red method was applied to thirty-eight samples of commercial turpentines collected from most of the central stills at various times during the season. The alpha Pinene content of these samples was found to be 61±6% and the beta Pinene content was found to be 30±4%. The percentage of beta Phellandrene was quite variable since there is little if any beta Phellandrene present in pure longleaf turpentine, and the samples were of mixed origin.

Palkin, Chadwick and Matlack[29] undertook the fractionation and chemical identification of steam distilled turpentine in 1937. The improvements in the methods of refining steam distilled turpentine had rendered the distinction between it and gum spirits less and less obvious by the usual methods. They felt that the identification of some of the minor constituents might form the basis of a chemical method for distinguishing the two, and furnish an analytical means of detecting reasonable quantities of one in the other.

On the basis of chemical examination of fractions of a fifteen kilogram sample the following compounds were reported. Alpha Pinene, (about eighty per cent of the turpentine) beta Pinene, Champhene, present in small quantity, others, Dipentene, Limonene, Terpinolene, para Menthane, Fenchyl alcohol, Borneol, alpha Terpineol, making up the balance of the turpentine, and several other compounds present only in traces, low boiling paraffin Hydrocarbons, Benzaldehyde, Furfural, Cineole, Sobrerol, Methyl Chavicol, and Phenols.

This is the only comprehensive examination published to date and was made on only one representative sample. There have been no analyses reported for a number of samples, but presumably the composition does not vary as much depending on the source as it does depending on the method of processing.

Grotlisch[17] reported a method for detecting adulteration of turpentine by analysing for sulfur and chlorine. This would detect the presence of sulfate wood turpentine. Steam distilled wood turpentine can be detected by the presence of benzaldehyde utilizing the method of Snider.[31]

Methods based on the optical rotation shown by turpentine are not of value directly for the analysis of turpentine due to the large variations found in turpentines,[5, 22, 26] although average values might be used to differentiate between gum turpentine from slash or longleaf pines. A recent paper describes the quantitative determination of alpha pinene by optical dilution.[21]

Gas chromatography and infra-red analysis is being widely applied in the analysis of essential oils in general, and a great number of papers have been published recently on this subject.

Essential oils of Pinus Sylvestris, (Scotch or Norway pine) a European species, the oil of which is used for scenting purposes, have been analyzed by gas chromatography and some of its terpenes identified.[7] Another recent paper[6] describes the isolation of some of the components of turpentine from Pinus Sylvestris growing in smoky areas. Pinus Sylvestris has been extensively investigated in the past fifty years. Its composition differs markedly from commercial American varieties.

Retention data has been reported for terpenes and related compounds relative to camphor by Bayer, Kupper, and Reuther,[3] and a compilation of infra-red and ultra-violet spectra was made by O'Connor and Goldblatt.[25] A recent paper by Westway and Williams[35] describes the analysis of monocyclic terpenes by gas chromatography using Apiezon M substrate together with infra-red identification of components.

Bernhard[4] used C-22 firebrick coated (40:100) with Silicone fluid (GE SF-96-40), Apiezon L stopcock lubricant, Di-N-octyl phthallate, glycerol, and carbowax 600. At 177°C and a helium flow rate of 23 ml/min, through a glass column, an artificial mixture of terpenes occurring in lemon oil was separated with the carbowax substrate. Carbowax 20,000 has also been reported[13] as being useful for the separation of terpenes occurring in turpentine. Terpene

analysis by gas chromatography was reported by Stanley and Mirov[32] at the 133rd ACS meeting in San Francisco in April, 1958. Ucon (trade mark of Wilkins Instrument Company) and a Silicon substrate were used.

An analysis of terpene hydrocarbons and related compounds by gas chromatography was reported by Zubyk and Conner.[36] They investigated the gas chromatographic properties of forty-four compounds and gave the relative retention data for two columns, didecyl phthallate and Carbowax 4000. During the course of the investigation they examined the suitability of five solid supports, Celite 545, Sil-O-Cel, C-22 firebrick, Chromsorb C-44857, and Chromsorb C-48560. They found the Chromsorb C-48560 to be most suitable.

The Authors of this paper reported that isomerization was caused by some of the solid supports and liquid substrates used, and also by the copper tubing used for their work. Accordingly, their work was done with stainless steel tubing. Some of the didecyl phthallate obtained commercially also was sufficiently active to isomerize alpha and beta pinene. As an application of their data they used a thirteen foot column filled with 25% didecyl phthallate coated on Cellite C-545 to separate the components of steam distilled wood turpentine. With a column temperature of 130°C and a helium flow rate of 45 ml/min they found the composition of a crude wood turpentine as follows: 1) alpha Pinene 77.5%, 2) Unidentified 2.9%, 3) alpha Fenchene 8.4%, 4) Camphene 6.2%, 5) trans Pinane, 3-p-Menthane, trans-p-Menthane, (any, or all three) 4.0% 6) 3-Carene 0.7%, 7) Dipentene 0.3%.

The identifications were made on the basis of relative retention ratios. Infra-red analysis of the same sample gave 79% alpha Pinene, 14% of a compound corresponding to Camphene, and 4% Tricyclene. The Authors supposed the absence of tricyclene in the chromatogram due to its being obscured by the large alpha Pinene peak.

A commercial detergent (Tide) was proposed and has been thoroughly investigated[9] as a general column packing, both as a support and as a support-substrate combination, for the separation of hydrocarbons. It has also been used[8] for the analysis of pyridines as a solid support.

The composition of rosin from various sources has been recently investigated by both mass spectrography[15] and gas chromatography[20] of its methyl esters.

EXPERIMENTAL

Gas Chromatography Apparatus

The work described here was performed on a Perkin-Elmer 154-C Vapor Fractometer (Fig. 1), using hot wire detecting elements. A separate power supply was used for these elements, and the current flow was maintained at a constant two hundred and fifty milliamperes. The air bath temperature was controlled within $\pm$ 0.5°C. The injection block was modified to incorporate a separate cartridge heater powered by a variable transformer. A thermocouple and a potentiometric pyrometer provided the means for temperature measurement. Optimum flow rate determinations were carried out using a bubble type flowmeter connected to the detector block outlet. A stopwatch was used to determine the rate of flow through this apparatus. Flow rates were maintained $\pm$ 0.3 milliliters per minute.

Sample Collection

Samples were collected using Luer syringe needles, 20 gauge, 2-3/4" in length, along with small three inch capillary tubes. The capillary tubes were drawn with a bulb at the lower end, and a slightly flared opening at the capillary end. The sealed end was pointed to allow all of the liquid to be completely withdrawn with a syringe. Alpha pinene was collected directly into these capillary


BEST-OF-ALL
LINSEED OIL
HOUSE PAINT
VAPOR FRACTOMETER RECORDER

tubes without solvent and used for isomerization determinations. Samples to be used to obtain infra-red spectra were collected in 0.1-0.2 ml of carbon disulfide. In both cases the capillary tubes were cooled in an ice bath.

Preparation of Column Packings

Solid Supports

Three separate types of solid supports were used. Two of these supports were commercial preparations (42-60 mesh Johns-Manville C-22 Firebrick, Areograph Corporation, and Chromsorb "W", F & M Scientific Company). The remaining support was prepared in this laboratory from a commercial detergent (Tide). In all cases the detergent was crushed and sifted (40-60 mesh) and then spread thinly on metal trays and heated for ten hours at 175°C. It was then extracted for ten hours with a solvent, the solvent used depending on the material with which it was to be coated. The material for the "B" column was prepared as above and then extracted with petroleum ether as described by Dexora and Dinneen[34] and used without coating. Detergent to be coated with rosin was extracted with acetone.

Liquid Substrates

Carbowax 20M, diisodecyl phthalate, and rosin were used as liquid substrates. The carbowax was a commercial product obtained from the Areograph Corporation. Diisodecyl phthalate was obtained already coated on Chromsorb "W" (F & M Scientific Company.)

Investigation of Rosin as a Liquid Substrate

A sample of commercial rosin was placed in an oven at 110°C for three one-hour periods. The sample initially weighed 1.1690 grams and at the end of the three heating periods the sample weight had become stable and the total loss in weight during the three hour period was 0.6%. The sample was replaced in the oven and the temperature was raised to 160°C. At the end of the two hours the total weight loss was 0.9%. (An additional 0.3% loss) The sample was replaced in the oven for four hours at 175°C. At the end of this period the total weight loss was 4.02%. The heating was continued for sixteen hours, and the total weight loss at the end of this time was 9.9%. After 48 hours at 175°C the total weight loss was 25.5%. A second batch of dried, distilled rosin was heated in the oven for five hours at 165°C and the loss in weight was 0.1%. This data on the heating of rosin in air seemed to indicate that rosin would be suitable as a liquid phase up to about 150°C.

One hundred grams of rather impure, dark amber rosin were placed in a vacuum distillation apparatus and distilled at less than one mm. pressure. The rosin began to distil at 220°C, the larger portion distilling up to 250°C. Above 250°C the distillation temperature rose rapidly to 295°C and at this temperature the residue underwent decomposition. About 90-95% of the original charge could be recovered below 250°C, and this material was used for column packing preparation.

To determine the loss of the liquid substrate under operating conditions, a sample of the packing was weighed into a glass tube plugged with glass wool. The tube was placed into a drying oven set at 130°C. The temperature control on this oven was only approximate. The tube was connected with rubber tubing to a tank of nitrogen and the flow rate through the tube maintained at roughly fifty milliliters per minute. At the end of one hour the weight loss was 0.43%. Placed in the oven for an additional two hours under the same conditions the weight loss averaged 0.007% per hour for these two hours. To determine whether the injection of rather large samples of turpentine would increase the weight loss, 0.5 ml of turpentine were injected at four 15 minute intervals. This resulted in no appreciable change in the sample weight under the same conditions. On the basis of this data it was concluded that the injection of turpentine did not cause the packing to bleed the liquid substrate at an increased rate. The steady state loss at 130°C indicated that the column packing could be used at 130 degrees centigrade for gas chromatography, and probably at higher temperatures.

Preparation of Columns

All columns were packed in one-quarter inch copper tubing six feet one-inch in length and plugged at each end with one-half inch of glass wool, giving an active length of six feet. Several test columns were made of glass tubing 42 inches in length to test

for isomerization by certain materials. In each case the granular packing was allowed to flow slowly into the tubing while a vibrator was passed up and down the column.

"A" Column--Ninety grams of acetone-extracted detergent (300 ml) were placed in a 1000 ml round bottom flask. Ten grams of prepared rosin were dissolved in 250 ml of reagent grade acetone and added to the flask containing the extracted detergent. This flask was attached to a rotating vacuum evaporator. As much acetone as possible was pulled off, then the flask was warmed to eighty degrees centigrade in a water bath. After approximately one hour the solid was removed, sieved, (40-60 mesh) and packed into the column.

"B" Column--One hundred and forty grams of detergent sieved 40-60 mesh were heated for 16 hours at 175^{o}C, resieved and extracted for 16 hours with petroleum ether in a Goldfinch extraction apparatus. After placing into a drying oven at 110^{o}C to drive off the remaining petroleum ether, a sample of the material was packed directly into one-quarter inch copper tubing without any further treatment.

"C" Column--The "C" Column was prepared from acetone extracted detergent coated by a process similar to that for the "A" column with 20% prepared rosin and packed in one-quarter inch copper tubing.

"D" Column--Twenty grams of C-22 Firebrick were placed in a nichrome wire filled round bottom flask. The purpose of the nichrome wire was to impart a tumbling action to the packing as the flask was rotated. Five grams of Carbowax 20M were dissolved in one hundred milliliters of methylene chloride and then added to the flask. The apparatus was connected to the vacuum pump and partially immersed in a water bath. When almost all of the methylene chloride was evaporated, very gentle heat was applied to the water bath. This procedure yielded twenty-three grams of 40-60 mesh packing.

"E" Column--C-22 Firebrick was coated with rosin dissolved in acetone (20%) by a procedure similar to that for the "A" column, and packed in one-quarter inch copper tubing.

"F" Column--The packing of the "F" column was a commercially prepared material, 20% diisodecyl phthalate coated on Chromsorb "W" (F & M Scientific Company). The "F" column was packed by the usual procedure.

RESULTS AND DISCUSSION

Column Performance

One of the major problems of analyzing a mixture of terpenes such as turpentine is the possibility of isomerization caused by heat and catalysis. Gas chromatography offers one rather obvious advantage over distillation. The separation can be performed rapidly on a gas chromatographic column, whereas an extended period of time is needed to carry out an analytical distillation. The materials for a gas chromatographic column are chosen from essentially inert materials in order to minimize isomerization.

Since gum turpentine is originally distilled from its oleoresin, the residue of which becomes rosin, it was of interest to see how rosin would perform as a liquid substrate in gas chromatography. If it caused isomerization, then it would seem possible that isomerization would occur when the turpentine is distilled from the oleoresin in the first place. It also seemed possible that isomerization would occur since rosin is a mixture of resin acids.

A chromatogram of gum turpentine on the "A" column is shown in figure two. While the peaks are separated, there is hardly enough separation to allow quantitative analysis using an automatic integrator. The column is of interest qualitatively, however, since

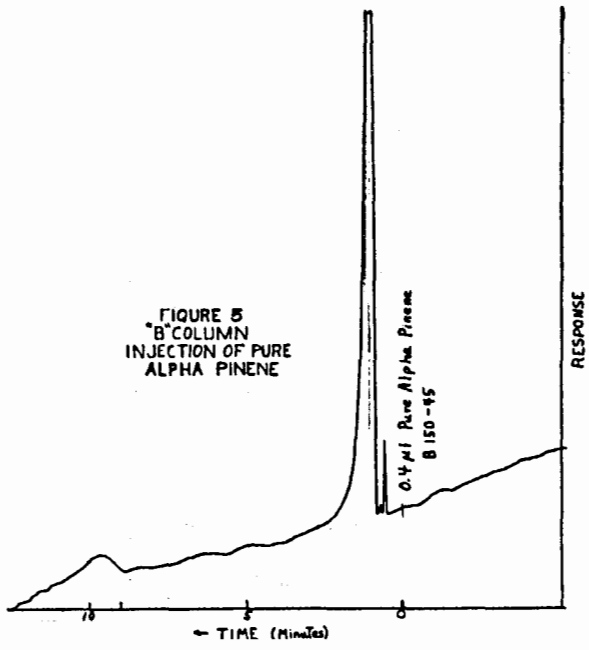


FIGURE 5
"B" COLUMN
INJECTION OF PURE
ALPHA PINENE
0.4 µl Pure Alpha Pinene
B 150-45
RESPONSE
TIME (Minutes)

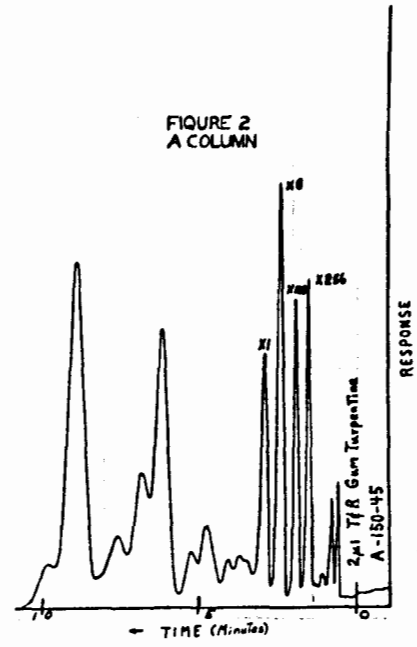


FIGURE 2
A COLUMN
X8
X256
X1
2µl T&R Gum Turpentine
A-150-45
RESPONSE
TIME (Minutes)

