The Catalytic Effect of the Support on Terpene Compounds in Gas-Liquid Chromatography

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At the present time, diatomaceous earth or its manufactures are in general use as the solid support of a packed column in gas-liquid chromatography because these materials are porous and, consequently, have a large surface area. In the gas chromatography of terpene compounds using these solid supports, unfortunately, erroneous results due to the catalytic action of the support have occasionally been observed. Naves,1) for instance, has observed dehydration, condensation and other reactions in the gas chromatography of essential oils by the use of diatomaceous earth or firebrick. Cartoni2) has observed the decomposition of oxygen-containing terpene compounds in gas chromatography using Sterchamol. Using diatomaceous earth, Celite 545, Chromosorb or C-22 firebrick, Vilkas,3) Zubyk,4) Lukes5) and the present authors6) have reported the isomerisation of α-pinene into such compounds as camphene, dipentene and p-cymene, and also of β-pinene into such compounds as α-pinene, camphene, dipentene and p-cymene. Besides, the liquid phase on diatomaceous earth or fireblick is also rapidly decomposed,7) as has been shown in Polyethylene glycol-1500 impregnated on Shimalite.8) Also, the authors6) have done preliminary research on the catalytic action of several solid materials (commercial supports, rock crystal, glass, etc.), using nearly fifty kinds of monoterpene compounds (hydrocarbons 16, alcohols 11, esters 13, carbonyl compounds 8, etc.); they have found a remarkable catalytic decomposition of terpene compounds by commercial solid supports.

Thus, in order to get a correct gas chromatogram it will be necessary to find a support possessing no catalytic action or a weak one.

In the present investigation, the catalytic action of several materials, such as glass, rock crystal, quartzite, sea sand and unglazed porcelain, and that of several commercial solid supports, such as Shimalite,*1 Diasolid-M,*2 Microsorb-W, -S, -SS and -F,*3 towards six monoterpene compounds and five sesquiterpene alcohols, which were chosen as representative compounds, will be examined in detail.

Experimental

Apparatus and Procedure. — A Shimadzu Gas Chromatograph GC-2 furnished with a katharometer-type detector was used. A coiled copper tube (3 m. long, 4 mm. inner diameter) packed with the

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1) Y.-R. Naves, *J. Soc. Cosmetic Chemists*, **9**, 101 (1958).

2) G. P. Cartoni and A. Liberti, *J. Chromatog.*, **3**, 121 (1960).

3) M. Vilkas and N. A. Abraham, *Bull. soc. chim. France*, **1959**, 1651.

4) W. J. Zubyk and A. Z. Connor, *Anal. Chem.*, **32**, 912 (1960).

5) V. Lukes, R. Komers and V. Herout, *J. Chromatog.*, **3**, 303 (1960).

6) T. Matsuura, T. Aratani, H. Komae and S. Hayashi, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **64**, 795 (1960).

7) A. Kele, R. Bate, B. Costa and P. Forman, *J. Chromatog.*, **8**, 157 (1962).

8) S. Akiyoshi, T. Matsuda and K. Akune, *Japan Analyst* (*Bunseki Kagaku*), **10**, 960 (1961).

[*1] Commercial solid support put on sale by Shimadzu Seisakusho Ltd., Kyoto, Japan.

[*2] Commercial solid support by the Nippon Kuromato Kôgyô Co., Ltd., Tokyo, Japan.

[*3] Commercial solid support by the Tôsin Gôsei Kôgyô Co., Ltd., Tokyo, Japan.

bare material (28—48 mesh) to be examined was connected between a sample injector and a separation column (Fig. 1). The operating temperature and the flow rate of the helium carrier were set as follows: 140°C and 60 ml./min. for the monoterpene compound, 200°C and 30 ml./min. for the sesquiterpene alcohol.

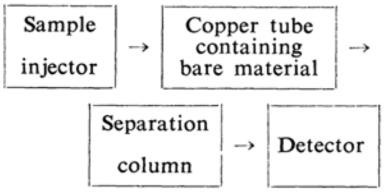

Fig. 1. Scheme of apparatus.

