ELSEVIER

Journal of Molecular Structure 596 (2001) 83–88

Journal of
MOLECULAR
STRUCTURE

www.elsevier.com/locate/molstruc

Determination of the aromatic compounds in plant cuticular waxes using FT-IR spectroscopy

Eligiusz N. Dubis*, Alina T. Dubis, J. Popławski

University of Białystok, Institute of Chemistry, Al. J. Piłsudskiego 11/4, 15-443 Białystok, Poland

Received 30 September 2000; revised 17 February 2001; accepted 17 February 2001

Abstract

The infrared study of the aromatic components of hops (*Humulus lupulus*) cuticular wax was performed. HATR FT-IR technique for fresh leaves and their extract analysis was applied. Phenylmethyl myristate, 2-phenylethyl myristate and docosyl benzoate were synthesized and used as reference standards. An absorption band in the range of 709–966 cm^{-1} indicates the presence of aromatic esters in plant cuticular waxes.

Keywords: IR spectroscopy; ATR; *Humulus lupulus*; Plant cuticular wax

1. Introduction

In our previous studies [1] we have applied horizontal attenuated total reflection HATR FT-IR technique for preliminary analysis of plant cuticular waxes. The aim of this work was to present HATR FT-IR studies of the aromatic components of plant cuticular waxes.

Plant cuticular waxes are composed of varying amounts of mainly long-chain alkanes, 1-alkanols, aldehydes, alkanoic acids, alkyl esters and additional classes of aliphatic substances. There are only a few reports in the literature [2,3,4,5] in which aromatic compounds were found as epicuticular wax components. Esters of phenylmethanol and 2-phenylethanol with a very long chain fatty acids (C_{20}–C_{32}) were found in small amounts (0.5–2%) in the wax of *Ginkgo biloba*, *Magnolia grandiflora*, *Liriodendron tulipifera*, *Jojoba*, *Humulus lupulus* and *Olea europaeum* leaves. Furthermore, benzoic acid esters with long chain alcohol (C_{22}–C_{26}) were detected [6].

2. Experimental

Infrared spectra were obtained with a Nicolet Magna 550 FTIR Series II Spectrometer. The spectra were collected in the wave number range 4000–400 cm^{-1}. For HATR (Spectra-Tech) measurements, a ZnSe crystal ($\theta = 45°$, $n_p = 2.4$) was used. All spectra were recorded at a resolution of 2 cm^{-1} and apodized with triangular function, and a zero-filling factor of 1 was applied. A total of 1000 scans were averaged for each sample. For in situ cuticular wax investigation, a fresh and not mutilated plant leaf was placed on top of the HATR crystal in close contact with its crystal surface. Transmittance spectra were

* Corresponding author. Tel.: +48-85-745-7580; fax: +48-82-745-7581.

E-mail address: dubis@noc.uwb.edu.pl (E.N. Dubis).

0022-2860/01/$ - see front matter
PII: S0022-2860(01)00691-3

converted to absorbance spectra, and baseline drift was removed using the instrument software (OMNIC 3.0). In some cases, smoothing procedure was required.

Lipid extract was prepared by the treatment of fresh leaves with chloroform (15 s), followed by the addition of drying agent (anhydrous $MgSO_4$), and evaporation of the solvent. Cuticular waxes were investigated in the HATR mode. A wax solution in acetone was deposited on the surface of the ZnSe crystal. Solvent was evaporated at room temperature and the sample was then heated to 80°C for 3 min in order to completely remove the solvent [7].

Fresh leaves of hops (*Humulus lupulus*) were collected from garden cultivation in Białystok.

3. Synthesis of phenylmethanol and 2-phenylethanol esters of C14 myristic acid

100 mg (4.3×10^{-4} mole) of myristic acid (C14 long) was dissolved in 2 ml of dichloromethane and cooled in an ice bath. 0.1 ml (8×10^{-4}mol) of oxalyl chloride and a drop of DMF were added and the resulting mixture was stirred until it was brought down to room temperature. After evaporation of the solvent (CH_2Cl_2), 0.1 g (8.6×10^{-4}mol) of phenylmethanol or 2-phenylethanol in pyridine was added. The mixture was warmed for 30 min. After cooling, the solvent was evaporated and the residue was subjected to column chromatography on aluminium oxide using CH_2Cl_2 as an eluent. Yield of pure ester was 80%.