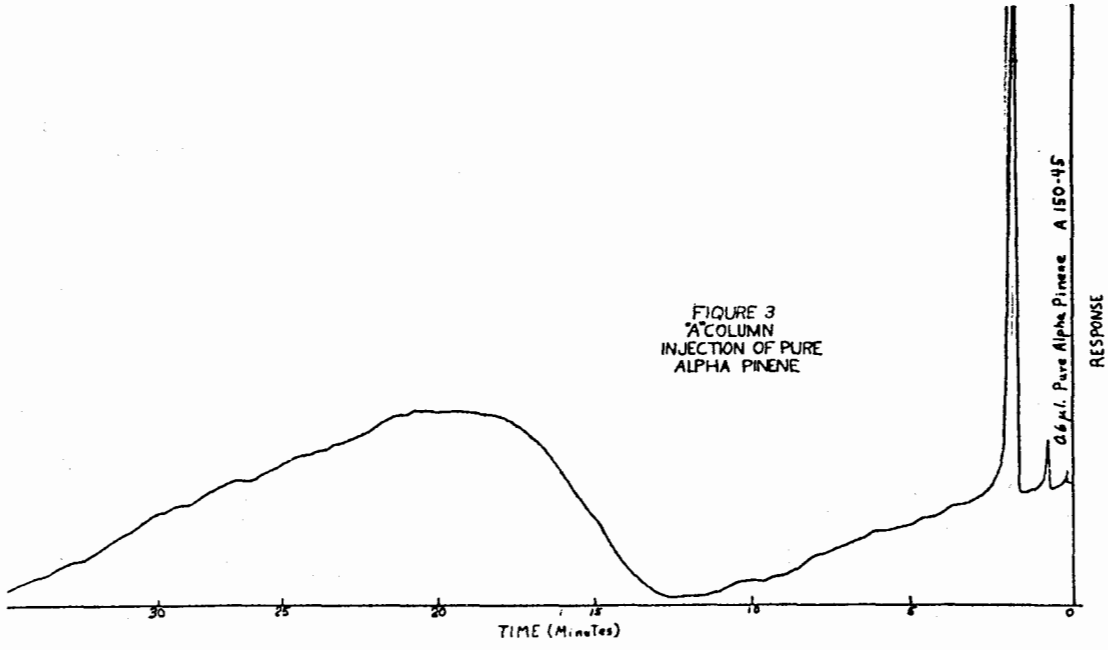


FIGURE 3
"A" COLUMN
INJECTION OF PURE
ALPHA PINENE
0.6 µl. Pure Alpha Pinene A 150-45
RESPONSE
TIME (Minutes)

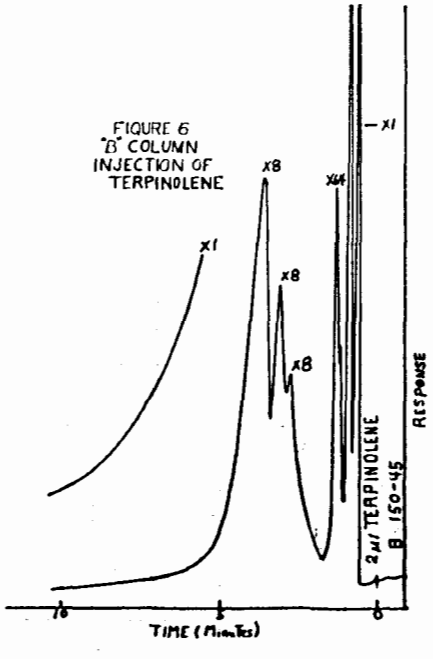


FIGURE 6
"B" COLUMN
INJECTION OF
TERPINOLENE
X1
X8
X64
X1
X8
X8
2µl TERPINOLENE
B 150-45
RESPONSE
TIME (Minutes)

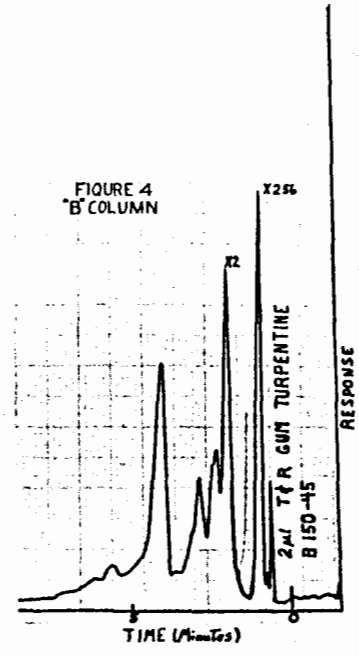


FIGURE 4
"B" COLUMN
X256
X2
2µl T&R GUM TURPENTINE
B 150-45
RESPONSE
TIME (Minutes)

it does separate alpha and beta pinene completely, and most of the other suspected compounds at least to some extent.

The isomerization of turpentine is not readily apparent from the chromatogram of the whole turpentine shown in figure two. About the only evidence which would lead one to suspect isomerization in this case would be the slight variation in the height of the peaks for some of the more sensitive compounds, and the tailing which occurs after the major peaks have emerged.

In order to further check the possibility of isomerization, a sample of alpha pinene was trapped from the "D" column, which was known to be stable to alpha pinene, and this pure, trapped material was injected on to the "A" column. Figure three shows the result of this experiment. The uncontaminated peak is seen first, then a large rounded and tailing peak. This peak is caused by the isomerization of alpha pinene. Since this column caused the isomerization of alpha pinene, the detergent column was not used for quantitative analysis.

The "B" column was constructed to determine the usefulness of prepared detergent as a solid support or as a solid support-liquid substrate combination for the separation of the components of turpentine. The material, as described in the experimental section, was prepared by the method described in the literature. The chromatogram of gum turpentine on this column (Figure four) shows no more apparent isomerization than does the chromatogram for column "A". The separation is somewhat less, which indicates that

rosin does affect the separation. Without the added rosin, alpha and beta pinene are not separated at all. The injection of purified alpha pinene (Figure five) shows that very little, if any, isomerization is caused by the detergent itself. The injection of a sample of the more sensitive terpinolene shows little isomerization, but a great deal of tailing (Figure six). The presence of the large number of peaks in this chromatogram is attributed to the impurity of the sample rather than isomerization. Extracted detergent gives satisfactory results as far as inertness is concerned; however, its fragility and non-uniformity have ruled against its use as a solid support. Just what the effects were of the extraction of the detergent with different solvents is not known, but weight losses on extraction with petroleum ether, acetone and methylene chloride were about the same (13%). It seems reasonable to suppose that the nature of the extracted material was not the same in all cases. The material extracted with methylene chloride was used in an attempt to coat it with Carbowax 20M. The preparation was attempted using both the vacuum evaporator and by evaporation of the solvent in an evaporating dish. Apparently the Carbowax film shrank while the solvent was evaporating, reducing the fragile packing to powder.

The "C" column (Figure seven) contained twice the amount of rosin that was used for the packing in the "A" column. This column retained the components of turpentine for a greater length of time, but the resolution suffered, isomerization was greater, and the efficiency measured in HETP was less.

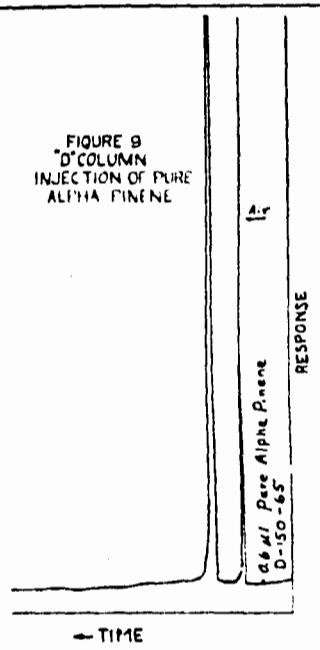


FIGURE 9
"D" COLUMN
INJECTION OF PURE
ALPHA PINENE

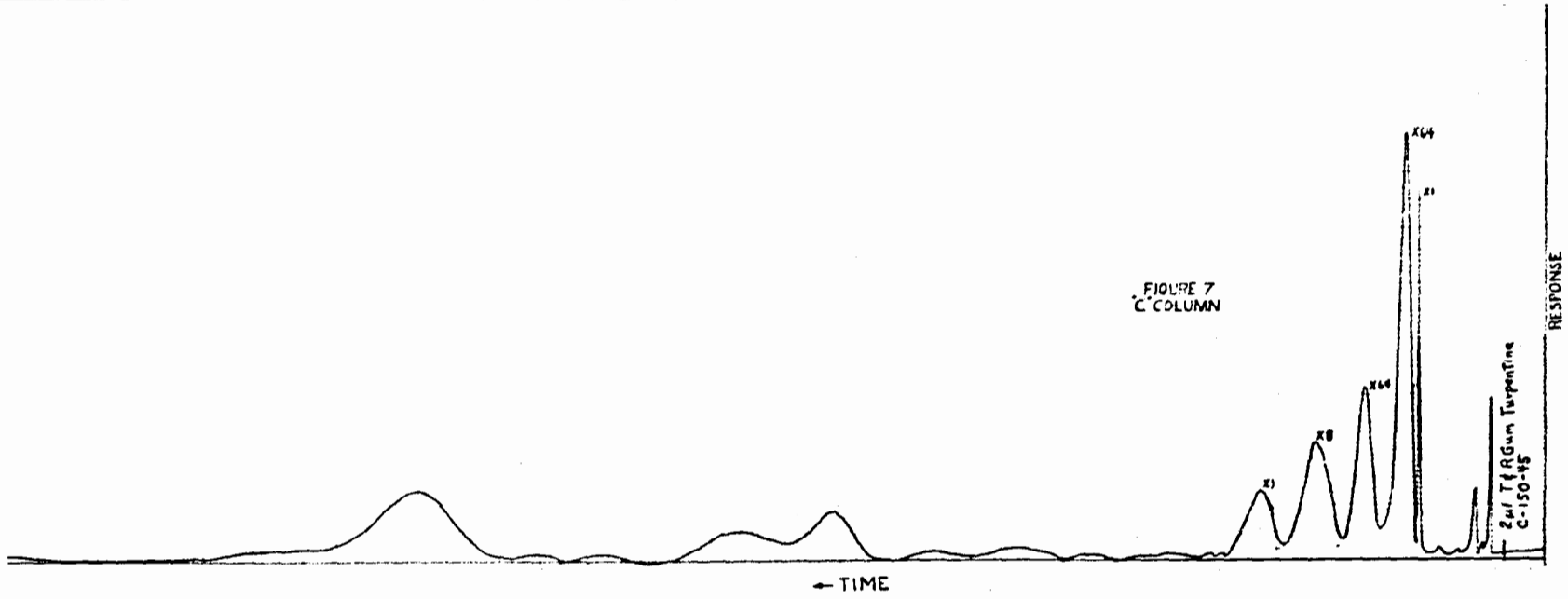


FIGURE 7
"C" COLUMN

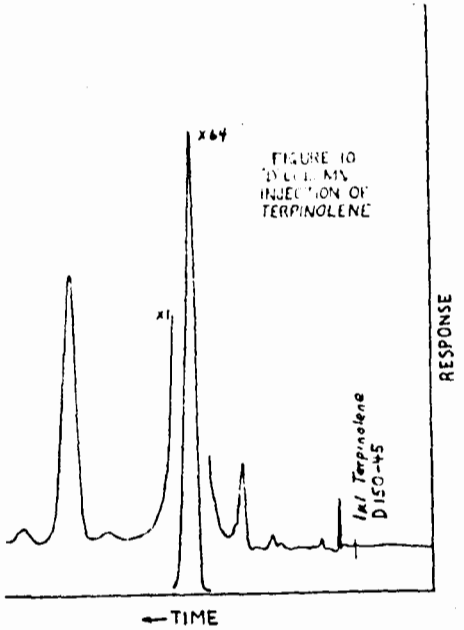


FIGURE 10
"D" COLUMN
INJECTION OF
TERPINOLENE

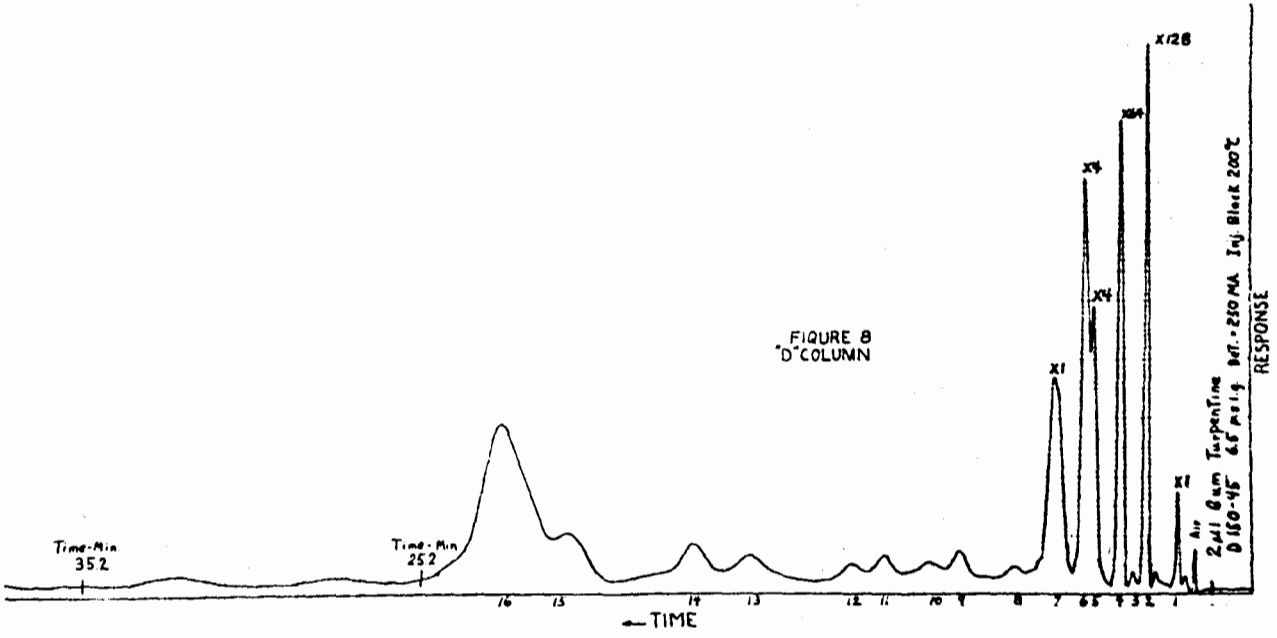


FIGURE 8
"D" COLUMN

The "D" column (Figure eight) showed the best overall separation of any column which was tested. Several molecular weight fractions of Carbowax have been used in work with terpenes. It has generally been considered a moderately polar material. The components of turpentine have a wide range of polarities. The advantages of a polar material may be lost by the failure of this packing to separate non-polar materials. Likewise, a non-polar material may fail to separate polar compounds. Carbowax 20M seems to be a compromise, in this case, between the two types of liquid substrates. Its high molecular weight lends non-polar characteristics, while the remaining OH groups lend a sufficiently polar nature to separate compounds requiring it.

There is no such thing as an "ideal" column packing for any chromatographic separation. Turpentine is a mixture of a number of compounds with many different types of structure. It is therefore unlikely that an "ideal" packing could be found for it. The "D" column showed the best overall separation of the components of turpentine in a reasonable length of time. The column did not isomerize alpha pinene or terpinolene (Figures nine and ten).

The "E" column was constructed in the course of the investigation of rosin as a liquid substrate. The chromatogram of gum turpentine (Figure eleven) on this column does not show good separation or any particular advantages; if anything the effects of isomerization are worsened here.

