# GC-FTIR Potential for Structure Elucidation

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Mostra-se neste trabalho que a correlação dos grupos de freqüências de espectros do infravermelho no vapor é uma contribuição importante às informações necessárias para a elucidação de estruturas moleculares de compostos voláteis ou volatizáveis. Como exemplo de aplicação apresenta-se a caracterização de um novo monoterpeno, 4-isopropilideno-1-ciclohexen-1-metanol.

We show in this paper that group frequency correlations from vapor-phase infrared spectrum are a valuable addition to the informations required to elucidate molecular structures of volatile or volatilizable compounds. As an example of application, the characterization of a novel monoterpenoid, 4-isopropylidene-1-cyclohexen-1-methanol, is also presented.

**Key words**: *isoperillyl alcohol.*

## Introduction

To date, GC-FTIR has been mostly applied to analyze complex mixtures of volatile or volatilizable components with help of vapor phase libraries. In the field of chemical ecology, like others dealing with natural products, by and large one have to characterize new structures based on various pieces of informations. In that case, the identification of group frequencies is much more valuable than library search, which has its great merit for identification of previously reported compounds.

Taking as an example our studies on chemical ecology of arthropods, even GC-MS, a much richer library, was a little help[1,2] because most of the target compounds were not found in the library[3-5] or they were novel structures[6-12]. However, the pieces of information taken from the interpretation of the mass spectrum were worth for structure elucidation. Likewise, group frequencies associated with certain structural units can be inferred by analyzing GC-FTIR and information that are complementary to and as rich in content as those derived from GC-MS can be obtained. We describe here certain features unique to vapor-phase IR, group frequencies correlations and, as an example of application, the characterization of a novel monoterpenoid based primarily on GC-FTIR spectrum.

## Experimental

The GC-FTIR system used was composed of a gas chromatograph (GC-15A), a Fourier transform infrared spectrometer (FTIR-4300) and a GC-FTIR interface (GFI-2, all Shimadzu). The splitless path flow was divided into FTIR and FID paths with a feeding ratio FTIR/FID of 10/1 regulated by a flexible quartz tube set before the FID. The fractional gas eluted from GC was connected to the light pipe unit by a gas feeding pipe (0.3 mm i.d. x 1.5 m length; flexible quartz). Neither on FID nor on the spectrometric detector make-up gas was used. The light pipe was a 0.12ml (1.2 mm i.d. x 110.4 mm length) pyrex glass with its inside surface coated by gold; the windows were KBr plates (8 mm diameter x 2 mm thick). Whenever possible the light pipe was operated at 150°C in order to increase sensitivity; the gas feeding tube was set at 250-280°C. At this condition, 250 ng of perillaldehyde was detected with S/N >15/1, the signal being the Y scale expanded to 10%T; the noise was calculated from the Y scale expanded to 0.6%T peak-to-peak at 2000-2500 $cm^{-1}$ interval. All infrared spectrum were recorded at a resolution of 8 $cm^{-1}$ with a mirror speed of 3 mm/sec. The gas chromatograph was equipped with a glass-insert in the injection port and an OV-1 bonded wide-bore column (0.53 mm x 25 m, df = 5μm) operated at a

temperature programed from 150 to 250°C at 10°C/min [150-250/10]. MS was recorded on a Hitachi M-80B operated at 70 eV and equipped with an OV-1 bonded-phase capillary column (0.25 mm x 25 m, Quadrex) that was operated at 100-250/5. Preparative GLC was carried out on an Ohkura 802GC equipped with a 25% FFAP packed column operated at 160°C. NMR was obtained by a Bruker (500 MHz) instrument using $CDCl_3$ solution; $^{13}C$ NMR was obtained by the completely decoupled and DEPT methods. The compounds utilized to generate the data of the tables were either synthesized by us[1-10] or purchased from various sources. Most of them were dissolved in hexane to give solution of 250-10000ppm; 1 µl was applied by splitless injection mode.

