

Investigation of vibrational theory of olfaction with variously labelled benzaldehydes

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Abstract

Vibrational theory of olfaction was investigated with the following three labelled analogues of benzaldehyde; ¹³C₆(ring)-benzaldehyde, ¹³CHO-benzaldehyde and benzaldehyde-d₆. Sensory analysis, by a trained panel of 30 subjects, using a duo-trio test, showed that the benzaldehyde-d₆ gave a statistically significant difference in odour perception ($P=0.002$) relative to the unlabelled benzaldehyde. The odour changes in the other substituted analogues were found to be less significant ($P=0.058$ for ring-¹³C₆ and $P=0.017$ for ¹³CHO). Analysis of the infrared spectra revealed that benzaldehyde-d₆ also experienced the most drastic shifts ($\Delta\lambda \sim 700 \text{ cm}^{-1}$) in the absorption frequencies of the aromatic and aldehydic C–H stretching bands (3085, 2819 and 2738 cm^{-1}), in addition to smaller shifts ($\Delta\lambda \sim 25\text{--}100 \text{ cm}^{-1}$) in the C–H bending or deformation bands (828, 745, 688 and 650 cm^{-1}). In addition, molecular modelling studies indicated that the shape of the molecule was retained after isotopic substitution but the molecular volume increased by less than 1%. The data suggested that one of the required vibrational bands for the perception of bitter almond aroma lies in the 3000 to 2500 cm^{-1} frequency range. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The molecular properties underlying the characteristic odour signature of aroma compounds are not really understood. Presently, the most widely accepted theories of olfaction are either based on molecular shape (Amoore, 1962; Moncrieff, 1967) or on vibrational properties of molecules (Dyson, 1938; Turin, 1996; Wright, 1977). The former assumes the simplistic notion that regardless of the type of functional groups present in a molecule, the overall shape of the molecule determines the characteristic aroma. The known limitations of shape–activity relationships of aroma compounds (Weyerstahl, 1994) are well illustrated by the presence of a common aroma in molecules having widely differing shapes. The bitter almond odour, for example, is shared by as many as 75 different molecules (Turin, 1996) including benzaldehyde and hydrogen cyanide. The molecular shape theory was later refined by Beets (1961) to include the functional groups as important features

of molecular recognition. On the other hand, Dyson (1938) suggested that molecular vibrations are the basis for odour specificity in different molecules, without proposing a mechanism of detection by olfactory receptors. Wright (1977), however, proposed that detection of vibrational frequencies by the receptors is mechanical in nature. This hypothesis limits the vibrational modes that could be detected, to those that could be excited only at physiological temperatures (below 500 cm^{-1}). The vibrational theory of olfaction was recently strengthened by the introduction of a new mechanism of biological transduction of molecular vibrations based on the concept of inelastic electron tunnelling (Turin, 1996). This eliminated one of the main shortcomings of the vibrational theory that restricted detection by the receptors to only vibrational modes excited at the body temperature (below 500 cm^{-1}). The most convincing evidence of vibrational theory of olfaction could be derived by comparison of odours of molecules generated by isotopic substitution such that they differ only in their vibrational frequencies but retain their identical molecular structures; however, no systematic study has been reported with human subjects. Turin (1996) reported differences in the smell of acetophenone and aceto-

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phenone-d₈ and Wright (1975) compared the smell of substituted naphthalenes, with no statistical details. To provide sensory evidence for the vibrational theory of olfaction, benzaldehyde and three of its labelled analogues; ¹³C₆(ring)-benzaldehyde, ¹³CHO-benzaldehyde and benzaldehyde-d₆ were subjected to sensory analysis by a trained panel of 30 subjects, using a duo-trio test (Poste, Mackie, Butler & Larmond, 1991), and the results were compared with their vibrational spectra acquired in the 4000–200 cm⁻¹ range.