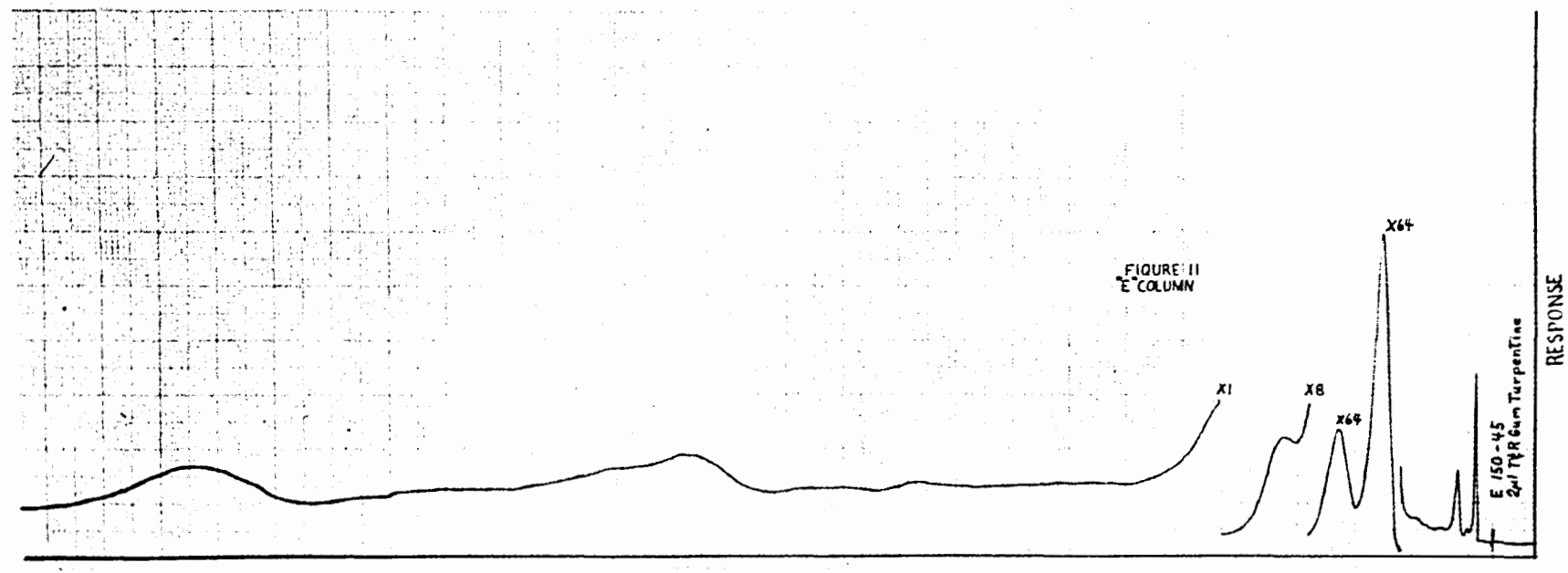


FIGURE 11
"E" COLUMN
X1
X8
X64
X64
E 150-45
2µl T&R Gum Turpentine
RESPONSE
TIME

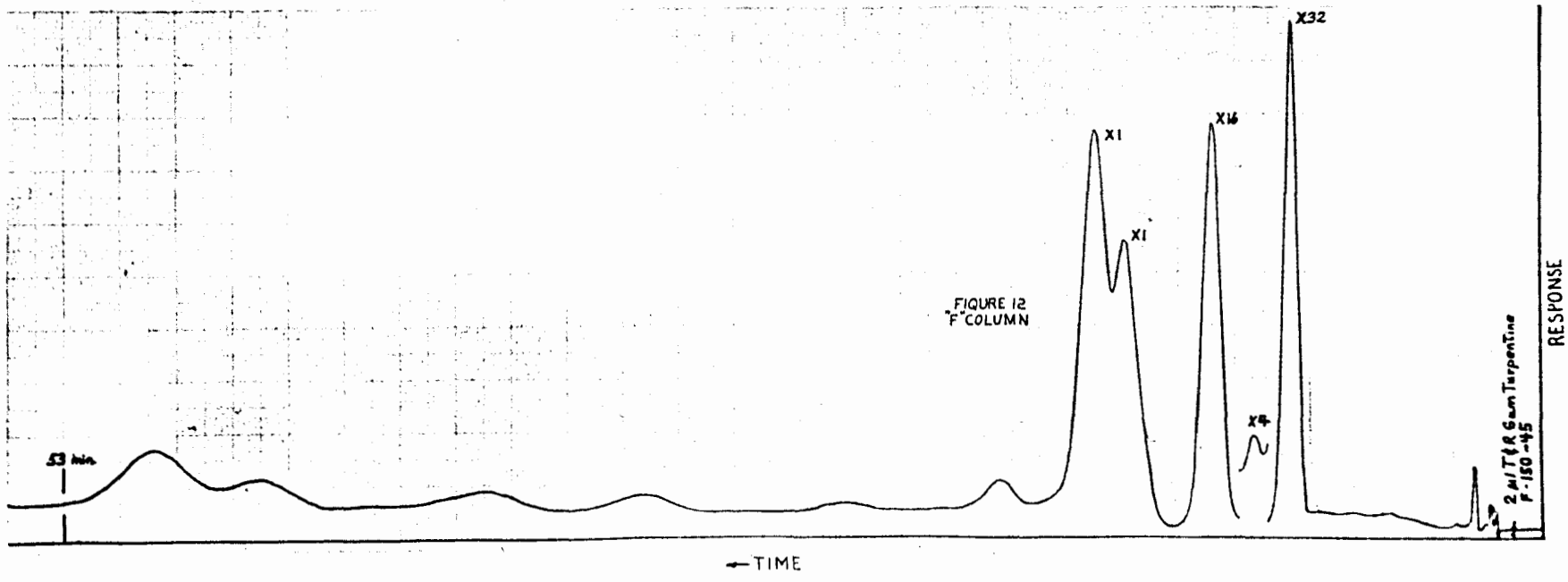


FIGURE 12
"F" COLUMN
X1
X1
X16
X4
X32
53 min
2µl T&R Gum Turpentine
F-150-45
RESPONSE
TIME

An extensive examination was made of the "F" column and the materials with which it was constructed. Diisodecyl phthalate and related materials have been used for the separation of terpene mixtures by several investigators. Under the conditions normally used in this work the commercial material used did not give good results.

The concentration of liquid substrate on this column packing was supposed to be 20%, according to the manufacturer. In later experiments to determine the causes of isomerization by this material, extractions were carried out with anhydrous ethyl ether and the concentration of diisodecyl phthalate was found to 24.93%. This error is not serious, since a variation of 5% in the concentration of the liquid substrate at this level (20-25%) should not make a great deal of difference in the overall separation, but would affect the retention times of samples chromatographed on it.

The overall separation of turpentine on this column was not good as can be readily seen (Figure twelve). All components of turpentine were retained too long. Terpinolene was isomerized by the column as is shown in Figure thirteen. Alpha pinene was not isomerized by the column.

A short length of glass tubing was packed with the same material as was used for the "F" column. As may be seen in Figure fourteen, isomerization still occurs and cannot be attributed solely to the use of copper tubing for the "F" column. The same length of

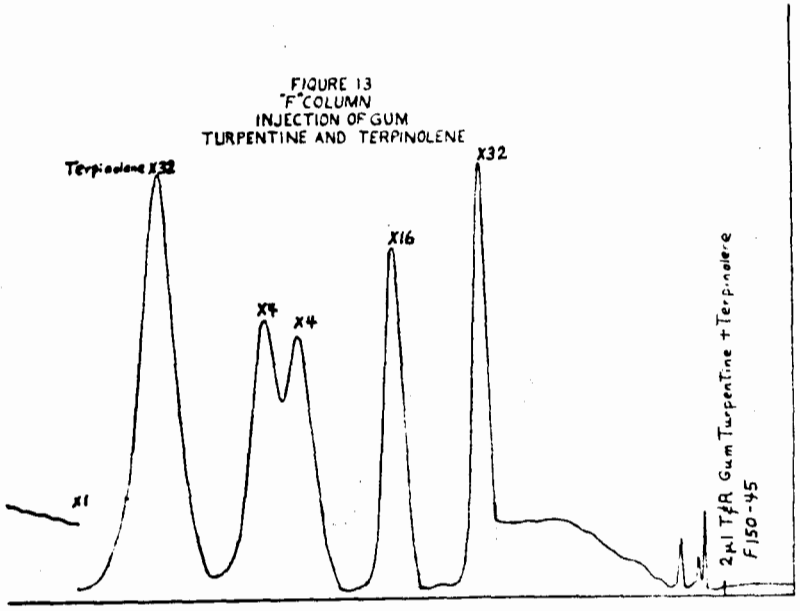


FIGURE 13
"F" COLUMN
INJECTION OF GUM
TURPENTINE AND TERPINOLENE

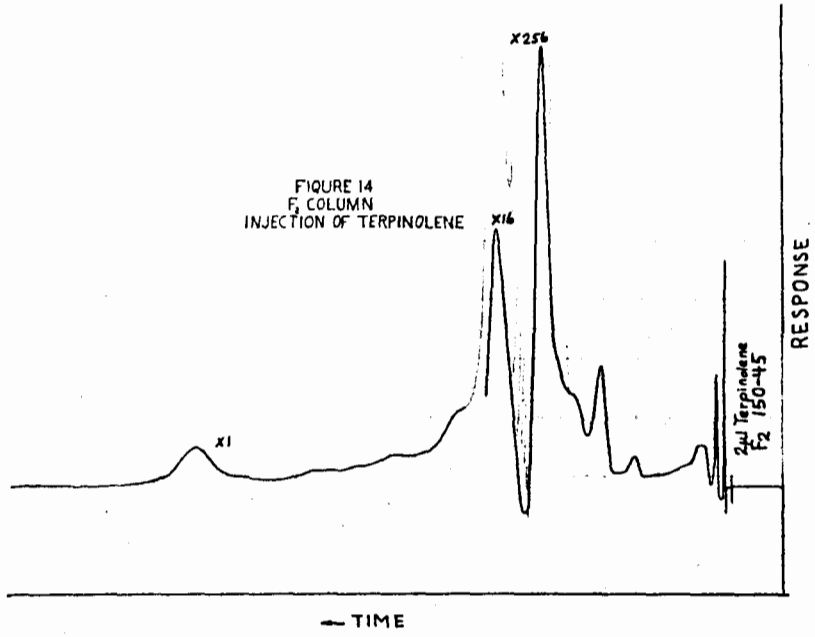


FIGURE 14
F_2 COLUMN
INJECTION OF TERPINOLENE

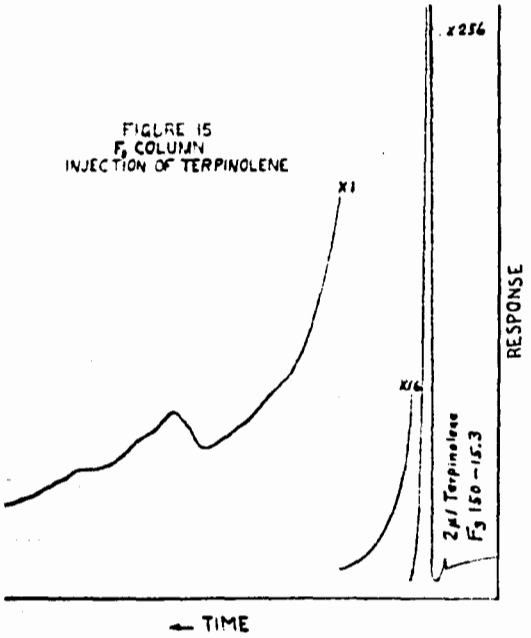


FIGURE 15
F_3 COLUMN
INJECTION OF TERPINOLENE

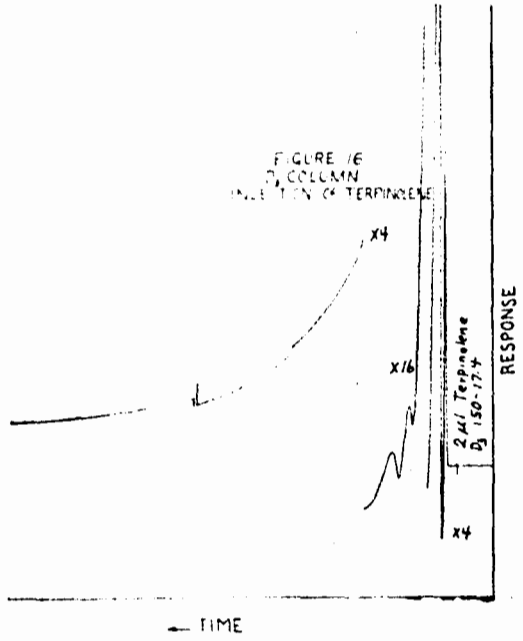


FIGURE 16
D_3 COLUMN
INJECTION OF TERPINOLENE

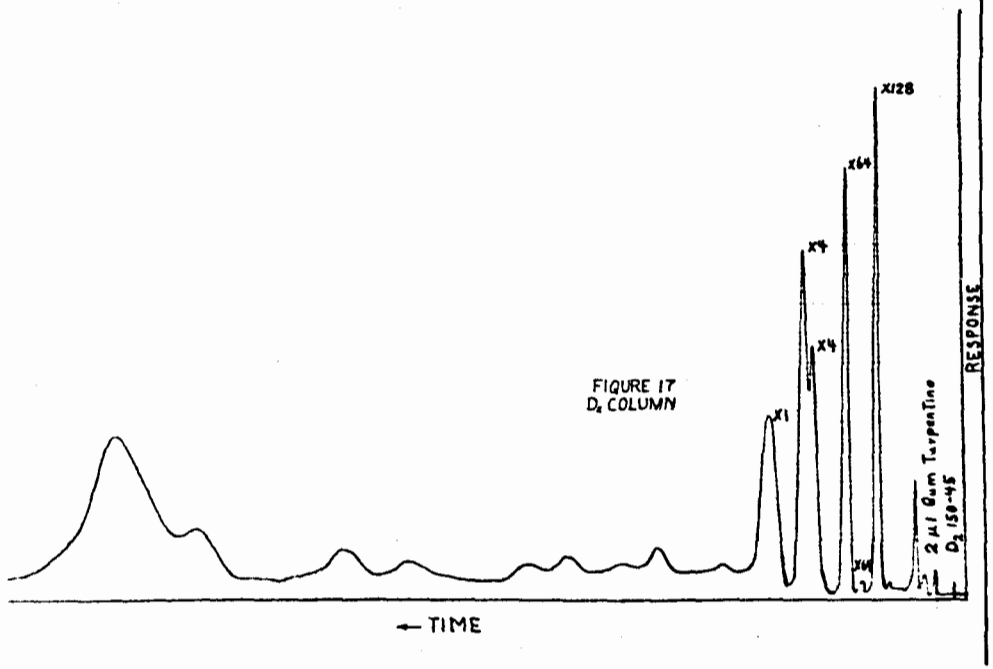


FIGURE 17
D_2 COLUMN

glass tubing was emptied and filled Chromsorb "W" from which the liquid substrate had been extracted. Injection of terpinolene (Figure fifteen) shows that the uncoated packing causes isomerization of this compound.

Chromsorb "W" is prepared by calcining it with about 3% sodium carbonate. This does reduce the ability of the solid support to isomerize terpinolene. This may be seen by comparing Figure fifteen with Figure sixteen, a chromatogram of column D_3, which was prepared by packing the same length of glass tubing with C-22 firebrick, which is essentially the same material except for the sodium carbonate treatment. The Carbowax column might be improved somewhat by preparing it with Chromsorb "W", however, this material was not available at the time.