Spectral data of isoperillyl alcohol. MS: m/z 152 (48%), 40(3.6), 41(67.4), 43(30.3), 44(8.7), 51(16), 52(9.4), 53(13.1), 55(5.5), 57(4.4), 65(14.7), 67(33.2), 68(2.1), 77(42), 78(6.1), 79(76.1), 80(9.6), 81(36.4), 91(38.5), 92(11.9), 93(30.9), 94(6.7), 95(8.6), 105(21.7), 107(9.8), 109(26.9), 117(5.7), 119(13.2), 121(100), 122(13.3), 149(1.7) and 153(6.3). $^1H$ NMR δ 5.66(1H, m), 4.00 (2H, s), 2.80(2H, br. s), 2.35 (2H, t, J=6.28 Hz), 2.10 (2H, br. t) 1.70 and 1.66 (3H each, s). $^{13}C$ NMR δ 137.73 (C), 127.19 (C), 122.70 (CH), 122.40 (C), 67.12 ($CH_2$), 29.24 ($CH_2$), 27.00 ($CH_2$), 26.12 ($CH_2$), 20.21 ($CH_3$) and 19.77 ($CH_3$).

## Results and Discussion

It is essential to have the knowledge of group correlation generated from vapor-phase to successfully apply GC-FTIR to the elucidation of molecular structures because the IR bands recorded in the condensed-phase are not generally coincidental with those of vapor-phase IR. If, for example, the νC=O band at 1709 $cm^{-1}$ of perillaldehyde were correlated with group frequencies from condensed-phase, it could be misassigned as a non-conjugated aldehyde!

**Table 1.** *Vapor-phase infrared frequencies for carbonyl group at various environments.*

| Functional group | Frequency | Example |
|---|---|---|
| **Ketone** | | |
| R-C(=O)-R | 1728-1740 | 4-decanone(1728), acetone (1740), 2-decanone (1732), methyl n-nonyl ketone (1732) |
| Six-member ring | 1736-1740 | 4-methylcyclohexanone (1736), 1,4-cyclohexanedione (1740), 1,3-cyclohexanedione (1736) |
| α, ß-unsaturated six - member ring | 1693-1709 | 2-cyclohexen-1-one (1709), isopiperitenone (1963), carvone (1697) |
| five-member ring | 1767 | cyclopentanone |
| α ß-unsaturated five - member ring | 1743 | 2-cyclopenten-1-one |
| Quinone | 1670-1678 | p-quinone (1678), methyl- and ethyl-quinone (1674), thymoquinone (1670), 2-hydroxy-1,4-naphthoquinone (1678) |
| Chelated quinone | 1655 | plumbagin (1655) |
| **Aldehyde** | | |
| R-C(=O)H | 1744 | citronellal (1744), ß-acaridial (non-saturated CHO, 1744), hexanal (1744) |
| R-CHO with and adjacent expoxide | 1736 | 2,3-epoxyneral (1736), 2,3-epoxygeranial (1736) |
| U-C(=O)H U= α, ß-unsaturation | 1696-1713 | farnesals (1969), neral (1697), geranial (1697), perilladehyde (1709), 2-(E)-hexenal and decenal (1713), α-acaridial (1712), ß-acaridial (conjugated CHO, 1712) |
| A-C(=O)H A=aryl | 1713-1720 | vanillin (1713), p-hydroxybenzaldehyde (1720), o-tolualdehyde (1716) |
| Chelated A-C(=O)H | 1670-1680 | 2-hydroxy-4-methylbanzaldehyde (1674), methyl 2-formyl-3-hydroxybenzoate (1670), 2-hydroxybenzaldehyde (1678) |
| **Acid** | | |
| R-C(=O)OH | 1778 | monanoic acid (1778), caprylic acid (1778), hexanoic acid (1778) |
| U-C(=O)OH | 1751-1759 | nerolic acid (1751), farnesoic acid (1752), 2-(E)-hexenoic acid (1759) |
| A-C(=O)OH | 1759-1770 | m-toluic acid (1763), m- anfd o-chlorobenzoic acid (1767 and 1770), p- and m-hydroxybenzoic acid (1759 and 1762) |
| A-C(=O)OH with an ortho-OH | 1716 | salicyilic acid |
| $(=CHCOOH)_2$ | 1767 | fumaric acid |
| **Anhydride** | | |
| Five-member ring | 1865-1875<br>1763-1813 | succinic anhydride (1875; 1813), methyl succinic anhydride (1875, 1813), dimethyl succinic anhydride (1763), maleic anhydride (1801), phthalic anhydride (1867, 1805) |
| Open anhydride | 1824<br>1763 | butyric anhydride (1824, 1767)<br>isobutyric anhydride (1824, 1763) |
| **Ester** | | |
| R-C(=O)OR | 1755-1763 | methyl undecanoate (1759), ethyl oleate (1755), dimethyl malate (1763) |
| R-O-C(=O)H | 1744-1747 | 8-hepthadecenyl formate (1747), 8,11-heptadecenyl formate (1747), neryl formate (1744) |
| R-O-C(=O)-$CH_3$ | 1759-1763 | 7,9-(E,Z)-dodecadienyl acetate (1763), perillyl acetate (1763), citronellyl acetate (1759) |
| A-C(=O)OR | 1740-1755 | methyl phthlate (1755), methyl 4-methyl benzoate (1743), methyl p-hydroxybenzoate (1743), hexyl 3-hydroxybenzoate (1740), methyl 3-formyl-4-hydroxybenzoate (1743) |
| A-C(=O)OR with an ortho OH | 1697 | methyl salicylate |
| **Lactones** | | |
| γ-lactone | 1813 | γ-decalactone (1813), γ octalactone (1813) |
| δ-lactone | 1774 | δ-decalactone |
| ε-lactone | 1770 | ε-caprolactone |