2. Materials and methods

2.1. Infrared analysis

Benzaldehyde (99.9%) was purchased from Sigma-Aldrich Canada Ltd., (ON, Canada). ¹³CHO-benzaldehyde (99%), ¹³C₆(ring)benzaldehyde (99%) and benzaldehyde-d₆ (98%) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Water was purified using a Milli-Q water purification system (Millipore Corp., USA). Spectra covering the mid infrared region were acquired with the Nicolet 8210 spectrometer using ZnSe windows containing neat samples with no spacers, except for ¹³C₆(ring)benzaldehyde (which exists as a solid) where a 5% KBr pellet was used. Far infrared analysis was conducted on the Bomem Michelson 102 spectrometer using CsI windows containing neat samples with no spacers, except for ¹³C₆(ring)-benzaldehyde where a 0.05×25 mm Teflon spacer was used to hold a 5% solution in methanol. Spectra were manipulated using GRAMS/386 software (Galactic Industries, New Hampshire).

2.2. Molecular modelling studies

Theoretical vibrational frequencies of labelled benzaldehyde, bond angles, dihedral angles and bond lengths were calculated using Gaussian 98 (SGI-G98RevA.3, Carnegie, PA). Molecular volumes were calculated using MMX force field of PCModel for Windows Version 6.0 (Serena Software, Burlington, CA).

2.3. Sensory analysis

Sensory analysis was conducted at the Agriculture and Agri-Food Canada research branch of the Food Research and Development Center in Ste-Hyacinthe, Quebec. Stock solutions (350 ml, 4.3×10³ M) of four benzaldehyde samples were prepared. Using clear screw-cap glass jars (6×5 cm), 5 ml portions of the unlabelled benzaldehyde solutions were placed in 90 jars while 5 ml portions of the three labelled analogues were placed in 30 jars each. The main discriminative test employed was the duo-trio test. This test can establish if a detectable

difference exists between samples. However, it does not indicate the size of the difference or whether the panelists' identifications of the odd sample were based on the same characteristic.

Three sample sets were presented to the panellists. In each set one sample was labelled R (reference), while the other two were coded with numbers (Table 1). One coded sample was identical with R in each set. The panellists were asked to sniff the samples once and identify the odd sample. The panellists were allowed 1 min of recovery between sniffs. For the purposes of this test 30 trained panellists were used and the samples were ordered as shown in Table 2.

Table 1
Blinding codes

Sample	Corresponding blinding codes
Benzaldehyde	1
¹³ CHO-benzaldehyde	2
¹³ C ₆ (ring)-benzaldehyde	3
Benzaldehyde	4
Benzaldehyde	5
Benzaldehyde-d ₆	6

Table 2
Sample presentation order for the duo-trio test^a

Panelist	Set 1	Set 2	Set 3
1	1-REF 1 2	3-REF 4 3	6-REF 6 5
2	1-REF 2 1	3-REF 3 4	6-REF 5 6
3	1-REF 1 2	3-REF 4 3	6-REF 6 5
4	1-REF 2 1	3-REF 3 4	6-REF 5 6
5	1-REF 1 2	3-REF 4 3	6-REF 6 5
6	1-REF 1 2	3-REF 4 3	6-REF 6 5
7	1-REF 2 1	3-REF 3 4	6-REF 5 6
8	1-REF 1 2	3-REF 4 3	6-REF 6 5
9	1-REF 2 1	3-REF 3 4	6-REF 5 6
10	1-REF 1 2	3-REF 4 3	6-REF 6 5
11	1-REF 1 2	3-REF 4 3	6-REF 6 5
12	1-REF 2 1	3-REF 3 4	6-REF 5 6
13	1-REF 1 2	3-REF 4 3	6-REF 6 5
14	1-REF 2 1	3-REF 3 4	6-REF 5 6
15	1-REF 1 2	3-REF 4 3	6-REF 6 5
16	2-REF 2 1	3-REF 3 4	5-REF 5 6
17	2-REF 1 2	3-REF 4 3	5-REF 6 5
18	2-REF 2 1	3-REF 3 4	5-REF 5 6
19	2-REF 1 2	3-REF 4 3	5-REF 6 5
20	2-REF 2 1	3-REF 3 4	5-REF 5 6
21	2-REF 2 1	3-REF 3 4	5-REF 5 6
22	2-REF 1 2	3-REF 4 3	5-REF 6 5
23	2-REF 2 1	3-REF 3 4	5-REF 5 6
24	2-REF 1 2	3-REF 4 3	5-REF 6 5
25	2-REF 2 1	3-REF 3 4	5-REF 5 6
26	2-REF 2 1	3-REF 3 4	5-REF 5 6
27	2-REF 1 2	3-REF 4 3	5-REF 6 5
28	2-REF 2 1	3-REF 3 4	5-REF 5 6
29	2-REF 1 2	3-REF 4 3	5-REF 6 5
30	2-REF 2 1	3-REF 3 4	5-REF 5 6