The preparation of the "D" column was reproducible as may be seen by comparing Figure seventeen with Figure eight. Column D_2 was prepared using the same samples of Carbowax 20M and C-22 firebrick.

Determination of Column Efficiency

In order to determine the efficiency of some of the columns which were otherwise suitable, and to provide some basis of comparison of columns, calculations of the height equivalent to one theoretical plate were made. The HETP determinations were carried out in the commonly accepted manner as described in Pecsok, Ed., Principals and Practice of Gas Chromatography:

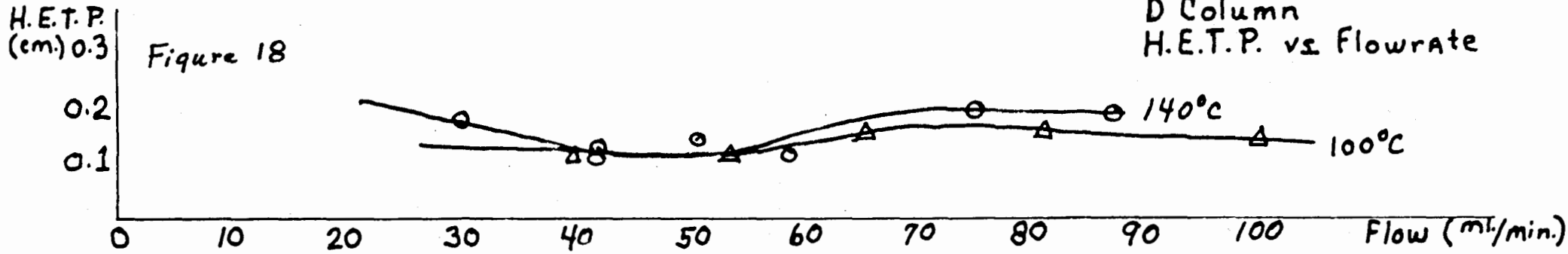


Figure 18

"D" Column
H.E.T.P. vs. Flowrate

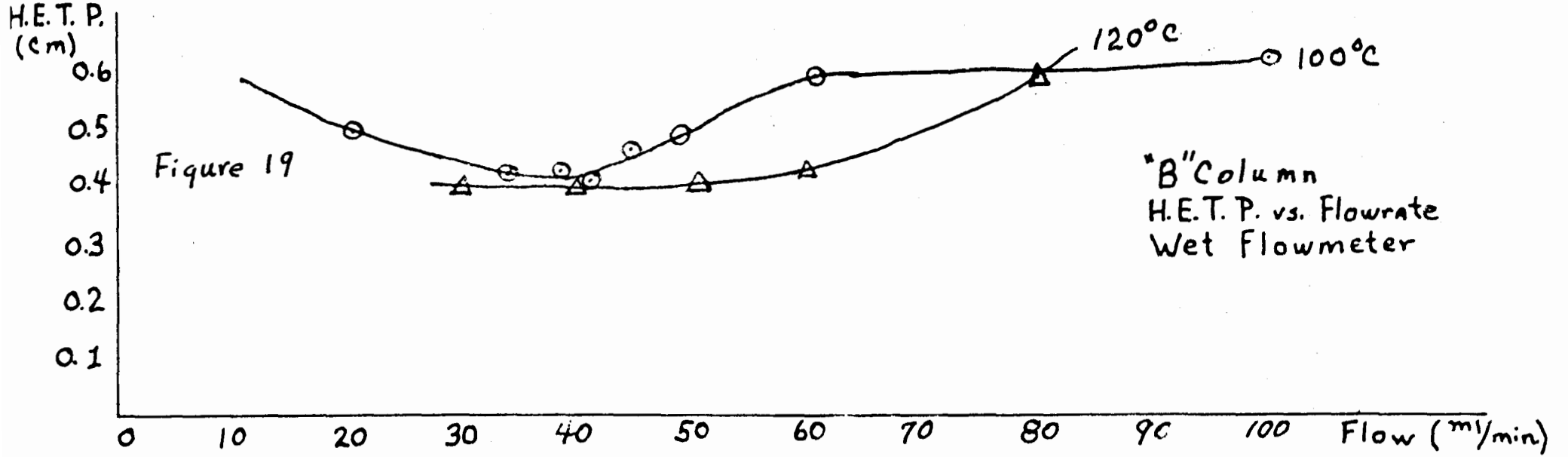


Figure 19

"B" Column
H.E.T.P. vs. Flowrate
Wet Flowmeter

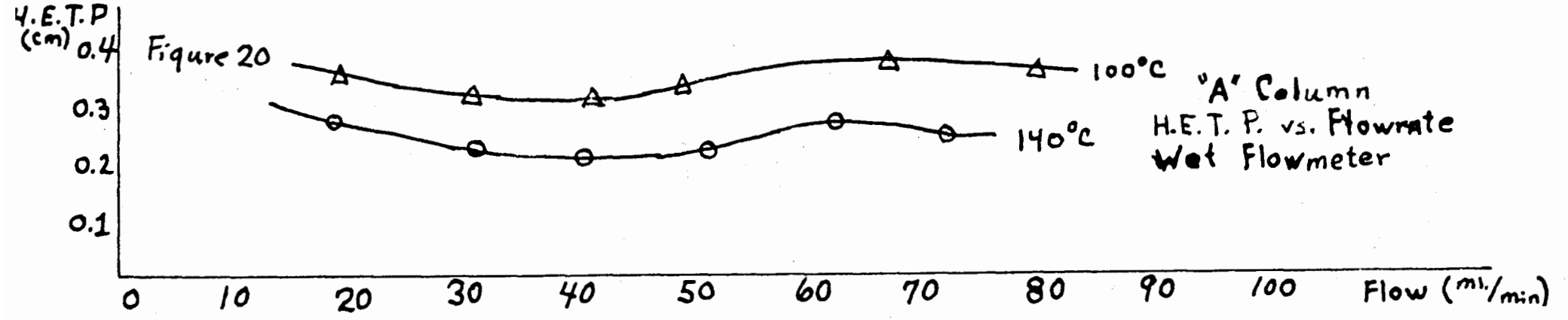
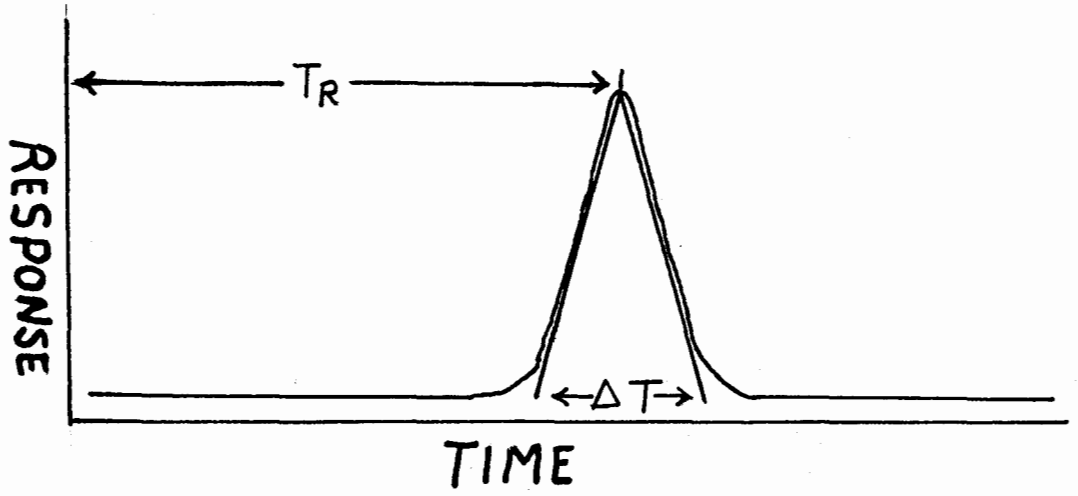


Figure 20

"A" Column
H.E.T.P. vs. Flowrate
Wet Flowmeter



$$n = 16\left(\frac{T_R}{\Delta T}\right)^2$$

n = No. Theoretical Plates

$$HETP = \frac{185}{n} \text{ cm/plate}$$

These determinations were made at several flow rates and for several temperatures, and these results were plotted (Figures eighteen through twenty). Examination of the curves shows that the greatest efficiency (Lowest value of HETP) is given by a flow rate between forty and fifty milliliters per minute.

The HETP determinations were made using alpha pinene since it was the compound to be used as the standard for relative retention data, and also because it had a convenient retention time on all three columns. The flow rates were accurately measured with a bubble type flowmeter and maintained $\pm$ 0.3 milliliters per minute.

On the basis of these results and those published by other investigators, the flow rate value of forty-five milliliters per minute was chosen for all further work on turpentine, except in the case of several short glass columns used to determine isomerization.

Qualitative Analysis

Gas chromatography is primarily a means of separation of sample components. Once the components of a sample have been satisfactorily separated, any suitable means may be used to identify them. The use of standard samples is one of the means most often applied. The known compounds are chromatographed and their retention times are compared with those of the sample mixture. The experimental conditions must be exactly the same here, or appropriate correction factors must be applied. Another, and perhaps simpler method, is to add the proper amount of the suspected component to the sample before it is chromatographed. This was the primary means of identification used in this work. Such a procedure requires some knowledge of the possible constituents of the sample in addition to a number of relatively pure samples. Fortunately, a reasonable amount of information is available on the composition of turpentine, and a number of the known or suspected sample components were available. This identification procedure is by no means absolute proof of the presence of a particular material, but for the major components, it is adequate.

Another means of identification which was used to a limited extent in this work was infra-red spectroscopy. For this method to be of value one must possess known spectra or these must be obtained using pure samples. The chromatographic peaks under study are then trapped into solvents and their infra-red spectra

obtained and compared with the known spectra. Quite often, as in this case, the amount of sample available is insufficient for this purpose. Another difficulty encountered was the purity of the samples versus that of the known, published spectra. The available data on terpenes was published before the advent of gas chromatography and it was found that the samples trapped from the gas chromatograph were considerably purer than the infra-red standards had been. Distilled fractions of terpenes of supposed high purity were shown to contain as high as twenty other components. The spectra of samples trapped to eliminate these impurities did not show as many peaks as the published spectra, and therefore the published spectra would not necessarily have been reliable as a means of identification even if all of the components could have been isolated in sufficient quantity.

Chemical reaction before chromatography may be applied to gain some idea of the composition of a sample. The sample may be treated with a reagent to remove some of the components of a mixture. The nature of the reaction will afford some idea of the structure of the compounds comprising peaks which are removed. This procedure may also uncover peaks which heretofore were hidden.

Samples for Testing

Samples of compounds known to be present in gum and steam distilled turpentine, as well as some related materials which might be present, were obtained from various sources. Samples of alpha

and beta pinene and camphene were obtained from the Glidden Company. These were commercial products whose purity was estimated at about 90-95%. They contained numerous impurities, but the pure compounds could be isolated by chromatographing and trapping the proper fractions.

The following terpene hydrocarbons were provided by Hercules Powder Company:

Camphene
3-Carene
Carvomenthene
Dipentene
Isocamphene
2,4(8)-p-Menthadiene
3,8-p-Menthadiene
cis-p-Menthane
trans-p-Menthane
3-Menthene
Myrcene
alpha-Phellandrene
cis-Pinane
alpha-Pinene
beta-Pinene
alpha-Pyronene
alpha-Terpinene
gamma-Terpinene
Terpinolene
Tricyclene

The following compounds were obtained from the Drug Laboratory of the Division of Chemistry and foods, Commonwealth of Virginia:

1,8 Cineole
Camphor
Geranyl Acetate
Bornyl Acetate
Carvone
Citral
l-Menthone
Citronellal
Furfural

Benzaldehyde was obtained from the University of Richmond, and Bornyl Alcohol was prepared from the above sample of Bornyl Acetate.

The hydrocarbon samples from Hercules Powder Company had been distilled using about 100 theoretical plates after preliminary isolation. Their purity was at least ninety per cent and in many cases better, depending on the individual sample. The samples from the Drug Laboratory were U. S. P. grade. All the samples used showed a number of impurities when chromatographed.

Relative Retention Ratios

Each sample was chromatographed by itself in order to determine its retention time and to determine the extent of impurity. The experimental conditions were maintained as closely as possible while this work was being carried out. Alpha pinene was frequently added to a small amount of the test compound in order to insure the accuracy of the retention time for this standard. If the retention time matched that of an unknown peak in the chromatograms of the turpentine samples, a trade of the compound was added to a small amount of either gum turpentine, or if appropriate, steam distilled wood turpentine. The mixture was adjusted to just cause a slight increase in the size of the unknown peak. An X-ray film viewer was found to be very useful in matching chromatograms of knowns with unknown peaks in chromatograms of turpentines. Identifications of peaks in commercial samples were made by this means

as often as by relative retention ratios. This method was just as accurate, if not more so, since the experimental conditions were quite reproducible. The comparison method also speeded identification work considerably.

A number of terpene hydrocarbons had retention times quite close together and determination of a reliable relative retention ratio by measurement of time elapsed on the chart paper was not accurate enough to provide satisfactory data even when dividers were used for the measurement. In these cases a stopwatch was used to time the emergence of the peaks. As the air peak reached its maximum, the stopwatch was started; it was stopped when the sample peak reached its maximum. This gave much more satisfactory results. For compounds with a retention time greater than five minutes the alpha pinene (standard) peak was timed but the sample peak was measured on the chart paper.

Retention ratios relative to alpha pinene and corrected for dead space are shown in table two. These peaks were measured or timed from the peak maximum of air to that of the sample component. Since air is not absorbed on the column this is a measure of the dead space, or inactive portion, of the column, injection block, and detector. Measurement of elution time from the air peak corrects for this dead space. The ratio of this value to the same value for a sample component is termed the relative retention ratio. The operating conditions are shown in table three.

