The Annals of the University *Dunarea de Jos* of Galati
Fascicle VI – Food Technology, New Series Year III (XXXIII)
Paper presented at the International Symposium *Euro - aliment 2009*, 9th – 10th of October 2009, Galati – ROMANIA

SYNTHESIS AND PHYSICO-CHEMICAL CHARACTERIZATION OF 2.4-DINITROPHENYL HIDRAZONES DERIVED FROM CARBONYL COMPOUNDS WITH SOME IMPORTANCE IN THE STUDY OF FOOD QUALITY

ZGHEREA GHEORGHE, STOIAN CRISTINA

Department of Chemistry
University "Dunărea de Jos" of Galati, 111 Domnească St., 800201 Galati, Romania
gzgherea@chem.ugal.ro, cstoian@ugal.ro

PERETZ SANDU

Institute of Physical Chemistry "I. Murgulescu", Department of Colloids, 202 Spl. Independentei, 060021, Bucharest, Romania

Received 31 July 2009
Revised 15 August 2009

In many foods, the carbonyl compounds have an important contribution to fragrance and aroma. The identity of carbonyl compounds is dependent on the foods nature; their concentrations are very small. It is not directly an analytical technology the one to carry through the study of carbonyl compounds. This study implies two distinct and successive stages. The first stage is of separation after the transformation stage. The second stage will be an analytical process of identification and dosage, for all compounds formed by all carbonyl compounds. The most elegant method is the transformation in a mixture of 2.4-dinitrophenylhidrayones water insoluble; the mixture is separated by filtration, washed in water to neutral medium and dissolved in appropriate organic solvent. The mixture of 2.4-dinitrophenylhidrazones is separated by HPLC method. The chromatographic process involves the preparation of standard compounds; they are not present in catalogs with offers of substances. The standard hydrazones must be physico-chemically studied.

Keywords: foods, carbonyl compounds, derivatization, filtration, physics-chemical and analytical characterizations

1. Introduction

An exhaustive study of foods assumes complete information about the carbonyl compounds. It is known that the carbonyl compounds are present only in foods, but not in the raw materials. There are two distinct reasons to study carbonyl compounds, as follows: the contribution to sensorial quality (fragrance and aroma) and the level of raw degradations. The concentrations of carbonyl compounds are very small (mg/kg), being very difficult their identification and dosage. Therefore, it is necessary a transformation process of separation and an analytical process of identification and dosage. Under the chemical properties of carbonyl compounds, this might be separates after specific precipitation.

The carbonyl compounds might be transformed in a mixture of bisulphite compounds, insoluble in water (Nenitescu, 1974); the mixture is separated by filtration, followed by washing with water or organic solvents to delete any other compounds. By acid hydrolyze or in sodium carbonate solution, the mixture of bisulphite compounds issued the mixture of carbonyl compounds; next, a chromatographic separation and identification with carbonyl compounds etalons is necessary.

Another way is the transformation of carbonyl compounds in a mixture of 2.4-dinitrophenylhydrazones, insoluble in water, with appropriate reactive in strongly acidic medium. First, the mixtures of hydrazones are separated by filtration and, secondly, it is washed with water until the effluent is neutral. In an organic solvent (methanol, acetonitrile, tetrahydrophuran etc) the mixture of yellow hydrazones has dissolved. The mixture of soluble hydrazones might be separated on a liquid-chromatographic method; for the identification it is necessary to have all the 2.4-dinitrophe-

nylhydrazones etalons; these have big molecules – molecular weight are higher than 200 g/mol. – being very stable in time (Liteanu, 1974, Zgherea, 1998, Gocan, 2002).

In this paper there is much information about any 2.4-dinitrophenylhydrazones provided by carbonyl compounds present in foods; there is also information about the synthesis, the description of purity and the compouns analytical behavior.

2. Materials and Methods

2.1 Materials

An apparatus and three categories of chemical compounds were being used, as follows: carbonyl compounds, solvents and specific reagents.

The carbonyl compounds

To prepare the 2.4-dinitrophenylhydrazones etalons, pure carbonyl compounds normally present in foods, were being used as follows: acetaldehyde, diacetyl (present in beer and other fermented foods), butiraldehyde (present in foods as degradation compounds), 2-heptanona and 2-nonanona (presents in special chesses). In addition, carbonyl compounds were being used as follows for comparison: formaldehyde, glioxal, propanona and propionaldehide.

