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Pentobarbital in tobacco

J. Jabłoński [a,*], E. Jabłońska [b], J. Moniuszko-Jakoniuk [a]

[a] Department of Toxicology, Medical University of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland
[b] Department of Immunology, Medical University of Białystok, Waszyngtona 15a, 15-274, Poland

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Abstract

The spectrometric analysis of extracts from tobacco and tobacco smoke revealed the presence of pentobarbital in the analyzed substances. Tobacco samples and tobacco smoke were extracted with chloroform, determinations were performed with the Perkin-Elmer Autosystem XL system, on a Turbo Mass spectrometer.

Subject to analysis were 4 cigarette brands manufactured in Poland and raw, unprocessed tobacco. The presence of pentobarbital in the analyzed samples was confirmed by the analysis of the mass spectrum of the substance, as well as by comparison of retention time with standard of pentobarbital.

The determined pentobarbital concentrations in tobacco amounted to 3–6 μg/cigarette, and in tobacco smoke they were approximately 45% lower.

In case of tobacco extracts it can with high probability be excluded that pentobarbital is synthesized during chromatographical analysis. The presence of pentobarbital in tobacco is thus beyond question.

Keywords: Tobacco; Pentobarbital; Concentration

1. Introduction

Over 4000 chemical compounds have been detected in tobacco and tobacco smoke. The majority of the compounds are toxic (IARC Monographs, 1986; Rusteimer et al., 2002; Swauger et al., 2002).

A significant number of detected chemicals contain in their molecules nitrogen atoms. The most important of them are nicotine, *N*-nitrosamines, dipyrydil, pyridyne and others. During the spectrometric analysis of extracts from tobacco and tobacco smoke we detected the presence of pentobarbital–a barbiturate characterized by high toxicity and addictiveness. Because of the side effects, pentobarbital is currently used in veterinary medicine (Grillmaier, 1976; Nabeshima and Ho, 1981).

The presence of pentobarbital in tobacco is significant due to its interactions with nicotine and addictive effect. (Modak and Alderete, 1983; Lemmonds et al., 2002). Due also to their ability to induce isoensymes of the cytochrome p-450 system, barbiturates may increase the risk of tumor disease in smokers (Habel et al., 1998).

2. Materials and methods

2.1. Sample preparation

Determinations were conducted in tobacco samples from cigarettes manufactured in Poland: Caro (Philip Morris, Kraków, Poland), Marlboro (Philip Morris, Kraków, Poland), Cristal (Scandinavian Tobacco, Myślenice, Poland) and Pall Mall (British-American Tobacco, Augustów, Poland), as well as in samples of raw, unprocessed tobacco of the Virginia type, grown in the region of the town of Augustów (Poland). Fife determinations from each cigarette brand were conducted.

Abbreviations: EI, electron ionization; GC, gas chromatograph; MS, mass detector; nist, National Institute of Standards and Technology; SIR, selected ion research.

* Corresponding author. Tel./fax: +48 85 7485604.
E-mail address: toksykologia@wp.pl (J. Jabłoński).

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Tobacco samples of the weight of 0.5 g, which corresponds to the approximate tobacco weight in a single cigarette, were extracted on a rotary agitator, using chloroform (Chempur, Piekary Śląskie, Poland), in the volume of 10 cm^3, in the time span of 40 min. Extracts were filtered on filtration columns (Bakerbond spe filtration columns). The seepage was evaporated to 1 cm^3 in the nitrogen stream with the help of the Baker spe 12 G system.

Tobacco smoke produced by burning 0.5 g was passed through a washer containing 10 cm^3 of chloroform. The burning time was 100 s. Chloroform was evaporated to the volume of 1 cm^3 in the nitrogen stream with the help of the Baker spe 12 G system.

To exclude the possibility of synthesizing pentobarbital in the chromatograph column during the analysis, a sample containing potential substrates for such synthesis was created. Because the solubility of urea (POCH, Gliwice, Poland) and malonic acid (Fluka, Steincheim, Switzerland) in chloroform is insignificant, a saturated chloroform solution of these compounds and malonyl aldehyde (Merck, Hohenbrunn, Germany) in the concentration of 5 mg/cm^3 was created. The analysis was conducted in conditions identical with those for the analysis of tobacco samples.

2.2. Chromatography assay

Extracts were analyzed by gas chromatography (GC), model Clarus, Perkin-Elmer, USA, equipped with a mass detector (MS) model Turbo-Mass, Perkin-Elmer, USA. An Elite-5MS capillary column (30 m/0.25 mm/1 μm) was used with helium as the carrier gas at flow rate of 1 cm^3/min. The injection port was set at 280 °C and the oven temperature was increased from 110 °C to 200 °C at 20 °C/min. The oven temperature was held for 1 min, then it was increased to 300 °C at 10 °C/min. The 1 μl of the sample was injected. The ionization potential of MS was 70 eV with electron ionization (EI) mode; the scan range was 30–300 *m/z*. The ionization temperature was 180 °C, the temperature of transfer line 250 °C. The identification was performed by comparison of mass spectral data with mass spectral database NIST (National Institute of Standards and Technology, Gaithersburg, USA). Pentobarbital was identified at the retention time of 11.04 min. Quantitative pentobarbital determination was done by selected ion research method (SIR) for main pentobarbital ions at 156 *m/z*. The results were read from calibration curve, made of the pentobarbital (Biowet, Puławy, Poland) solutions in chloroform.

