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Short communication

Characterization of a chemical artifact in the liquid chromatographic determination of 3-butyn-2-one using the 2,4-dinitrophenylhydrazine method

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Abstract

This study reports the identification of a chemical artifact occurring in the liquid chromatographic analysis of 3-butyn-2-one by means of the 2,4-dinitrophenylhydrazine (DNPH) method. Besides the expected derivatization reaction to the corresponding butynone DNPhydrazone, a rearrangement was observed, thus leading to the formation of 3-methyl-1-(2′,4′-dinitrophenyl)pyrazol (DNPP). Although the rearrangement product and the hydrazone can easily be separated by means of liquid chromatography, problems arise from coelution of the pyrazol with the formaldehyde DNPhydrazone. Identification of the artifact by means of UV–Vis spectroscopy using dual wavelength or diode array detection is discussed.

Keywords: Derivatization, LC; 3-Butyn-2-one; 2,4-Dinitrophenylhydrazine; 3-Methyl-1-(2′,4′-dinitrophenyl)pyrazol

1. Introduction

A well-established international standard method for the determination of airborne aldehydes and ketones is based on 2,4-dinitrophenylhydrazine as derivatizing reagent. In most cases, the obtained carbonyl derivatives are separated by means of HPLC with subsequent UV–Vis detection [1–4]. For the quantification of α,β-unsaturated carbonyl compounds, e.g., acrolein [5,6] and crotonaldehyde [7], the expected hydrazones and additional peaks in the chromatograms of eluted DNPH coated test tubes have been found in recent years. Due to the reactivity of a double bond close to a carbonyl function, unsaturated aldehydes and ketones are assumed to easily undergo various addition or rearrangement reactions, thus leading to the formation of by-products. The occurrence of additional peaks in chromatograms of acrolein or crotonaldehyde samples may result in incorrect quantification results [5–7]. Although having structural and functional similarities, the analysis of aldehydes and ketones with a triple bond in α-position to the carbonyl function has

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not been subject to detailed investigations yet [4]. As 3-butyn-2-one and 2-butynal are structurally analogous to methylvinylketone and crotonaldehyde, the chromatographic properties of the derivatives are similar [4]. A detailed study has been carried out, and its surprising results are presented in this paper.

2. Experimental

2.1. Chemicals

All chemicals were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available. Sulfuric acid was obtained from Merck (Darmstadt, Germany). Acetonitrile for HPLC was from Merck and was of gradient grade. The sampling tubes were purchased from Supelco (Deisenhofen, Germany): cartridge length = 7.4 cm, filled with 350 mg of silica gel (chromatographic grade); particle size = 150–200 μm (60/100 mesh); DNPH loading was 0.29% (1 mg/cartridge). Quartz wool was obtained from Fleischhacker (Schwerte, Germany).

2.2. Synthesis

The DNPhydrazones of 3-butyn-2-one and 2-butynal diethylacetale were prepared according to a procedure based on the work of Behforouz et al. [8]. For the synthesis of 3-butyn-2-one DNPhydrazone, recrystallization should be done very carefully in order to avoid the cyclization reaction to the pyrazol (see below). The synthesis of the 3-methyl-1-(2′,4′-dinitrophenyl)pyrazol standard was performed according to the work of Henbest [9]. The products were characterized by UV–Vis, ^{1}H-NMR and IR spectroscopy, mass spectrometry, melting point analysis and elemental analysis. The respective data of all products coincide well with literature data [10–12].

2.3. UV–Vis absorption measurements

The UV–Vis measurements were performed with a concentration of $2.6\cdot10^{-5}$ mol/l of 3-methyl-1-(2′,4′-dinitrophenyl)pyrazol, $2.7\cdot10^{-5}$ mol/l of 3-butyn-2-one DNPhydrazone and $2.4\cdot10^{-5}$ mol/l of 2-butynal DNPhydrazone in acetonitrile. The spectra were recorded in a range from 190 to 500 nm with a HP 8453 diode array spectrophotometer (Hewlett-Packard, Waldbronn, Germany) and software HP Chem Station 845x-biochemical UV–Vis system.

2.4. Air sampling

Air sampling was performed using a personal air sampler pump model I.H. from A.P. Buck (Orlando, FL, USA) with the corresponding calibrator, also from A.P. Buck. Sampling of a defined amount of the analyte was performed by pipetting 50 μl of the solution of 3-butyn-2-one ($3.8\cdot10^{-3}$ mol/l) in acetonitrile on quartz wool which was placed into the test tube in front of the collecting layer. Afterwards, a constant air stream (sampling rate, 1 l/min) was pumped through the tube for 10 min. After sampling, the tubes were eluted with 10 ml of acetonitrile. Aliquots of 10 μl of this solution were injected into the LC system.