The solvents

The main solvent utilized was bi distilled water ($\gamma = 2\mu S \cdot cm^{-1}$), prepared in our laboratory with GFL Glass Water Stills type 2302. The methanol, acetonitrile and tetrahydrophuran were used to solve the powder of 2.4-dinitrophenylhydrazones and as a mobile phase in liquid-chromatographic separations.

The specific reagent

A strong acid (sulphuric acid 98%, Merck) solution in water of 2.4-dinitrophenylhydrazine (2.4-DNPH, LOBA-Chemie-Austranal) was utilized to precipite the etalons hydrazones, starting from etalons of carbonyl compounds. In addition, 0.1 M citric acid and 0.2 M $Na_2HPO_4 \cdot 12H_2O$, in water were utilized; by mixing a blank solution [H_2O:CH_3OH = 4:1 (v/v)] and ten buffer solution with pH = 2.6-7 were obtained.

The apparatus

The experimental analysis used: a spectrometer FT-IR, Bruker type Tensor 27, a unichanel spectrophotometer (type T60 V UV-Vis spectrophotometer supplied by PG Instrument Limited with UVWin5.0 analysis software), Pye Unicam Philips liquid-chromatograph type LC-XPD and a computer Windows Vista Home Premium as operating systems.

2.2 Methods and analysis

The reagent preparation

Fill an appropriate conical glass vial of a 500 ml volume with approximately 10 g powder of 2.4-dinitrophenylhydrazine (2.4-DNPH). Pour 150 ml water and, in drops of 1-2 ml, 100 ml sulphuric acid concentrate, continuously mixing until the content has been homogenized. 2.4-DNPH must be complete dissolved when all the acid has been added. The hot mixture is cooled down to ambient temperature by means of an external water jet and the content is then poured into a 1000 ml grade vial. Wash successively the little vial with 25 ml water and add in the 1000 ml vial. Up to the grade methanol is added; at the end must be a clear solution, reddish-brown solution (Zgherea, 1998).

The 2.4-dinitrophenylhydrazones etalons

Mix approximately 50 g of carbonyl compounds with 50 ml water in a 500 ml conical vial. At room temperature, add in drops the reagent - ensuring an excess - and mix until homogenization for carbonyl compound to become 2.4-dinitrophenylhydrazone. 2.4-DNPH-one is separated by dilution

with bi distillated water, as a colored precipitate; the precipitate is submitted, in the upper part of the conical vial appearing a yellow solution, slightly opalescent. The precipitate is successively washed with water and is separated by filtration at low pressure. Wash the precipitate with water, until the effluent has a neutral character. Dry the colored precipitate and keep it in an exicator.

The physico-chemical behavior of 2.4-dinitrophenylhydrazone etalons
2.4-dinitrophenylhydrazone was physically studied and checked by the analytical process behavior. The following determinations were made: elemental analysis, spectrophotometric (IR and UV-Vis) analysis and liquid-chromatographic separations.

3. Results and Discussion

3.1 The elemental analysis

Eight 2.4-DNPH-ones provided by carbonyl compounds with slowly molecular weight was analyzed by elemental analysis method; the Dumas method of nitrogen percentage. Table 1 presents information about the identity of 2.4-DNPH-ones and nitrogen percentage, theoretical and experimental data.

Table 1. The characterization of any 2.4-DNPH-ones provided by carbonyl compounds with C_1-C_4

No.	Carbonyl compound	2.4-DNPH-one (molecular formula and abbreviation)	Molar weight	% nitrogen	
				theoretical	experimental
1	Formaldehyde	$C_7H_6N_4O_4$; 2.4-DNPHF	210	26.66	26.46
2	Acetaldehyde	$C_8H_8N_4O_4$; 2.4-DNPHA	224	25.00	25.16
3	Propionaldehyde	$C_9H_{10}N_4O_4$; 2.4-DNPHAP	238	23.52	24.00
4	Propanona	$C_9H_{10}N_4O_4$; 2.4-DNPHP	238	23.52	23.38
5	Izobutiraldehyde	$C_{10}H_{12}N_4O_4$; 2.4-DNPHiB	252	22.22	22.35
6	Butiraldehyde	$C_{10}H_{12}N_4O_4$; 2.4-DNPHB	252	22.22	22.18
7	Glioxal	$C_{14}H_{10}N_8O_8$; 2.4-DNPHG	418	26.79	26.46
8	Diacetyl	$C_{16}H_{14}N_8O_8$; 2.4-DNPHD	446	25.11	25.84