If an injected terpene compound suffers isomerization and/or decomposition by the bare material, its isomerization and/or decomposition products are separated by the next separation column and recorded on a chromatogram. Accordingly, by a comparison of this chromatogram with the chromatogram obtained from a blank test performed without the connection of a column packed with the bare material, one can get information about the catalytic activity and the adsorptivity of this bare material.

Crushed rock crystals (150—200 mesh) coated with 5% Thermol-1[*4] were employed in the separation column throughout this research. The sample size to be injected at one run was in the range from 0.5 to 1.5 μl.; the value was varied according to the extent of the isomerization and/or the decomposition of the sample. The electric current of the detector cell and the sensitivity of the recorder were set at 200 mamp. and 2 mV. respectively.

The Preparation of the Solid Materials to be Examined. — For the preparation of the solid materials (supports) to be examined, the following substances were used: soda-lime glass tubing, scraps of rock crystal from which the quartz oscillator has been cut out, quartzite (SiO_2 99.88%, snow-white color) dug out in Yamanashi Prefecture, Japan, and unglazed porcelain tubing. When these substances were crushed separately in a stainless-steel mortar, 28—48 mesh particles were collected. To these particles ten portions (in volume) of aqua regia were added, and the mixture was boiled for two hours in order to dissolve any metallic impurities that entered during the crushing process. The cleaned particles were washed with water, dried, and heated at 300°C for two hours.

Sea sand (SiO_2 99.02%, Al_2O_3 0.52%, Fe_2O_3 0.32%, trace of CaO, light brown beads), collected in Kam-ranh Bay, Viet Nam, was treated in the same way as above.

All the commercial solid supports examined are manufactured from diatomaceous earth. Of these Shimalite belongs to a usual type, Diasolid is a support which has been treated at a high temperature in order to diminish the adsorptivity for oxygen-containing compounds, and Microsorb is a firebrick precoated with Polyflon,[*5] presumably in order to diminish its adsorptivity and its catalytic activity.

The Preparation of Terpene Compounds. — Six monoterpene compounds, that is, α-pinene, limonene, linalool, α-terpineol, linalyl acetate, and geranyl acetate, were chosen from the standpoint of their sensitivity to the catalytic action. Five sesquiterpene alcohols, that is, α- and β-santalol, α-cadinol, patchouli alcohol and elemol, were adopted because they were easily available in our laboratory.

α-Pinene (b. p. 156°C, d_4^{25} 0.8544, n_D^{25} 1.4638, $[\alpha]_D$ $-39.51°$) was isolated from a commercial turpentine oil, while limonene (b. p. 175.5°C, d_4^{25} 0.8397, n_D^{25} 1.4711, $[\alpha]_D$ $+117.6°$) was isolated from an essential oil of Citrus Natsudaidai Hayata. These two compounds were then each purified by repeated fractional distillation. The other monoterpene samples were commercial materials supplied by the Abrack Co., Ltd. and were used without further purification.

α-Santalol and β-santalol were isolated from an essential oil of the sandal-wood tree through their phthalic ester; each was purified by repeated fractional distillation. The infrared absorption spectrum of α-santalol (b. p. 160°C (10 mmHg), d_4^{25} 0.9722, n_D^{25} 1.4998) obtained here coincided with that reported by Herout[10] and Chakravarti[11]. β-Santalol (b. p. 164°C (10 mmHg), d_4^{25} 0.9703, n_D^{25} 1.5063) was recognized to be contaminated with a small amount of α-santalol by a comparison of its infrared spectrum here with that obtained by Chakravarti.[11]

α-Cadinol (b. p. 147°C (8 mmHg), d_4^{25} 0.9375, n_D^{25} 1.4960, $[\alpha]_D$ -23.63) was isolated from an essential oil of the hinoki tree,[12] by preparing its sodium alcoholate, and then fractionated. The infrared spectrum of this sample coincided with that reported by Motl et al.[13]