TABLE II

RELATIVE RETENTION RATIOS

Compound	Boiling Point	T_r	RTR
Tricyclene	152	1.60	0.96
trans-p-Menthene	170	1.60	0.96
alpha-Pinene	156	1.65	1.00
alpha-Pyronene	157	1.65	1.00
cis-p-Menthene	171	1.75	1.07
3-Menthene	169	1.85	1.12
cis-Pinane	168	2.00	1.21
Isocamphane	164	2.33	1.25
Camphene	159	2.07	1.29
Myrcene	167	2.42	1.48
beta-Pinene	165	2.45	1.49
Carvomenthene	175	2.60	1.58
3-Carene	170	2.85	1.70
alpha Phellandrene	169	3.00	1.79
alpha terpinene	175	3.10	1.84
Dipentene	177	3.40	2.06
1,8 Cineole	174	3.60	2.18
gamma-Terpinene	182	4.03	2.40
3,8-p-Menthadiene	184	4.35	2.64
Terpinolene	186	4.75	2.81
2,4(8)-p-Menthadiene	186	5.10	3.00

TABLE II (Cont'd)

Compound	Boiling Point °C	T_r	RTR
Furfural	162	9.60	5.8
Citronellal	105	9.8	5.9
l-Menthone	207	10.7	6.5
Benzaldehyde	180	13.1	7.9
Camphor	204 subl.	14.5	8.8
Bornyl Acetate	114 22 mm.	16.4	9.9
Geranyl Acetate (trans?)	245	20.5	12.4
alpha Terpineol	219	22.6	13.7
Borneol	211	23.6	14.3
Citral	229	27.9	16.9
Carvone	230	29.9	18.1

TABLE III

OPERATING CONDITIONS

Instrument	Perkin-Elmer 154C Vapor Fractometer
Recorder Range	0-1 millivolt
Response Time	one second
Chart Speed	30 inches per hour
Solid Support	C-22 Firebrick
Liquid Phase	Carbowax 20M (20%)
Helium Flow Rate	45 milliliters minute
Column Length	six feet
Temperature	150°C
Pressure Drop	6.5 p.s.i.g. to Atmospheric

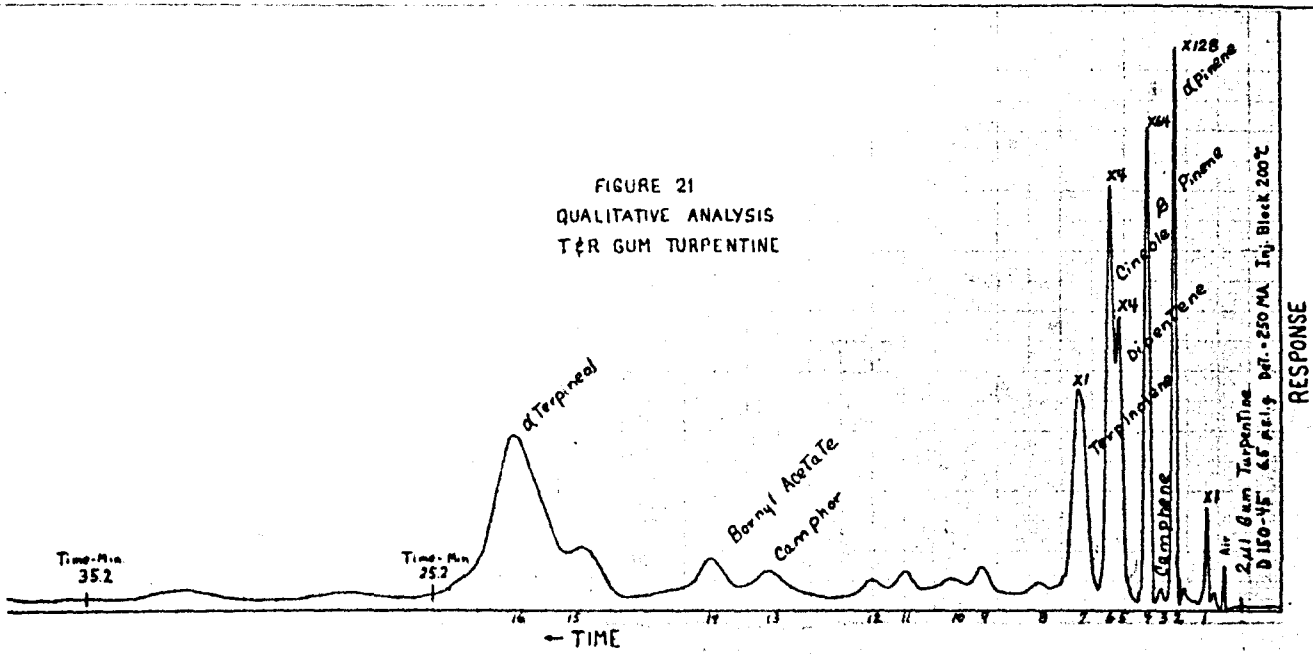


FIGURE 21
QUALITATIVE ANALYSIS
T&R GUM TURPENTINE

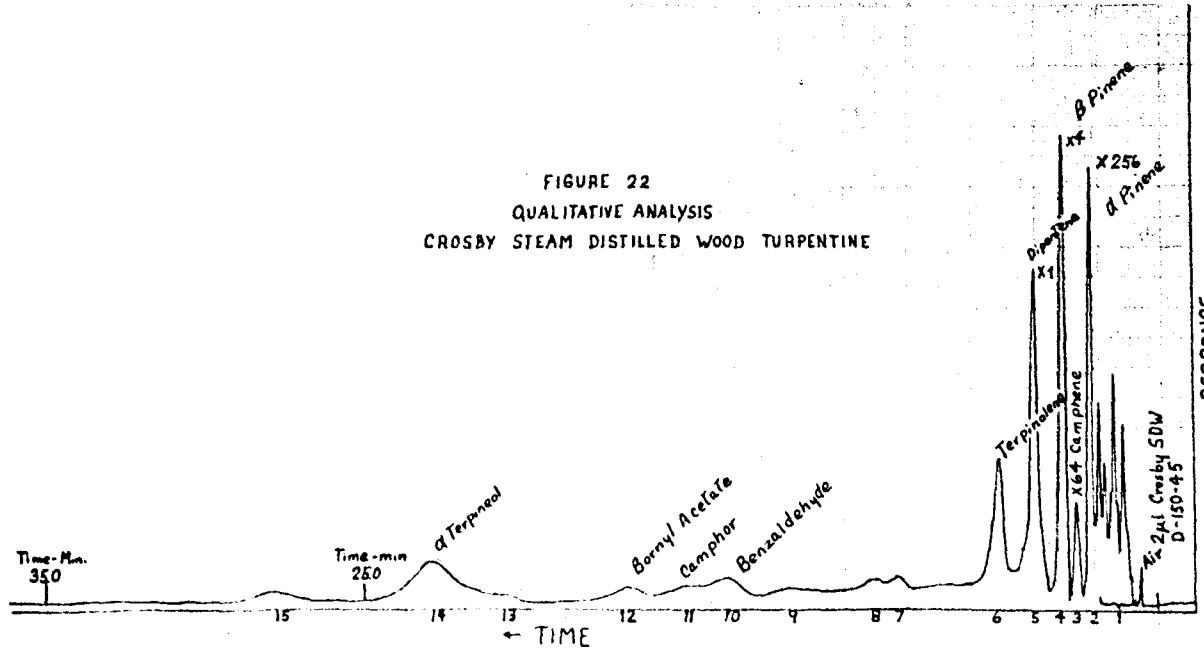


FIGURE 22
QUALITATIVE ANALYSIS
CROSBY STEAM DISTILLED WOOD TURPENTINE

The retention volume of alpha pinene corrected for dead space and pressure drop (V_R^o) was 59.5 milliliters per minute.

Qualitative Results

Application of Retention Data

A. Gum Turpentine A representative sample of gum turpentine (Turpentine and Rosin Factors, Inc.) was used for the purpose of qualitative analysis. This same sample was used to determine the characteristics of the various experimental columns. Under the conditions previously selected for analysis, this sample gives sixteen major peaks (Figure twenty-one). Using the retention times above, and information from the literature, the following identifications were made:

Peak number one has not been identified. It is believed to be a hydrocarbon, probably a fenchene. Peak number two is alpha pinene. There is often the possibility that a very large peak may cover up other compounds which occur only in small amounts. Other terpene hydrocarbons may be present in this peak, since a number have retention times in this area. Samples which were trapped and rechromatographed on this and other columns showed no evidence of this, however. Peak number three is caused by camphene. Peak four is beta pinene, with no known contamination. The fifth peak contains the limonenes, the d and l form if present, and the dl form,

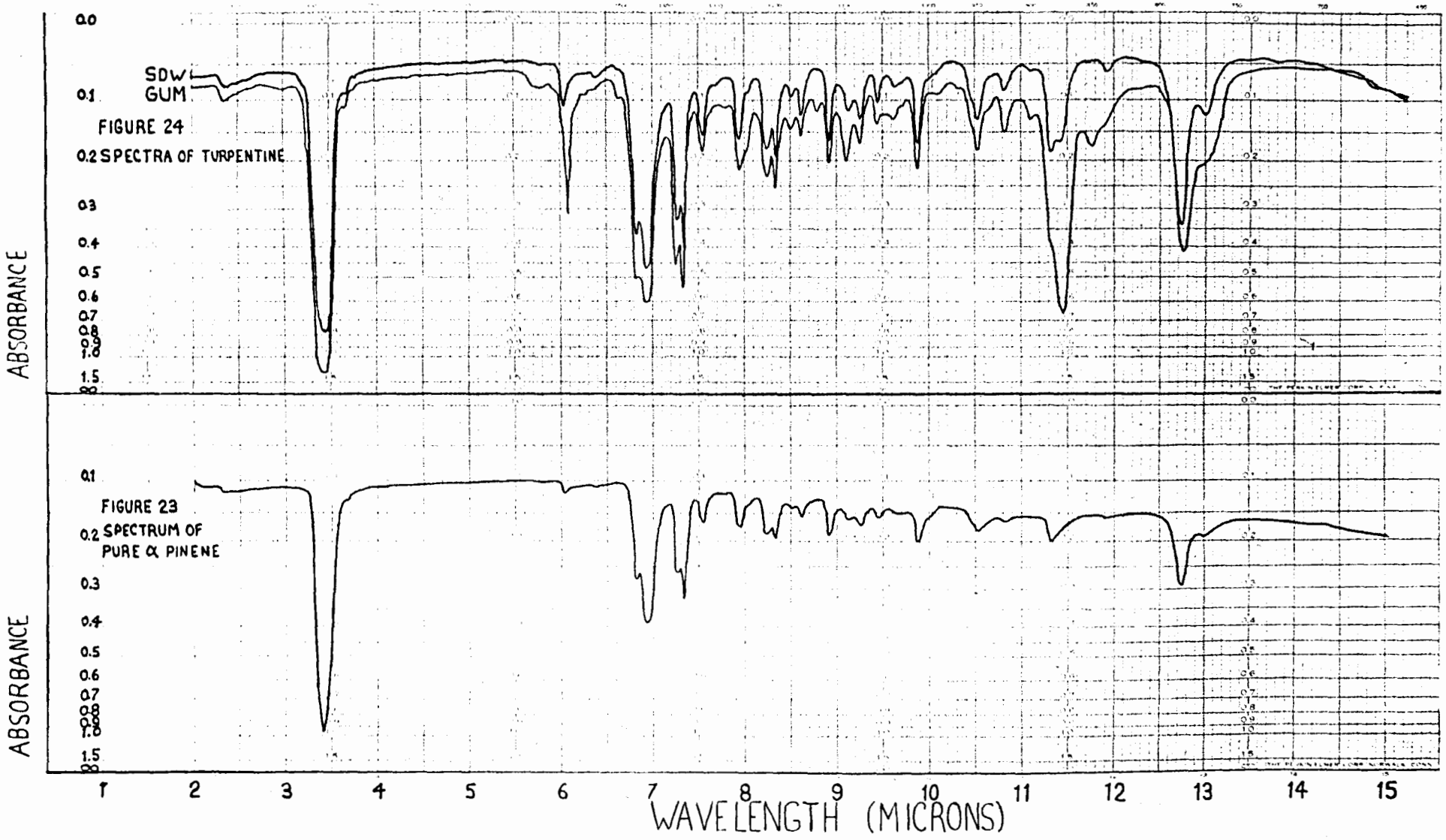
dipentene. Dipentene was the only sample used experimentally since all forms of this material would have the same retention time. Since all turpentines show optical rotation, it is assumed that the optically active forms of limonene are present also. Peak six was shown to contain some cineole. There is a strong possibility that this peak also contains beta-Phellandrene. This compound has been reported in gum turpentine and interpolation from published data indicates that its peak would occur in this position. A sample of this compound has not been obtained to date. Peak seven is known to be a mixed peak, the largest part of which is terpinolene. The other component may be p-cymene although this compound was not among the samples available.

Peaks eight through twelve have not been identified. Several of the compounds tested had retention times which corresponded to peaks in this area, however, the agreement in these cases was not as positive as for the other identified peaks. Peak thirteen was shown to be camphor; peak fourteen is bornyl acetate. Peak fifteen has not been positively identified, but may contain cis or trans gernyl acetate. Peak sixteen is almost entirely alpha terpineol which appears to cover a small amount of bornyl alcohol.

There were two peaks which could be observed with retention times greater than alpha terpineol. These were only apparent on injection of large (10 microliter) samples. They comprised less than 0.10% of the sample and were not identified.

B. Steam Distilled Wood Turpentine A single representative sample of steam distilled wood turpentine was used for qualitative analysis (Crosby Chemicals, Inc.). The chromatogram of this sample shows 15 recognizable peaks. (Figure twenty-two). All of the hydrocarbon components of this sample are placed together in collective peak one. The identification of these peaks was not undertaken. The peaks with retention times shorter than alpha pinene are often caused by naptha since this material is sometimes used in the solvent process for the production of steam distilled wood turpentine. Peak number two was shown to be alpha pinene. Here again,other trace components may be masked by the large alpha pinene peak. Peak number three consists of camphene. Peak four is beta pinene. Peak five is caused by dl-limonene or dipentene. Peak seven has been shown to contain terpinolene but as in the case of gum turpentine, this is a mixed peak. Peaks seven, eight, and nine remain unidentified. Peak ten is given by benzaldehyde. Camphor is responsible for peak eleven and bornyl acetate for peak twelve. Peak thirteen has not been identified, and neither has peak fifteen. Peak fourteen is alpha terpineol and/or bornyl alcohol.

Infra-red Data Infra-red spectra of the turpentines are shown in Figure twenty-three. The only major differences shown by these two spectra are caused by beta pinene at 6.4 and 11.45 microns. Samples of alpha and beta pinene were purified by trapping from the "D" column and their infra-red spectra were then obtained (Figures twenty-four and twenty-five). Figure twenty-six shows the spectrum


SDW
GUM
ABSORBANCE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.5
2.0
FIGURE 24
SPECTRA OF TURPENTINE
ABSORBANCE
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.5
2.0
FIGURE 23
SPECTRUM OF
PURE α PINENE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH (MICRONS)