For the analysis of IR spectrum, one first examine the presence of a carbonyl stretching band, which in vapor

phase appear in the range 1655-1875 $cm^{-1}$ (Table 1). Aliphatic ketones exhibit in the interval 1728-1740 $cm^{-1}$; $\nu_{c=0}$ frequencies for cycloalkanones decrease with the increase in ring size: hexanones, 1736-1740 $cm^{-1}$, cyclopentanone, 1767$cm^{-1}$. An α,ß-unsaturation decrease the frequency by 25$cm^{-1}$. Quinones display the $\nu_{c=0}$ in the region 1670-1678 $cm^{-1}$, whereas chelation decrease the absorption frquency by 23$cm^{-1}$.

The aldehydes $\nu_{c=0}$ band display in the region 1670-1744 $cm^{-1}$; the saturated ones exhibit in the upper frequency (1744 $cm^{-1}$); an epoxide adjacent to the aldehyde decrease the $\nu_{c=0}$ frequency by 8 $cm^{-1}$. α,ß-Unsaturated aldehydes exhibit in the interval 1696-1713 $cm^{-1}$, a decrease of ca. 32 $cm^{-1}$ compared to the saturated counterparts. Benzaldehydes display in the region 1713-1720 $cm^{-1}$, 28$cm^{-1}$ lower than the saturated aldehydes. Chelation gives rise to a 40 $cm^{-1}$ decrease in the frequency, which lays in the region 1670-1680 $cm^{-1}$. Two aldehydic C-H bands appear in the region 2723-2731 $cm^{-1}$ and 2808-2834 $cm^{-1}$. 2-Hydroxybenzaldehyde displays at 2746 $cm^{-1}$ probably due to the internal hydrogen bonding.

**Table 2.** *Vapor-phase OH stretching frequencies.*

| Functional group | Frequency | Example |
|---|---|---|
| Acid | 3572-3587 | nonanoic acid (3576), hexanoic acid(3576), fumaric acid (3584), m-toluic acid (3587), m- and o-chlorobenzoic acid (3584 and 3572), p- and m-hydroxy-benzoic acid (both 3587), cinnamic acid (3583), saliccylic acid (3580), nerolic acid (3580), w-(E)-hexenoic acid (3583) |
| Alcohol/Phenol | 3591-3668 | 2-decanol (3657), 2,4-pentanediol (3653; 3591), cinnamyl alcohol (3637), 9,11-(E,E)-tetradecadienol (3668), nerol (3652), perillyl alcohol (3653), 5-isopropyl-3-methylphenol (3653), -naphtol (3649), p- and m-hydroxybenzoic acid (3645 and 3652) |
| o-methoxyphenol | 3567-3595 | 2-methoxy-4-methylphenol (3595), vanillin (3567) |
| "phenol" with an CHO group in the ortho position | 3201-3271 | salicylic acid (3271), methyl salicylate (3259, 2-hydroxybenzaldehyde (3201) |