Patchouli alcohol was separated in the crystalline form by cooling commercial Patchouli oil in a refrigerator; it was then recrystallized three times from *n*-hexane. This preparation melted at 59—60°C, a slightly higher point than that shown in Büchi's reported (55—56°C).[14]

Elemol was isolated from Citronella oil by fractional distillation; it was then purified by elution chromatography using silica gel and *n*-hexane. This sample melted at 48—49°C, a value which coincided with that reported by Halsall,[15] and its phenyl urethane melted at 111—112°C, a value which

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[*4] Commercial stationary liquid put on sale by Shimadzu Seisakusho, Ltd. Thermol-1 has been confirmed by the present authors to be excellent for the separation of terpene alcohols.[9]

9) T. Matsuura, T. Aratani, H. Komae and S. Hayashi, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **64**, 799 (1961).

[*5] Tetrafluoroethylene polymer (Teflon) produced at the Osaka Kinzoku Co., Ltd., Osaka, Japan.

10) V. Herout, V. Jarolim and J. Pliva, *Collection Czech. Chem. Commun.*, **22**, 773 (1957).

11) K. K. Chakravarti, *Perfumery Essent. Oil Record*, **46**, 330 (1955).

12) S. Hayashi, K. Yano and T. Matsuura, This Bulletin, **37**, 474 (1964).

13) O. Motl, V. Sýkora, V. Herout and F. Šorm, *Collection Czech. Chem. Commun.*, **23**, 1297 (1958).

14) G. Büchi, R. E. Erickson and N. Wakabayashi, *J. Am. Chem. Soc.*, **83**, 927 (1961); **84**, 3205 (1962).

15) T. G. Halsall, D. W. Theobald and K. B. Walshaw, *J. Chem. Soc.*, **1964**, 1029.

