JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# High-Field Nuclear Magnetic Resonance (NMR) Study of Truffles (*Tuber aestivum vittadini*)

LUISA MANNINA,[†,§] MICHELA CRISTINZIO,[†] ANATOLI P. SOBOLEV,*[,§] PIETRO RAGNI,[§] AND ANNALAURA SEGRE[§]

Department STAAM, University of Molise, 86100 Campobasso, Italy, and Institute of Chemical Methodologies, CNR, 00016 Monterotondo Stazione, Rome, Italy

A high-field NMR technique was used to analyze aqueous and organic extracts of truffles (*Tuber aestivum vittadini*) to characterize their chemical composition. Water-soluble metabolites belonging to different classes such as sugars, polyols, amino acids, and organic acids were almost completely assigned by means of one- and two-dimensional experiments ($^1$H−$^1$H COSY, TOCSY, $^1$H−$^{13}$C HSQC, $^1$H−$^{13}$C HMBC, and $^1$H−$^{31}$P HMBC). The $^1$H spectral assignment of the cell membrane components such as lipids, sterols, and fatty acids extracted in organic solvents was also performed.

**KEYWORDS: Truffles; *Tuber aestivum vittadini*; chemical composition; metabolites; NMR**

## INTRODUCTION

Truffles are the fruiting bodies of the hypogeous fungus that grows in symbiosis with trees such as oaks. Due to their typical organoleptic characteristics, the "black diamonds" have a relevant gastronomic interest and are widely used in European and Chinese cooking. Owing to the commercial value of truffles and the actual interest in the naturally occurring bioactive metabolites of fungi, in past years most research (*1*, *2*) has been focused not only on the formation, the morphology, and the ultrastructure of the truffles (*3*, *4*) but also on the truffle chemical composition, namely, on sterolic and lipidic compositions (*5*).

Different methodologies such as chemical analysis and chromatographic and nuclear magnetic resonance (NMR) techniques are usually used to analyze food. High-resolution NMR is a powerful tool to characterize and to assess the quality and the shelf life of food (*6*−*13*). For instance, it has been shown that a high-resolution $^{13}$C NMR technique can provide valuable information about the fatty acids' positional distribution in the glycerol moiety of triglycerides (*14*, *15*): this information can be of crucial importance in the cultivar characterization of olive oils (*16*), in the analytical characterization of edible oils, and also in the prevention of frauds. On the other hand, the application of high-resolution $^1$H NMR allowed us to suggest new methodologies for the characterization of olive oils (*17*, *18*), coffee (*11*), and tomato juice (*6*).

Recently, we have been working on a research project funded by the Italian Ministry of Research aimed to enhance by an ionizing radiation treatment the safety and wholesomeness of fresh products subject to the bacterial contamination (*19*). Specifically, we have monitored by NMR and ultraviolet (UV) techniques the effect of the irradiation treatment on *Tuber aestivum vittadini* extracts. Preliminary results suggested that a low irradiation level (<1.5 kGy) did not modify the truffle chemical composition: indeed, the $^1$H NMR spectra of aqueous and organic extracts of non-irradiated and low-dose-irradiated truffles did not show significant differences, showing only small variations in the intensity of some unassigned NMR resonances. However, an accurate analysis of the metabolites present in truffle extracts seems to be interesting and necessary.

In this paper, a detailed study of the $^1$H NMR spectra of the organic and aqueous extracts of *T. aestivum vittadini* truffles is reported, to our knowledge, for the first time. A large number of compounds such as amino acids, organic acids, carbohydrates, fatty acids, sterols, and lipids have been identified.

## MATERIALS AND METHODS

**Sample Extraction.** *T. aestivum vittadini* truffles originating from middle-southern Italy were collected. Samples for NMR measurements were prepared according to the following procedure: Truffles, ∼8 g, were cut and put into a ceramic mortar, adding 24 mL of a mixture chloroform/methanol (1:1 v/v). The sample was ground and homogenized with a ceramic pestle, and 8 mL of purified water was added. All operations were carried out under $N_2$ flow to avoid oxidation processes. The homogenate was centrifuged at a speed of 10000 rpm for 20 min at 4 °C. A three-phase system was obtained: the two liquid phases, that is, chloroform and water/methanol phases, were separated and dried. The two dry residues were placed into 5 mm tubes and dissolved in $CDCl_3$ (0.6 mL) and $D_2O$ (0.6 mL); the aqueous extract had a neutral pH value. Finally, the tubes were flame-sealed.

**NMR Measurements.** NMR spectra of truffle extracts were recorded at 300 K on a Bruker AVANCE AQS600 spectrometer operating at the proton frequency of 600.13 MHz and equipped with a Bruker multinuclear *z*-gradient inverse probehead capable of producing gradients in the *z* direction with a strength of 55 G cm$^{-1}$. Proton spectra were referenced to the 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (TSP), signal ($\delta = 0.00$ ppm) in $D_2O$ and to the $CH_3$ resonance of

* Corresponding author (telephone +39 0690672385; fax +39 0690672477; e-mail anatoli.sobolev@imc.cnr.it).
† University of Molise.
§ CNR.

10.1021/jf048917v CCC: $27.50 
Published on Web 12/04/2004

saturated fatty chains ($\delta = 0.823$ ppm) in $CDCl_3$, whereas $^{13}C$ spectra were referenced to the $CH_3$ resonance of alanine (16.8 ppm) in $D_2O$ and to the $CH_3$ resonance of the oleic fatty chain (14.1 ppm) in $CDCl_3$.

The molar ratio of a given metabolite was calculated using the corresponding integral. The detection limit of a given metabolite, analyzed in a 5 mm tube using one-dimensional (1D) $^1H$ NMR spectroscopy at high field (11−16 T), is ∼100 $\mu$M (*20*).

The $^1H$ spectrum of the aqueous extract was acquired by co-adding 512 transients with a recycle delay of 3 s. The water signal was suppressed using a solvent presaturation (NOESY-presat scheme) during the relaxation delay and the mixing time (*21*). The experiment was carried out by using 90° flip angle pulses of 15.5 $\mu$s, 32K data points, and a mixing time of 160 ms. The $^1H$ spectrum of the $CDCl_3$ extract was obtained using 64K data points, a recycle delay of 2 s, and a 90° flip angle pulse of 10 $\mu$s.

The $\{^{31}P\}^1H$-decoupled spectrum was obtained using a GARP pulse sequence for the $^{31}P$ decoupling with a 90° $^{31}P$ pulse of 120 $\mu$s and a recycle delay of 2 s.

The $\{^1H\}^{31}P$ NMR spectrum was performed using a 5 mm broadband probehead by co-adding 512 transients with a recycle delay of 7 s, a 20 kHz spectral width, 8K data points, a GARP pulse sequence for proton decoupling, and a 90° $^{31}P$ pulse of 10 $\mu$s. Chemical shifts for the $^{31}P$ spectrum are given in parts per million with respect to an external standard of 85% $H_3PO_4$.

Two-dimensional (2D) NMR experiments, that is, $^1H-^1H$ COSY, $^1H-^1H$ TOCSY, $^1H-^{13}C$ HSQC, and $^1H-^{13}C$ HMBC (*22*) were performed using the same experimental conditions previously reported (*6*).