Three bands characterize the spectrum of acids, namely, OH, C=O, and C-O stretching bands. The $\nu_{c=0}$ band appear in the region 1767-1778 $cm^{-1}$. Aliphatic acids exhibit at 1778 $cm^{-1}$, while α,ß-unsaturated ones do at 27 $cm^{-1}$ lower frequency (1751-1759 $cm^{-1}$). The $\nu_{c=c}$ bands of these acids are also strong and appear at ca. 1656 $cm^{-1}$. Benzoic acids exhibit at 15 $cm^{-1}$ lower frequencies than the aliphatic counterparts, 1759-1770 $cm^{-1}$. Intramolecular hydrogen bonding due to an OH in the ortho position causes a drecrease of 44 $cm^{-1}$ in the carbonyl band absorption frequency. Fumaric acid exhibit at 1767 $cm^{-1}$, but maleic acid seems to form an equilibrium acid:anhydride. A band at 1743 $cm^{-1}$, probably acid $\nu_{c=0}$, is displayed along with the major carbonyl band at 1801 $cm^{-1}$ (from maleic anhydride). It was not possible to obtain vapor-phase spectrum of phthalic acid in our system as dehydration occurred leading to phtalic anhydride. That the degradation took place in the injection port rather than in the light pipe was reasoned by the identity of "phthalic acid" and phthalic anhydride retention times.

Anhydrides exhibit two carbonyl stretching bands in the region 1865-1875 and 1763-1813 $cm^{-1}$. The occurrence of two bands is very clear in open anhydrides, but the lower frequency band is much more intense or the sole $\nu_{c=o}$ band of the examined five-member ring anhydrides.

Esters of the formula R-C(=O)OR exhibit in the interval 1755-1763 $cm^{-1}$, whereas benzoates do within 1740-1743 $cm^{-1}$ (methyl phthalate display at 1755 $cm^{-1}$). The effect of an orthopositioned OH is the same as in benzoic acids, and $\nu_{c=0:H0}$ band appear at 1697 $cm^{-1}$. Formates exhibit in the interval 1744-1747 $cm^{-1}$, the most intense band of the spectrum being due to νC-O (see below). Acetates display in the range 1759-1763 $cm^{-1}$. The $\nu_{c-o}$ band of acetates is also intense of their spectrum. Lactones exhibit in the range 1770-1813 $cm^{-1}$.

The OH stretching band of aliphatic carboxylic acids (Table 2) exhibit in the range 3565-3584 $cm^{-1}$, whereas benzoic and α,ß-unsaturated acids display in the interval 3572-3587$cm^{-1}$.

The phenolic OH stretching exhibit in the range 3645-3653 $cm^{-1}$, except for ortho-substituted "phenols", which display in the interval 3201-3271 $cm^{-1}$. The decrease in the $\nu_{OH}$ frequencies depends on the substituent; the greatest effect is due to an -CHO group (ca 448 $cm^{-1}$) and the smallest is own to an -COOH group (374 $cm^{-1}$). Interactions with an ortho methoxy group also decrease the νOH frequencies, although to a lesser extent (< 100 $cm^{-1}$).

We found that alcohols exhibit approximately in the reported ranges[13] of primary (3670-3680), secondary (3658-3670) and tertiary (3640-3648 $cm^{-1}$). Allylic alcohols display in the interval 3648-3668 $cm^{-1}$, out of the reported range[13].

The sensitivity of our system to alcohols was poor, generating spectrum with low S/N ratio, especially in the region above 3000 $cm^{-1}$. In order to obtain better spectrum, acetylation of natural alcohols were carried out, whenever possible. As an example, citronellyl acetate gave an IR with S/N 5.5 times greater that of citronellol.

The C-O stretching frequencies in alcohols is not easily predictable, but it can be stated that allylic alcohols exhibit in the range 990-1018 $cm^{-1}$ (Table 3), which distinguish them from other alcohols (even when the νOH band is lost).

In phenols, the $\nu_{c-o}$ appear in the interval 1138-1269 $cm^{-1}$, but most of the time OH bending adsorption (1072-1161 $cm^{-1}$) has stronger intensity.

The C-O stretching frequencies due to ethers exhibit in the region 1131-1141 $cm^{-1}$ for R-O-R and anisoles do within 1273-1276 $cm^{-1}$; vinyl butyl ether display somewhere in-between the two regions.

The $\nu_{c-o}$ bands of acids appear in the range 1134-1184 $cm^{-1}$, except for α,ß-unsaturated acids such as nerolic and farnesoic acids, which display at ca. 1108 $cm^{-1}$. A band of medium intensity due to δOH is displayed in the interval 1334-1369 $cm^{-1}$.