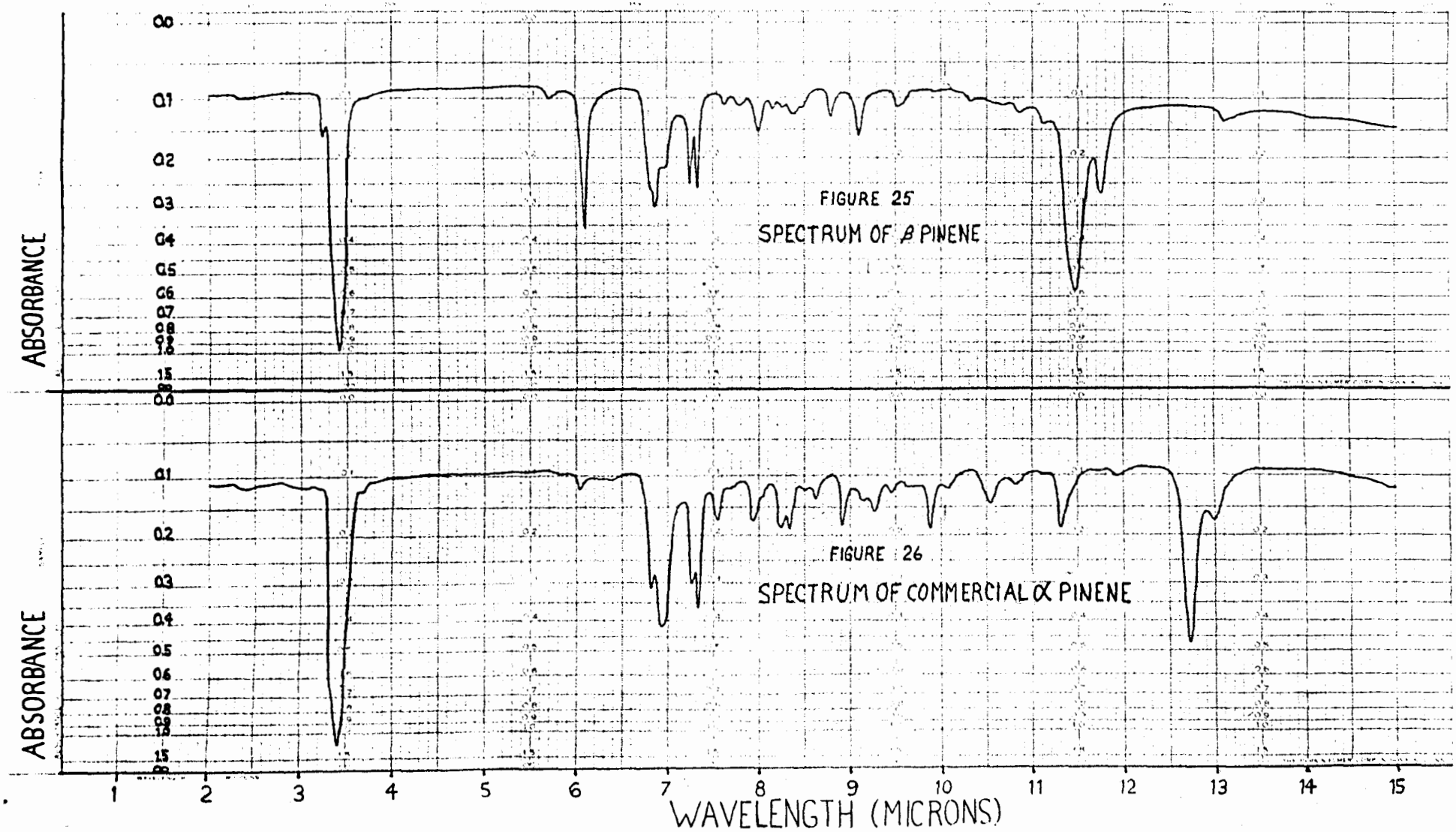
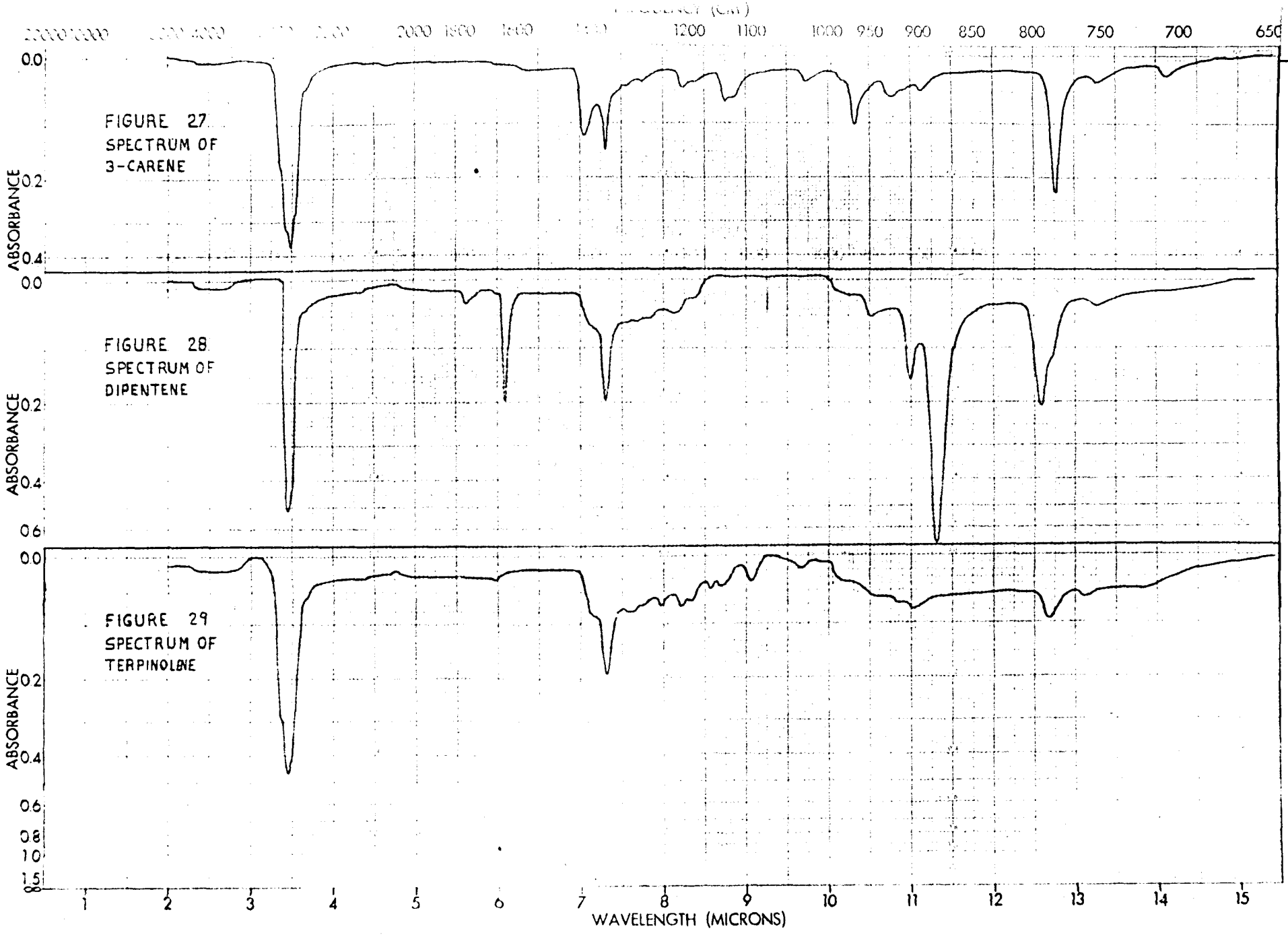


FIGURE 25

SPECTRUM OF β PINENE

FIGURE 26

SPECTRUM OF COMMERCIAL α PINENE


1200 1100 1000 950 900 850 800 750 700 650
0.0
0.2
0.4
ABSORBANCE
FIGURE 27
SPECTRUM OF
3-CARENE
0.0
0.2
0.4
0.6
ABSORBANCE
FIGURE 28
SPECTRUM OF
DIPENTENE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
ABSORBANCE
FIGURE 29
SPECTRUM OF
TERPINOLENE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

of distilled alpha pinene. At least twenty minor components were removed by gas chromatography and the differences in the spectra of the two samples are evident upon comparison of Figure twenty-four with Figure twenty-six. Note the difference in the peaks at 12.75 microns in particular. The peak at 11.32 microns is caused by camphene and is larger in the distilled sample.

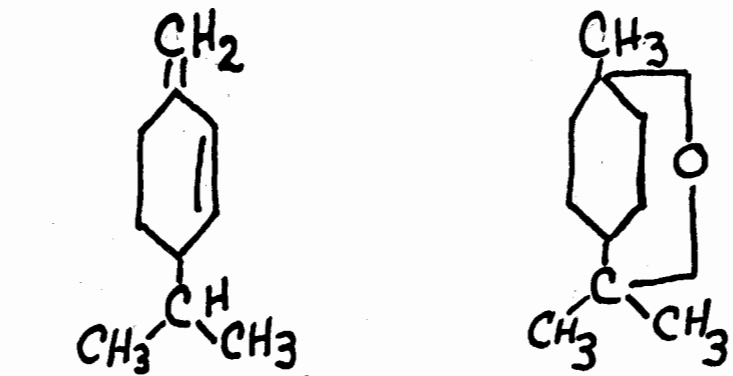
Other spectra are shown in Figures twenty-seven through twenty-nine. These are representative of some of the compounds which were purified by gas chromatography, trapped and the infra-red spectra compared with published data. Comparison with published values will show the absence of a number of the peaks in the spectra of chromatographed samples.

Alpha and beta pinene were shown to be present on the basis of infra-red data, but other than these two, the poor comparison of these infra-red data with those published, and the difficulty involved in trapping samples of minor components for use in 1 mm. cells, ruled against further work with infra-red identification.

Chemical Reaction Since infra-red identification did not yield adequate information for more positive identification of some of the minor constituents of gum turpentine, particularly peak six (Figure twenty-two), chemical means were used to remove some of the peaks.

Several of the turpenes in gum turpentine have a conjugated diene structure. These may be removed by reaction with maleic

anhydride. Maleic anhydride will also remove alcohols and cause some isomerization. In order to discover which compounds had been removed by the diene reaction and which were removed by other mechanisms, the same reaction was carried out with phthalic anhydride. Peak six (Figure twenty-two) may contain both cineole and beta-phellandrene. No sample of beta-phellandrene was available, consequently further identification could not be made on the basis of retention time. Beta-phellandrene, however, should undergo the diene reaction, whereas cineole would not be expected to. The structures of these two compounds are shown below.



These two reactions were carried out as follows:

Maleic Anhydride Reaction --Eighty milliliters of gum turpentine were placed in the flask of a soxhlet extraction apparatus and five grams of maleic anhydride added. This corresponded to approximately one tenth of a mole of maleic anhydride per mole of turpentine. The molecular weight of turpentine was based upon that of its major constituent, alpha pinene. Several glass beads were added for boiling chips and the solution was placed on a hot plate to reflux.

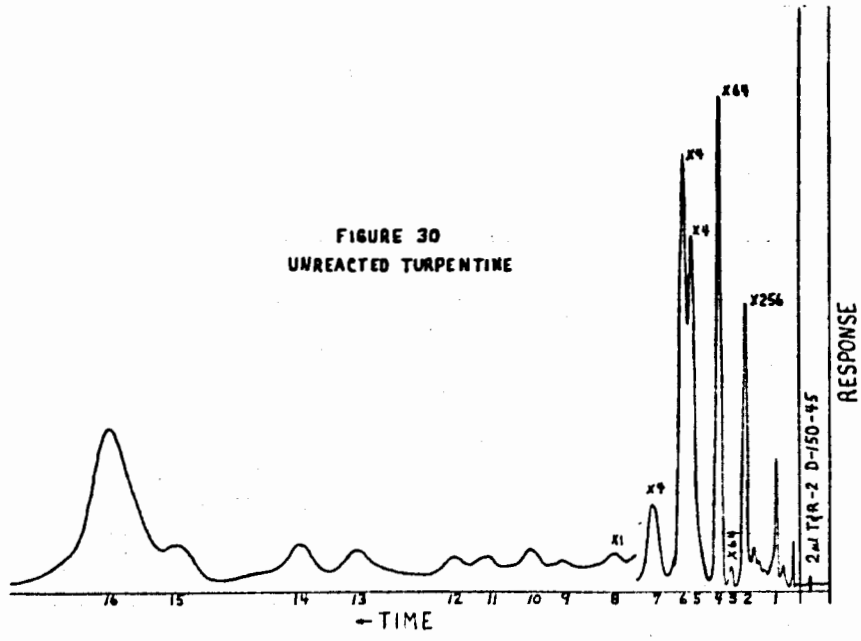


FIGURE 30
UNREACTED TURPENTINE

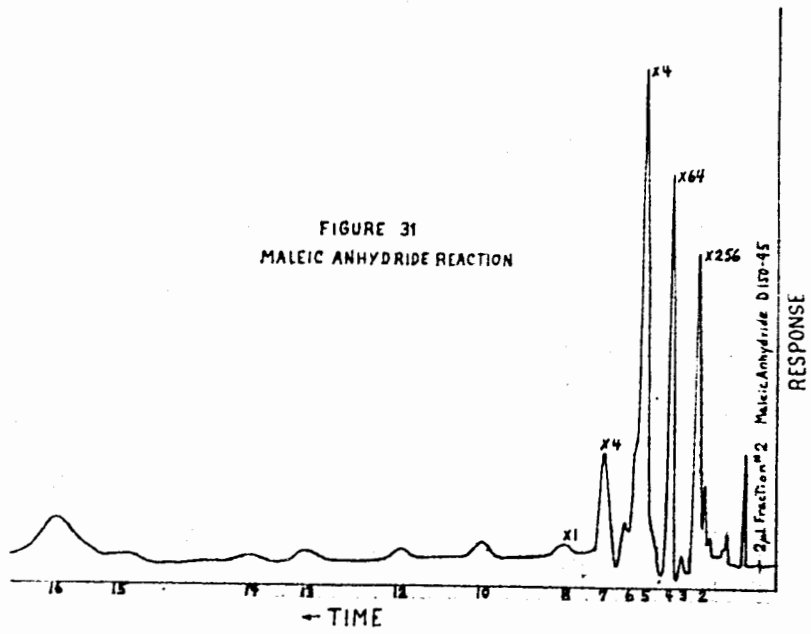


FIGURE 31
MALEIC ANHYDRIDE REACTION

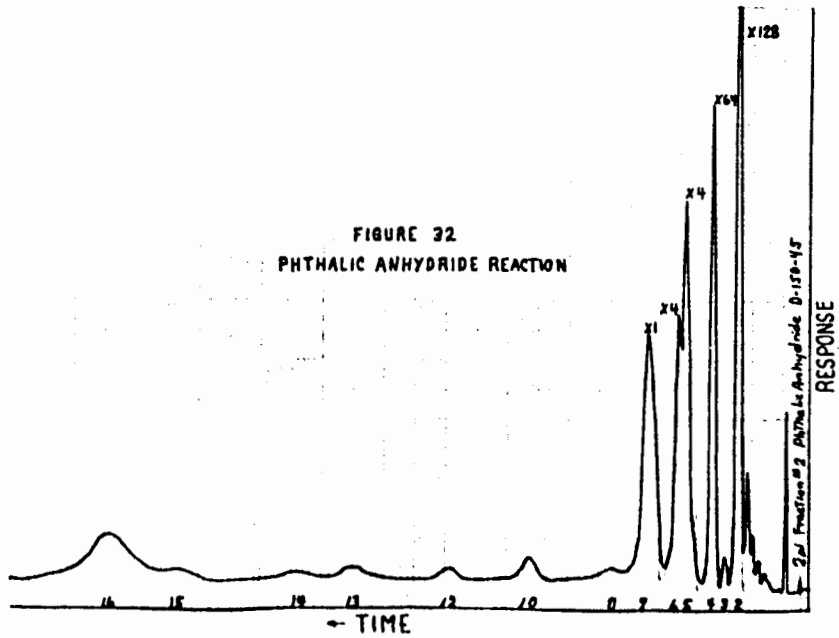


FIGURE 32
PHTHALIC ANHYDRIDE REACTION

Samples were taken through the condenser from the soxhlet trap and chromatographed immediately. They were taken from the initial boiling mixture, after one-half hour, at one hour and fifteen minutes, and again at the end of two hours.

Phthallic anhydride reaction--Eighty milliliters of the same turpentine were reacted with five grams of phthalic anhydride. Exactly the same procedure was used as above.

The gum turpentine used for these reactions did not come from the same sample as used for the identification by retention times, but it did come from the same source and its composition was almost identical. A chromatogram of the starting material is shown if Figure thirty. Figures thirty-one and thirty-two show chromatograms of the maleic anhydride and phthalic anhydride reactions.

The samples taken after one-half hour are shown above simply because the chromatograms were neat and well matched. There was little variation after the first sample was taken. What little there was could easily be explained on the basis of isomerization and by the variation in the amount of liquid in the soxhlet trap when the samples were taken from it.

It will be immediately noticed that peak six has been almost completely removed by the maleic anhydride reaction (Figure thirty-one), but not by the reaction involving phthalic anhydride (Figure thirty-two). Peaks nine, eleven and a substantial part of sixteen have also been removed. Peaks thirteen, fourteen and fifteen have been greatly reduced by both reactions.

It is concluded that the removal of peak six was principally a result of the diene reaction and therefore peak six must at least be partially composed of a conjugated diene terpene such as beta phellandrene. Peaks eight, ten, and twelve are suspected of being isomerization products on the basis of these reactions and their frequent occurrence for other reasons.