The $^1H-^{31}P$ HMBC spectrum (*22*) was obtained using a recycle delay of 2 s, a 90° $^1H$ pulse of 15.5 $\mu$s and a 90° $^{31}P$ pulse of 15 $\mu$s and 6 and 9 kHz spectral widths in proton and phosphorus dimensions respectively, 1K data points in $F_2$, 512 increments in $F_1$, and a linear prediction up to 1K points in $F_1$. The experiment was processed using unshifted sinusoidal window functions in both dimensions. The delay for the evolution of long-range couplings in $^1H-^{13}C$ HMBC and $^1H-^{31}P$ HMBC experiments was 80 ms.

$^1H$ diffusion filter-edited NMR spectra were obtained using a stimulated echo pulse sequence incorporating bipolar gradient pulses and a longitudinal eddy current delay (BPP-LED) (*23*) with a $\Delta$ of 800 ms, a $\delta/2$ of 2.1 ms, and a longitudinal eddy current delay of 25 ms. A gradient pulse recovery time of 0.1 ms, 50 and 95% of the maximum gradient intensity, was used. One hundred and sixty scans were accumulated in a 32K time domain. The $T_1$ value for different metabolites in aqueous extract is in the range of 0.5−2 s.

## RESULTS AND DISCUSSION

The NMR characterization of the extracts in aqueous solution and in organic solvent will be discussed separately.

**NMR Analysis of the Aqueous Extract.** The $^1H$ NMR spectrum of the truffle extract in $D_2O$ (see **Figure 1**) is rather complex, showing a strong signal overlapping, especially in the region between 3.0 and 4.0 ppm.

To assign the $^1H$ spectrum and, therefore, to identify the different metabolites present in the aqueous mixture, 1D experiments ($^1H$ and $^{31}P$), 2D conventional experiments ($^1H-^1H$ COSY, $^1H-^1H$ TOCSY, $^1H-^{13}C$ HSQC, $^1H-^{13}C$ HMBC, and $^1H-^{31}P$ HMBC) as well as $^1H$-diffusion-edited experiments were performed. Literature chemical shift data were used as guidelines for assignments (*20*).

*Carbohydrates*. In the aqueous extract of truffles, many saccharidic compounds are present, even though their concentrations are quite low (*24*). This is clearly evidenced by the 2D HSQC map (see **Figure 2**), which shows many cross-peaks inside the $^1H$ anomeric region (4.4−5.7 ppm) and the corresponding $^{13}C$ resonances (90−110 ppm). Only the saccharidic compounds present in a significant amount were identified, namely, glucose in the $\alpha$ and $\beta$ configurations and D-trehalose (see **Table 1**). D-Trehalose was identified by means of its characteristic spin system in the TOCSY map, which allowed us to assign the glucopyranosyl units and literature data (*20*). D-Trehalose occurs widespread in nature (*25*) and plays an important role in the protection of cells from adverse environmental conditions. Besides, this disaccharide represents an important reserve of carbohydrates during periods of carbon starvation.

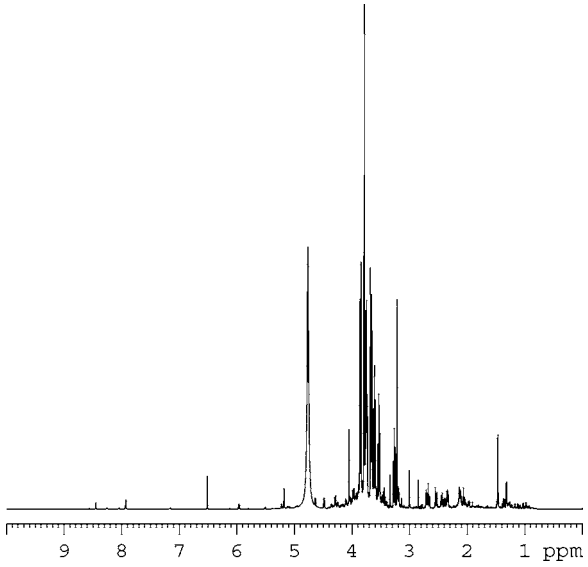


**Figure 1.** 600.13 MHz $^1H$ NMR spectrum of the aqueous truffle extract at 300 K.

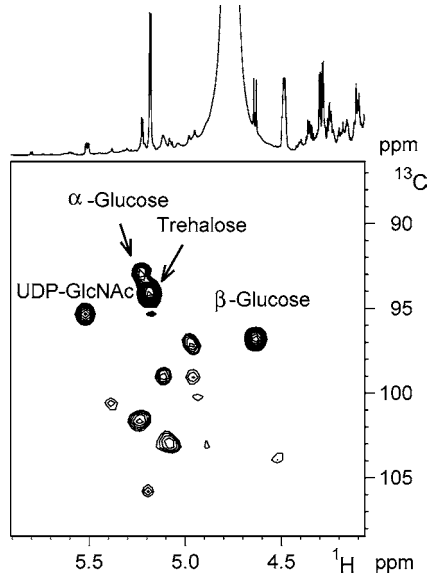


**Figure 2.** Anomeric spectral region of the $^1H$–$^{13}C$ HSQC map of the aqueous truffle extract.

Due to the signal overlapping and their low concentration, high molecular weight polysaccharides are not easily detectable in the $^1H$ spectrum (see **Figure 3a**): in fact, many resonances are either barely observable that is, observable only after a significant vertical expansion (**Figure 3b**) or completely hidden under other strong signals. However, the presence of polysaccharides can be evidenced by means of $^1H$-diffusion-filter-edited experiments (*23*). In these experiments, the application of strong magnetic field gradients allows the contribution of low molecular weight metabolites to be suppressed: the resulting $^1H$ spectra show only the resonances due to compounds with a high molecular weight. In **Figure 3c**, two $^1H$-diffusion-filter-edited spectra performed with gradients of different strengths are

