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2-METHYLISOBORNEOL, IMPROVED SYNTHESIS AND A QUANTITATIVE GAS CHROMATOGRAPHIC METHOD FOR TRACE CONCENTRATIONS PRODUCING ODOR IN WATER

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SUMMARY

Naturally occurring 2-methylisoborneol, responsible for objectionable odor in some domestic water supplies, has been shown to exist in the enantiomeric form related to *d*-camphor. The synthesis by the action of methyllithium or methyl-magnesium halides on *d*-camphor has been improved by the demonstration that the large amount of *d*-camphor in the crude product can be eliminated readily as the oxime. A method has been developed for the quantitation of 2-methylisoborneol in water down to the threshold-odor concentration of 0.1 μg/l. Camphor is added to the water as internal standard and the solution is extracted with 2% (v/v) of methylene chloride. The extract is concentrated to very small volume and analysed by gas chromatography using a flame ionization detector.

INTRODUCTION

2-Methylisoborneol (Fig. 1), a product of actinomycetes[1–6], is responsible for some of the earthy-musty odor found in public water supplies in the U.S.A.[2], The Netherlands[5], and Japan[4]. The odor is detectable down to 0.1 μg/l, but can be removed by addition of activated carbon to the water or the passage of the water through beds of activated carbon. Unfortunately, little is known concerning the efficient use of carbon in this way for the removal of 2-methylisoborneol or indeed of other trace substances. As part of our study of the problem of the removal of objectionable and harmful trace substances from public water supplies, we are making a detailed laboratory investigation into the adsorption of 2-methylisoborneol from water onto activated carbon. We intend to determine the influence on the adsorption of naturally occurring substances and any concurrent biological activity.

In this report we show that naturally occurring 2-methylisoborneol exists in the enantiomeric form related to that of *d*-camphor, and we describe an improved synthesis from *d*-camphor. Also, we describe the first method for the quantitation of 2-methylisoborneol in natural and domestic waters down to the threshold-odor concentration.
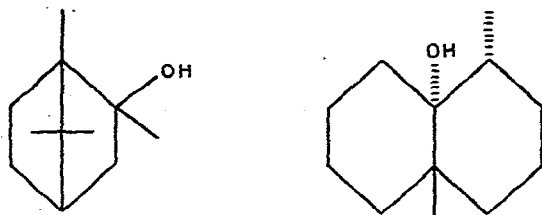
2 - methylisoborneol   geosmin

Fig. 1. Structures of bicyclic tertiary alcohols responsible for water odor.

EXPERIMENTAL

Reagents and solvents

d-Camphor, m.p. 178–180°, was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.), methyllithium, 1.7 M in diethyl ether, from Ventron (Beverly, Mass., U.S.A.), diethyl ether, anhydrous analytical reagent (a.r.), and hydroxylamine hydrochloride, analytical reagent, from Mallinckrodt (St. Louis, Mo., U.S.A.), hexane (non-spectro), distilled in glass, and methylene chloride, distilled in glass, from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). Methylene chloride, reagent grade, was used in the synthesis of 2-methylisoborneol.

Adsorbents

Silica gel, 0.05/0.2 mm, non-activated, was supplied by Brinkmann (Westbury, N.Y., U.S.A.) and Florisil, 100–200 mesh, non-activated, by Fisher Scientific (Pittsburgh, Pa., U.S.A.).

Water samples

The tap water used was that from the Civil Engineering Building, and well water that from a well in the basement. Surface water was that taken from a polluted stream running through the center of Urbana and lake water that taken from a small lake just outside the town.

Polarimetry

The specific rotation of both natural and synthetic 2-methylisoborneol was determined on a Bendix-NPL automatic polarimeter which employs the Faraday electro-optic effect to measure optical rotation. To measure the very small rotation observed from the small sample of natural 2-methylisoborneol, the instrument had to be operated at high sensitivity. Full scale deflection of the meter read-out needle to the right or the left then corresponded to a rotation of plus or minus 0.1°, respectively, and rotations could be read to within 0.001°. With 0.1° sucrose the instrument was found to have a calibration factor of 0.952. The 1-cm cell used in this work would not fit the usual cell holder for the instrument, so a cardboard holder was constructed that fitted in the recess in the base of the cell compartment. In this way the cell could be positioned inside the compartment in the same way every time, and a reading from a particular solution could be reproduced exactly.

The specific rotation of the synthetic 2-methylisoborneol was also determined using a Carl Zeiss polarimeter.

Thin-layer chromatography

Thin-layer chromatography (TLC) was performed on Merck precoated plates of silica gel GOF$_{254}$ (0.25 mm). (Stockists may supply the earlier version of these plates from old stocks, but results with these are distinctly inferior in terms of sensitivity and resolution.) Samples were spotted in amounts up to 200 μg. To develop the plates, suitable mixtures of ethyl acetate and hexane were used. Spots were visualized by examination under UV light, treatment with iodine vapor, and by spraying with 1% vanillin in sulfuric acid. Sensitivity with the spray was 1 μg for 2-methylisoborneol, 5 μg for camphor oxime, and 10 μg for camphor. Visualization of camphor spots was quite sensitive to the manner of spraying and sometimes required several hours of standing. Best results with camphor were obtained by a very light spraying followed by a second spraying after 15 min. Spray reactions and some typical R_F values are shown in Table I.

TABLE I

TLC OF 2-METHYLISOBORNEOL AND SOME OTHER CAMPHOR DERIVATIVES
Developing solvent, ethyl acetate–hexane (1:4).