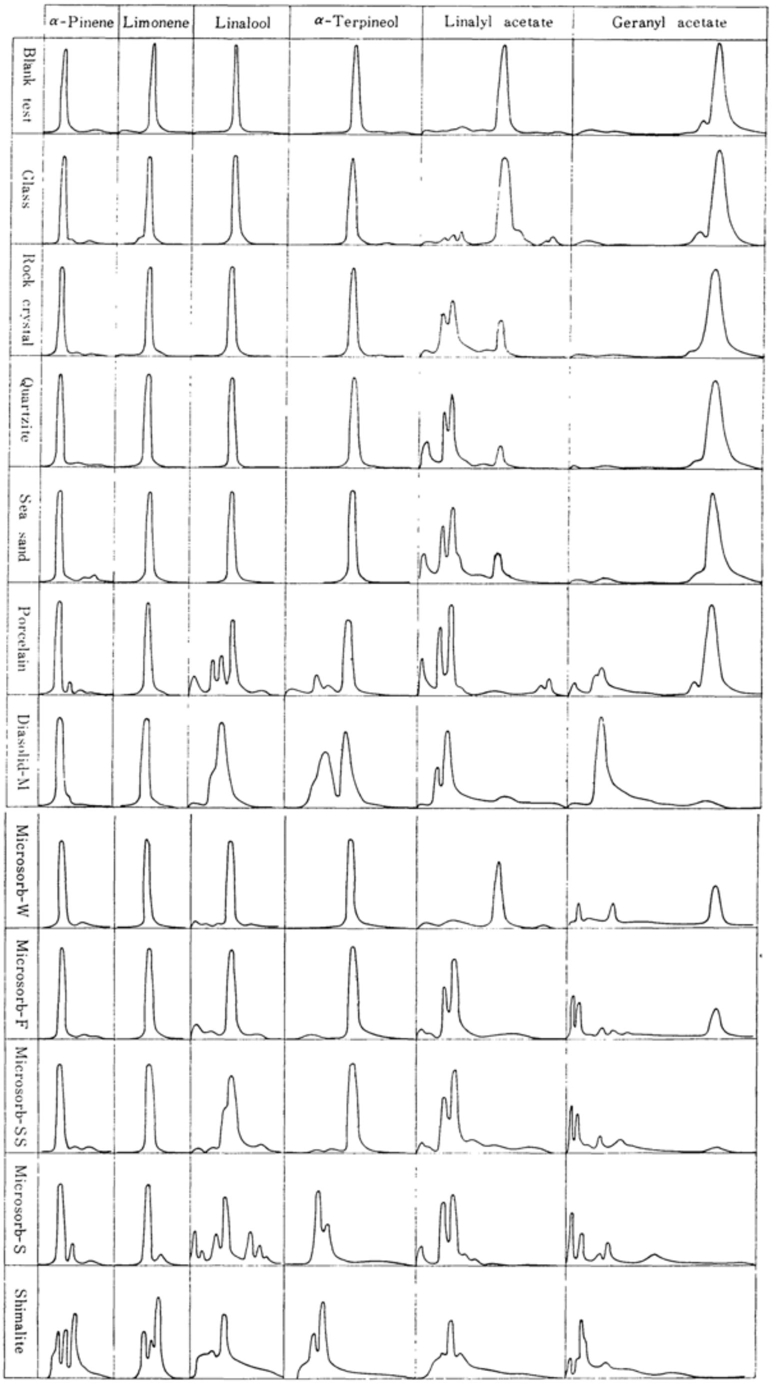

Fig. 2. Catalytic effect of bare supporting material toward monoterpene compound.
Column packed with bare material: 4 mm. × 3 m.
Separation column (Thermol-1 on crushed rock crystal): 4 mm. × 3 m.
Operating temperature: 140°C
Flow rate of helium: 60 ml./min.