**Table 1.** Summary of the Metabolites Identified in the 600 MHz $^1$H Spectrum of the Aqueous Extract of Truffles[a]

| compound | assignment | $^1$H (ppm) | multilicity: *J* (Hz) | $^{13}$C ($^{31}$P)[b] (ppm) | molar ratio (%) |
|---|---|---|---|---|---|
| | | Carbohydrates | | | |
| $\beta$-glucose ($\beta$-Glc) | H-1 | 4.64 | d: 7.9 | 96.6 | 1.9 |
| | H-2 | 3.24 | | 74.8 | |
| | H-3 | 3.48 | | 76.6 | |
| | H-4 | 3.40 | | 70.2 | |
| | H-5 | 3.45 | | 76.6 | |
| | H-6 | 3.72 | | 61.3 | |
| | H-6′ | 3.87 | | 61.3 | |
| $\alpha$-glucose ($\alpha$-Glc) | H-1 | 5.23 | d: 3.8 | 92.7 | 1.1 |
| | H-2 | 3.52 | | 72.8 | |
| | H-3 | 3.71 | | 73.6 | |
| | H-4 | 3.41 | | 70.2 | |
| | H-5 | 3.82 | | 72.8 | |
| | H-6 | 3.82 | | | |
| | H-6′ | | | | |
| D-trehalose | H-1 | 5.18 | d: 3.8 | 93.9 | 2.0 |
| | H-2 | 3.63 | | | |
| | H-3 | 3.84 | | | |
| | H-4 | 3.43 | | | |
| | H-5, H-6 | | | | |
| | | Sugar Alcohols and Choline | | | |
| *myo*-inositol | H-1 | 4.04 | t: 2.9 | 72.9 | 16.6 |
| | H-2, 5 | 3.53 | dd: 10.0; 2.9 | 71.7 | |
| | H-3, 6 | 3.61 | t: 10.0 | 73.2 | |
| | H-4 | 3.27 | t: 10.0 | 75.1 | |
| mannitol | H-1,6 | 3.85 | dd: 11.8; 2.9 | 64.0 | 43.4 |
| | H-1′,6′ | 3.67 | dd: 11.8; 6.2 | 64.0 | |
| | H-2,5 | 3.75 | ddd: 8.5; 6.2; 2.9 | 71.5 | |
| | H-3,4 | 3.79 | d: 8.5 | 69.9 | |
| choline | $N(CH_3)_3^+$ | 3.22 | s | 54.6 | 2.9 |
| | $\alpha$-$CH_2$ | 4.48 | m | 63.0 | |
| | $\beta$-$CH_2$(OH) | 3.75 | | | |
| | | UDP Sugars | | | |
| uridine 5′-(diphospho-*N*-acetylglucosamine) | C═O (2) | | | 152.3 | 0.6 |
| | H-5 | 5.96 | d: 8.0 | 102.9 | |
| | H-6 | 7.93 | d: 8.0 | 142.2 | |
| | C═O (4) | | | 166.9 | |
| | H-1′ | 5.97 | d: 4.5 | 88.9 | |
| | H-2′ | 4.35 | m | 74.4 | |
| | H-3′ | 4.28 | | | |
| | H-4′ | 4.18 | | | |
| | H-5′a, H-5′b | 4.03 | | | |
| | H-1″ (glucose) | 5.51 | dd: 7.2 ($J_{P-H}$); 3.5 | 95.0 | |
| | H-2″, H-3″, | 3.99, 3.95, | | | |
| | H-4″, H-5″ | 3.80, 3.55 | | | |
| | O–P(O)$_2$–O | | | (–12.0)[b] | |
| | O–P′(O)$_2$–O | | | (–10.2)[a] $J_{P-P} = 20$ | |
| uridine 5′-(diphosphoglucosamine) | H-1″ (glucose) | 5.60 | dd: 7.2 ($J_{P-H}$); 3.5 | | |
| | H-2″ | 3.55 | | | |
| | O–P(O)$_2$–O | | | (–11.9)[b] | |
| | O–P′(O)$_2$–O | | | (–10.5)[b] $J_{P-P} = 20$ | |
| | | Organic Acids | | | |
| A. malic (Mal) | $\alpha$-CH | 4.29 | dd: 9.7; 3.1 | 71.0 | 6.8 |
| | $\beta$-CH | 2.66 | dd: 15.3; 3.2 | 43.1 | |
| | $\beta$′-CH | 2.37 | dd: 15.3; 9.9 | 43.1 | |
| | COOH | | | 180.3 | |
| | COOH | | | 181.5 | |
| A. citric | $\alpha$, $\gamma$-CH | 2.54 | d: 16.1 | 45.6 | 3.9 |
| | $\alpha'$, $\gamma'$-CH | 2.69 | d: 16.1 | 45.6 | |
| | $\beta$-C | | | 76.5 | |
| | 1,5-COOH | | | 179.3 | |
| | 6-COOH | | | 182.1 | |
| A. fumaric | $\alpha$,$\beta$-CH═CH | 6.51 | s | 135.9 | 1.0 |
| | COOH | | | 175.2 | |
| A. formic | HCOOH | 8.45 | s | | |
| A. succinic | $\alpha$,$\beta$-$CH_2$ | 2.40 | s | 34.7 | |
| | 1,4-COOH | | | 182.5 | |
| A. acetic | $\alpha$-$CH_3$ | 1.91 | s | 24.2 | |
| | COOH | | | 182.1 | |

**Table 1.** (Continued)

| compound | assignment | $^1$H (ppm) | multilicity: *J* (Hz) | $^{13}$C ($^{31}$P)[b] (ppm) | molar ratio (%) |
|---|---|---|---|---|---|
| | | Amino Acids | | | |
| valine (Val) | α-CH | 3.62 | | | 0.3 |
| | β-CH | 2.26 | m | 29.8 | |
| | γ-$CH_3$ | 0.98 | d: 7.0 | 17.3 | |
| | γ′-$CH_3$ | 1.03 | d: 7.0 | 18.7 | |
| | COOH | | | 175.0 | |
| alanine (Ala) | α-CH | 3.79 | | 51.3 | 4.5 |
| | β-$CH_3$ | 1.47 | d: 7.3 | 16.8 | |
| | COOH | | | 176.5 | |
| isoleucine (Ile) | α-CH | 3.68 | | 60.4 | 0.2 |
| | β-CH | 1.97 | | 36.6 | |
| | γ-$CH_3$ | 1.00 | d: 7.1 | 15.4 | |
| | γ-CH | 1.26 | | 25.2 | |
| | γ′-CH | 1.47 | | | |
| | δ-$CH_3$ | 0.93 | t: 7.6 | | |
| | COOH | | | | |
| glutamate (Glu) | α-CH | 3.75 | m | 55.1 | 4.4 |
| | β-CH | 2.05 | m | 27.6 | |
| | β′-CH | 2.11 | m | | |
| | γ-CH | 2.34 | m | 34.2 | |
| | δ-COOH | | | 181.8 | |
| | COOH | | | 175.2 | |
| glutamine (Gln) | α-CH | 3.77 | | 55.1 | 3.1 |
| | β-CH | 2.13 | m | 26.8 | |
| | β′-CH | 2.13 | m | 26.8 | |
| | γ-CH | 2.44 | m | 31.6 | |
| | δ-CO($NH_2$) | | | 178.2 | |
| | COOH | | | 174.5 | |
| phenylalanine (Phe) | α-CH | 3.99 | | 55.0 | |
| | β-CH | 3.17 | | 39.0 | |
| | β′-CH | 3.27 | | 39.0 | |
| | C-1, ring | | | 131.0 | |
| | C-2,6, ring | 7.31 | m | 130.0 | |
| | C-3,5, ring | 7.41 | m | 129.6 | |
| | C-4, ring | 7.36 | m | 128.5 | |
| | COOH | | | 174.5 | |
| aspartate (Asp) | α-CH | 3.90 | dd: 8.6; 3.8 | 53.0 | |
| | β-CH | 2.68 | dd: 17.3; 8.6 | 37.2 | |
| | β′-CH | 2.80 | dd: 17.4; 3.8 | 37.2 | |
| | γ-COOH | | | 179.9 | |
| | COOH | | | 170.0 | |
| threonine (Thr) | α-CH | 3.60 | m | 61.1 | 1.5 |
| | β-CH | 4.27 | | 66.7 | |
| | γ-$CH_3$ | 1.32 | d: 6.4 | 20.5 | |
| | COOH | | | 173.4 | |
| glycine (Gly) | α-$CH_2$ | 3.55 | s | 42.2 | 3.5 |
| | COOH | | | 173.0 | |
| histidine (His) | α-CH | 4.0 | | | 0.3 |
| | β-CH | 3.22 | | 28.0 | |
| | β′-CH | 3.22 | | | |
| | C-2, ring | 8.04 | s | 136.6 | |
| | C-4, ring | 7.15 | s | 117.8 | |
| | C-5, ring | | | 131.3 | |
| | COOH | | | | |
| tyrosine (Tyr) | α-CH | 3.94 | | 55.7 | |
| | β-CH | 3.08 | | | |
| | β′-CH | 3.14 | | | |
| | C-1, ring | | | | |
| | C-2,6, ring | 6.88 | | 116.5 | |
| | C-3,5, ring | 7.18 | | 131.5 | |
| | COOH | | | | |
| | | Phosphodiesters | | | |
| glycerophosphoryl-ethanolamine (GPE) | | | | (1.16)[b] | |
| serine–ethanolamine phosphate (SEP) | | | | (0.84)[b] | |
| glycerophosphorylcholine (GPC) | | | | (0.61)[b] | |
| threonine–ethanolamine phosphate (TEP) | | | | (0.69)[b] | |

[a] $^1$H, $^{13}$C, and $^{31}$P chemical shifts are reported with respect to TSP signal ($\delta$ = 0.00 ppm), to $CH_3$–Ala resonance at 16.8 ppm, and to 85% $H_3PO_4$ resonance, respectively. The molar ratio (%) of major components is also reported. [b] $^{31}$P chemical shifts are given in parentheses.