Compound	R_F	Spot formed with 1% vanillin in sulfuric acid
Camphor oxime	0.30	blue-gray, developing slowly
Borneol	0.34	sharp translucent, developing slowly
Unknown from HBr on 2-methylisoborneol	0.38	bright crimson developing immediately
Isoborneol	0.41	yellow-brown, turning blue
2-Methylisoborneol	0.49	bright crimson, developing immediately
Camphor	0.59	translucent, developing very slowly

Gas–liquid chromatography

Gas–liquid chromatography (GLC) was performed at 140° on a 183 × 0.2 cm I.D. glass column containing Supelcoport (60–80 mesh) coated with 3% SP2100 using a F & M instrument (Model 810) equipped with a flame ionization detector. The glass column was treated initially with 10% dimethyldichlorosilane in toluene for 2 h, washed with methanol, dried, packed with the stationary phase (Supelco, Bellefonte, Pa., U.S.A.), and conditioned at 300° for 2 h. Flow-rates were: nitrogen carrier, 35 ml/min; hydrogen, 30 ml/min; and air 350 ml/min. Injections were made on-column at 270° with a Hamilton 10-μl syringe set at 2 μl with a Chaney adapter. Typical retention times were: camphor, 1.4 min; 2-methylisoborneol, 1.7 min; and camphor oxime, 3.4 min.

GLC proved invaluable for analysing and for monitoring the formation of the camphor oxime, the course of liquid–liquid extractions, and the eluants from the silica gel and Florisil columns. Before injection, small aliquots of both aqueous and organic solutions were diluted 1:250 with hexane.

Specific rotation of natural 2-methylisoborneol

    Natural 2-methylisoborneol investigated in this work originated from *Strepto-myces* sp. CWW3 isolated from Lake Michigan. It was contained in a by-product kindly sent to us by the Environmental Protection Agency from the production of geosmin by Dr. Nancy Gerber of Rutgers University for the Agency[3]. Geosmin (Fig. 1) is another bicyclic tertiary alcohol also responsible for odor in some natural waters[2-5,7]. The by-product, a dark tar, was found by GLC to contain traces of what were probably olefins (0.6 min and 0.7 min), about 35 mg of geosmin (4.6 min), and only 14.0 mg of 2-methylisoborneol (1.5 min).

    The 2-methylisoborneol was isolated by liquid chromatography (LC) of the by-product on silica gel. Though probably not essential to the operation, advantage was taken of its convenient availability to use modern LC equipment. A stainless-steel column (60 × 0.71 cm I.D.) was packed with silica gel and connected with stainless-steel fittings to a Waters (Model M 6000 A) pump, through a Waters (Model U 6 K) loop injection. Methylene chloride was pumped through the column at 2 ml/min and the by-product was injected in about 1 ml of the same solvent. Fractions of 10 ml were collected and analysed without dilution by GLC; results are shown in Table II. Total recovery of 2-methylisoborneol was estimated as 14.5 mg. Fractions 7-9, that were homogeneous in 2-methylisoborneol by GLC, were combined and found to be homogeneous by TLC also. Removal of methylene chloride was carried out on a rotary evaporator with the bath temperature at 30° and the evaporation terminated as soon as it appeared that all solvent had been removed. The residue was a clear gum weighing about 17 mg.

TABLE II

ISOLATION OF 2-METHYLISOBORNEOL BY COLUMN CHROMATOGRAPHY OF A NATURAL PRODUCT

Adsorbent, silica gel; Eluant, methylene chloride; fractions, 10 ml.

Fraction No.	Peak height by GLC			
	Unknown ($R_t = 0.6$ min)	Unknown ($R_t = 0.7$ min)	2-Methylisoborneol ($R_t = 1.5$ min)	Geosmin ($R_t = 4.6$ min)
2	7.5	9.6		
3	3.4	4.4		
4				15.0
5				48.4
6			5.7	3.1
7			48.4	
8			25.4	
9			4.0	
10			0.1	

    The residue was transferred with ethanol to a quartz cell (1 × 1 cm I.D.) and made up to a calibration mark of 0.905 ml on the sidearm. The cell was sealed with a PTFE stopper and an air bubble in the sidearm manipulated around the entire cell to produce a homogeneous solution. The observed rotation was −0.023°, and allowing for the ethanol blank of −0.003° and the calibration factor of 0.952, this corresponded

to a corrected rotation of $-0.019°$. The concentration of 2-methylisoborneol in the cell solution was found to be 0.0136 g/ml after a 9.81-μl portion had been diluted to 1.0 ml with ethanol and analysed by GLC.

$$\text{Thus } [\alpha]_{20}^{D} = -\frac{0.019}{0.1 \times 0.0136} = -14°$$

Mass spectrometry

The mass spectrum of 2-methylisoborneol was recorded on a Finnigan Model 3300 quadrapole instrument using the solid probe.

Infrared spectroscopy

IR spectra were recorded on a Beckman Model IR-20A spectrometer from samples as potassium bromide pellets (1 mg in 200 mg) or 10% solutions in chloroform.