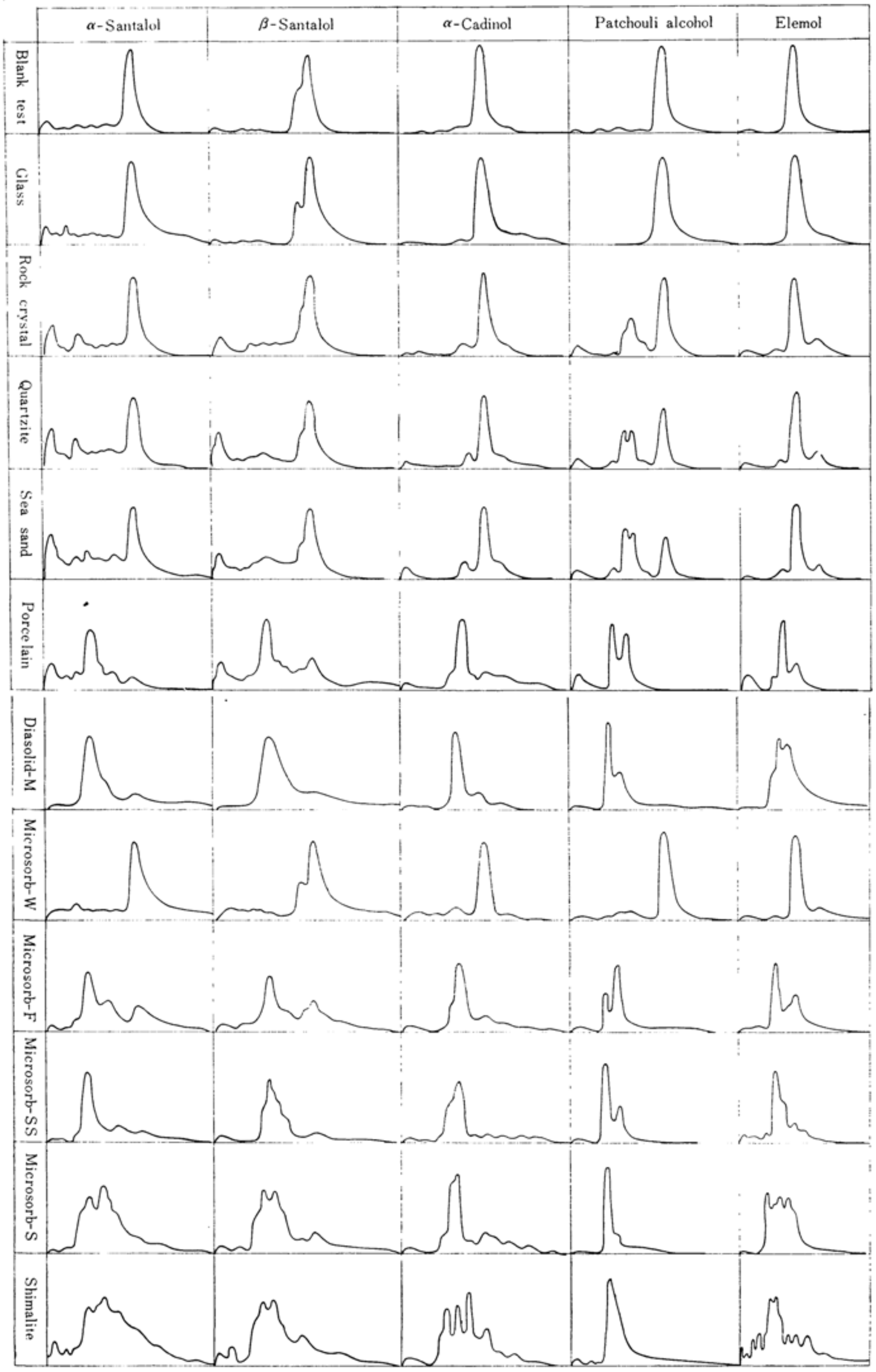

Fig. 3. Catalytic effect of bare supporting material toward sesquiterpene alcohol.
Column packed with bare material: 4 mm.×3 m.
Separation column: 4 mm.×3 m.
Operating temperature: 200°C
Flow rate of helium: 30 ml./min.

agreed with the melting point reported in the literature.[16)]

Results and Discussion

Gas chromatograms of monoterpene compounds and sesquiterpene alcohols, which were obtained by an apparatus furnished with a column containing the bare material to be examined, are shown in Figs. 2 and 3. Each run was carried under the same operating conditions. However, the same component did not show an identical retention time in each chromatogram on account of the difference in adsorptivity, depending on the kind of bare material. Every chromatogram shown in Figs. 2 and 3 was corrected, for the sake of convenience, by multiplying a certain factor, one which was special for each material, since the peak of the same component could be seen at an identical position in each gas chromatogram.

In these figures, the top lines represent the results of the blank test, that is, gas chromatograms of terpene compounds obtained without a column packed with the examined bare material. Therefore, if gas chromatograms regarding a certain examined material are compared with those of the corresponding blank test, information about the catalytic activity and the adsorptivity of this material can be obtained.

Glass. — As will be seen in Figs. 2 and 3, chromatograms obtained using a connection of a column packed with glass are simpler than chromatograms of the cases employing other bare materials. Besides, the chromatograms where glass was used closely resemble those of the blank test except for the cases of linalyl acetate and of α-santalol. In the chromatograms of linalyl acetate and α-santalol, several small peaks appear in addition to the main peak; these small peaks are probably due to the catalytic action of glass.

From these observations, the catalytic activity of glass seems to be the feeblest among the examined materials.

Rock Crystal.—When a rock crystal-packed column was used, each chromatogram of a monoterpene compound is almost identical with that obtained in the case of glass or the blank test except for the case of linalyl acetate; chromatograms of linalyl acetate and of sesquiterpene alcohols are compared with those in the case of glass. In the chromatogram of linalyl acetate, the peaks, which are small in shape in the case of glass, appear large. In the case of patchouli alcohol, new moderate-size peaks appear besides the main peak. These newly-formed or newly-enlarged peaks are perhaps due to the catalytic action of the bare material.

Therefore, the catalytic activity of rock crystal is slightly stronger than that of glass.