^a See Table 1 for the blinding codes.

3. Results

3.1. Sensory analysis

The results of the duo-trio tests shown in Table 3 indicated that 60% (18/30) of the subjects were able to distinguish between $^{13}\text{C}_6$ -(ring)-benzaldehyde and the reference ($P=0.181$). Furthermore, 63% (19/30) of the subjects were able to distinguish between ^{13}CHO -benzaldehyde and the reference ($P=0.100$) and 77% (23/30) of the subjects were able to distinguish between benzaldehyde- d_6 and the reference ($P=0.008$).

Using a standard critical P -value of 0.05 it was then concluded that there was a statistically significant difference between the odour of the benzaldehyde and benzaldehyde- d_6 (Table 4).

However, due to the 50% probability of guessing right, it was necessary to calculate the percentage of panellists who actually saw a difference without guessing. Table 4 shows that a difference was still found among the three samples with the d_6 sample showing 47% recognition. An interesting pattern was discovered when the sample presentation order and the corresponding results were examined (Table 5). The ordering of the samples seemed to have influenced the results. Consequently, the affected sequences were omitted and the probability was re-calculated.

The probability for benzaldehyde- d_6 was found to markedly improve to a value of $P=0.002$ (Table 3). The sensory analysis indicated that an odour change had occurred when all the hydrogen atoms of benzaldehyde were replaced with deuterium atoms.

Table 3
Statistical results of the sensory analysis

Labelled compound	No. of correct identifications	Corresponding P -values ^a
$^{13}\text{C}_6$ -(ring) benzaldehyde	18/30	0.181 (0.058)
^{13}CHO -benzaldehyde	19/30	0.100 (0.017)
Benzaldehyde- d_6	23/30	0.008 (0.002)

^a Values in parentheses represent adjusted P -values to take into account the influence of the order of presentation of the samples (Table 5).

Table 4
Comparison of percent correct identifications and actual true responses

Test compound	Correct identifications (%)	Actual true responses (%) ^a
$^{13}\text{C}_6$ -(ring) benzaldehyde	60	20±35%
^{13}CHO -benzaldehyde	63	27±35%
Benzaldehyde- d_6	73	47±32%

^a Refers to the corrected responses to eliminate the effect of 50% probability of guessing right.