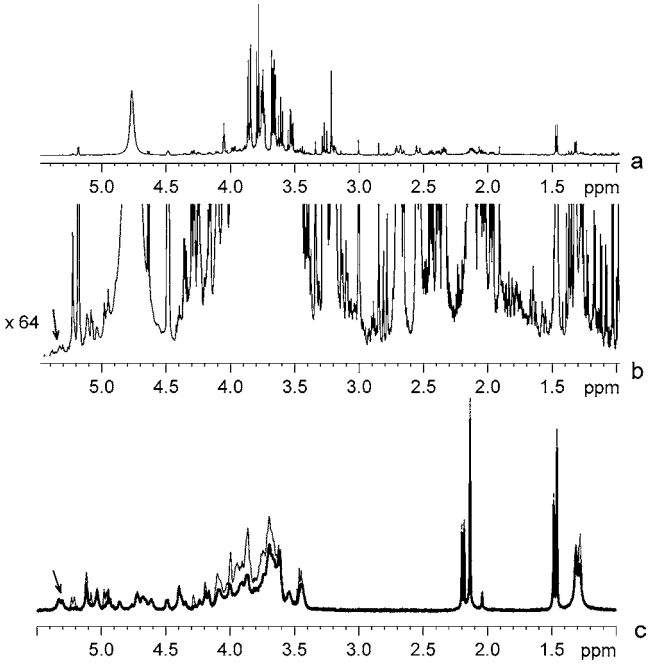


**Figure 3.** (**a**) 600.13 MHz $^1$H NMR spectrum of the aqueous truffle extract; (**b**) the same spectrum with a strong vertical expansion; (**c**) a stacked plot of two $^1$H NMR spectra performed on the same sample with diffusion filter (BPP-LED pulse sequence): 50% (thin line) and 95% (thick line) of the maximum gradient strength were used.

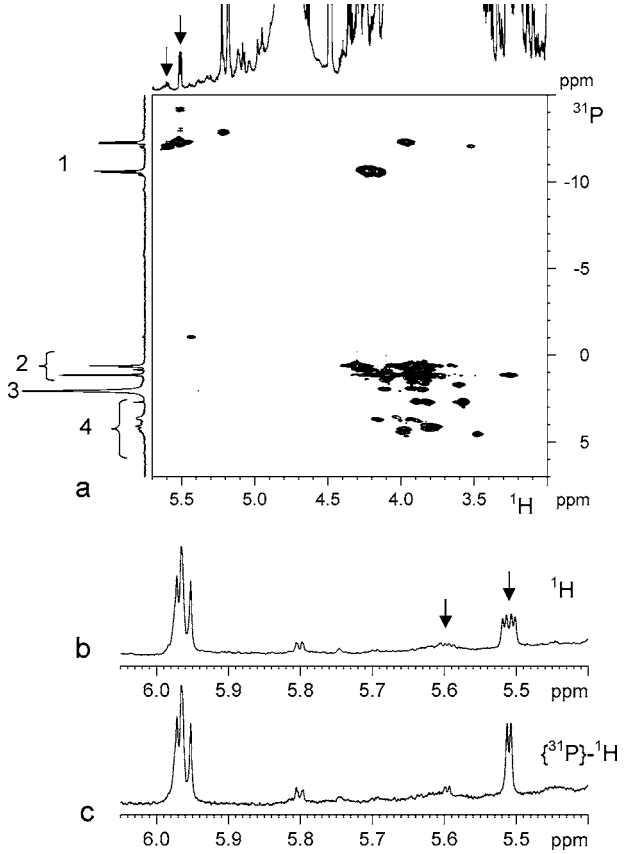


**Figure 4.** (**a**) $^1$H–$^{31}$P HMBC experiment performed on the aqueous truffle extract; $^1$H (horizontal projection) and $^{31}$P{$^1$H}(vertical projection) spectra are also reported ($^{31}$P spectral assignment: 1, UDP-GlcNAc; 2, phosphodiesters; 3, inorganic phosphate; 4, phosphomonoesters); (**b**) Expansion of UDP sugar region of $^1$H spectrum and (**c**) {$^{31}$P}$^1$H spectrum of the same aqueous truffle extract.

reported. These spectra show some differences suggesting the presence of a few high molecular weight metabolites with different diffusion coefficients. The resonances in the 2.0−2.4 ppm spectral range could arise from acetyl groups in the saccharidic moiety (*26*), whereas those at 1.3 ppm are from methyl groups of pectic moieties (*27*).

*Sugar Alcohols and Choline.* Polyols, namely, mannitol and *myo*-inositol, were also identified by means of 2D experiments (see **Table 1**). Mannitol, the most abundant polyol in many sporocarps (*24*), is an osmoticum as well as an energy source. The presence of choline was indicated by its characteristic resonance at 3.22 ppm due to the $N^+(CH_3)_3$ group and was confirmed by adding the standard compound to the sample.

*Uridine Diphosphate (UDP) Sugars*. UDP sugars constitute an important energy source; they were identified in the aqueous extract by means of $^1$H, {$^{31}$P} $^1$H, and $^1$H−$^{31}$P HMBC experiments. Panels **b** and **c** of **Figure 4** show the same 5.40−6.05 ppm spectral region of the $^1$H spectrum performed with (**c**) and without (**b**) the $^{31}$P decoupling: the double doublet present at 5.51 ppm (major component) and 5.60 ppm (minor component) in the $^1$H spectrum became doublets in the $^{31}$P decoupled one. In the 2D $^1$H−$^{31}$P HMBC map, (see **Figure 4a**), the $^1$H resonances at 5.51 and 5.60 ppm, labeled with arrows, show cross-peaks with the $^{31}$P doublets at −12.0 and −11.9, respectively, which, in turn, show cross-peaks with the $^1$H resonance at 3.99 and 3.55 ppm. The $^{31}$P doublets at −12.0 and −11.9 are *J* coupled to the $^{31}$P doublets at −10.2 and −10.5 ppm, which, in turn, correlate to $^1$H resonances at 4.1−4.2 ppm (see **Figure 4a**). These results suggest the presence of a sugar1−O−P(O)$_2$−O−P(O)$_2$−O−sugar2 structure. Finally, 2D TOCSY and $^1$H−$^{13}$C HSQC experiments allowed us to identify the major of two components as uridine-5′-(diphospho-*N*-acetylglucosamine) (UDP-GlcNAc; see **Table 1**). The N-acetylation was in position 2 of glucose, as suggested by the $^1$H downfield shifted resonance of the H-2″ at 3.99 ppm (*20*). The minor compound can be tentatively assigned to uridine diphosphate bound to a non-N-acetylated glucose ring (*28*). It has the H-2″ resonance at 3.55 ppm; this value is typical for nonsubstituted glucose rings.