The Adsorpitivity of Glass Support. — There have been several papers[17)] which have recommended glass beads as a solid support for impregnation with a liquid phase in a low concentration. The present authors[9)] and Takayama[18)] have, however, pointed out that glass is not suitable as a solid support, because the oxygen-containing compound has shown a broad peak and a remarkable tailing phenomenon when glass has been employed as a solid support, Takeuchi,[19)] who has introduced the "tailing coefficient" as a measure to indicate quantitatively the degree of peak tailing, recently compared several solid supports by the tailing coefficient as calculated from the peak of butyl alcohol. According to his results, the tailing coefficient of glass is not small.

The uncorrected retention time of the main peak of each chromatogram involving glass and rock crystal (cf. Figs. 2 and 3) is listed in Table I. In the case of the glass-packed column, the retention time of each compound is ca. 1.5 times as large as that in the case of the rock crystal-packed column, in which the retention time is practically the same as in the blank test.

The peak width measured at half-height is shown in Table II. The peak width of each of the hydrocarbons and the esters is nearly the same in both glass and rock crystal, whereas

TABLE I. COMPARISON OF RETENTION TIME (min.) OF TERPENE COMPOUNDS

Compound	Bare supporting material connected to the separation column		
	Blank test	Glass	Rock crystal
α-Pinene	2.4	3.5	2.4
Limonene	3.7	5.3	3.6
Linalool	4.1	6.7	4.3
α-Terpineol	6.7	11.1	6.8
Linalyl acetate	8.2	14.0	8.5
Geranyl acetate	14.9	25.1	14.0
α-Santalol	9.4	15.2	9.8
β-Santalol	10.6	17.1	11.0
α-Cadinol	8.8	13.8	8.6
Patchouli alcohol	10.2	15.7	10.2
Elemol	6.6	10.1	6.4

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16) V. Sýkora, V. Herout, J. Plíva and F. Šorm, *Collection Czech. Chem. Commun.*, **19**, 124 (1954).

17) C. Hishta, J. P. Messerly and R. F. Reschke, *Anal. Chem.*, **32**, 1730 (1960); P. R. Datta and H. Susi, ibid., **34**, 1028 (1962); D. H. Frederick, B. T. Miranda and W. D. Cooke, ibid., **34**, 1521 (1962); J. W. Ashley and C. N. Reilley ibid., **34**, 1537 (1962).

18) Y. Takayama, *J. Japan Chem.* (*Kagaku no Ryoiki*), **15**, 369 (1961).

19) T. Takeuchi and T. Setsuda, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **66**, 1648 (1963).

TABLE II. COMPARISON OF PEAK WIDTH (min.) MEASURED AT ONE HALF OF PEAK HEIGHT

Compound	Bare supporting material connected to the separation column		
	Blank test	Glass	Rock crystal
α-Pinene	3.4	4.2	3.5
Limonene	3.6	4.8	3.8
Linalool	4.7	5.6	4.6
α-Terpineol	5.4	7.8	5.7
Linalyl acetate	6.6	8.7	6.8
Geranyl acetate	10.4	14.6	10.4
α-Santalol	13.0	21.0	13.0
β-Santalol	20.0	25.0	20.1
α-Cadinol	13.0	19.7	12.8
Patchouli alcohol	9.0	11.6	9.0
Elemol	7.5	8.8	7.3

the peak width of each alcohol is very much larger in glass than in rock crystal. Generally, the peak width in rock crystal is the same as in the blank test.

Such prolongation of the retention time and broadening of the peak width observed in the case of glass are probably due to the adsorption of the oxygen-containing compound on the surface of the glass used as the bare material. In fact, when the bare glass-packed column was set between the injector and the separation column, a considerable tailing phenomenon was observed in the chromatography of oxygen-containing terpene compounds, as compared with the results in the blank test.

Glass showed practically no catalytic action on terpene compounds, but it did show the adsorption or the tailing phenomenon to some extent on oxygen-containing terpene compounds. A further examination of the glass support is being attempted in our laboratory.

Quartzite and Sea Sand.—The chromatograms obtained by using quartzite and those in the case of sea sand are almost identical. Besides, the chromatograms in both these cases closely resemble the chromatograms obtained using rock crystal. Therefore, of rock crystal, quartzite and sea sand, it may be said that there is little difference in catalytic activity.