3.1. Phenylmethyl myristate (PMM) — spectroscopic data:

^{1}H NMR (ppm): 7.36 (5H, m, Ar–H); 5.12 (2H, s, Ar–CH_2–); 2.36 (2H, t, –$COCH_2$–); 1.65 (2H, dt, –CH_2–); 1.26 (20H, m, $(CH_2)_{10}$); 0.85 (3H, t, –CH_3). ^{13}C NMR (ppm): 173.69(C); 136.11 (CH); 129.51 (CH); 128.13 (CH); 66.02 (CH_2); 34.31 (CH_2); 31.90 (CH_2); 29.64 (CH_2); 29.62 (CH_2); 29.57 (CH_2); 29.42 (CH_2); 29.33 (CH_2); 29.23 (CH_2); 29.10 (CH_2); 14.10 (CH_3). IR ($CHCl_3$, cm^{-1}): 3091, 3066, 3034, 2923, 2853, 1738, 1655,1498, 1456, 1161, 733, 696.

3.2. 2-Phenylethyl myristate (2-PEM) — spectroscopic data:

^{1}H NMR (ppm): 7.26 (5H, m, Ar–H); 4.30 (2H, t, Ar–CH_2–); 3.01 (2H, t, –$COCH_2$–); 2.29 (2H, t, –CH_2–); 1.60 (2H, dt, –CH_2–);1.27 (20H, m, $(CH_2)_{10}$); 0.86 (3H, t, –CH_3). ^{13}C NMR (ppm): 173.74(C); 137.03(CH);128.85(CH); 128.40 (CH); 126.46 (CH_2); 64.63 (CH_2); 34.28 (CH_2); 31.88 (CH_2); 29.65 (CH_2); 29.62 (CH_2); 29.57 (CH_2); 29.41 (CH_2); 29.33 (CH_2); 29.23 (CH_2); 29.07 (CH_2); 14.09 (CH_3). IR ($CHCl_3$, cm^{-1}): 3087, 3064, 3029,2923, 2853, 1736, 1655, 1605, 1497, 1466, 1455, 747, 723, 699.

4. Synthesis of docosyl benzoate

Benzoil chloride was added to a stirred solution of docosyl alcohol (C22 long) in pyridine. The reaction mixture was then heated for 1 h. After cooling and removing the solvent, the residue was then subjected to column chromatography on silica gel using a hexane: ether (93:7; V:V) mixture as an eluent. Yield of pure ester was 75%.

4.1. Docosyl benzoate – spectroscopic data:

^{1}H NMR (ppm): 8.07 (2H, d, Ar–H); 7.40 (3H, m,Ar–H); 4.32 (2H, t,–$COCH_2$–); 1.77 (2H, dt, –CH_2–); 1.26 (18H, m, $(CH_2)_9$); 0.86 (3H, t, –CH_3). ^{13}C NMR (ppm): 166.67(C); 132.76(CH); 129.52(CH); 128.29(CH); 65.14(CH_2); 31.92(CH_2); 29.69(CH_2); 29.58(CH_2); 29.53(CH_2); 29.36(CH_2); 29.29(CH_2); 28.71(CH_2); 14.12 (CH_3). IR ($CHCl_3$, cm^{-1}): 3090, 3061, 3033, 2954, 2916, 2849, 1720, 1602, 1585, 1469, 1275, 1111, 709.

5. Results and discussion

In our research, we performed spectroscopic studies of fresh hops (*Humulus lupulus*) leaves and their lipid extract, which is known to contain aromatic compounds [4]. Reference standards of the aromatic esters phenylmethyl mirystate, 2-phenylethyl mirystate and docosyl benzoate were synthesized and analysed using the HATR technique. For the analysis of aromatic components, the C–H out-of-plane