Evaporations

Analytical extracts of methylene chloride were concentrated using the apparatus shown in Fig. 2. The concentration vessels were made from 25-ml round-bottom flasks and are unavailable commercially. Micro Snyder distillation columns[8] like the one shown are supplied by Kontes (Vineland, N.J., U.S.A.). The heating bath used consisted of a pan completely filled with water held at 75° by means of a small hot plate. The cover was a round aluminum plate (0.5 × 25 cm diameter) with a small hole in the center fitted with a thermometer and several holes (4 cm in diameter) to
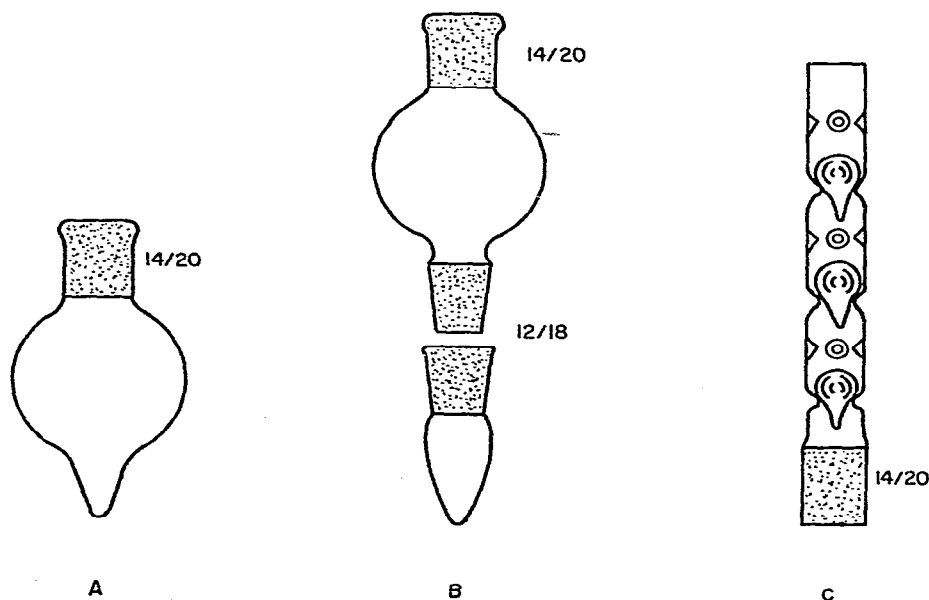

Fig. 2. Concentration apparatus. A and B are concentration vessels, C is a micro Snyder distillation column.

accept concentration vessels. The holes held the vessels securely around the midpoint of the bulb and no additional support was necessary.

Extracts were added to concentration vessels together with a small boiling chip. Glass joints were sealed with a thin film of water. After about 5 min on the water-bath, evaporation was complete and the vessel was cooled in a stream of cold tap water or by allowing it to stand at room temperature before removing the Snyder column. The condensate was about 200 μl. Where maximum sensitivity by gas chromatography (GC) was desired, this solution was further concentrated to about 20 μl using a rotary evaporator without a heating bath. To avoid solutions of this small volume evaporating to dryness on standing, about 20 μl of ethanol were sometimes added.

Eluates from columns used in the purification of synthetic and natural 2-methylisoborneol were evaporated to dryness using a rotary evaporator with a bath temperature at 30°.

Stock solutions of 2-methylisoborneol

Stock solution A consisted of 100 μg methylisoborneol/ml ethanol, B of 10 μg/ml, C of 1 μg/ml, and D of 0.1 μg/ml. A was prepared by making 10.00 mg up to 100 ml with ethanol. The other solutions were prepared by successive 1 in 10 dilutions with ethanol. A and B were mixed with similar camphor solutions to form reference mixtures. C and D were used to make additions of 2-methylisoborneol to water at 0.1–10 μg/l during recovery experiments.

Stock solutions of d-camphor, the internal standard

Stock solution A' consisted of 80 μg d-camphor/ml ethanol, B' of 8 μg/ml, C' of 0.8 μg/ml, and D' of 0.08 μg/ml. The preparation was similar to that described for the 2-methylisoborneol solutions.

Reference mixtures of 2-methylisoborneol and d-camphor

Usually solution A was mixed with A' in the following ratios by volume: 2:1, 1:1, 1:2, 1:5, and 1:10, respectively.

Method for 2-methylisoborneol in water

An exact volume of internal standard solution was added to 1 l of the water sample. For an expected 2-methylisoborneol concentration of 1–10 μg/l, 5.0 ml of C' were usually used and for 0.1–1 μg/l, 5.0 ml of D'. The solution was extracted with methylene chloride (1 × 25 ml and 1 × 10 ml) and the extracts run directly into a concentration vessel. The total volume of the combined extracts was 20 ml, since about 15 ml of methylene chloride dissolved in the water. After concentration to about 20–200 μl, the extracts were analysed by GC, using a reference mixture with a similar mixture of 2-methylisoborneol to camphor. Peak height ratios were determined by dividing the peak height of the 2-methylisoborneol by that of the camphor.

Now, assuming response of 2-methylisoborneol relative to camphor is constant

$$h_1 = k \frac{W_1}{w_1}$$

$$h_2 = k \frac{W_2}{w_2}$$

where

h_1 and h_2 = peak height ratios for sample and reference mixture, respectively

W_1 = weight of 2-methylisoborneol in sample

w_1 = weight of camphor added to sample

W_2 = weight of 2-methylisoborneol per ml of A used to make reference mixture

w_2 = weight of camphor added to reference mixture per ml of A

k = constant.