3.2. Infrared analysis

Figs. 1 and 2 show, respectively, mid- and far-infrared spectra of benzaldehyde (solid line) superimposed on spectra of labelled analogues (dotted line). The spectrum of ^{13}CHO -benzaldehyde showed the least variation from the normal spectrum. The major shift, as expected, was that of the carbonyl band (shifted from 1702 to 1665 cm^{-1}). $^{13}\text{C}_6$ -(ring)-benzaldehyde exhibited more noticeable changes involving aldehydic CH stretching vibrations (2820 and 2737 cm^{-1}) that were shifted to 2665 and 2547 cm^{-1} ; in addition, the carbonyl band also experienced a slight shift to 1692 cm^{-1} , the aromatic double bond stretching bands (1597, 1583 and 1455 cm^{-1}) shifted to 1547, 1530 and 1426 cm^{-1} . In the far IR region, the CH bending vibrations (828, 745, 688, 650, 451 cm^{-1}) were shifted to 776, 712, 640, 520 and 416 cm^{-1} , respectively. However, the most dramatic shifts were experienced by the benzaldehyde- d_6 analogue. Aldehydic CH stretching vibrations (2820 and 2737 cm^{-1}) were shifted to 2103 and 2066 cm^{-1} ; the carbonyl band was shifted to 1684 cm^{-1} and aromatic CH (3085 cm^{-1}) band was shifted to 2280 cm^{-1} . In the far IR region the CH bending vibrations (688, 650, 451 cm^{-1}) were shifted to 623, 542 and 393 cm^{-1} , respectively. The observed vibrational shifts due to isotopic substitution and their relative intensities were consistent with those predicted by theoretical calculations using Gaussian 98 force field.

4. Discussion

According to Turin (1996), volatile compounds can elicit specific aroma sensations if their vibrational modes can excite the receptor bands in the frequency regions associated with a particular aroma. For example, the smell of bitter almond is classified by Turin as a “bichromatic odor” since, in order to be perceived, it requires excitation of receptor bands at two frequency ranges that define bitter almond aroma. This hypothesis is consistent with a recent finding that more than one receptor is involved in generating a specific aroma sensation (Malnic, Hirono, Sito & Buck, 1999) and that

Table 5
Influence of the order of presentation of benzaldehyde- d_6 on the sensory analysis^a

	AA'B	ABA'	BB'A	BAB'
Total	5	10	5	10
Correct	5 (100%)	7 (70%)	2 (40%)	9 (90%)
Incorrect	0	3	3	1

^a In each set, the primed letters represent R (reference) and corresponding unprimed letters represent coded samples identical with R (A and A' represent benzaldehyde- d_6 ; B and B' represent benzaldehyde).