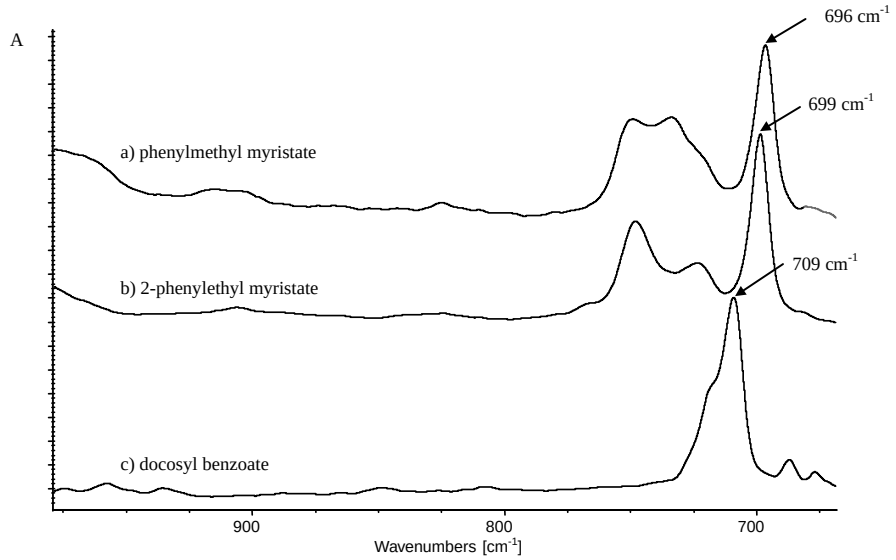

Fig. 1. The aryl C–H δ_{CH}^{oop} bands of phenylmethyl-, 2-phenylethylmyristate and docosyl benzoate.

vibrations close to 650–900 cm^{-1} were selected [8]. Unfortunately, broad absorption due to water overlaps the aromatic absorption bands around 3100–3000 and 1650–1450 cm^{-1}, making it difficult to use them for the analysis. It was found that the main absorption band of phenylmethanol and 2-phenylethanol esters, and benzyl acid esters is located at 696, 699 and 709 cm^{-1}, respectively (Fig. 1). The detection limit of the δ_{CH}^{oop} band was determined. HATR spectra of pure eicosane and eicosane with 2 and 4% admixture

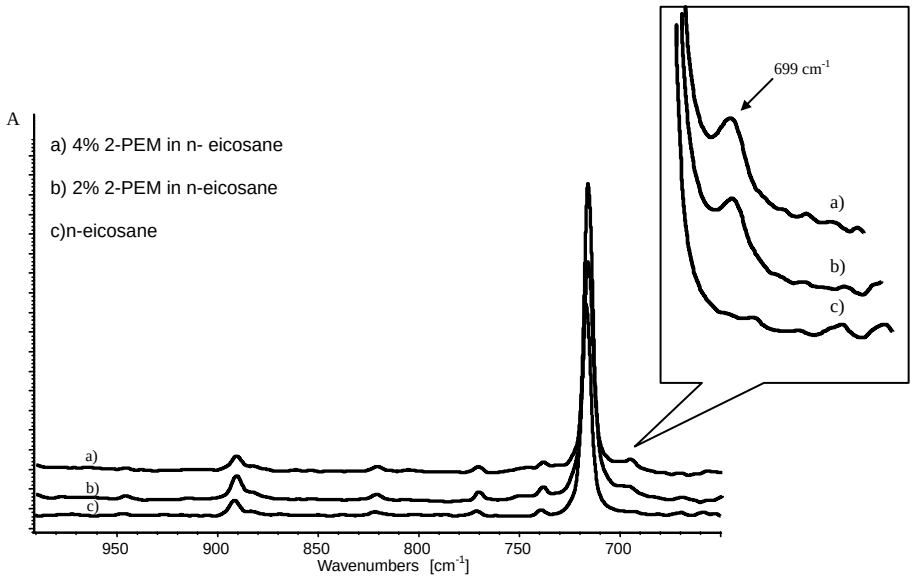

Fig. 2. HATR FT-IR spectra of *n*-eicosane (c), *n*-eicosane with 2% admixture of 2-PEM (2phenylethyl myristate) (b) and with 4% of 2-PEM (a). Enlargement shows δ_{CH}^{oop} band of 2PEM.