Eliminating k and rearranging

$$W_1 = \frac{h_1}{h_2} \times \frac{w_1}{w_2} \times W_2$$

$$= \frac{h_1}{h_2} \times \frac{cdv}{cr} \times W_2$$

$$= \frac{h_1}{h_2} \times \frac{dv}{r} \times W_2$$

where

c = concentration of camphor/ml of A'

d = dilution factor for the particular stock solution of camphor used with the sample

v = number of ml of camphor stock solution added to sample

r = ratio of A' to A in the reference solution

Thus, when 5 ml of C' ($d = 0.01$) were used

$$\text{2-methylisoborneol} = \frac{h_1}{h_2} \times \frac{0.01 \times 5}{r} \times 100 = \frac{h_1}{h_2} \times \frac{5}{r} \, \mu g/\mu l$$

When 5 ml of D' ($d = 0.001$) were used

$$\text{2-methylisoborneol} = \frac{h_1}{h_2} \times \frac{0.001 \times 5}{r} \times 100 = \frac{h_1}{h_2} \times \frac{0.5}{r} \, \mu g/l$$

Synthesis of 2-methylisoborneol

d-Camphor (60 g) in about 80 ml of diethyl ether was added dropwise with magnetic stirring to about 260 ml of 1.7 M methyllithium in diethyl ether at such a rate as to maintain the solution under gentle reflux. Before use, the reaction flask was flushed with dry nitrogen fed in through a three-way stopcock in the top of the reflux condensor in one arm and out through the dropping funnel in another arm. Since *d*-camphor dissolves readily in diethyl ether, the solution was prepared in the dropping funnel, so as to avoid exposure of the ether to the atmosphere. After completion of the camphor addition, the solution was refluxed for a further 2 h and allowed to stand at room temperature overnight. The solution, containing a white precipitate, was then poured onto about 400 g of crushed ice and adjusted to pH 6 with glacial acetic acid. The ether layer was collected by decantation, and the aqueous

layer was extracted with more diethyl ether (2 × 10 ml). The combined ether solutions were dried over sodium sulfate and evaporated to dryness using a rotary evaporator.

To the residue in 200 ml of ethanol were added 40 g of hydroxylamine hydrochloride in 100 ml of water and 64 g of sodium hydroxide in 100 ml of water to yield two clear layers of approximately equal volume. The solution was refluxed for 8 h and allowed to stand at room temperature overnight. Water (about 230 ml) was then added with shaking, resulting at first in the formation of a homogeneous solution and finally in the appearance of a slight permanent precipitate. The solution was extracted with hexane (2 × 100 ml) and the combined extracts were washed first with 2 N sodium hydroxide (15 × 500 ml) and finally with water (2 × 50 ml). The hexane was dried over sodium sulfate and evaporated on a rotary evaporator to yield 24.87 g (37.5%) of 2-methylisoborneol as a white crystalline solid.

The product was purified by chromatography on a column of silica gel (6 × 34 cm, 500 g) using methylene chloride as eluant. Elution of 2-methylisoborneol (23.04 g) commenced after 1 l of eluate had been collected and was complete after 3 l. Only the first and last 3 g of eluated material showed trace impurities by GC. The remaining material was homogeneous by both GC and TLC.

The mass spectrum of the product was closely similar to that already published[1], with a parent peak at m/e 168 and a very strong base peak at m/e 95. The IR spectrum was also closely similar to that published[1]. Specific rotation at 20° for the D line using the Carl Zeiss polarimeter was −14.7° (c15.5, ethanol) and using the Bendix polarimeter was −14.9° (c10.76, ethanol) and −3.2° (c10.0, hexane).

RESULTS AND DISCUSSION

Enantiomeric form of natural 2-methylisoborneol

2-Methylisoborneol can exist in two optically active forms with the current Chemical Abstracts names[9] of (1-R-*exo*)- and (1-S-*exo*)-1,2,7,7-tetramethylbicyclo-[2.2.1]heptan-2-ol (Fig. 3; R and S, respectively). These are derived from *d*- and *l*-camphor, respectively. In the literature natural 2-methylisoborneol has always been depicted as the S form, although no evidence for this has ever been reported. Since we were interested in studying not only the adsorption of the natural form on carbon beds but also in examining for any concurrent biological activity, we needed to determine which of the two forms occurred naturally so as to synthesize the appropriate one for our experiments.
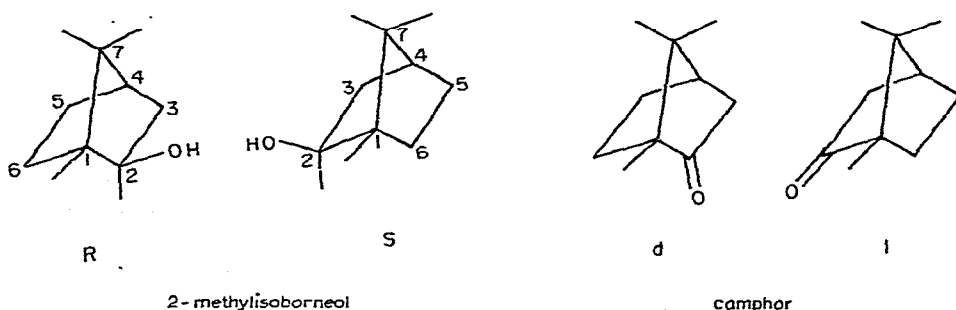

Fig. 3. Stereochemical structures of 2-methylisoborneol and camphor enantiomers.