The diacetyl is a very important carbonyl compound for foods obtained by fermentation. Therefore, the 2.4-DNPH-one of diacetyl was thoroughly studied, obtaining the results: 42.86% C (43.049%, theoretically) and 3.09% H (3.139%, theoretically). It is a favorable conclusion: both carbonyl groups of diacetyl react with 2.4-DNPH.

3.2 The IR spectrophotometry analysis

The spectrometer FT-IR, Bruker type Tensor 27 was used; the 2.4-DNPH-one powder were mixed with anhydrous kalium bromide and modeled as tablet, by compression using high pressure.

In the domain 400-1500 cm^{-1} the absorption signals, due to specific particularity of each 2.4-DNPH-one (Balaban, 1983, Avram, 1966) are present. The 2.4-DNPH-one provided by formaldehyde has the most simple spectra, having the most simple structure. The IR spectra of 2.4-DNPHF-one have only two signals in the domain 1375-1385 cm^{-1}. The NO_2 groups on aromatic structure provide the two specific signals, according to the literature (Mager, 1983, Bacaloglu, 1985). In the IR spectra of upper 2.4-DNPH-ones, the two slow signals are covered by signals of structural particularity.
In the domain 1300-4000 cm^{-1}, the studied 2.4-DNPHAF-ones have two distinct categories of signals, as follows.

a) Small wavelength, λ = 3000-3600 cm^{-1}. There are many absorption bands, the highest having 3500 cm^{-1}. The authors (Bacaloglu, 1985, Hendrickson, 1976) considers that the signal is provided by structural detail amino – NH – without basic character, on account of actions of the vicinal groups. The catalogue of IR spectral bands (Szymansky, 1963) describes many bands in this domain. All the signals are due to NH group bonded to an aromatic inner with two NO_2 groups in *orto*

and *para* positions; all the studied 2.4-DNPH-ones have this structural detail, present in the structure of the reactive.

b) Large wavelength, λ = 1500-1700 cm^{-1}

Figure 1 presents these spectra regions. The absorption band at λ = 1500 cm^{-1} might be attributed to NO_2 independent group, without participation to a conjugated process (Bacaloglu, 1985). The same signal might be attributed to an aromatic inner with two nitro groups in *meta* positions (Balaban, 1983, Avram, 1966).

The absorption band at λ = 1600-1620 cm^{-1} might be attributed to an aromatic ring, being a characteristic signal (Bacaloglu, 1985, Hendrickson, 1976, Szymansky, 1963). The absorption band at λ=1630-1690 cm^{-1} might be attributed to specific –C=N– structural detail; this common structural detail is due to the condensation process, between the carbonyl group (C=O) and the amino group (-NH_2). Depending on the neighbors, the absorption band can appear on different positions. It is known that this absorption band is at λ = 1630 cm^{-1}, if the carbon atom has a direct relation with an aromatic ring (C_6H_5-C=N-). If the carbon atom has a direct relation with an aliphatic catena, the band is at λ = 1670 cm^{-1}.