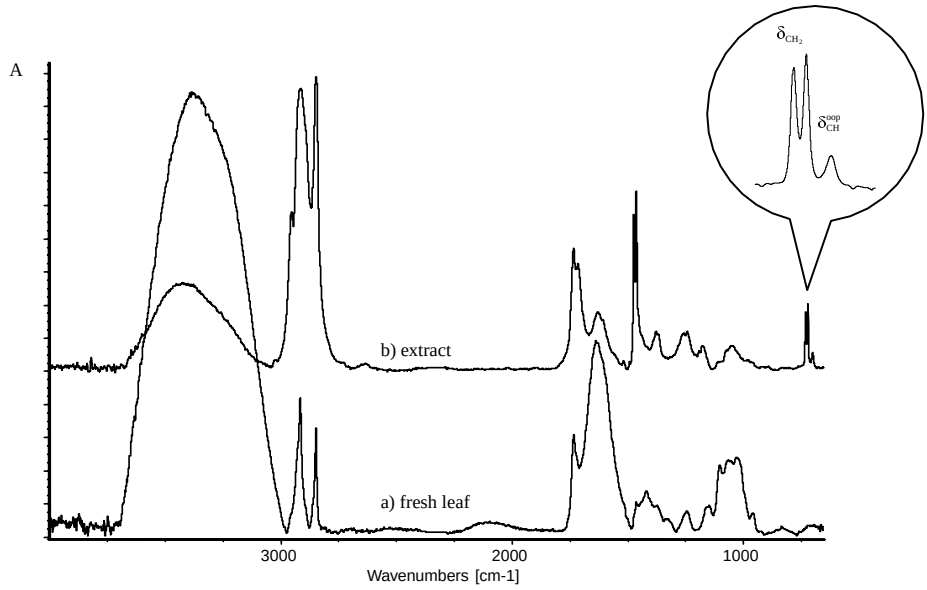

Fig. 3. HATR FT-IR spectra of hops (*Humulus lupulus*) fresh leaves (a) and their epicuticular wax extract (b). Enlargement shows aliphatic δ_{CH_2} and aromatic δ_{CH}^{oop} bands.

of phenylmethyl and 2-phenylethyl ester (Fig. 2) were recorded. Plant lipids consist mainly of aliphatic long-chain compounds and for this reason *n*-eicosane was chosen. The content of these esters in prepared mixtures is similar to their natural concentration in epicuticular leaf waxes. Recorded spectra revealed that the δ_{CH}^{oop} band is noticeable when the ester content comes above 2%.

Fig. 3 shows HATR spectra of the fresh leaves of hops (*Humulus lupulus*) and their epicuticular wax

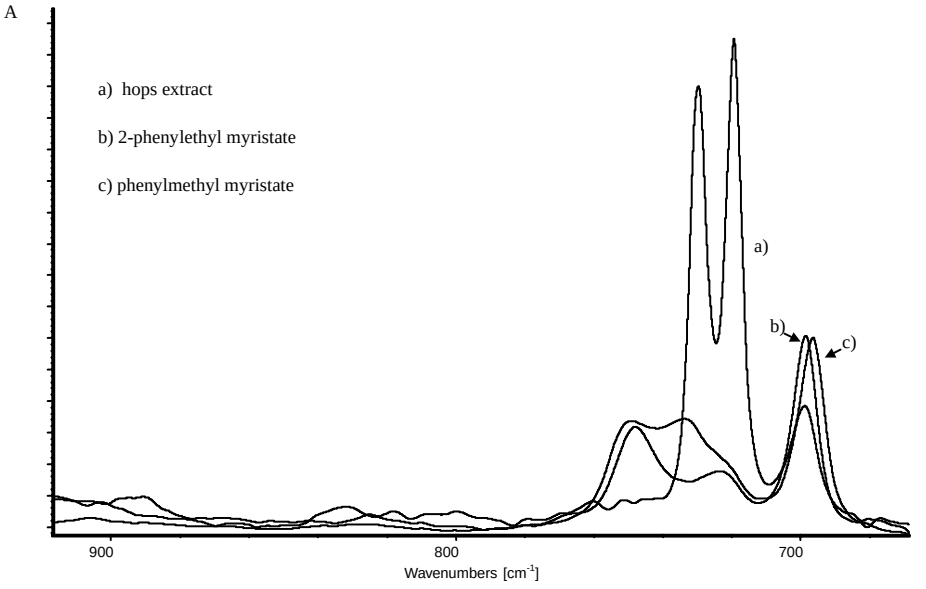

Fig. 4. HATR FT-IR spectra of hops wax extract, 2-PEM and PMM in the range of 900–650 cm^{-1}.