The {$^1$H}$^{31}$P spectrum, reported as a projection in **Figure 4a**, shows the presence of other $^{31}$P resonances that we assigned according both to literature data (*28*−*30*) and to the $^1$H−$^{31}$P map; specifically, the four distinct resonances at 1.16, 0.84, 0.61, and 0.69 ppm are due to phosphodiesters, namely, glycerophosphorylethanolamine (GPE), serine−ethanolamine phosphate (SEP), glycerophosphorylcholine (GPC), and threonine−ethanolamine phosphate (TEP), respectively; the strong resonance at 2.3 ppm is due to inorganic phosphate, whereas the resonances in the 2.8−5.0 ppm spectral range can be attributed to phosphomonoesters (*30*).

*Organic Acids*. Some organic acids such as malic, citric, fumaric, formic, succinic, and acetic acids were assigned in the proton spectrum (see **Table 1**).

*Amino Acids*. The amino acid composition of mushrooms is strongly dependent on the species (*31*). Eleven essential amino acids were detected in the aqueous extract of truffles using 2D experiments and literature data (*20*). The most abundant amino acids are Ala, Glu, Gln, and Thr, whereas the others are present only in a small amount (see **Table 1**).

*Unknown Compounds.* Few unassigned resonances were observed at 1.36 ppm (a doublet of triplets with $J_{HH}$ = 11.5 and 12.6 Hz), 1.97 ppm (a doublet of doublets of doublets, $J_{HH}$ = 12.6, 5.3, and 1.5 Hz), 3.21, 3.48, 3.55, 3.66, and 3.99 ppm. According to the TOCSY experiment, all of these resonances

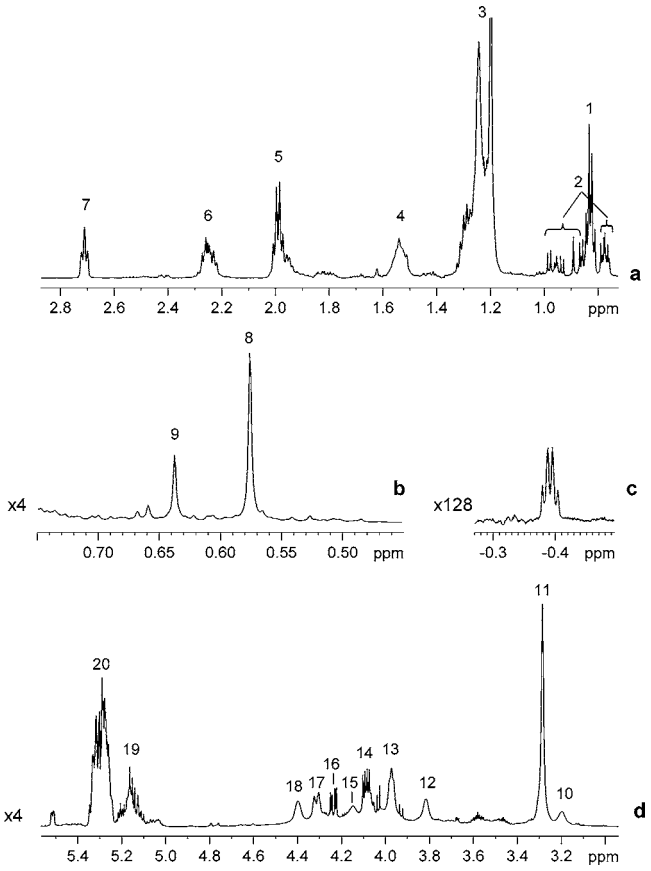


**Figure 5.** 600 MHz $^1$H NMR spectrum of the organic extract: (**a**) fatty chain region (1, $CH_3$, fatty acid chains; 2, $CH_3$, sterols; 3, $CH_2$ fatty acid chains; 4, –$\underline{CH_2}CH_2COO$– fatty acid chains; 5, –$\underline{CH_2}CH{=}CH$–; 6, –$\underline{CH_2}$-COO–; 7, CH=CH$\underline{CH_2}$CH=CH); (**b**) $CH_3$-18 sterolic region (8, $CH_3$-18 of ergosterol; 9, $CH_3$-18 of brassicasterol); (**c**) –0.27 to –0.50 ppm region; (**d**) phospholipidic and double-bond region (10, –$\underline{CH_2}NH_2$, PE; 11, –$N(CH_3)_3$ of PG; 12, –$\underline{CH_2}$N of PC, –$\underline{CH_2}$O–, sphingosine; 13, *sn*3 $CH_2$ of glycerol PC and PE, *CH*–$NH_2$, sphingosine; 14, *sn*1 $CH_2$ of glycerol PC and PE, –CHOH–CH=CH, sphingosine; 15, $CH_2OP$, PE; 16, *sn*1,3 $CH_2$ of glycerol, triglycerides; 17, *sn*1 $CH_2$ of glycerol PC and PE; 18, $CH_2OP$, PC; 19, *sn*2 CH of glycerol PC and PE; 20, CH=CH fatty acid chains).

belong to the same spin system, suggesting that the corresponding compound may be a deoxy sugar.

**NMR Analysis of the Organic Extract.** *Sterols.* The biosynthesis and the distribution of sterols in mushrooms have been reported in many papers (*32*). Using a gas chromatographic method, ergosterol and brassicasterol have been identified as the predominant sterols in fungi and truffles (*1*). According to Crosmun and Carlson (*33*), the $^1$H resonance of the methyl in position 18 of sterols always appears in the 0.6−0.7 ppm narrow range, well separated from all of the other resonances; therefore, it is possible to get information about the sterolic composition in food (*9*) by observing this useful small spectral region. In the case of the organic truffle extract (see **Figure 5b**), two major resonances due to the $CH_3$-18 group of ergosterol and brassicasterol are present; the assignment was confirmed by 2D experiments and by literature data (*2*). Other sterols are also present in very low concentrations ($\leq$100 $\mu$M); some of these sterols have been tentatively identified in the literature as (*2*, *32*) ergost-7-en-3$\beta$-ol, ergosta-7,22-dien-3$\beta$-ol, and ergosta-5,7-dien-3$\beta$-ol.

*Fatty Acids.* The $^1$H NMR technique allows us to obtain the determination only at a class level, that is, monounsaturated, distinct from diunsaturated, distinct from polyunsaturated, and distinct from saturated ones (*8*). In the case of *T. aestivum vittadini*, saturated, mono- and diunsaturated fatty chains were easily identified by some characteristic $^1$H resonances such as the resonances of the methyl groups in 0.8−0.9 ppm spectral region and the resonances of the allylic methylene groups in the 2.6−2.8 ppm spectral range. The assignment, reported in **Table 2**, was confirmed by 2D experiments and by literature data (*5*, *8*, *20*). However, the most abundant mono- and diunsaturated and saturated fatty chains can be identified as oleic, linoleic, and palmitic fatty chains according to the gas chromatographic data reported for mushrooms (*34*, *35*).