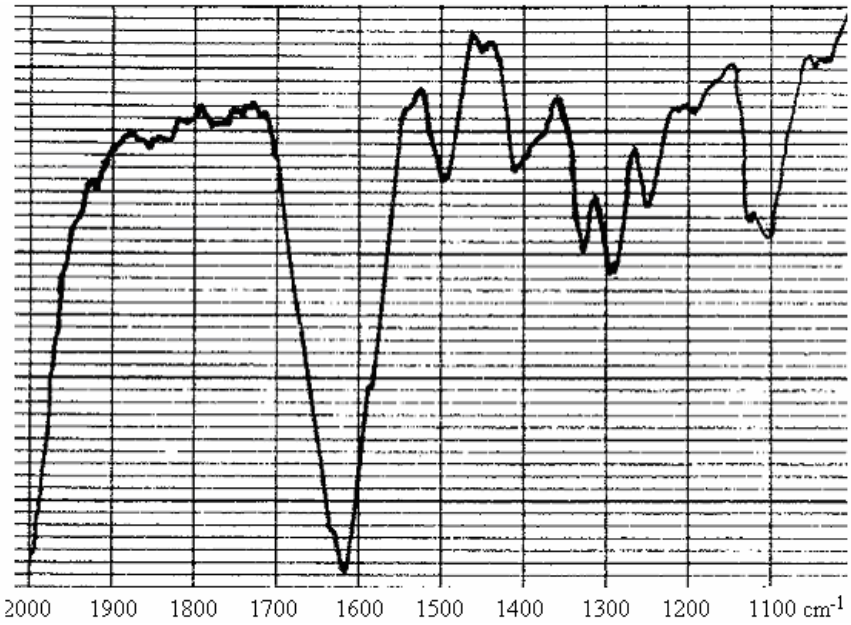
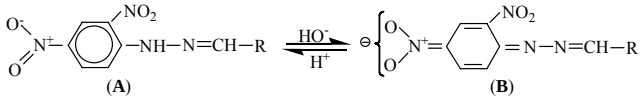


Figure 1. The IR spectra in the domain λ = 1100-2000 cm^{-1} for 2.4-DNPH-ones of carbonyl compounds with C_1-C_4

3.3 The UV-Vis spectrophotometry analysis

A dissolved 2.4-DNPH-one may be in a structure A (acid medium, yellow solution) or B (basic medium, red solution). Considering the conditions of liquid chromatographic separations – the mobile phase must have pH = 2-7.0 – this work makes a study of absorbance values versus the pH of 2.4-DNPH-ones solutions.



(**A**) (**B**)

For all 2.4-DNPH-ones solutions the absorption spectra in the domain 340-380 nm was drawn, using cells of 1 cm. For the 2.4-DNPHD ($0.9 \cdot 10^{-5}$ M) the maxim values of absorbance were at λ = 365 nm. Figure 2 presents the absorbance values versus pH, having a very specific evolution.