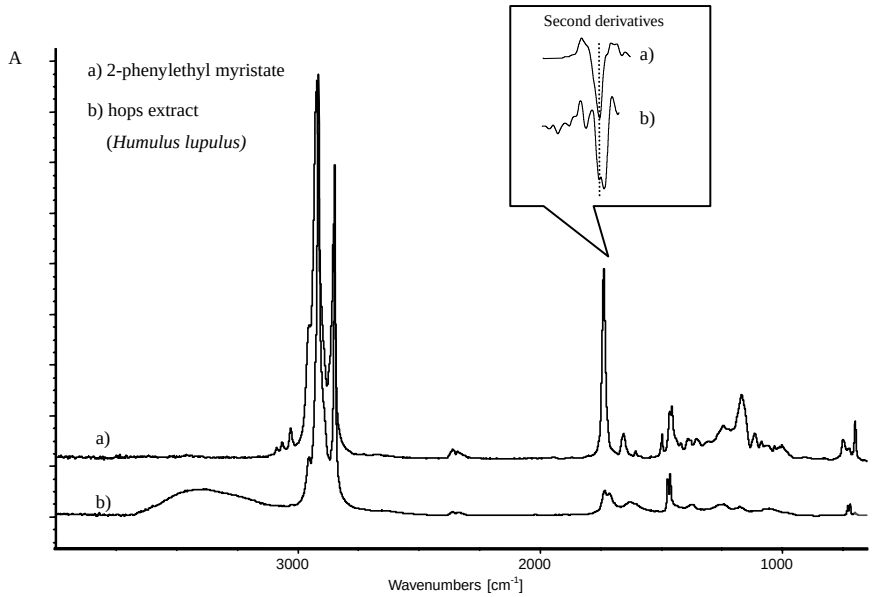

Fig. 5. HATR FT-IR spectra of reference aromatic standard (2-PEM) and hops (*Humulus lupulus*) epicuticular wax extract.

extract in the wavenumber range 4000–600 cm^{-1}. The bands at 3400 cm^{-1} (ν_{OH}) and 1650 cm^{1} (δ_{H_2O}) were characteristic for water. Bands at 2916, 2849, 729 and 719 cm^{-1} were assigned to the asymmetric (ν_{as}), symmetric (ν_s) CH_2 stretching and methylene rocking (δ_{CH_2}) vibrations, respectively. In addition, carbonyl absorption in the range of 1740–1700 cm^{-1} was observed. It clearly reveals (Fig. 3) that aromatic components might be recognized in case of extract analysis alone. The absence of the 699 cm^{-1} band in the spectrum for the fresh leaf strongly suggests that aromatic components are distributed in a deeper part of the wax layer than that which is penetrated by the infrared beam. Aromatic compounds were found in plant epicuticular wax in small amounts (0.5–2.5%) and for this reason visibility of aromatic absorption is poor. Near the δ_{CH_2} bands (729, 718 cm^{-1}) of the aliphatic chain, a weak aromatic band δ_{CH}^{oop} (699 cm^{-1}) is located. The diagnostic meaning of this band is of great importance because it allows the recognition of aromatic compounds in lipid mixtures. Fig. 4 shows spectra of hops leaf extract and reference aromatic standards in the range of 900–650 cm^{-1}. An absorption band at 699 g cm^{-1}, characteristic for 2-phenylethyl esters, was found in the hops spectrum. This indicates that hops cuticular wax extract contains such compounds, and in accordance with standard spectra (Fig. 2) theirs concentration is above 2%.

Furthermore, the carbonyl range provides valuable analytical information [1]. Due to the relatively broad carbonyl ν_{CO} band in the hops extract spectrum, the second derivative function [9] was applied (Fig. 5). This transformation displayed two absorption bands at 1738 and 1736 cm^{-1}, which are assigned to the reference standard 2-phenylethyl ester and to the wax esters, respectively.

Thus, this study has shown that detailed analysis of the δ_{CH}^{oop} and ν_{CO} bands could point to the presence of phenyl esters in plant waxes. It should be taken into consideration that the wax may change in composition during the ontogeny of the plant organ, and data obtained from this study may represent only a momentary average composition of these lipids.

6. Conclusions

HATR provides a simple means of the direct handling of plant material. Through the comparison with synthesized reference standards, it makes the detection of aromatic components of epicuticular waxes possible.

References

[1] E.N. Dubis, A.T. Dubis, J.W. Morzycki, J. Mol. Struct. 511–512 (1999) 175.
[2] P.G. Gülz, F.J. Marner, Z. Naturforsch. 41c (1986) 673.
[3] P.G. Güz, F.J. Marner, S. Guth, Z. Naturforsch. 47c (1992) 516.
[4] P.G. Gülz, E. Muller, T. Herrmann, Z. Naturforsch. 48c (1993) 689.
[5] G. Bianchi, G. Vlahov, C. Anglani, C. Murelli, Phytochemistry 32 (1993) 49.
[6] R.J. Hamilton (Ed.), Waxes: Chemistry, Molecular Biology and Function Plant WaxesThe Oil Press, Dundee, 1995 Chapter 5.
[7] S. Merk, A. Blume, M. Riederer, Planta 204 (1998) 44.
[8] R.M. Silverstein, G.C. Bassler, T.C. Morrill, Spectrometric Identification of Organic Compounds, 5th ed., Wiley, New York, 1991.
[9] M.K. Ahmed, J. Daun, D. DeClercq, Appl. Spectrosc. 52 (1998) 990.