3. Results

On Fig. 1 is presented the chromatogram of standard solution of pentobarbital in the concentration of 25 μg/cm^3, and the chromatogram obtained from tobacco extract.

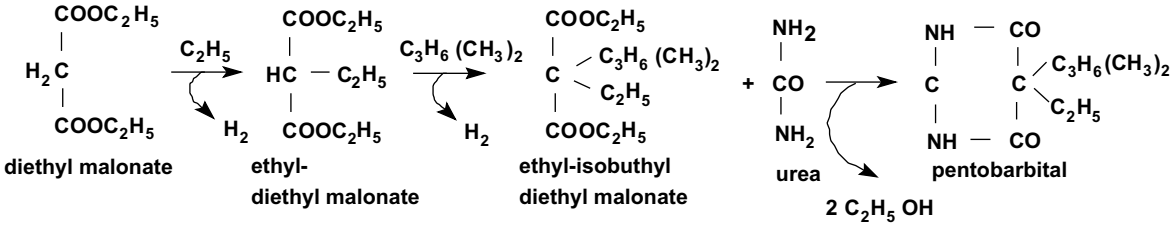
On Fig. 2 is presented the referential mass spectrum of pentobarbital form the NIST library, and the mass spectrum obtained from the examined tobacco sample.

The obtained results of pentobarbital in tobacco and tobacco smoke extracts are presented in Table 1.

4. Discussion

The results of the analysis suggest that pentobarbital can be found in tobacco and tobacco smoke.

Pentobarbital can emerge as a result of the malonic acid ester reacting with urea or malonic acid ester with urea.



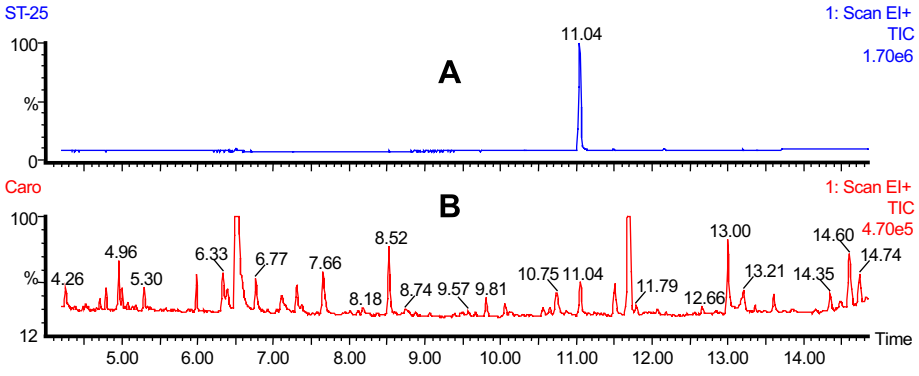


Fig. 1. Chromatograms obtained from standard solution of pentobarbital in the concentration of 25 μg/cm^3 (A), and from tobacco extract from Caro cigarette brand (B). Scan range 30–300 *m/z*, retention time 11.04.

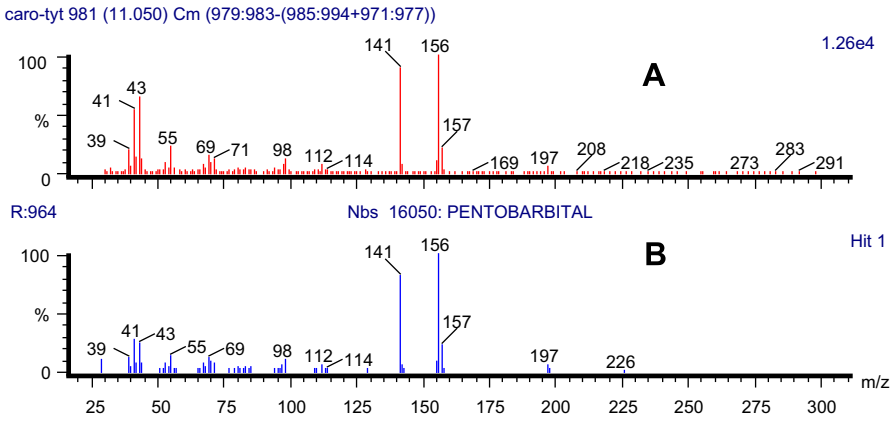


Fig. 2. Mass spectrum of the evaluated signal, obtained from the examined tobacco sample (A), and the referential mass spectrum of pentobarbital from the NIST library (B).