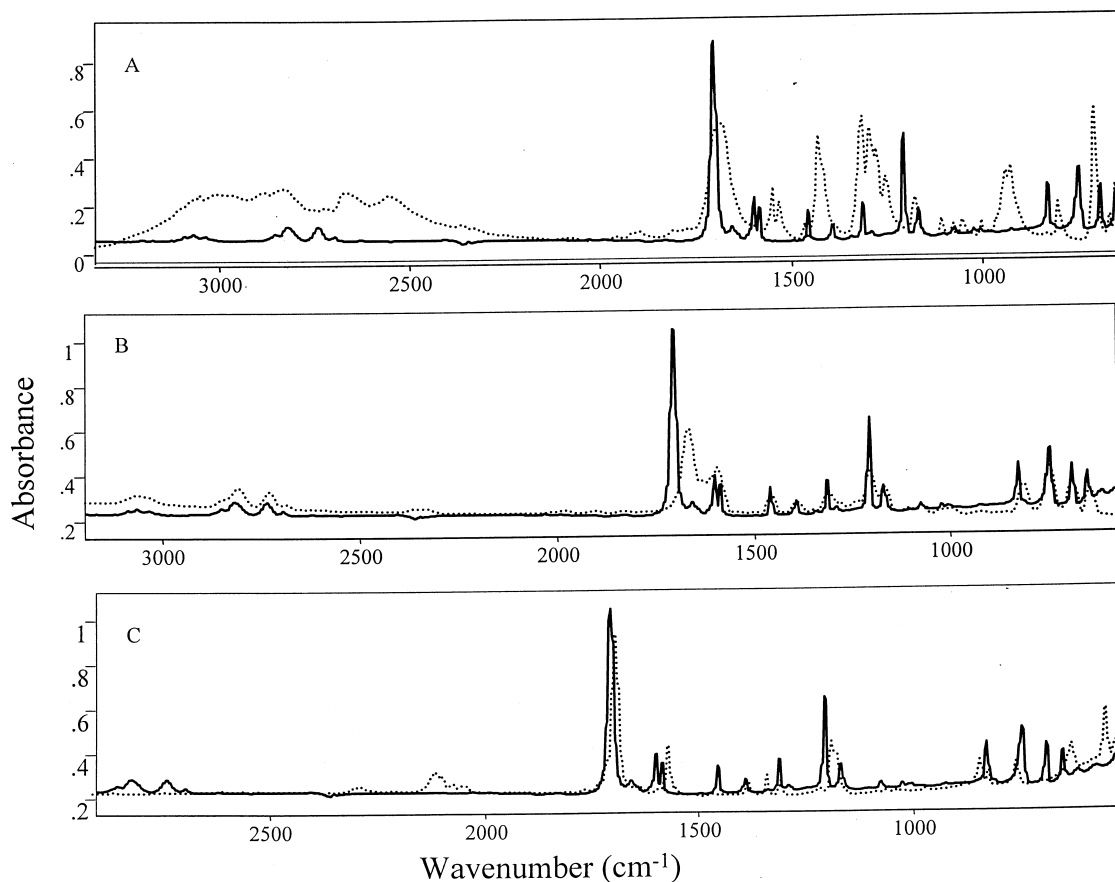


Fig. 1. Mid-infrared spectra of neat benzaldehyde (solid line) superimposed on spectra of labelled analogues (dotted line). (A) $^{13}\text{C}_6$ (ring)-benzaldehyde (KBr), (B) ^{13}CHO -benzaldehyde (neat), (C) benzaldehyde- d_6 (neat).

each receptor generates only part of the codes that define the aroma, referred to as “combinatorial receptor codes”. It could be proposed that the language of the receptor codes is based on a “vibrational alphabet” where specific frequency ranges constitute its letters. Each receptor responds to a particular frequency stimulation. When odour molecules bind to all the receptors necessary to complete the code, then the odour is perceived.

Isotope replacement studies, in principle, can verify Turin’s hypothesis, if the shift in frequency caused by isotopic substitution is sufficient enough to move the band out of the range of detection by the particular receptor. In addition, the shifted band should be located in the frequency range that defines the particular aroma. Therefore, whether a band shift will cause an effect on the perceived aroma will depend on the wavelength and on the extent of the shift. According to Turin (1996) olfactory receptors constitute biological spectrometers with poor resolution ($\sim 400\text{ cm}^{-1}$). If a vibrational frequency necessary to elicit a sensation lies close to the high frequency end of a receptor band, then a minimum shift of 400 cm^{-1} is required to bring the frequency out of the range of detection by the receptor. On the other hand, if a vibrational frequency necessary to elicit a

sensation lies closer to the low frequency end of a receptor band, then a smaller shift can bring the frequency out of the range of detection by the receptor. It is therefore expected that deuterium substitution (mass change from 1.0078 to 2.0141) would provide a better chance of altering aroma perception, due to a larger resulting frequency shift compared to substitution of ^{12}C atoms by ^{13}C atoms (mass change from 12.0000 to 13.0034).

Three isotopically substituted derivatives of benzaldehyde (^{13}CHO -benzaldehyde, $^{13}\text{C}_6$ -ring-benzaldehyde and benzaldehyde- d_6) were utilized to investigate the validity of vibrational theory as modified by Turin (1996). The test compounds were subjected to sensory and infrared analysis. Sensory analysis, by a trained panel of 30 subjects and using a duo-trio test, showed that the benzaldehyde- d_6 gave a statistically significant difference in odour perception ($P=0.002$) relative to the unlabelled benzaldehyde (Table 3). The observed odour change was found to be similar to a previous observation (Wright, 1975) involving the isotopic substitution of naphthalene, where the general odour was always retained, but an odour change was detected by 4 out of 6 (67%) panellists. The odour changes in the other substituted analogues were found to be less significant