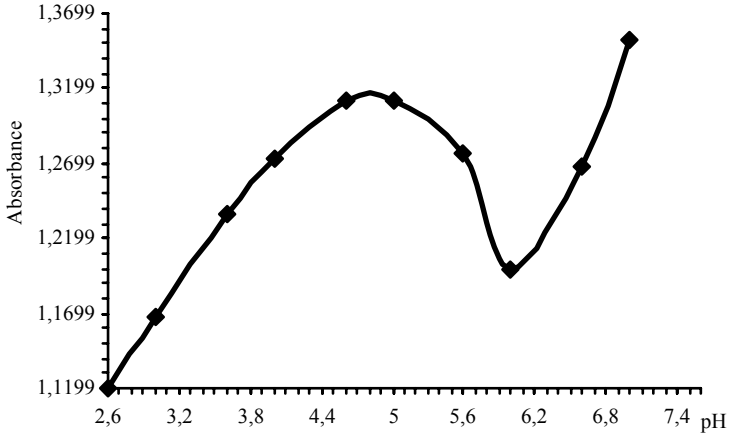


Figure 2. The absorbance values versus pH for 2.4-DNPHD solution

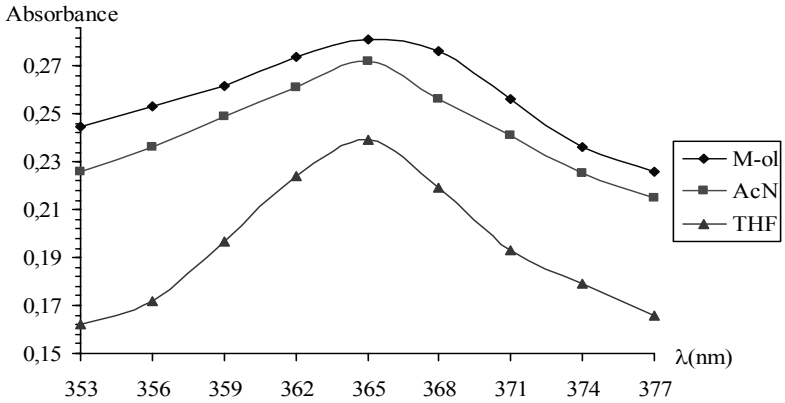


Figure 3. The absorption spectra of 2.4-DNPHH-one in three different solvents

The absorbance value increases if pH increases; if pH = 4.6-5.0, the absorbance rate is of maximum values. For the values of pH = 5-5.6, the absorbance value decreases, and increases again for pH>5.6, having maxim value (A=1.3531) at pH = 7.0. The 2-heptanone and 2-nonanone provide two 2.4-DNPH-ones with similar behaviors, abbreviated 2.4-DNPHH and 2.4-DNPHN. Figure 3. shows the absorption spectra of 2.4-DNPHH in three solvents utilized frequently as mobile phases in liquid-chromatography. The absorption curves have different images, but the same maxim absorbance value at λ=365 nm; the solution of 2.4-DNPHN in methanol has the highest values of absorbance.

3.3 The liquid-chromatographic separations

The liquid-chromatographic separation was used to study all the 10^{-4} M solutions in acetonitrile of 2.4-DNPH-ones provided by carbonyl compounds with a maxim of four carbon atoms and the two ketones.

First the maxim values of absorbance to the LC-XPD liquid-chromatograph were identified, working with a solution of 0.1 mg 2.4-DNPHD in 100 ml methanol. The circuit of mobile phase was minimized, deleting the separation columns, the injector being coupled with the LC-UV detector; the electrical signal of the detector was transformed in arbitrary units by DP 101 computing integrator. The transporter fluid is a mixture of methanol and water degassed, 80:20 (v:v); the flow of fluid was 1 mL/min. With metering pump in function, will be transferred 10 μL-injected volumes in LC-UV

detector, directly. Table 2 shows the results of measurements in the domain of λ=205-380 nm, in steps of 5 nm.

The first maxim value of signal is at λ=255 nm, $\bar{x}$=6.08 a.u., due to $n \rightarrow \pi *$ the available transition in the chromophore group C=N-. The appropriate value is for the second maxim value, corresponding to λ=365 nm, characterized by the higher means ($\bar{x}$ = 7.79 a.u.) and the small standard deviation (s =0.131); the appropriate value identified justifies the spectral measurements. Secondly, the solutions 10^{-4} M of 2.4-DNPH-ones in acetonitrile, was liquid-chromatographically separated, by the mechanism of the reverse phase, in the following conditions:

- Volume of sample: 10 μL;
- Separation column with Spherisorb 5 ODS: L = 25 cm, inner diameter = 4.6 mm;
- Mobile phase: a mixture of methanol and water, degassed, in ratio = 80:20 (v:v);
- Flow mobile phase = 0.7 ml/min;
- LC-UV detector with λ=365 nm, coupled with DP101 computing integrator (Spectra Physics) and potentiometer recorder type PM 8251 (Philips).

Table 2 The experimental data for the identification of the appropriate values for incident radiation

No.	λ (nm)	Date	$\bar{x}$ (a.u.)	s	No.	λ (nm)	Date	$\bar{x}$ (a.u.)	s
1	205	32	0.29	0.650	19	295	11	2.86	0.355
2	210	15	0.88	0.505	20	300	15	2.28	0.390
3	215	16	1.30	0.608	21	305	15	2.29	0.334
4	220	15	1.96	0.562	22	310	15	2.79	0.320
5	225	10	3.78	0.542	23	315	15	3.22	0.361
6	230	15	4.15	0.565	24	320	15	3.84	0.365
7	235	10	5.52	0.545	25	325	15	4.76	0.291
8	240	15	5.75	0.470	26	330	15	5.41	0.275
9	245	10	5.90	0.459	27	335	15	6.03	0.310
10	250	15	5.97	0.412	28	340	15	6.17	0.305
11	255	15	6.08	0.473	29	345	21	6.22	0.344
12	260	15	5.87	0.460	30	350	15	6.54	0.296
13	265	15	5.43	0.315	31	355	15	6.89	0.325
14	270	15	5.26	0.399	32	360	15	7.42	0.209
15	275	12	4.62	0.462	33	365	15	7.79	0.131
16	280	15	3.80	0.465	34	370	15	7.41	0.215
17	285	13	3.24	0.426	35	375	15	7.21	0.214
18	290	15	3.01	0.476	36	380	12	7.05	0.220

50 μL of pure acetonitrile have a chromatogram with two insignificant positive peaks, in the domain 420-480 seconds, as retention times; between these peaks, there are other two negative peaks.