In many mushrooms an extremely high degree of unsaturation is present (*5*, *34*), mostly due to the linoleic fatty acid, which is the precursor of the 1-octen-3-ol known as "mushroom alcohol". The percentage of linoleic fatty acid in many fungi is 60−80%, whereas the percentage of oleic fatty chain is <30%. In the case of *T. aestivum vittadini* the fatty acid distribution is rather different because a significant amount of oleic fatty acid is also present. The integration of suitable resonances in the $^1$H spectrum allowed the percentage of saturated, mounsaturated (oleic), polyunsaturated (linoleic), and total unsaturated fatty chains to be determined. In detail, the amount of total unsaturated fatty chains (A, % mol) was calculated using the ratio of integrals of the $-\underline{CH_2}CH{=}CH$ (1.90−2.05 ppm) resonances to that of the $-\underline{CH_2}COO-$ resonances (2.18−2.31 ppm). The amount of polyunsaturated fatty chains (B) was calculated using the ratio of integrals of the $CH{=}CH\underline{CH_2}CH{=}CH-$ (2.66−2.75 ppm) resonances to $-\underline{CH_2}COO-$ resonances (2.18−2.31 ppm). The amount of saturated (C) and monounsaturated (D) fatty chains was therefore a matter of a simple subtraction:

$$C = 100 - A; \qquad D = A - B \tag{1}$$

Saturated and monounsaturated fatty chains resulted to be about 9 and 37% of the total fatty chains, respectively. Polyunsaturated fatty chains constituted ~54%. The amount of total unsaturated fatty chains was ~91%.

*Di- and Triglycerides and Diacylglycerophospholipids.* The $^1$H spectrum of the organic extract shows the characteristic pattern of the glycerol moiety of triglycerides at 4.09 and 4.24 ppm (see **Table 2** and **Figure 5d**). On the other hand, the *sn*1,3-diglycerides, present only in a small amount, were identified by means of the HSQC experiment as reported in **Table 2**.

Different diacylglycerophospholipids are present in the truffle organic extracts (see **Figure 5d** and **Table 2**). The $^1$H resonances of the glycerol moiety of all diacylglycerophospholipids are in the same spectral region: the *sn*2 resonances are in the 5.1−5.2 ppm spectral range, the *sn*1 resonances are at 4.08 and 4.31 ppm, whereas the *sn*3 protons are at 3.97 ppm. A few different diacylglycerophospholipids, namely, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), can be distinguished by the $^1$H resonances of their tail (or headgroup) (*5*). In fact, PC was identified by the characteristic $^1$H singlet of the $-N(\underline{CH_3})_3$ group at 3.29 ppm; moreover, the other methylene groups of the choline tail, namely, $-\underline{CH_2}-N$ and $-\underline{CH_2}-O-P$, were easily assigned resonances at 3.81 and 4.40 ppm, respectively, by 2D spectra and literature data (*5*, *20*). On the other hand, PE was identified by the characteristic signal of the $-\underline{CH_2}-N$ group at 3.20 ppm, which is *J*-coupled to the protons at 4.15 ppm due to the $\underline{CH_2}-O-P$ group.

The molar percentages of triglycerides, PC, and PE, calculated by integrating the $^1$H resonances at 4.24 ppm (TG), 3.29 ppm (PC), and 3.20 ppm (PE) with respect to the total sum are 23, 47, and 30%, respectively.

**Table 2.** Summary of the Metabolites Identified in the 600 MHz $^1$H Spectrum of the $CDCl_3$ Extract of Truffles[a]

| compound | assignment | $^1$H (ppm) | multiplicity: *J* (Hz) | $^{13}$C (ppm) |
|---|---|---|---|---|
| | | Sterols | | |
| ergosterol | C1–C2 | | | |
| | CHOH-3 | 3.58 | m | 70.5 |
| | C4–C5 | | | |
| | CH-6 | 5.52 | dd | 119.7 |
| | CH-7 | 5.33 | m | 116.3 |
| | C8–C17 | | | |
| | $CH_3$-18 | 0.58 | s | 12.1 |
| | $CH_3$-19 | 0.90 | s | 16.3 |
| | CH-20 | 1.99 | m | |
| | $CH_3$-21 | 0.98 | d: 6.5 | 21.1 |
| | CH-22 | 5.16 | m | 132.0 |
| | CH-23 | 5.12 | m | 135.6 |
| | CH-25 | 1.42 | m | |
| | $CH_3$-26 | 0.87 | d: 6.5 | 17.7 |
| | $CH_3$-27 | 0.79 | d: 6.5 | 19.7–19.9 |
| | $CH_3$-28 | 0.77 | d: 6.5 | 19.7–19.9 |
| brassicasterol | C1, C2 | | | |
| | CHOH-3 | 3.47 | m | 71.8 |
| | C4, C5 | | | |
| | C6 | 5.30 | | |
| | C7–C17 | | | |
| | $CH_3$-18 | 0.64 | s | 12.1 |
| | $CH_3$-19 | 0.96 | s | 19.3 |
| | C-20 | | | |
| | $CH_3$-21 | 0.96 | d: 6.5 | 1.01 |
| | CH-22 | 5.15 | m | 132.0 |
| | CH-23 | 5.12 | m | 135.6 |
| | C-25 | | | |
| | $CH_3$-26 | 0.86 | d: 6.5 | 17.7 |
| | $CH_3$-27 | 0.78 | d: 6.5 | 19.7–19.9 |
| | $CH_3$-28 | 0.77 | d: 6.5 | 19.7–19.9 |
| | | Lipids: Fatty Acid Chains | | |
| saturated fatty chain | C1(p,*s*) | | | 176.5, 173.5 |
| (p, palmitic acid; | C2(p,*s*) | 2.22 | | 34.1 |
| *s*, stearic acid) | C3(p,*s*) | 1.54 | m | 25.1 |
| | C4–C13(p) or C4–C15(*s*) | 1.198 | m | 29.2–29.7 |
| | C14(p) or C16(*s*) | 1.198 | m | 31.9 |
| | C15(p) or C17(*s*) | 1.198 | m | 22.6 |
| | C16(p) or C18(*s*) | 0.823 | t: 6.8 | 14.1 |
| oleic fatty chain | C1 | | | 176.5, 173.5 |
| | C2 | 2.22 | | 34.1 |
| | C3 | 1.54 | | 25.1 |
| | C4–C7 | 1.24 | m | 29.4–29.9 |
| | C8 | 1.95 | m | 27.3 |
| | C9–C10 | 5.29 | | 130 |
| | C11 | 1.95 | m | 27.3 |
| | C12–C15 | 1.24 | m | 29.4–29.9 |
| | C16 | 1.24 | m | 31.9 |
| | C17 | 1.24 | m | 22.6 |
| | C18 | 0.823 | t: 6.8 | 14.1 |
| linoleic fatty chain | C1 | | | 176.5, 173.5 |
| | C2 | 2.22 | | 34.1 |
| | C3 | 1.54 | | 25.1 |
| | C4–C7 | 1.28 | | 29.3–29.7 |
| | C8 | 1.99 | q: 7.0 | 27.3 |
| | C9 | 5.30 | m | 130.2 |
| | C10 | 5.30 | m | 128.0 |
| | C11 | 2.71 | t: 7.0 | 25.7 |
| | C12 | 5.30 | m | 128.0 |
| | C13 | 5.30 | m | 130.2 |
| | C14 | 1.99 | q: 7.0 | 27.3 |
| | C15 | 1.28 | m | 29.3 |
| | C16 | 1.28 | m | 31.6 |
| | C17 | 1.28 | m | 22.7 |
| | C18 | 0.83 | t: 7.0 | 14.1 |
| | | Lipids: Di- and Triglycerides | | |
| triglycerides (TG) | CH *sn*2 | 5.26 | m | 68.9 |
| | $CH_2$ *sn*1,3 | 4.24 | dd: 11.7; 4.4 | 62.1 |
| | $CH_2$ *sn*1,3 | 4.09 | dd: 11.7; 6.0 | 62.1 |
| diglycerides (DG) | CH *sn*2 | 5.03 | | 72.1 |
| | $CH_2$ *sn*1 | 4.28; 4.15 | | 62.1 |
| | $CH_2$ *sn*3 | 3.67 | | 61.6 |