Reaction with Sodium Bisulfite-- Both samples of turpentine were tested for reaction with sodium bisulfite. One gram of sodium bisulfite was added to about ten milliliters of each turpentine and shaken for one-half hour. The sodium bisulfite was allowed to settle and the supernatant liquid was decanted through filter paper. Two microliters of each sample was chromatographed.

The treatment caused no changes in the chromatogram of gum turpentine, however, peak ten was removed from steam distilled wood turpentine. This peak had been tentatively identified as benzaldehyde on the basis of retention data.

Summary of Information used for Identification-- Table four is a summary of the information used for the identification of the major components of gum and steam distilled wood turpentine.

TABLE IV

SUMMARY OF INFORMATION USED FOR IDENTIFICATION

No.	Compound	Lit.	RTR	IR	React.
1	Alpha Pinene	X	X	X	
2	Camphene	X	X		
3	Beta Pinene	X	X	X	
4	Dipentene	X	X		
5	Cineole	X	X		X
6	Beta Phellandrene	X	?		X
7	Terpinolene	X	X		
8	Camphor	X	X		
9	Bornyl Acetate	X	X		
10	Alpha Terpineol	X	X		
11	Benzaldehyde	X	X		X

Quantitative Analysis

Three samples of T & R Gum Turpentine were analyzed by gas chromatography using the automatic printing integrator. The integrator was used in its manual function; that is, as a peak was about to appear on the chromatogram the integrator was caused to print by pushing the manual printing button. When the pen of the recorder returned to the lowest point, or zero, the button was again pushed. If necessary, the attenuation of the recorder was changed and/or the integrator was zeroed at the base line for the next peak. Some adjustment of the integrator zero was usually necessary for the remaining peaks after peak seven in order to assure highest accuracy for these minor components. For a sample of Gum Turpentine such as the T & R sample used here, the attenuations were; times 1 for the low boiling peaks, times 256 for alpha pinene, times 32 for Camphene, times 64 for beta pinene, and times 4 for dipentene and cineole. The remaining peaks were all run at an attenuation of 1. A representative chromatogram is shown in Figure thirty-three. For the sample of Steam distilled wood turpentine the attenuations were; times one for the low boiling hydrocarbons, times 256 for alpha pinene, times 32 for camphene, times 8 for beta pinene, and times one for the remaining peaks. A representative chromatogram is shown in Figure thirty-four.

Tables of data for the analyses (Tables five and six) show the peak number, the number of counts given for a particular peak in each analysis, the appropriate multiplication factor for each peak, the total number of counts, the area per cent for each peak, and an average for the three analyses.

The method used for calculation of the per cent of a peak was that of internal normalization of peak areas. In this method it is assumed that the total area under a chromatogram represents all of the sample components. Using the automatic integrator, the number of counts for each peak is divided by the number of counts given for the entire sample. The use of such a method without corrections is, of course, subject to errors in relative response, mechanical error in the integrator, detector, and recorder. These might be corrected for if need be, but were considered outside the scope of the present work.

Tables seven and eight give information on the precision of the method. These data include the average value, the deviation from the mean for each peak for the three runs, and the average deviation.

The use of the automatic integrator for this work requires the operator to be somewhat familiar with the sample he is working with. It is sometimes necessary to run the sample once to determine the attenuations to be used for the various peaks.

The precision of the method using the automatic integrator is improved substantially as the operator gains experience in using

TABLE V

ANALYSIS OF T & R GUM TURPENTINE

	COUNTS				TOTAL COUNTS			AREA %			AVERAGE
Peak	I	II	III	X	I	II	III	I	II	III	
1	78	82	92	1	78	82	92	0.04	0.06	0.05	0.05
2	408	338	396	256	104448	86528	101376	60.22	58.44	59.11	59.29
3	59	42	40	32	1888	1344	1280	1.09	0.91	0.75	0.91
4	855	769	894	64	54720	49216	57216	31.55	33.24	33.36	32.68
5	720	628	692	4	2880	2512	2768	1.66	1.70	1.61	1.66
6	1005	907	1026	4	4020	3628	4104	2.32	2.45	2.39	2.39
7	1192	1141	1033	1	1192	1141	1033	0.69	0.77	0.60	0.69
8	140	18	24	1	140	18	24	0.08	0.01	0.01	0.04

TABLE V (Cont'd)

ANALYSIS OF T & R GUM TURPENTINE

	COUNTS				TOTAL COUNTS			AREA %			AVERAGE
Peak	I	II	III	X	I	II	III	I	II	III	
9	122	38	48	1	122	38	48	0.07	0.03	0.03	0.03
10	172	56	55	1	172	56	55	0.10	0.04	0.03	0.06
11	239	61	82	1	239	61	82	0.14	0.04	0.05	0.08
12	120	28	49	1	120	28	49	0.07	0.02	0.03	0.04
13	216	211	117	1	216	211	117	0.12	0.14	0.07	0.11
14	269	278	191	1	269	278	191	0.16	0.19	0.11	0.15
15	447	373	407	1	447	373	407	0.26	0.25	0.24	0.25
16	2488	2544	2670	1	2488	2544	2607	1.43	1.72	1.56	1.57
		TOTALS			173439	148058	171512	100.00	99.98	100.00	100.00

TABLE VI

ANALYSIS OF CROSBY SDW TURPENTINE

	COUNTS				TOTAL COUNTS			AREA %			AVERAGE
Peak	I	II	III	X	I	II	III	I	II	III	
1	1515	1498	1467	1	1515	1498	1467	0.62	0.61	0.60	0.61
2	847	854	850	256	216832	218624	217600	88.70	88.79	88.76	88.75
3	551	557	547	32	17632	17824	17504	7.21	7.24	7.14	7.20
4	622	620	611	8	4976	4960	4888	2.04	2.01	1.99	2.01
5	1242	1175	1200	1	1242	1175	1200	0.51	0.48	0.49	0.49
6	645	595	840	1	645	595	840	0.26	0.24	0.34	0.28
7 8	146	149	123	1	146	149	123	0.06	0.06	0.05	0.06

TABLE VI (Cont'd)

ANALYSIS OF CROSBY SDW TURPENTINE

	COUNTS				TOTAL COUNTS			AREA %			AVERAGE
Peak	I	II	III	X	I	II	III	I	II	III	
9	32	38	32	1	32	38	32	0.00	----	----	----
10	326	310	362	1	326	310	362	0.13	0.12	0.15	0.13
11											
12	95	103	80	1	95	103	80	0.04	0.04	0.03	0.04
13	885	818	876	1	885	818	876	0.36	0.33	0.36	0.35
14											
15	114	171	169	1	114	171	169	0.05	0.07	0.07	0.06
	TOTALS				244440	246227	245141	99.98	99.99	99.98	99.98

TABLE VII

PRECISION OF ANALYSIS OF GUM TURPENTINE

Peak No.	AREA %				DEVIATIONS			
	1	2	3	Average	1	2	3	Average
1	0.04	0.06	0.05	0.05	0.01	0.01	0.00	0.01
2	60.22	58.44	59.11	59.29	0.93	0.85	0.18	0.65
3	1.09	0.91	0.75	0.91	0.18	0.00	0.16	0.11
4	31.55	33.24	33.36	32.68	1.13	0.56	0.68	0.79
5	1.66	1.70	1.61	1.66	0.00	0.04	0.05	0.03
6	2.32	2.45	2.39	2.39	0.07	0.06	0.00	0.04
7	0.69	0.77	0.60	0.69	0.00	0.08	0.09	0.06
8	0.08	0.01	0.01	0.04	0.04	0.03	0.03	0.01
9	0.07	0.03	0.03	0.03	0.04	0.00	0.00	0.01
10	0.10	0.04	0.03	0.06	0.04	0.02	0.03	0.03
11	0.14	0.04	0.05	0.08	0.06	0.04	0.03	0.04
12	0.07	0.02	0.03	0.04	0.03	0.02	0.01	0.02
13	0.12	0.14	0.07	0.11	0.01	0.03	0.04	0.03
14	0.16	0.19	0.11	0.15	0.01	0.04	0.04	0.03
15	0.26	0.25	0.24	0.25	0.01	0.00	0.01	0.01
16	1.43	1.72	1.56	1.59	0.14	0.15	0.00	0.10

TABLE VIII

PRECISION OF ANALYSIS OF SDW TURPENTINE

					DEVIATIONS			
	#2	#1	#3	Average	#2	#1	#3	Average
1	0.61	0.62	0.60	0.61	0	.01	.01	.01
2	88.70	88.76	88.75	.04	.04	.05	.01	.03
3	7.24	7.21	7.14	7.20	.04	.01	.06	.04
4	2.01	2.04	1.99	2.01	0	.03	.02	.02
5	0.48	0.51	0.49	0.49	.01	.02	0	.01
6	0.24	0.26	0.34	0.28	.04	.02	.06	.04
7	0.06	0.06	0.05	0.06	0	0	.01	0
8								
9	----	----	----	----				
10	0.12	0.13	0.15	0.13	.01	0	.02	.01
11								
12	0.04	0.04	0.03	0.04	0	0	.01	0
13	0.33	0.36	0.36	0.35	.02	.01	.01	.01
14								
15	0.07 / 99.99	0.05 / 99.98	0.07 / 99.98	0.06 / 99.98	.01	.02	.02	.02

FIGURE 35

Analysis of Sample No. 3875

Peak No.	Compound	Area %
1	low boiling	1.19
2	α Pinene	80.83
3	Camphene	11.50
4	β Pinene	1.77
5	Unknown	1.16
6	Dipentene	1.81
7	Terpinolene	1.29
8	Total Other	0.96
		100.01

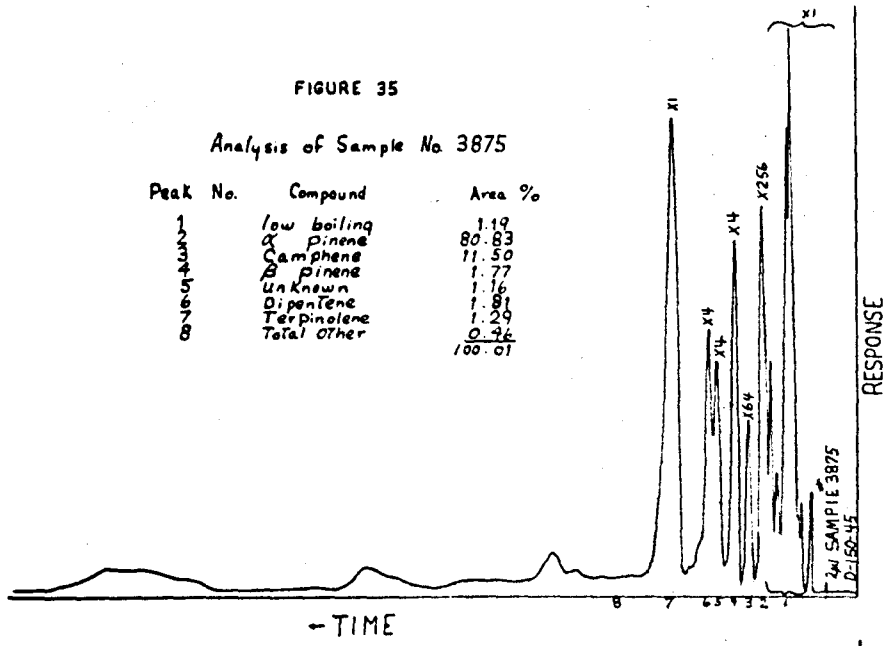


Figure 39

Quantitative Analysis

T&R Gum Turpentine

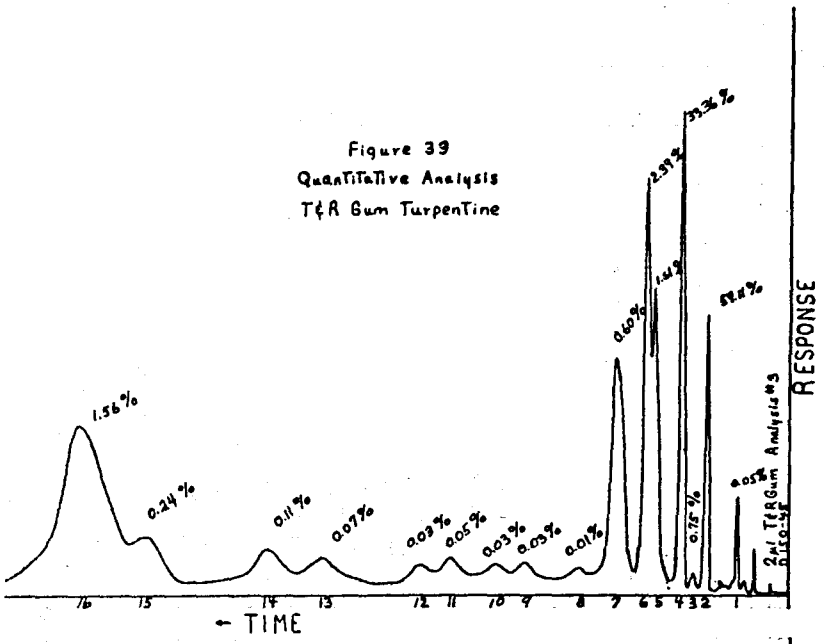


FIGURE 36

Analysis of Sample No. 3876

Peak No.	Compound	Area %
2	α Pinene	59.20
3	Camphene	8.63
4	β Pinene	32.69
5	Dipentene	1.55
6	Cineole	2.86
7	Terpinolene	0.81
	Total Other	2.26
		100.00