2.5. Liquid chromatographic instrumentation and analysis

A liquid chromatograph consisting of the following components (all from Shimadzu, Duisburg, Germany) was used: two LC-10AS pumps, SPD-M10AVP diode array detector, SIL-10A autosampler, Class LC-10 software Version 1.4 and CBM-10A controller unit. The injection volume was 10 μl. The column material was Merck LiChroSpher RP-18ec (Merck, Darmstadt, Germany) in ChromCart cartridges (Macherey-Nagel, Düren, Germany); particle size = 5 μm; pore size = 100 Å; column dimensions = 250×3 mm; guard column = 8×3 mm. For separation, a binary gradient consisting of acetonitrile and water was selected. The profile of the gradient is shown in Table 1.

Table 1
The acetonitrile and water binary gradient profile (concentrations in %, v/v)[a]

Time (min)	0	1	6.5	8	11.5	14.5	19.5 (stop)
c(CH_3CN) (%)	49	49	65	80	80	49	49

[a] Flow-rate = 0.62 ml/min.

3. Results and discussion

The sampling of α,β-unsaturated alkynones and alkynals turned out to show artifact formation only for the derivatization reaction of 3-butyn-2-one, where an additional peak was observed in the chromatogram (Fig. 1). Cyclization reactions of the butynone DNPhydrazone had already been used for the preparative scale synthesis of a pyrazol in the literature, thus leading to the formation of a product with a UV–Vis absorption maximum of 321 nm (Scheme 1) [11]. The formed side product was identified as 3-methyl-1-(2′,4′-dinitrophenyl)pyrazol (DNPP) by means of spectroscopic investigations. The reaction product is similar to, but not identical with, the product obtained in the reaction between malondialdehyde and DNPH, where the formation of a pyrazol as the main derivatization product had been reported earlier [13,14]. For sampling of 3-butyn-2-one on DNPH coated silica gel test tubes with immediate elution and injection of the solution into the HPLC system, no additional peak, but the 3-butyn-2-one DNPhydrazone was observed. After 12 h of storage time at room temperature before injection, the liquid chromatographic separation revealed the appearance of one additional peak at 7.4 min (Fig. 1) which was identified as DNPP. Repeated identical experiments resulted in the finding that the formation of the DNPP peak is badly reproducible and hardly predictable. In some cases, its formation is already observed during sampling, while in other cases, the pyrazole is not even formed when refluxing the 3-butyn-2-one hydrazone as a solution in acetonitrile. Even in the presence of high concentrations of mineral acids, the occurrence of the DNPP peak varies strongly from one approach to another that is identical. A possible explanation for this effect might be trace impurities with catalytical properties on the cyclization.

Under the given chromatographic conditions, the formaldehyde DNPhydrazone elutes at the same retention time as DNPP. Using a diode array detector, the UV–Vis spectrum of the artifact peak showed an absorption maximum of 321 nm, while

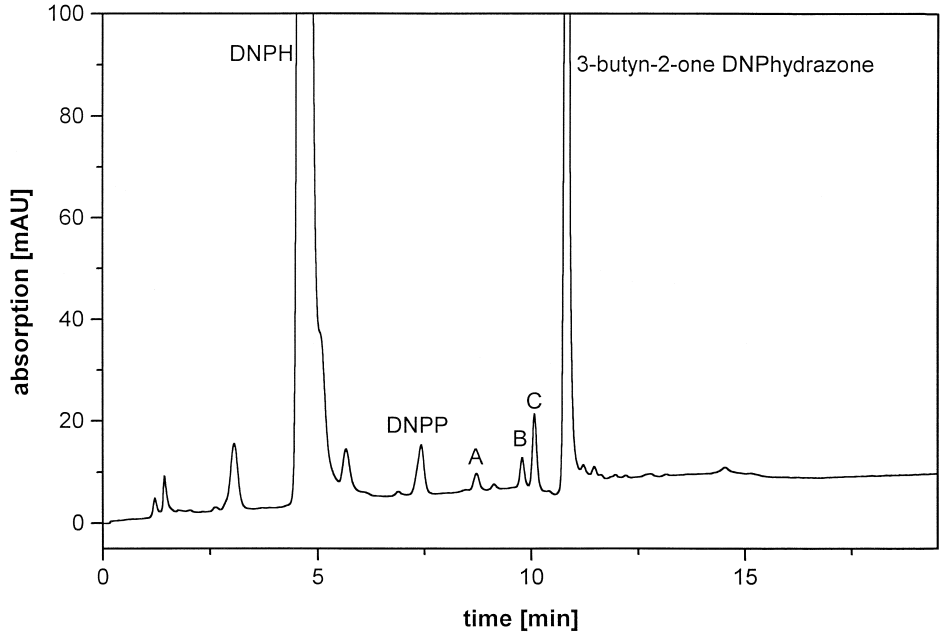
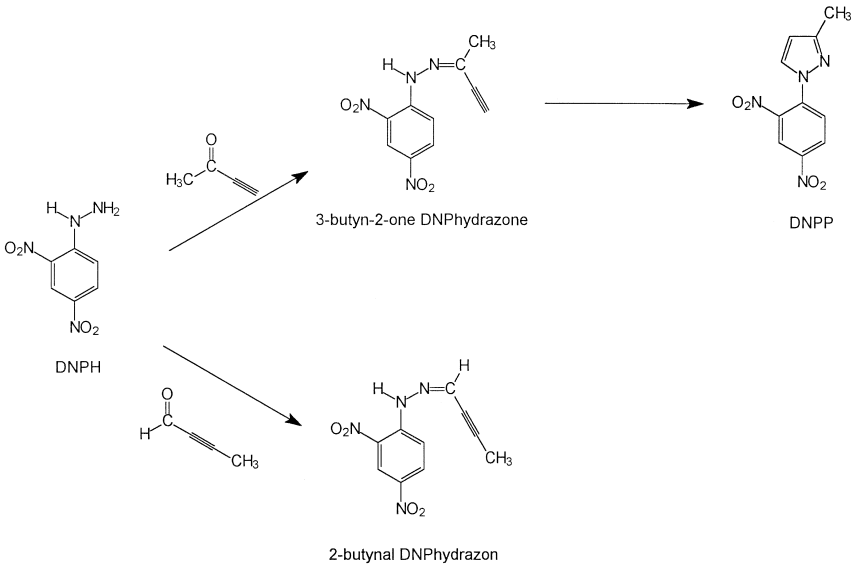