**Table 2.** (Continued)

| compound | assignment | $^1$H (ppm) | multiplicity: *J* (Hz) | $^{13}$C (ppm) |
|---|---|---|---|---|
| | Lipids: Diacylglycerophospholipids | | | |
| phosphatidylcholine (PC) | CH *sn*2 | 5.15 | | 70.6 |
| | $CH_2$ *sn*1 | 4.31; 4.08 | | 62.4 |
| | $CH_2$ *sn*3 | 3.97 | | 64.7 |
| | $CH_2OP$ | 4.40 | | 60.5 |
| | $CH_2N$ | 3.81 | | 66.3 |
| | $N(CH_3)_3$ | 3.29 | | 54.7 |
| phosphatidylethanolamine (PE) | CH *sn*2 | 5.15 | | 70.6 |
| | $CH_2$ *sn*1 | 4.31; 4.08 | | 62.4 |
| | $CH_2$ *sn*3 | 3.97 | | 64.7 |
| | $CH_2OP$ | 4.15 | | 62.9 |
| | $CH_2N$ | 3.20 | | 40.4 |
| | $-NH_3^+$ | 8.24 | | |
| | Sphingolipids | | | |
| sphingosine | $-NH_2$ | 7.60 | | |
| | CH=CH | 5.69 | | 134.0 |
| | CH=CH | 5.40 | | 129.0 |
| | $CH_2$–CH=CH | 1.99 | | |
| | CHOH–CH=CH | 4.09 | | |
| | CH–$NH_2$ | 3.99 | | |
| | $CH_2O$– | 3.79 | | |

[a] $^1$H and $^{13}$C chemical shifts are reported with respect to the $CH_3$ resonance of fatty chains at 0.823 and 14.1 ppm, respectively.

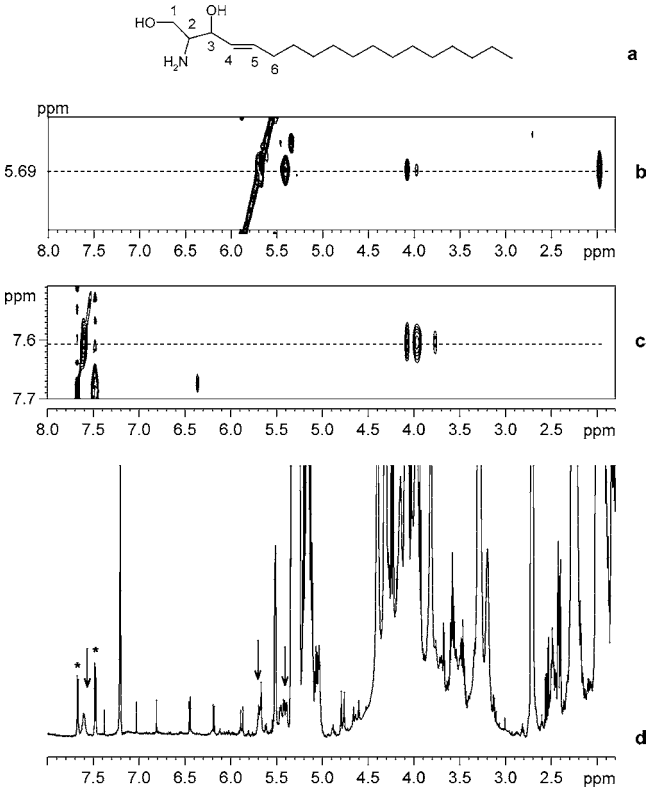


**Figure 6.** (**a**) Chemical structure of sphingosine; (**b**, **c**) slices of TOCSY spectrum of truffle organic extract; (**d**) the $^1$H NMR spectrum. The arrows indicate the resonances due to sphingosine; asterisks denote impurities in the solvent.

*Sphingolipids.* Sphingolipids are defined by their characteristic 1,3-dihydroxy-2-aminoalkane (sphingoid base) backbones. The presence of this class of compounds in mushrooms has been previously reported (*5*). Sphingosine was identified by its specific spin system observed in the TOCSY experiment (see **Figure 6a**−**d**); the $NH_2$ group at 7.60 ppm shows correlation peaks with protons at 4.09, 3.99, and 3.79 ppm due to CHOH−CH=CH, CH−$NH_2$, and $CH_2O$−, respectively. The resonance at 5.69 ppm of the CH-4 group of the double bond shows correlations with protons at 5.40 ppm (CH-5, double bond), 4.09 ppm (CHOH−CH=CH), 3.99 ppm (CH−$NH_2$), and 1.99 ppm ($CH_2$-6) (see **Figure 6b**). The signals labeled with asterisks in **Figure 6d** do not relate to truffle metabolites, because they are also present as impurities in the $^1$H spectrum of the solvent.

*Unknown Compounds.* Few unassigned resonances were observed in the $^1$H spectrum of the $CDCl_3$ extract. In particular, we observed an AB spectrum consisting of two doublets ($J_{HH}$ = 8 Hz) at 6.19 and 6.44 ppm, directly correlated with $^{13}$C signals at 130.8 and 135.5 ppm, respectively. Both doublets showed long-range correlations in the HMBC experiment with two quaternary carbons at 79.5 and 82.0 ppm. This information suggests the presence of a compound with a nonsymmetric conjugated *cis* −C−HC=CH−C− fragment or an *ortho* aromatic compound in which quaternary carbons are bound to heteroatoms.

Another weak resonance, even more unusual, was present at −0.39 ppm (see **Figure 5c**). This resonance was one bond coupled to a C atom resonating at 11.0 ppm and showed a complex pattern possibly due to a coupling with a $CH_2$ ($J \approx$ 3.5−4 Hz) and a CH ($J \approx$ 4.5−5 Hz), as shown in **Figure 5c**. The extremely low concentration of the corresponding compound and the presence of diagnostic correlations only in a very crowded spectral region (TOCSY: −0.39; 0.51; 0.59; 1.08; 1.31 ppm) allowed us only to hypothesize the presence of a compound with a three-membered ring.

With the aim of obtaining small amounts of the unassigned compounds and to obtain their structure, the fractionation of truffles is currently in progress.

## ABBREVIATIONS USED

UDP-GlcNAc, uridine-5′-(diphospho-*N*-acetylglucosamine); GPE, glycerophosphorylethanolamine; SEP, serine−ethanolamine phosphate; GPC, glycerophosphorylcholine; TEP, threonine−ethanolamine phosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

## ACKNOWLEDGMENT

Thanks are due to Prof. Raffaele Coppola of the University of Molise, Dep. STAAM for the valuable discussions and support.

The morphological characterization of the studied truffles was performed by Prof. Corrado Fanelli of the University of Rome.

## LITERATURE CITED


(1) Harki, E.; Klaebe, A.; Talou, T.; Dargent, R. Identification and quantification of *Tuber melanosporum Vitt*. sterols. *Steroids* **1996**, *61*, 609−612.

(2) Jinming, G.; Lin, H.; Jikai, L. A novel sterol from Chinese truffles *Tuber indicum*. *Steroids* **2001**, *66*, 771−775.

(3) Parguey-Leduc, A.; Montant, C.; Kulikaj, M. Morphologie et structure de l'ascocarpe adulte du *Tuber melanosporum* Vitt. *Cryptogamie Mycol.* **1987**, *8*, 173−202.