Accordingly, we have measured the specific rotation of a natural sample of 2-methylisoborneol and so determined its enantiomeric form. We isolated the sample by LC on silica gel of a product obtained by Dr. Nancy Gerber of Rutgers University from the culture of *Streptomyces* sp. CWW3 (ref. 3). The product was mostly geosmin (II), another odor-inducing substance of similar structure, and only 14 mg of pure 2-methylisoborneol were obtained. By measuring the optical rotation in ethanol to within 0.001°, a specific rotation of −14° was obtained, in good agreement with the −14.8° reported in the literature[10] for 2-methylisoborneol obtained from *d*-camphor. Thus natural 2-methylisoborneol exists in the R form.

Synthesis of 2-methylisoborneol from d-camphor

Since the first preparation was reported in 1901 (ref. 11), 2-methylisoborneol has been prepared many times by the action of methylmagnesium halides or methyllithium on camphor[1,2,10,12–14]. The difficulty with this seemingly easy preparation is that much of the camphor reacts to form an enolate and during the work-up this reverts to camphor, which until now has been difficult to eliminate from the product.

Direct separation of the 2-methylisoborneol from the camphor by chromatography has been reported. LC on alumina[10,12] has been used, though it is apparently difficult to eliminate the camphor entirely[15,16]. Preparative GLC[1,15,16] apparently yields a pure product but is limited to the preparation of milligram quantities.

Another approach[10] to the problem has been to repeatedly treat the crude product with more reagent until the camphor is reduced to such a small proportion that it can be eliminated by recrystallization.

Finally, 2-methylisoborneol has been separated from the camphor through its chromate ester[13,14]. Although reduction of the ester with hypophosphorous acid or saporification yielded impure 2-methylisoborneol[13], reduction with lithium aluminum hydride on a small scale appeared to yield pure material[14].

In our synthesis of 2-methylisoborneol we chose methyllithium as reagent rather than a methylmagnesium halide, partly because this was conveniently available commercially, and partly because we hoped it would give the higher yield. Fig. 4 shows the gas chromatogram from the crude product obtained from 60 g of camphor and 1.3 equivalents of methyllithium after reaction at reflux for 2 h and overnight at room temperature. Since the response of 2-methylisoborneol relative to camphor was 0.90, the observed peak height ratio of 0.50 corresponds to 67% of unchanged camphor. When the amount of reagent was increased to 2.0 equivalents, the observed peak height ratio increased to 0.56 corresponding to only a slightly decreased amount of 64% of unchanged camphor. Decreasing the reaction time to 2 h without reflux had no effect on the amount of unchanged camphor. Malkonen[10] has reported only 37% of unchanged camphor using 3.0 equivalents of methylmagnesium iodide. Thus, although the Grignard reagents may not be as convenient to use as methyllithium, they appear to give better yields of 2-methylisoborneol.

To eliminate the large amount of camphor in our crude product we decided to react selectively the camphor to form quantitatively a derivative that would be more easily separated than the camphor itself. Many reagents were tested but, as had been previously found[16], most were unsuitable because of the particularly unreactive nature of camphor. The exception was alkaline hydroxylamine at reflux. Using the reaction conditions of Lenz[17] and monitoring the reaction mixture by GC, it was
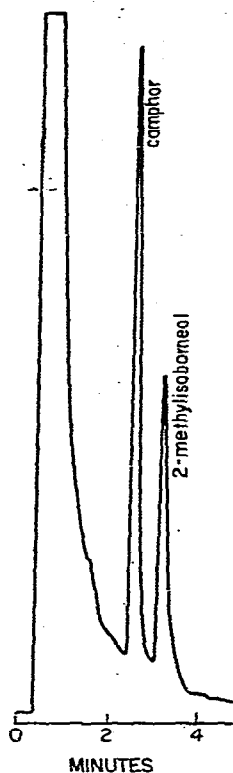
Fig. 4. Gas chromatogram from the product of methyllithium on *d*-camphor.
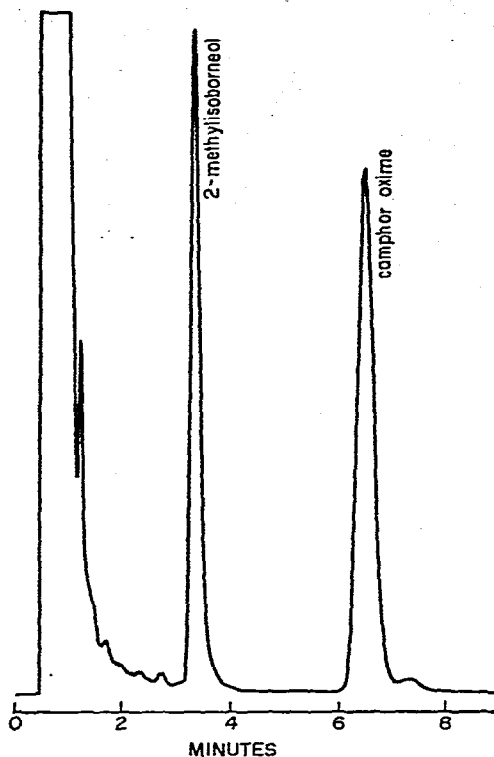
Fig. 5. Gas chromatogram from the product of methyllithium on *d*-camphor after treatment with alkaline hydroxylamine.

found that 19% camphor remained after 2 h but reaction to the oxime was complete after 7 h. A chromatogram of the reaction product is shown in Fig. 5.