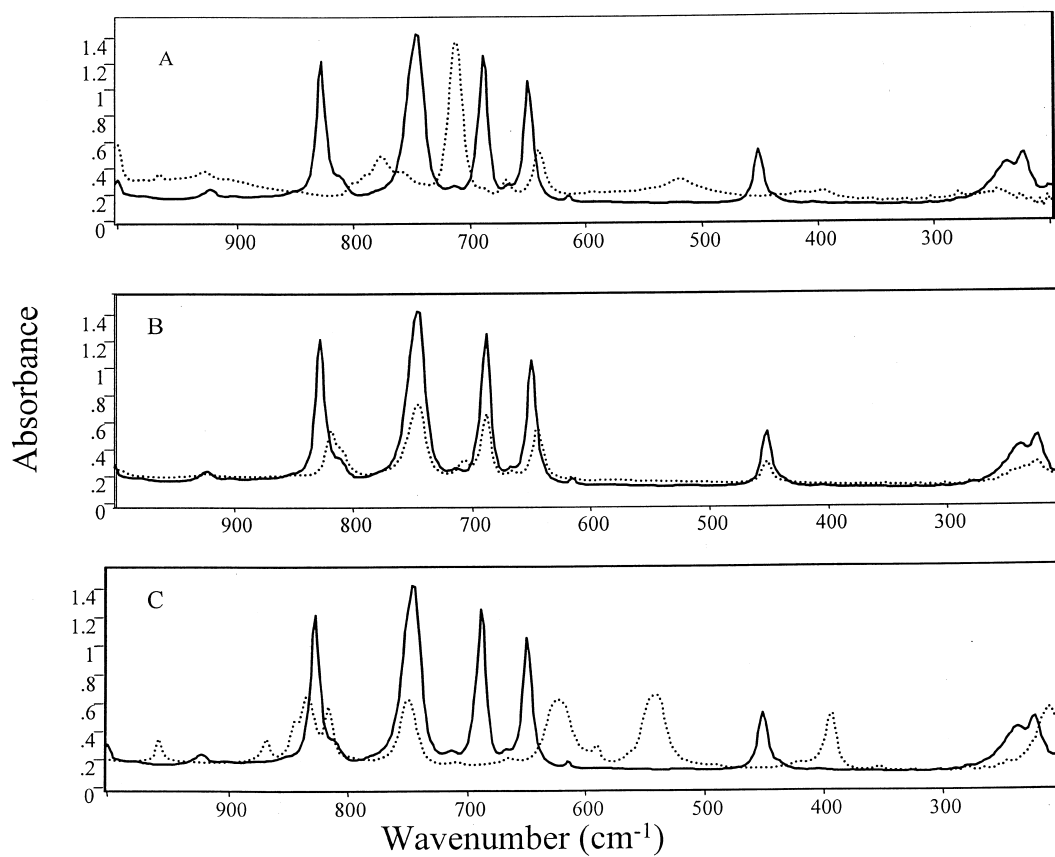


Fig. 2. Far-infrared spectra of neat benzaldehyde (solid line) superimposed on spectra of labelled analogues (dotted line). (A) $^{13}\text{C}_6(\text{ring})$ -benzaldehyde (KBr), (B) ^{13}CHO -benzaldehyde (neat), (C) benzaldehyde- d_6 (neat).