The solutions of prepared 2.4-DNPH-ones have chromatograms with two peaks. The first one is small, and is due to solvent; the second peak is due to the 2.4-DNPH-one. The retention time has the following values (in seconds, after abbreviation name): 2.4-DNPHF-756, 2.4-DNPHG-775, 2.4-DNPHD-792, 2.4-DNPHA-858, 2.4-DNPHAP-1074, 2.4-DNPHP-1128, 2.4-DNPHiB-1170 and 2.4-DNPHB-1188. The 2.4-DNPH-ones of studied ketones have big values of retention times (Iordan, 2002).

4. Conclusions

Based on the experimental data, the following conclusions can be formulated:

1. All the 2.4-DNPH-ones etalon, by mixing reagents at room temperature may be easily synthesized.
2. The elemental analysis, by Dumas method, justifies the high purity of 2.4-DNPH-ones, by the percentage of nitrogen.

3. The IR analysis, justifies the purity of 2.4-DNPH-ones, by IR specter without spectral band to the carbonyl group.
4. The 2.4-DNPH-ones have two structures, according to the solution medium.
5. All the etalon 2.4-DNPH-one absorbs better the energy of electromagnetic radiation characterized by λ=365 nm.
6. In solutions with pH = 2.6-7.0 the absorbance values of 2.4-DNPHD-one have two domain of increase, according to the two molecular structures. The first domain is of a pH =2.6-5.0, with a maxim value at the end of the domain; this is the domain of interest for the liquid-chromatography separation.
7. The 2.4-DNPH-ones may be easily prepared and obtained in a pure state, being used only as liquid-chromatographic etalons.
8. Using the liquid-chromatographic separations with a reverse phase mechanism, a mixture of carbonyl compounds is analyzed as a mixture of 2.4-DNPH-ones.

References

Avram, M., Mateescu, Gh. D., 1966, *Spectroscopia în IR. Aplicatii în chimia oganică*, Editura Tehnică, Bucuresti.

Bacaloglu, R., Csunderlich, C., Cotarcă. L., Glatt, H.H., 1985, *Structura si proprietătile substantelor organice*, Editura Tehnică, Bucuresti.

Balaban, A.T., Banciu, M., Pogany, I.,1983. *Aplicatii ale metodelor fizice în chimia organică*, Editura Stiintifică si Enciclopedică, Bucuresti.

Hendrickson, J.B., Cram, D.J., Hammond, G.S., 1976. *Chimie Organică*, Editura Stiintifică si Enciclopedică, Bucuresti.

Iordan, M., Banu, C., Zgherea, Gh., 2002. *The methyl-ketones (2-heptanone and 2-nonanone) formation in complex biosynthesis media, by addition of purified exogen lipaze*, Simpozion internațional "Biochimie şi Biotehnologii în Industria Alimentară", 67-69, ISBN 9975-63-174-6, Chişinău, Moldova, Editura "Tehnica-Info".

Liteanu, C., Gocan, S., Hoditan, T., Natcu, H., 1974. *Cromatografia de Lichide*, Editura Stiintifică, Bucuresti, 196-201.

Mager, S., 1983, *Analiza structurală organică*. Editura Stiintifică si Enciclopedică, Bucuresti.

Nenitescu, C.D., 1974, *Chimie Organică*, vol I, Editura Didactică si Pedagogică, Bucuresti, 675-676, 692-696.

Szymansky, H.A., 1963, *Infrared band handbook*. Plenum Press, New York.

Zgherea, Gh., 1998, *Cercetări prin H.P.L.C. privind dinamica unor compusi carbonilici din produse alimentare obtinute la prelucrarea cerealelor*, Teză de doctorat, Universitatea „Dunărea de Jos" din Galati.