With the camphor in the form of the oxime it was rather easily eliminated by taking advantage of its acidic properties. First the alkaline reaction solution was diluted with water and extracted twice with a small proportion of hexane. GC on the residual solution (Fig. 6) and the combined extracts (Fig. 7) showed that less than 50% of the oxime and more than 98% of 2-methylisoborneol were in the extracts. Finally repeated washing of the extracts with aqueous sodium hydroxide gradually removed all of the oxime without loss of 2-methylisoborneol. The product was essentially pure by GC (Fig. 8).

In another experiment the reaction solution containing the oxime was not diluted with water as above but extracted directly with hexane. Partitioning into the hexane layer was reduced but selectivity improved. Four extractions removed a total of 92% of the 2-methylisoborneol and only 20% of the oxime from the reaction solution, so that complete elimination of the oxime was achieved with far fewer washings of aqueous sodium hydroxide.

TLC, in addition to GC, was used to monitor the above work. Table I gives details for 2-methylisoborneol and related camphor derivatives. Note that the bright
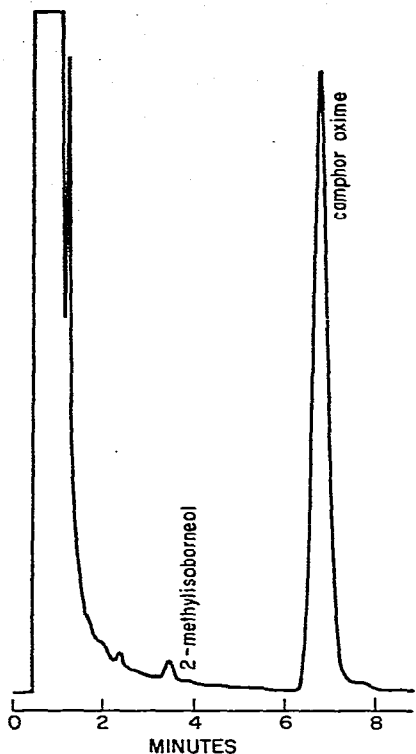
Fig. 6. Gas chromatogram from the residual aqueous solution from the hydroxylamine reaction after hexane extraction.
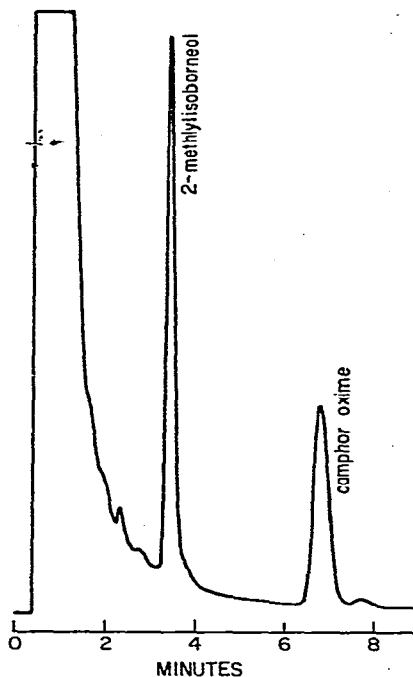
Fig. 7. Gas chromatogram from the hexane extract after the hydroxylamine reaction.

crimson color reaction of 2-methylisoborneol is quite characteristic and developes immediately with a sensitivity of less than 1 μg. Unfortunately camphor gives only an uncharacteristic translucent spot that developes erratically over a long period with a sensitivity of 10 μg at best. (Other sprays for camphor give even poorer sensitivities[18].) Camphor oxime does give a characteristic color reaction but this developes only slowly and again with a sensitivity of 10 μg. Despite the limitations with camphor and its oxime, TLC was useful in that it detected impurities in the 2-methylisoborneol not revealed by GC, and thus indicated the need for final purification of the product by LC.

LC on alumina has usually been used for the purification of 2-methylisoborneol, but our attempt to use basic aluminum oxide (Woelm) led to complete decomposition. This also occurred when an old preparative column of silica gel was used. Even on Fluorisil there appeared to be slight decomposition as elution was delayed for 10 h. This was evidenced by the appearance of several very slow developing translucent spots on the thin-layer chromatograms of the product. However, 2-methylisoborneol homogeneous by TLC was obtained by chromatographing on fresh silica gel and eluting with methylene chloride within 2 h.
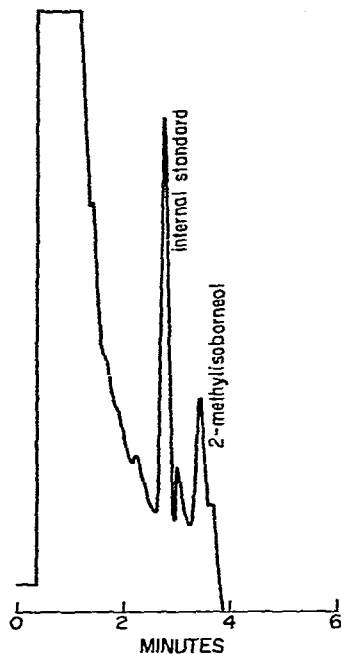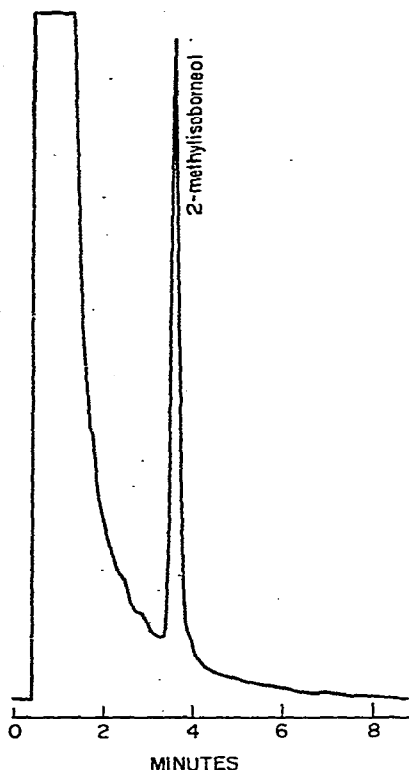
Fig. 8. Gas chromatogram from the hexane extract in Fig. 7 after exhaustive washing with 2 N sodium hydroxide.