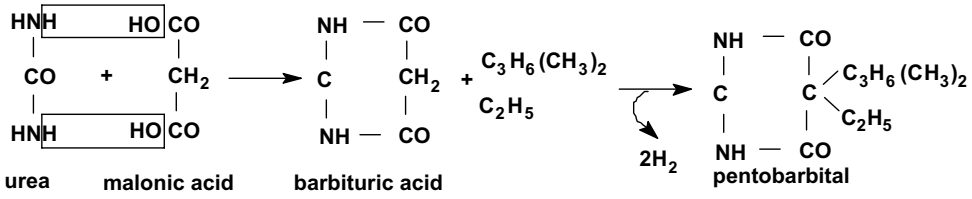
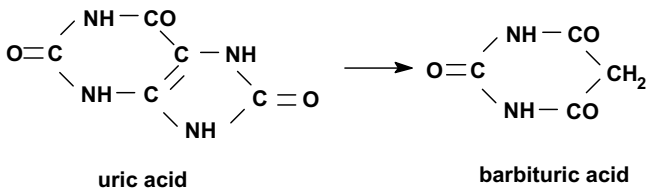


Table 1
The mean concentration of pentobarbital and standard deviations in the examined samples of tobacco and tobacco smoke ($n = 5$)

Cigarette brand	Concentration in tobacco (μg/cigarette)	Concentration in tobacco smoke (μg/cigarette)
Raw tobacco	6.21 ± 1.07	3.9 ± 0.70
Caro	4.36 ± 0.89	2.91 ± 0.67
Pall Mall	3.64 ± 0.75	1.98 ± 0.38
Cristal	2.93 ± 0.84	2.01 ± 0.64
Marlboro	3.52 ± 0.53	1.81 ± 0.66

The analysis of mass spectrums obtained from the determined samples showed the presence of malonic aldehyde and various esters of malonic acid. The source of urea may be the nitric fertilizers, commonly used in tobacco growing. Urea is also added to the tobacco during the cigarette production process. (Cai and Qian, 2003; Armitage et al., 2004; Henningfield et al., 2004).

In the examined samples the presence of uric acid was identified. As a consequence of transformations of this compound the barbituric acid – substrate in the pentobarbital synthesis – may be produced.



However, the presence of pentobarbital in raw, unprocessed tobacco indicates that the fact that pentobarbital can be found in tobacco has a natural background and the presence of this compound is not the result of technological processes carried out during the cigarette production.

Ions characteristic for pentobarbital were not shown on the chromatogram obtained from the sample containing potential substrates for pentobarbital synthesis, in the retention time area for this compound. This allows to exclude, with high probability, the possibility of its synthesis under the influence of temperature during analysis (Fig. 3).

However, there is a possibility that chemicals included in tobacco may, in certain conditions, mainly when exposed to high temperature, catalytically influence the pentobarbital synthesis during the analysis. If there is some possibility that pentobarbital may be synthesized during the analysis of tobacco extracts, the fact that high temperature is an inherent part of the burning process should erase any doubts as to the presence of this compound in tobacco smoke.

Additionally, there is a variety of compounds in tobacco and tobacco smoke that contain nitrogen in their molecules. There is a possibility that pentobarbital synthesis may take place with their assistance, especially in high temperature. However, no information concerning this issue has been found in the literature.

The mean pentobarbital concentration in tobacco smoke amounted to 2–3 μg/cigarette. The therapeutic pentobarbital concentration in serum amounts to 1–3 μg/cm^3

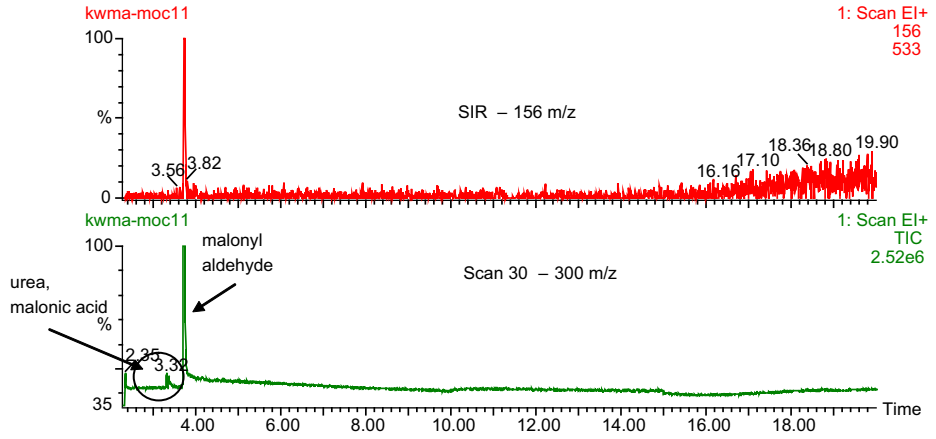


Fig. 3. Chromatograms obtained from the sample containing saturated chloroform solution of malonic acid, urea and malonyl aldehyde in the concentration of 5 mg/cm^3.

(Winek et al., 2001). Assuming that the daily count of cigarettes for an average smoker is 20, it can be also assumed that the organism takes up to 60 μg of pentobarbital. This amount is small and the sedative influence of this compound will probably be minor.

The results presented here are the first news about the presence of pentobarbital in tobacco and tobacco smoke, hence it is only a signal of the problem. Establishing precise synthesis mechanisms of this compound requires further research.

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