Fig. 1. Chromatogram of the eluted fraction of a DNPH test tube after a subsequent storage time of 12 h. The tube had been used for the air sampling of 3-butyn-2-one (for air sampling conditions see Section 2). Detection wavelength = 360 nm. (A) Acetaldehyde DNPhydrazone, (B) acetone DNPhydrazone, (C) propanal DNP-hydrazone. c(DNPH) = $4.8 \cdot 10^{-4}$ mol/l, c(DNPP) = $3.5 \cdot 10^{-6}$ mol/l, c(3-butyn-2-one DNPhydrazone) = $1.6 \cdot 10^{-5}$ mol/l.



Scheme 1. Reaction of 2,4-dinitrophenylhydrazine with 3-butyn-2-one and 2-butynal, respectively.

the formaldehyde hydrazone shows a maximum of 349 nm (Fig. 2). Dual wavelength detection at 360 and 300 nm allows an easy differentiation between both coeluting compounds, as peak area ratios of 5.5 for the formaldehyde DNPhydrazone [4] and of only 0.4 for the pyrazol are observed.

In analogous investigations for 2-butynal, the rearrangement was not observed. This is due to the

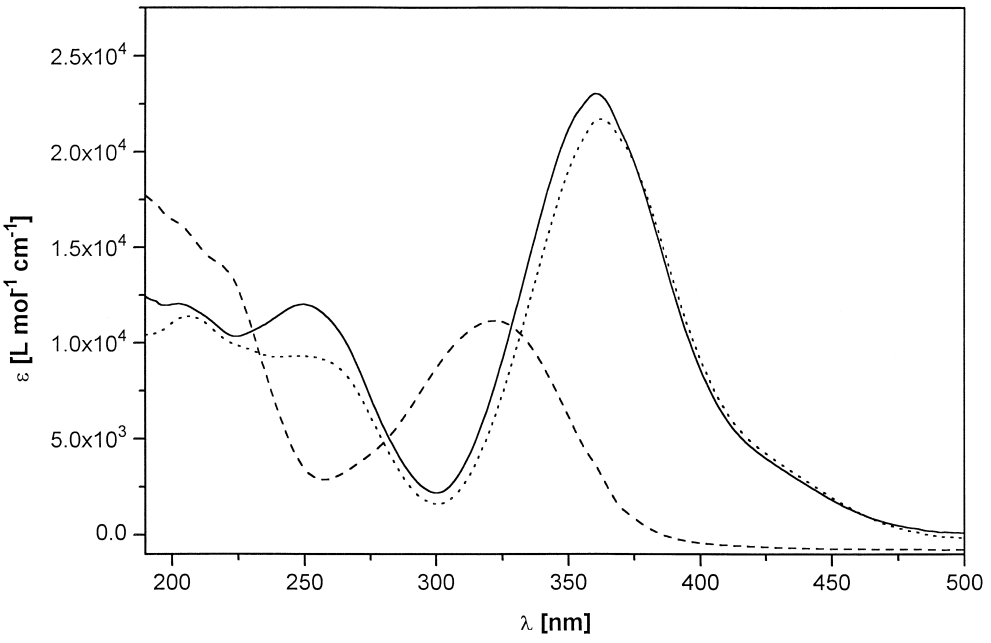


Fig. 2. UV–Vis spectra of DNPP (---), λ_{max} = 321 nm; 3-butyn-2-one DNPhydrazone (····), λ_{max} = 367 nm; 2-butynal DNPhydrazone (—), λ_{max} = 361 nm (for concentrations see Section 2).

fact that in contrast to 3-butyn-2-one, the acetylenic position is substituted by a methyl group, thus avoiding a cyclization reaction (compare to Scheme 1).

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