Fig. 9. Gas chromatogram obtained in a recovery of 2-methylisoborneol added to tap water at 0.1 μg/l. The internal standard used in this case was 0.8 μg and so the attenuation was reduced fourfold between the peaks.

Attempted preparation of electron-capturing derivatives from 2-methylisoborneol

    Since we were interested in analysing aqueous solutions of 2-methylisoborneol down to 0.1 μg/l by GC, the use of the sensitive electron-capture detector seemed appropriate. Accordingly, attempts were made to prepare a suitable electron-capturing ester.

    Heptafluorobutyric anhydride alone did not react significantly with 2-methylisoborneol at room temperature, and even at 60°, 12 h were required before most of the 2-methylisoborneol had reacted. The product was a mixture of two substances whose retention times by GC relative to that of 2-methylisoborneol (0.56 and 0.60) indicated that they were olefins. In benzene in the presence of triethylamine at room temperature reaction was virtually complete in 1 h but a similar product resulted. TLC showed only one major spot (R_F 0.94), which had a bright crimson appearance. After 24 h the initially predominant substance (relative retention time 0.60) appeared to have partially converted to the other substance (relative retention time 0.56).

    Hydriodic acid (49%) reacted immediately with a solution of 2-methyliso-borneol in acetone to give a product that showed a single crimson spot (R_F 0.96) on

TLC. The product in chloroform showed IR absorption at 3080 (medium), 1740 (weak), 1655 (medium), and 880 cm^{-1} (strong), corresponding to a methylene with standard absorption at 3080 (medium), 1800 ~ 1750 (medium), 1655 (medium), and 890 cm^{-1} (strong)[19]. Again an olefin was produced rather than the desired ester.

In contrast, hydrobromic acid (22%) reacted almost completely with 2-methylisoborneol in ethanol in 30 min at room temperature to produce little olefin. The major product had a GC retention time relative to that of methylisoborneol of 1.10 and gave a crimson spot on TLC with a slightly smaller R_F (0.42) than that of 2-methylisoborneol (0.49). The major change in the IR absorption in potassium bromide was a shift of the C–O stretching band from 1095 cm^{-1} in 2-methylisoborneol to 1030 cm^{-1} in the product, and this indicated transformation of the tertiary alcohol to a secondary[19]. Both the chromatographic and the IR evidence indicate rearrangement of the 2-methylisoborneol to a secondary alcohol, possibly 4-methylisoborneol[20].

These failures to develop a suitable derivative to use with the electron-capture detector meant that we were forced to use the flame ionization detector. To get the desired sensitivity for trace concentrations of 2-methylisoborneol in water, the techniques explained below had to be developed to make extreme concentrations of water extracts.

Extraction

2-Methylisoborneol resembles its precursor camphor in volatility, in GC retention times, and in having extremely high distribution ratios for partitioning into organic solvents from water. Camphor then quickly became our choice as internal standard in the analysis of 2-methylisoborneol.

The favorable partitioning into organic solvents meant that only usually small proportions of extractant (1–2%) were necessary to extract 2-methylisoborneol from water, and this helped both to reduce the amount of coextractives and also the degree of concentration necessary before analysis. However, considerable concentration was still necessary and this severely limited the choice of solvent for extraction. 2-Methylisoborneol co-distills with some solvents and with others the grade readily available has impurities that concentrate sufficiently to cause interference with the analysis. Chloroform was quite satisfactory for analysis down to about 1 μg/l but below this solvent impurities tended to interfere. Methylene chloride was entirely satisfactory and in addition was readily removed because of its outstanding volatility.

Concentration of extracts

To achieve the desired sensitivity of 0.1 μg/l for 2-methylisoborneol in water, it was evident that the extract from 1 l of water would have to be concentrated to as little as 20 μl before GC. In view of the fact that both 2-methylisoborneol and camphor readily sublime, special attention had to be given to the method of the concentration.

Removal of solvent through a micro Snyder distillation column (Fig. 2) was first tried. This method has the advantage that several samples can be concentrated simultaneously with little attention and at the end of evaporation the apparatus is self-rinsing[8]. Concentration vessels of the types A and B shown in Fig. 2 were used. These were fabricated from 25 ml round-bottomed flasks and confortably held the 20 ml of solvent from the extraction of 1 l of water for 2-methylisoborneol. With type A access to the concentrate with a syringe was a little awkward but it was gener-

ally preferred because of its simplicity. Type B had to be assembled with water as a seal on the lower joint to avoid small losses during the concentration, but was advantageous in that the lower tube could be removed later for easy access to the concentrate. The smallest volume attainable with this apparatus was about 200 μl, which was sufficiently low to allow analyses down to about 1 μg/l.