(4) Parguey-Leduc, A.; Montant, C.; Kulikaj, M. Structure et évolution *Tuber melanosporum* Vitt. (Truffe noire du Périgord). *Botanique* **1984**, *299*, 315−320.

(5) Bonzom, P. M. A.; Nicolaou, A.; Zloh, M.; Baldeo, W.; Gibbons, W. A. NMR lipid profile of *Agaricus bisporus*. *Phytochemistry* **1999**, *50*, 1311−1321.

(6) Sobolev, A. P.; Segre, A.; Lamanna, R. Proton high field NMR study of tomato juice. *Magn. Reson. Chem.* **2003**, *41*, 237−245.

(7) Amato, M. E.; Ansanelli, G.; Fisichella, S.; Lamanna, R.; Scarlata, G.; Sobolev, A. P.; Segre, A. Wheat flour enzymatic amylolysis monitored by in situ $^{1}$H NMR spectroscopy. *J. Agric. Food Chem.* **2004**, *52*, 823−831.

(8) Mannina, L.; Segre, A. High-resolution nuclear magnetic resonance: from chemical structure to food authenticity. *Grasas Aceites* **2002**, *53*, 22−33.

(9) Mannina, L.; Sobolev, A. P.; Segre, A. L. Olive oil as seen by NMR and chemometrics. *Spectroscopy* **2003**, *15*, 6−14 and references therein.

(10) Charlton, A. J.; Farrington, W. H. H.; Brereton, P. Application of $^{1}$H NMR and multivariate statistics for screening complex mixtures: quality control and authenticity of instant coffee. *J. Agric. Food Chem.* **2002**, *50*, 3098−3103.

(11) Bosco, M.; Toffanin, R.; Palo, D.; Zatti, L.; Segre, A. High-resolution $^{1}$H NMR investigation of coffee. *J. Sci. Food Agric.* **1999**, *79*, 869−878.

(12) Gil, A. M.; Duarte, I. F.; Delgadillo, I.; Colquhoun, I. J.; Casuscelli, F.; Humpfer, E.; Spraul, M. Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **2000**, *48*, 1524−1536.

(13) Shintu, L.; Ziarelli, F.; Caldarelli, S. Is high-resolution magic angle spinning NMR a practical speciation tool for cheese samples? Parmigiano Reggiano as a case study. *Magn. Reson. Chem.* **2004**, *42*, 396−401.

(14) Mannina, L.; Luchinat, C.; Emanuele, M. C.; Segre, A. Acyl positional distribution of glycerol tri-esters in vegetable oils: a $^{13}$C NMR study. *Chem. Phys. Lipids* **1999**, *103*, 47−55.

(15) Mannina, L.; Luchinat, C.; Patumi, M.; Emanuele, M. C.; Rossi, E.; Segre, A. Concentration dependence of $^{13}$C NMR spectra of triglycerides: implication for the NMR analysis of olive oils. *Magn. Reson. Chem.* **2000**, *38*, 886−890.

(16) Mannina, L.; Dugo, G.; Salvo, F.; Cicero, L.; Ansanelli, G.; Calcagni, C.; Segre, A. L. Study of the cultivar-composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. *J. Agric. Food Chem.* **2003**, *51*, 120−127.

(17) Mannina, L.; Patumi, M.; Proietti, N.; Segre, A.; P. D. O. (Protected Designation of Origin) geographical characterization of Tuscan extra virgin olive oils using high-field $^{1}$H NMR spectroscopy. *Ital. J. Food Sci.* **2001**, *13*, 53−63.

(18) Mannina, L.; Patumi, M.; Proietti, N.; Bassi, D.; Segre, A. Geographical characterization of Italian extra virgin olive oils using high-field $^{1}$H NMR spectroscopy. *J. Agric. Food Chem.* **2001**, *49*, 2687−2696.

(19) Adamo, M.; Capitani, D.; Mannina, L.; Cristinzio, M.; Ragni, P.; Tata, A.; Coppola, R. Truffles decontamination treatment by ionizing radiation. *Radiat. Phys. Chem.* **2004**, *71*, 167−170.

(20) Fan, T. W. M. Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. *Prog. Nucl. Magn. Reson. Spectrosc.* **1996**, *28*, 161−219.

(21) Guéron, M.; Plateau, P.; Decorps, M. Solvent signal suppression in NMR. *Prog. Nucl. Magn. Reson. Spectrosc.* **1991**, *23*, 135−209.

(22) Braun, S.; Kalinowski, H.-O.; Berger, S. In *150 and More Basic NMR Experiments*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 1998.

(23) Johnson, C. S., Jr. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203−256.

(24) Sanmee, R.; Dell, B.; Lumyong, L.; Izumori, K.; Lumyong, S. Nutritive value of popular wild edible mushrooms from northern Thailand. *Food Chem.* **2003**, *82*, 527−532.

(25) Elbein, A. D. The metabolism of α,α-trehalose. *Adv. Carbohydr. Chem. Biochem.* **1974**, *30*, 227−256.

(26) Crescenzi, V.; Francescangeli, A.; Taglienti, A.; Capitani, D.; Mannina, L. Synthesis and partial characterization of hydrogels obtained via glutaraldehyde cross-linking of acetylated chitosan and of hyaluronan derivatives. *Biomacromolecules* **2003**, *4*, 1045−1054.

(27) Schols, H. A.; Voragen, A. G. J.; Colquhoun, I. J. Isolation and characterization of rhamnogalacturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. *Carbohydr. Res.* **1994**, *256*, 97−111.

(28) Teleman, A.; Richard, P.; Toivari, M.; Penttilä, M. Identification and quantitation of phosphorus metabolites in yeast neutral pH extracts by nuclear magnetic resonance spectroscopy. *Anal. Biochem.* **1999**, *272*, 71−79.

(29) Thebault, M. T.; Kervarec, N.; Pichon, R.; Nonnotte, G.; Gal, Y. Le. A $^{31}$P nuclear magnetic resonance study of the hydrothermal vent tube worm *Riftia pachyptila*. *C. R. Acad. Sci. Paris, Sci. Vie* **1999**, *322*, 537−541.

(30) Rasmussen, N.; Lloyd, D. C.; Ratcliffe, R. G.; Hansen, P. E.; Jakobsen, I. $^{31}$P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. *Plant Soil* **2000**, *226*, 245−253.

(31) Manzi, P.; Gambelli, L.; Marconi, S.; Vivanti, V.; Pizzoferrato, L. Nutrients in edible mushrooms: an inter-species comparative study. *Food Chem.* **1999**, *65*, 477−482.

(32) Yokokawa, H.; Mitsuhashi, T. The sterol composition of mushrooms. *Phytochemistry* **1981**, *20*, 1349−1351.

(33) Croasmun, W. R.; Carlson, R. M. K. Steroid structural analysis by two-dimensional NMR. In *Two-Dimensional NMR Spectroscopy: Application for Chemists and Biochemists*, 2nd ed.; Croasmun, W. R., Carlson, R. M. K., Eds.; VCH Publishers: New York, 1994; pp 785−832.

(34) Sancholle, M.; Weete, J. D.; Kulifaj, M.; Montant, C. Change in lipid composition during ascocarp development of the truffles. *Mycologia* **1988**, *80*, 900−903.

(35) Diez, V. A.; Alvarez, A. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chem.* **2001**, *75*, 417−422.


**Received for review July 1, 2004. Revised manuscript received October 5, 2004. Accepted October 5, 2004. This work was funded by the Ministero della Istruzione, Università e Ricerca, within the project PROFSICURI.**

JF048917V