Unglazed Porcelain.—All the chromatograms in the case of unglazed porcelain are remarkably complicated compared with those of glass, rock crystal, quartzite and sea sand. Especially, linalyl acetate, α-santalol, α-cadinol and patchouli alcohol do not show the peak corresponding to the original sample in their chromatograms. On the basis of such results, it is obvious that all monoterpene and sesquiterpene compounds suffered an intense action by unglazed porcelain. That is, the catalytic activity of this material is certainly very strong, in spite of the recommendation of Lukes[5] that it has a weak activity.

Commercial Solid Supports.—Three groups of commercial solid supports, Shimalite, Diasolid and Microsorb, show chromatograms with characteristic features.

The chromatograms obtained with the Shimalite-packed column are the most complicated; they consist of many peaks projected on a large mountain. In these chromatograms, however, the peak corresponding to the original sample is not observed except in the chromatograms of α-pinene and limonene.

On the basis of such complex chromatograms, it may be assumed that all the terpene compounds examined suffered greatly from various catalytic actions, such as dehydration, dehydrogenation, decarboxylation, isomerization, and the cleavage of a carbon-carbon bond.

On the other hand, the chromatograms obtained by using the Diasolid-packed column show simple features, with only a few peaks, although the catalytic action of Diasolid is obviously vigorous, because the peak corresponding to the original sample is not observed in the chromatograms obtained using this material, except in the case of α-pinene and limonene. Although Diasolid has been subjected to the high temperature-treatment to diminish the catalytic activity of diatomaceous earth, it is not yet satisfactory for the gas chromatography of terpene compounds.

Regarding Microsorb, four manufactures of different grades of activity, W, F, SS and S, were examined. These materials commonly show less-tailling chromatograms than the case with Shimalite or Diasolid. Of these, Microsorb-W gives the simplest chromatograms in shape, that is, the number of peaks is the least; largely the peak corresponding to the original sample is left in these chromatograms. Therefore, the catalytic action of Microsorb-W is assumed to be the weakest among the commercial solid supports examined, and to be nearly the same as that of glass on the basis of a comparison of the two kinds of chromatograms.

The Gas Chromatography of Terpene Compounds Using Microsorb-W. — Microsorb-W, which showed the feeblest catalytic action among the commercial solid supports examined, was used for the gas chromatography of terpene compounds.

This support, impregnated with 7% Thermol-1, was packed into a column (4 mm. in internal diameter, 3 m. long), and the conditioning of the column was carried out at 140°C for five hours. Two chromatograms of the mixture which was prepared from ten oxygen-containing compounds are shown in Figs. 4A and 4B.

4A is a chromatogram which has been obtained immediately after the conditioning, while 4B is a chromatogram which has been obtained after 40 hr. In A, there is a large, gently sloping hill; it has been confirmed to be attributable to a trace of water contained in the terpene mixture. On the other hand, no such hill is to be seen in B. Besides, in B the peak resolution is very good, and the retention times of the peaks are also remarkably shortened in comparison with the corresponding peaks in A.

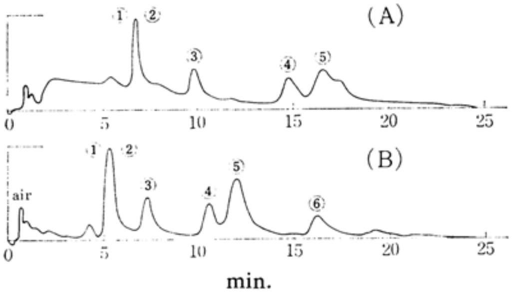

Fig. 4. Gas chromatography of terpene compounds using Microsorb-W.
A: After conditioning at 140°C for 5 hr.
B: After the use of 40 hr.
Stational liquid: Thermol-1 (7%)
Column temp.: 160°C
Flow rate of helium: 40 ml./min.
(1) Ethyl valerianate (2) Benzyl alcohol
(3) Linalool (4) Camphor
(5) Menthol (6) α-Terpineol
(7) Pulegone (8) Hydroxycitronellal
(9) α-Terpinyl acetate (10) α-Pinene

The difference observed between the two chromatograms, of A and of B, is assumed to result from an increase in the surface area of the stationary phase and from a decrease in its thickness, as a result of the evaporation and/or the decomposition of the precoating material. In such a support as Microsorb-W, the activity centers will gradually be exposed from the precoating material. Thus, this support appears unsatisfactory for the gas chromatography of terpene compounds.