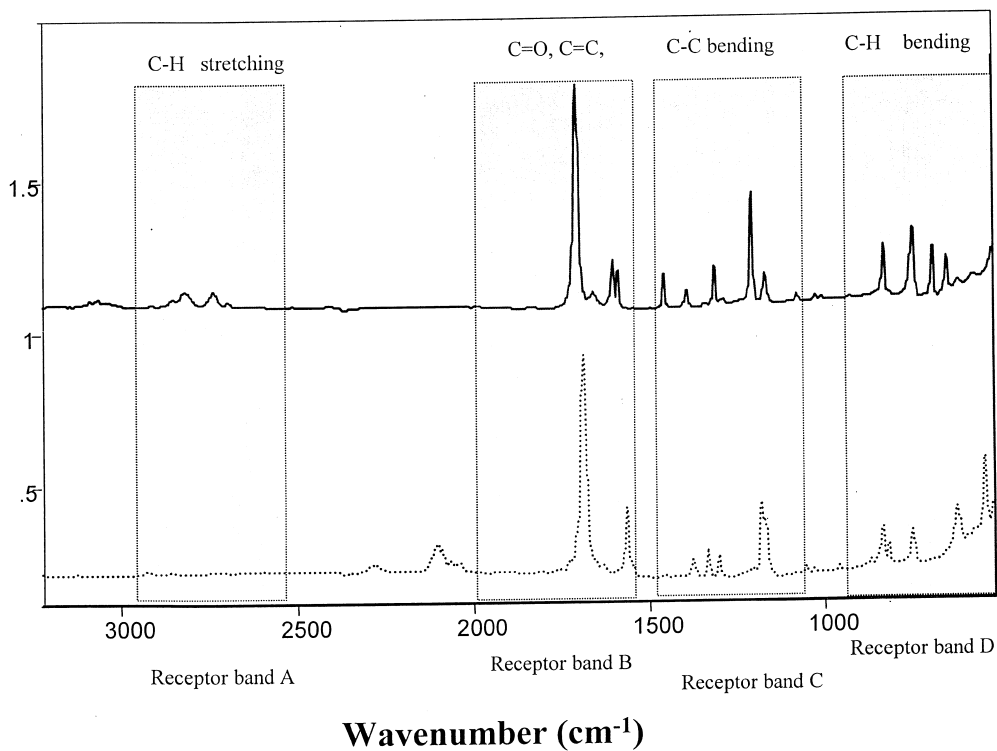


Fig. 3. Hypothetical receptor bands (shaded areas) characteristic of bitter almond aroma. Benzaldehyde (solid line) and benzaldehyde- d_6 (dotted line).

($P=0.058$ for $^{13}\text{C}_6(\text{ring})\text{benzaldehyde}$ and $P=0.017$ for $^{13}\text{CHO-benzaldehyde}$). As expected, benzaldehyde- d_6 also exhibited the largest frequency shifts (see Fig. 1C) in aldehydic and aromatic C–H stretching vibrations ($\Delta\lambda\sim 700\text{ cm}^{-1}$) as well as in the C–H bending region ($\Delta\lambda\sim 25\text{--}100\text{ cm}^{-1}$). In principle, the former shift can remove the band to well outside the resolution of the receptor (Fig. 3), confirming that at least one of the required vibrations for bitter almond aroma lies within the $3000\text{--}2500\text{ cm}^{-1}$ frequency range. Fig. 3 shows a conservative estimate of possible receptor bands and their frequency ranges involved in the transduction of bitter almond odour.

Although isotopic substitution is not expected to alter the molecular shape, it may alter the molecular volume. Theoretical calculations using Gaussian 98 have indicated that all the bond lengths, bond angles and dihedral angles in both benzaldehyde and benzaldehyde- d_6 were identical, confirming the retention of molecular shape of benzaldehyde after isotopic substitution. In addition, molecular modelling studies have also indicated that the change in molecular volume, due to substitution of hydrogen atoms of benzaldehyde with deuterium, was less than 1% (from 139 to 140 \AA^3). Consequently, the observed odour modifications due to the isotopic substitutions in benzaldehyde can result mainly from changes in the vibrational frequencies.

5. Conclusion

The sensory data in this study provided additional evidence in favour of the vibrational theory of olfaction.

Furthermore, the spectral analysis identified at least one band, in the $3000\text{--}2500\text{ cm}^{-1}$ region, necessary to elicit bitter almond aroma.

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