**Table 3.** *Vapor-phase C-O stretching frequencies data.*

| Functional group | Frequency | Example |
|---|---|---|
| **Alcohol** | 1002-1130 | 9,11-(E,E)-tetradecadienol (1049), 8,10-(E,E)-dodecadienol (1049), nerol (1002), perillyl alcohol (1007), 2,3-epoxynerol (1034), linalool (1107), 2-decanol (1130), 2,4-pentanediol (1130), cinnamyl alcohol (1018), citronellol (1034) |
| **Phenol** | 1138-1269 | α-naphtol (1138), 2-methoxy-4-methylphenol (1240; δOH 1149), 5-isopropyl-3-methylphenol (1269; δOH 1157), p-hydroxybenzaldehyde (1256, δOH 1157), vanillin (1184, δOH 1157), salicylic acid (1184; δOH 1072), p- and m-hydroxybenzoic acid (1265; δOH 1080 and 1260, δOH 1080), methyl p-hydroxybenzoate (1192; δOH 1107), methyl salicylate (1254; δOH 1161) |
| **Ether** | 1203-1227 | vinyl butyl ether (1203); butyl ether (1131), ethyl ether (1141), vanillin (1277), 2-methoxy-4-methylphenol (1273) |
| **Acid** | | 1107-1227 m-toluic acid (1165; δOH 1358) nanoic acid (1138; 1103), caprylic acid (1134; 1105), hexanoic acid (1137; 1099), 2-(E)-hexenoic acid (1138; δOH 1362), m- and o-chlorobenzoic acid (1184; δOH 1362 and 1184; δOH 1340), salicylic acid (1149; δOH 1362), benzoic acid (1180; δOH 1346), p- and m-hydroxybenzoic acid (1161; δOH 1366 and 1169; δOH 1334), fumaric acid (1122; δOH 1369), cinnamic acid (1119); δOH 1362), nerolic acid (1107) |
| **Anhydrides** | 1018-1257<br>891-1165 | butyric anhydride (1030), isobutyric anhydride (1018), succinic anhydride (1053; 906), methyl succinic anhydride (1218); 995), dimethyl succinic anhydride (1215; 1165), maleic anhydride (1230; 891), phthalic anhydride (1257; 910) |
| **Ester** | | |
| C-C(=O)-O | 1773-1307 | methyl undecanoate (three bands pattern with the most intense at 1173), dimethyl |
| O-C-C | 1107-1222 | malate (three bands; 1261, 1215 and 1176), diethyl mthylmalonate (four bands from 1245 to 1157), methyl 3-formyl-4-hydroxybenzoate (1277; 1215), methyl p-hydroxybenzoate (1273; 1165), methyl phthalate (1284; 1130), methyl 3-hydroxybenzoate (1288; 1222), methyl salicylate (1307; 1215), ethyl oleate (1242; 1176) |
| **Formate** | 1161-1169 | neryl formate (1161), 8-heptadecenyl formate (1169) |
| **Acetate** | | |
| C-C(=O)-O | 1231-1234 | erillyl acetate (1231;1022), |
| O-C-C | 1022-1053 | 7,9-(Z,E)-dodecadienyl acetate (1231; 1045), citronellyl acetate (1234; 1053) |
| **Lactone** | 1161-1230 | γ-decalactone (1164; 1038), γ-octalactone (1161; 1026), ε-caprolactone (1165; 1076), δ-decalactone (1230; 1140) |

Two C-O-C stretching bands appear in the spectrum of anhydrides in the region 891-1165 and 1018-1275 $cm^{-1}$; in the open chain anhydrides only one band appeared, which was the strongest in the spectrum.

Esters exhibit two $\nu_{c-o}$ bands due to C-C(=O)-O and O-C-C stretchings. In the benzoate esters they appear in the region 1273–1307 and 1165–1222 $cm^{-1}$, respectively. Aliphatic esters display the two bands in the interval 1157-