The Catalytic Action of the Stationary Phase Prepared from Diasolid-M.—The controlling of the catalytic activity on Microsorb by precoating with a high polymer has been shown in the above section. Geranyl acetate, even when Shimalite is used, has been gas-chromatographed without decomposition by the impregnation of

TABLE III. CATALYTIC ACTION OF STATIONARY PHASE PREPARED FROM DIASOLID-M

Compound	Stationary liquid			
	Termol-1 (20%)	Polycarbonate (5%) + PEG-6000 (5%)	PEG-6000 (5%)	Result in Figs. 2 and 3
		Monoterpene		
Column temp.	140°C	140°C	140°C	140°C
Citronellal	○	+	+	−−
Hydroxycitronellal	○	○	+	−−
Nerol	+	++	++	−−
Geraniol	+	++	+	−−
Linalool	+	+	+++	+++
Citronellol	+	+	++	−−
Phenyl ethyl alcohol	○	+	+	−−
α-Terpineol	○	○	○	++
Geranyl acetate	+	+	+	++++
Linalyl acetate	++	++	++	++++
		Sesquiterpene		
Column temp.	180°C	160°C	160°C	200°C
α-Santalol	++	+++	+++	++++
β-Santalol	+	+	++	++++
α-Cadinol	○(?)	+++	+++	++++
Elemol	○	○	○	++
Patchouli alcohol	+(?)	+(?)	+	++++

○: No decomposition nor isomerization
+: Slight decomposition
++: D is roughly equal to U, where D is total area depending upon decomposition components, and U is peak area of unchanged original sample.
+++: D is larger than U.
++++: Complete decomposition with no peak of original sample.

a large quantity of Polyethylene glycol-1500.[20)]

In order to clarify the extent of the controlling, several monoterpene compounds were separatly gas-chromatographed using three kinds of stationary phases prepared from Diasolid-M, that is, by the impregnation of 5% Polyethylene glycol-6000 or 20% Thermol-1 into Diasolid-M, and by the impregnation of 5% Polyethylene glycol-6000 into Diasolid-M precoated with 5% polycarbonate.[*6] The results are listed in Table III.[*7] In the right-hand column of this table, the results regarding Diasolid-M, which has been illustrated in Figs. 2 and 3, are represented using the same symbols for convenience in comparison.

Judging from the results in Table III, it seems difficult to prepare the stationary phase, which is adequately suitable for the gas chromatography of every terpene compound, even by the impregnation of a large quantity of a stationary liquid on Diasolid-M.

Summary

The catalytic action of several solid supports, that is, glass, rock crystal, quartzite, sea sand, unglazed porcelain and commercial supports, have been examined. Glass shows practically no catalytic action in the examined terpene compounds, except for linalyl acetate and α-santalol, which were decomposed and/or isomerized slightly, but this support does show an adsorption of oxygen-containing compounds. The catalytic action of rock crystal is slightly greater than that of glass, but the adsorptivity of rock crystal is extremely small. Commercial diatomaceous earth supports show a remarkable catalytic action.

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20) G. Indo and H. Yukawa, *J. Japan Chem.* (*Kagaku no Ryoiki*), Addendum for Gas Chromatography, No. 3, Nankodo, Tokyo (1962), p. 105.

[*6] Presented by the Teijin Kasei Co., Ltd., Matsuyama, Japan.

[*7] This experiment was carried out using a flame ionization-type gas chromatograph, because Diasolid is manufactured to use when impregnated with a low concentration of a stationary liquid.