Both relative and absolute recoveries for the evaporation were determined using 1–10 μg of 2-methylisoborneol in 20 ml of various solvents. Absolute recoveries were determined as near as possible from the volume of concentrate as calculated from its weight. Disappointingly, pentane and hexane gave only about 50% absolute recoveries and relative recoveries were very erratic. However, methylene chloride, chloroform, and carbon tetrachloride gave quantitative absolute and relative recoveries. Of these latter solvents, methylene chloride was the obvious choice for the analysis because of its greater volatility and availability in pure grades.

To attain the highest sensitivity desired, it was necessary to devise how to reduce the initial concentrate of 200 μl to about 20 μl without loss of 2-methylisoborneol. This was attempted initially in the lower tube of vessel B by equipping this tube with a small Snyder column. Although relative recoveries were excellent, absolute losses occurred which largely nullified the effect of solvent removal.

However, concentration of the initial concentrate in vessel A or the lower tube of B on a rotary evaporator without a heating bath was successful without loss of 2-methylisoborneol or camphor. The same result was possible by simply connecting the vessels to a water aspirator, but then there was the risk of losing the sample by bumping.

Evaporation of column eluates

Before carrying out the purification of the few milligrams of natural 2-methylisoborneol available by LC, losses were anticipated during the evaporation of the column eluate to dryness. However, preliminary tests with synthetic 2-methylisoborneol showed that, although 2.5 mg in 50 ml of hexane were evaporated to dryness on a rotary evaporator at 45° with a recovery of only 30%, similar evaporation at 35° using methylene chloride gave nearly quantitative recovery. Accordingly, the LC was worked out on the basis of methylene chloride as eluant.

Gas chromatography

The initial parts of this work were carried out by GC using a commercially prepared glass column of 3% OV-1. First tests on the use of camphor as internal standard gave satisfactory results in the determination of 2-methylisoborneol in water at 100 μg/l. However at 10 μg/l unfavorable adsorption effects were observed with the smaller amount of internal standard used. Peak height response relative to that of 2-methylisoborneol decreased markedly and peak width and retention time increased to the point that resolution from 2-methylisoborneol was mostly destroyed.

The column was therefore replaced with one of DMCS-treated glass packed with 3% SP2100, a similar stationary phase to that of 3% OV-1 but with improved characteristics. No untoward adsorption effects were noted with the column. Providing it was not subjected to harsh heat treatment, this type of column proved to be very stable in performance over a period of many months.

In the quantitation of 2-methylisoborneol in this work, peak height relative

to camphor for the sample was compared with that of a standard mixture showing a similar ratio. Standard mixtures in ethanol were prepared by mixing a stock solution of 2-methylisoborneol (10 mg/100 ml) with that of camphor (8 mg/100 ml) in various proportions. When stored in stoppered volumetric flasks at room temperature, they proved to be very stable over a period of many months. It was assumed that there was a linear relationship between the amount of 2-methylisoborneol in the sample and the peak height ratio, and that this ratio was unaffected by dilution of the solution.

To substantiate these assumptions the response factor was determined for 2-methylisoborneol from some standard reference mixtures and their corresponding 1:10 dilutions, as shown in Table III. Evidently the response factor is not appreciably affected by dilution. Also, although there is a slight fall in the response factor with the undiluted mixtures as the proportion of 2-methylisoborneol is reduced greatly, this is not significant enough to affect accuracy if widely differing peak height ratios are not compared.

TABLE III

RESPONSE FACTOR OF 2-METHYLISOBORNEOL FOR VARIOUS PROPORTIONS WITH THE INTERNAL STANDARD AND AMOUNTS GAS CHROMATOGRAPHED

Ratio of 2-methylisoborneol stock solution to that of internal standard in reference mixture*	Amount chromatographed (pg)**		Peak height of 2-methylisoborneol (H) relative to that of internal standard (H_i)	Response factor $\dfrac{H}{H_i} \times \dfrac{W_i}{W}$
	2-Methylisoborneol (W)	Internal standard (W_i)		
2	133.3	53.33	2.15	0.860
	13.33	5.333	2.14	0.856
1	100.0	80.0	1.04	0.832
	10.00	8.000	1.05	0.840
0.2	33.33	133.3	0.196	0.784
	3.333	13.33	0.211	0.844

* Stock solution of 2-methylisoborneol (A) = 10.0 mg/100 ml in ethanol; internal standard solution (A') = 8.0 mg/100 ml.
** Two microliters of each reference mixture were chromatographed both undiluted and as a 1:10 dilution with ethanol.

Recoveries from various types of water

Recoveries of 2-methylisoborneol added to various types of water in a small amount of ethanol are given in Table IV. Blanks equivalent to 0.1 μg/l were carried out for these types of water and also for a sample of lake water, but no interference was noted. A typical chromatogram from a recovery at 0.1 μg/l is shown in Fig. 9.

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TABLE IV

RELATIVE RECOVERY OF 2-METHYLISOBORNEOL FROM VARIOUS TYPES OF WATER

Added, $\mu g/l$		Relative recovery of 2-methylisoborneol (%)			
2-Methylisoborneol	Camphor (internal standard)	Distilled	Tap	Well	Polluted stream
10	4	100.1	97.8	98.2; 97.5	99.0
5	4		97.7; 94.9	97.4; 97.3	103.7
1	4	100.4	100.6	100.7	
1	0.4		96.6		
0.5	0.4		100.1		
0.1	0.8		101.8		
Mean relative recovery, % ± standard deviation		100.3 ± 0.2	98.5 ± 2.4	98.2 ± 1.4	101.4 ± 3.3

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