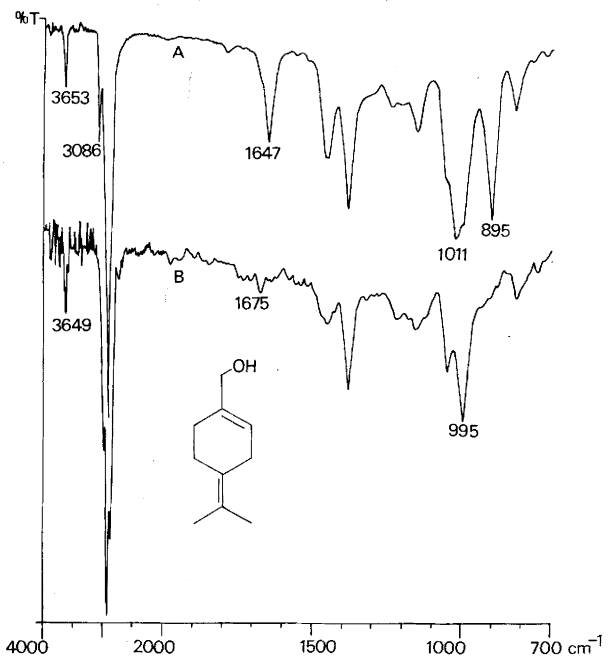


**Figure 1.** *Vapor-phase infrared spectrum of perillyl (A) and isoperillyl alcohol (B).*

1261 $cm^{-1}$. The most intense band in the spectrum of formates is $\nu_{c-o}$ band in the range 1161-1169 $cm^{-1}$. Likewise, in acetates they appear in the interval 1231-1234 $cm^{-1}$ along with a $\nu_{o-c-c}$ in the interval 1022-1053 $cm^{-1}$. Lactones exhibit two bands in the intervals 1161-1230 $cm^{-1}$ and 1026-1140$cm^{-1}$.

The geometry of double bonds may be assigned by vapor phase IR spectrum; the E-configuration of RCH=CHR exhibit in the range 980-984 $cm^{-1}$, except for trans-stilbene (957 $cm^{-1}$). That band of 8,10-(E,E)-dodecadienol, 9,11-(Z,E)-tetradecadienol and 7,9-(E,Z)-dodecadienyl acetate at 984, 980 and 980 $cm^{-1}$, respectively. The trans absorbance band has been used for the quantification of trans unsaturation in fatty acid methyl esters[14]. On the other hand, the cis configuration[12] gives rise to a band at ca. 3020 $cm^{-1}$.

Commercialy available perillyl alcohol (Aldrich) contains ca. 85% of that terpenoid along other hitherto unknwon structure, which appeared at longer retention time. Perillyl alcohol generated an IR spectrum (Fig. 1) displaying a νOH band at 3653 together with $\nu_{c=o}$ and $\nu_{c-o}$ at 1647 and 1011$cm^{-1}$, respectively. $CH_3$ and $CH_2$ bending bands appeared at 1450 and 1377 $cm^{-1}$, respectively. A vinylidene (exo-$CH_2$) band was displayed at 895 $cm^{-1}$. That the unknown compound was an alcohol was supported by the the bands at 3649 ($\nu_{co}$) and 995 $cm^{-1}$ ($\nu_{c-o}$). The following features strongly suggested that the alcohol was an isomer of perillyl alcohol: the vinilidene olefinic adsorption bands (=CH, 3086; C=C, 1647 and C-H, 895 $cm^{-1}$) did not appear, but istead a very weak band of a tetrasubstituted olefinic νC=C did at 1675 $cm^{-1}$ along with a νC-O (< 1020 $cm^{-1}$) of an allylic alcohol. Therefore, the structure was reasoned as an isomer of perillyl alcohol having a double bond in-between carbons 4 and 8, C(4)=C(8). GC-MS also supported the structure: the more intense molecular ion at m/z 152 (48%) than that of perillyl alcohol (12%) characterized a more stable structure. Futhermore, the occurrence of a C(4)=C(8) double bond did not favor retro Diels-Alder rearrangement like in perillyl alcohol, which displayed the base peak at m/z 68; the base peak of isoperillyl alcohol appeared at m/z 121 due to the loss of $CH_2OH$. The assignment was corroborated by $^1H$ and $^{13}C$ NMR spectrum (see Experimental) of the new compound isolated by preparative GLC. Therefore, the monoterpenoid was elucidated to be 4-isopropylidene-1-cyclohexen- 1-methanol, for which we gave the trivial name isoperillyl alcohol. Likewise, GC-FTIR has been demonstrated to be worth for the identification of various natural products of biological significance[1,2,9,10,15,16]. The occurrence of a weak band at 1622 $cm^{-1}$ was a striking feature which led to the identification of a new type of insect sex pheromone.

## Conclusions

Despite of the recent improvements in terms of sensitivity, the main limitation of GC-FTIR compared to the more prominent separation and identification method of **GC-MS** is the lack of sensitivity for low concentration components.

The knowledge of vapor-phase group frequencies correlations is a valuable addition to the pieces of informations required for the structural elucidation of volatile compounds. In certain fields of applications, the cost of a GC-FTIR system may be tremendously reduced by the elimination of a library. In that case, the informations we presented here may serve as a basis for the interpretation of the spectrum.

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