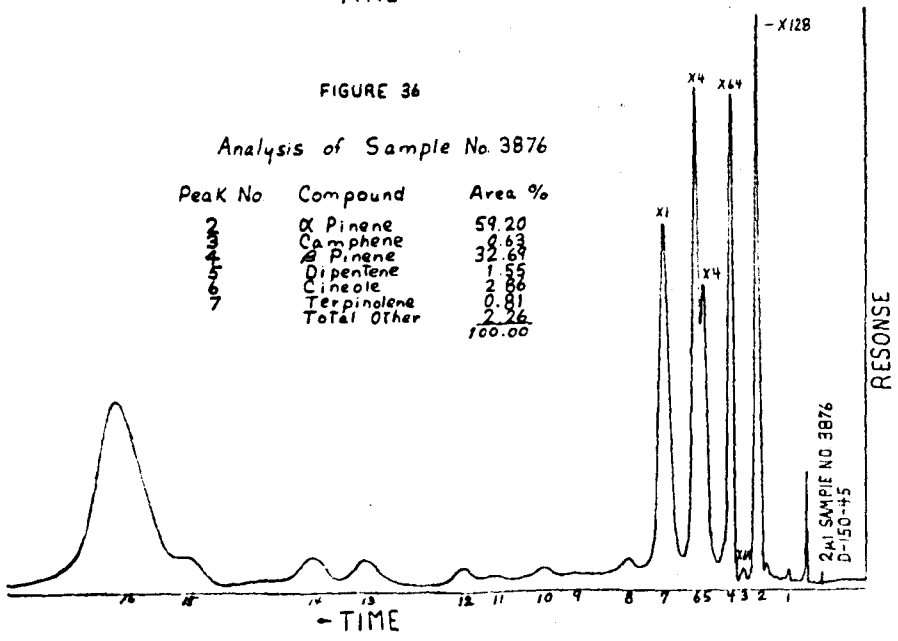


Figure 34

Quantitative Analysis

Crosby Steam Distilled Wood Turpentine

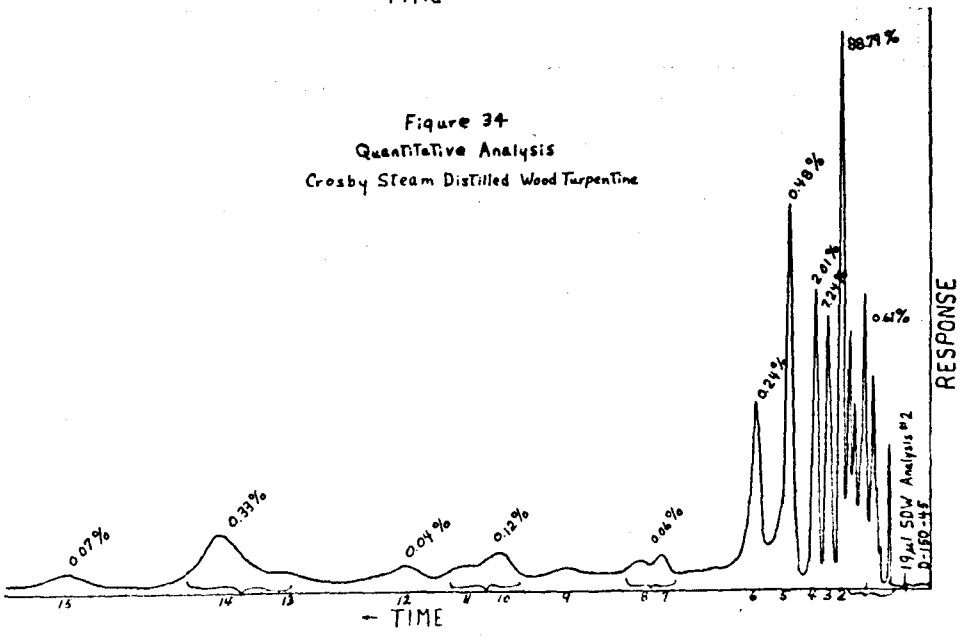


FIGURE 39

Analysis of Sample No. 3879

Peak No.	Compound	Area %
1	low boiling	1.29
2	α Pinene	80.68
3	Camphene	7.47
4	β Pinene	2.73
5	Dipentene	1.47
6	Terpinolene	2.40
7	Furfural ?	0.12
8	Mixed	0.24
9	α Terpineol	0.30
		100.00

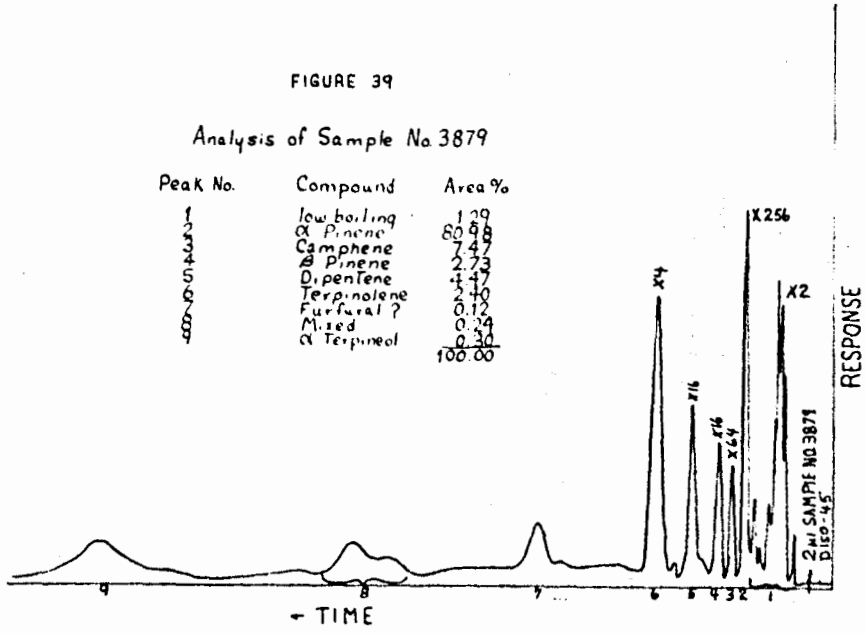


FIGURE 37

Analysis of Sample No. 3877

Peak No.	Compound	Area %
1	low boiling	0.03
2	α Pinene	57.85
3	Camphene	0.91
4	β Pinene	31.50
5	Dipentene	1.62
6	Cineole	3.83
7	Terpinolene	1.29
	Total Other	2.97
		100.00

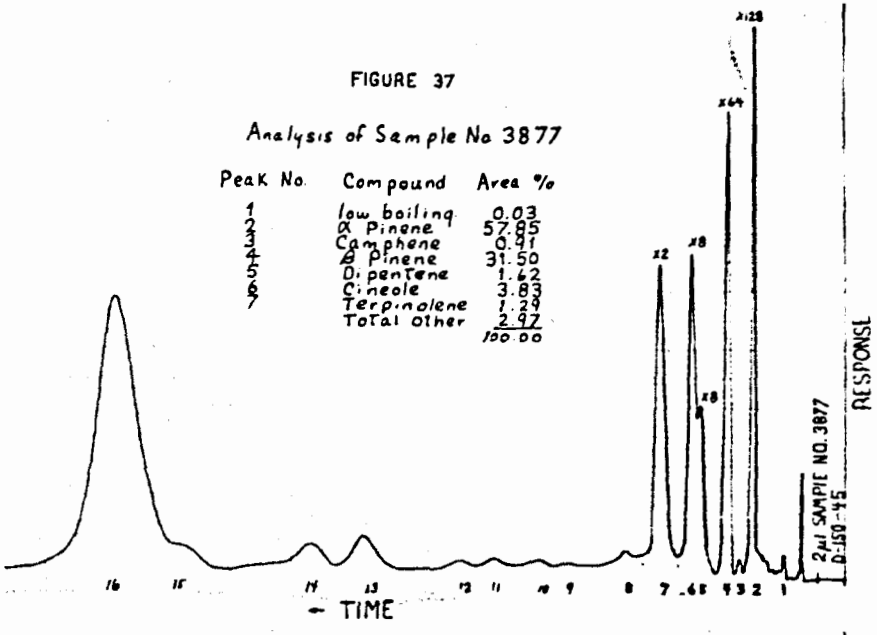


FIGURE 40

Analysis of Sample No. 3880

Peak No.	Compound	Area %
1	low boiling	0.71
2	α Pinene	69.98
3	Camphene	3.86
4	β Pinene	17.93
5	Dipentene	3.00
6	Cineole	1.46
7	Terpinolene	1.63
	Total Other	1.42
		99.99

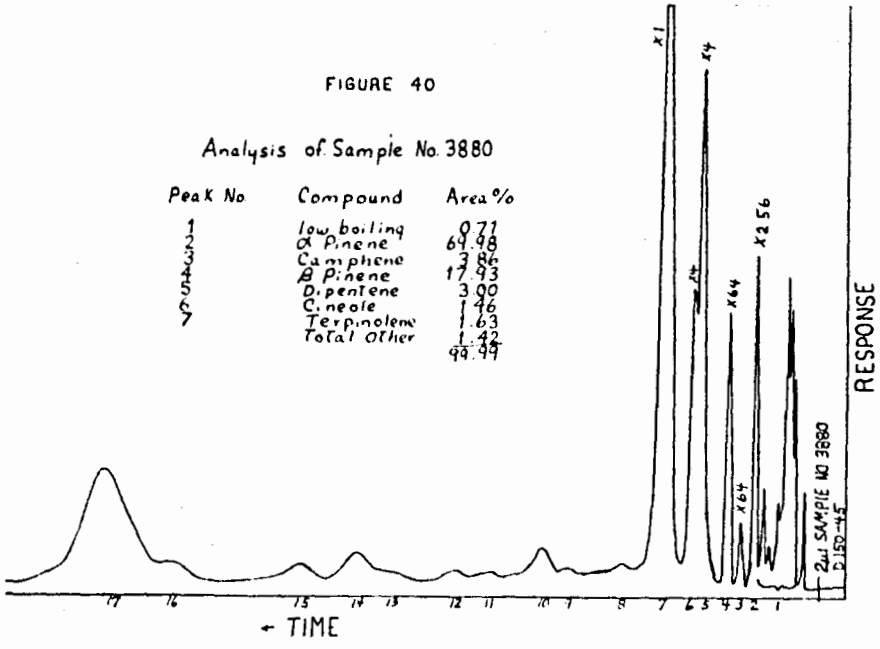
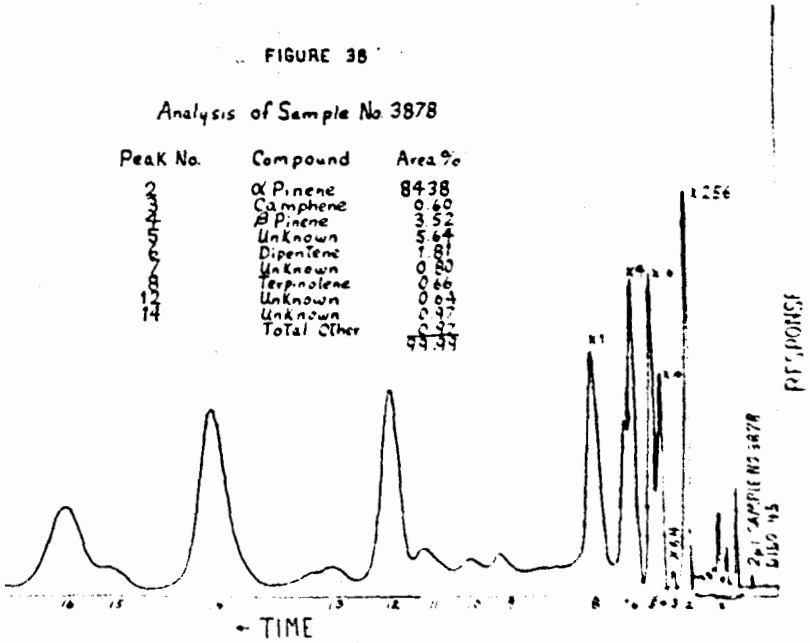


FIGURE 38

Analysis of Sample No. 3878

Peak No.	Compound	Area %
2	α Pinene	84.38
3	Camphene	0.60
4	β Pinene	3.52
5	Unknown	5.64
6	Dipentene	1.81
8	Unknown	0.80
10	Terpinolene	0.66
12	Unknown	0.04
14	Unknown	0.47
	Total Other	2.08
		99.99



the equipment. The attenuation must be changed several times during each analysis, the number of counts taken, the peaks and integrator tape marked, and the integrator zeroed. At the start of the analysis, when peaks are emerging in rapid succession, the operator must move from one function to another swiftly. This task, once mastered, is not difficult, but may require several trials before it is learned. The precision is also improved by treating two incompletely separated peaks as one peak, as was done for the analysis of SDW.

Six turpentine samples were prepared by the Paint Laboratory of Department of Agriculture, Commonwealth of Virginia. These samples were in one-half pint bottles marked only with an identifying number. It was known that these samples were of commercial turpentines and could be gum or steam distilled wood turpentine or a mixture of the two.

The chromatograms and analysis data for these samples are shown in Figures thirty-five through forty. The following evaluations were made on the basis of what has been published on the composition of turpentine, the over all "fingerprint" of the sample, identification of peaks by retention times, and the quantitative data obtained.

Sample # 3875 - One may easily note the similarity of this sample to the known steam distilled wood turpentine (Figure twenty-two). The percentage of alpha pinene is that reported for steam distilled wood turpentine, and the absence of a large peak for

beta pinene further indicates that this sample could not be gum turpentine. The amount of low boiling material in this sample is larger than that present in the known sample. Comparison of these low-boiling peaks with a chromatogram of naptha indicates that the turpentine was most likely produced by the solvent process, in which the woody material is chipped and then extracted with a petroleum hydrocarbon solvent before distillation. The percentage here is too low to be deliberate adulteration since this would hardly be commercially feasible.

Sample # 3876 can readily be identified as gum turpentine. Its marked resemblance to the known gum turpentine lead to the conclusion that it might possibly be the same brand. Comparison of the values for alpha and beta pinene with the known analysis is quite remarkable.

Sample # 3877 is also gum turpentine. Note that peaks five, six and seven are at an attenuation twice that of the known sample and also the previous sample.

Sample # 3878 appears to be steam distilled wood turpentine at first glance, but closer examination reveals several inconsistencies. Peak four is rather large, but within reason. However, peak five in this chromatogram does not occur in any other chromatogram. Its identity is not known. Peaks twelve and fourteen are larger than might be considered "normal" on the basis of limited experience. This might be attributed to the fact that the sample may not have been fractionated quite as closely as others which

have been run. With these qualifications the sample appears to be steam distilled wood turpentine.

Sample # 3879 is quickly identified as steam distilled wood turpentine. With minor differences it is similar to sample # 3875. Note that the hydrocarbon peaks are more numerous and are run at an attenuation of two in this case. These peaks are attributed to naptha.

Sample # 3880 would appear to be a mixture as indicated by the values of 69.95% for alpha pinene and 18.01% for beta pinene. The high boiling peaks shown by gum turpentine are present, but reduced in size. The amount of low boiling hydrocarbons is large. It was known that if this were a mixture it was made up of the turpentine samples analyzed here. With this knowledge the chromatograms were again examined, and as may be seen, the sample is a mixture of samples 3877 and 3879. This is shown by the size of peak seven in the chromatogram of sample # 3877 and by the size of the hydrocarbon peaks in sample # 3879. Note that both these peaks are at an attenuation of two in the chromatograms of the original samples. This conclusion concerning the mixture may be verified by the examination of other peaks. The arrangement of collective peak one and peak seven offered the opportunity to calculate the percentages of the two turpentines in the mixture. This was done and the values were 53.03% wood turpentine on the basis of peak one, and 52.61% on the basis of peak seven. When the information on the actual composition of this sample was made available it was found

that the sample had been rather roughly mixed 50-50 in a graduate cylinder.

All the identifications in the above report were correct according to the claims made on the original containers of the samples except in the case of sample # 3878. This sample was claimed to be pure spirits of gum turpentine. The claim would be considered untrue promptly, if it were not for the fact that it came from a western location (El Paso, Texas). Although it was not known that any turpentine was being produced in that area, it does not rule out the possibility that this may in fact be gum turpentine from western varieties such as the bristlecone pine (Pinus aristata), the Coulter pine (Pinus coulteri), the Pinyon, (Pinus edulis), the Limber pine (Pinus flexilis), the Torrey and Fremont pine (Pinus monophylla), and the Ponderosa pine (Pinus ponderosa). The turpentine content, physico-chemical properties, and chemical composition of these pines may be found in Guenther, The Essential Oils, vol. VI. It is almost certainly a mixture, and not chiefly any one variety, based on the information in the above reference. This sample may also have been imported from Mexico. The container was not stamped with a code, and identification of the source by the manufacturer might be difficult.

SUMMARY

Three liquid substrates and three solid supports were investigated for the separation of the components of turpentine. A combination of twenty per cent Carbowax 20M on C-22 Firebrick was found to give satisfactory separation at a column temperature of one hundred and fifty degrees centigrade and a helium flow rate of forty-five milliliters a minute.

The retention times of thirty-one terpenes and related materials were determined and these retention times used for the identification of the constituents of a sample of gum turpentine and a sample of steam distilled wood turpentine. Trapping and infra-red analysis were also used to a limited extent for qualitative analysis. Chemical reaction of turpentine with maleic anhydride and sodium bisulfite was used to gain additional information on the structure of several components.

An automatic integrator was used for quantitative analysis of the separated peaks. The precision was found to be good using this instrument, and the values obtained compared well with analyses reported using distillation and infra-red methods.

Identification and analysis of several commercial turpentines indicated that the method could be used for routine analysis.

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