

INVESTIGATION OF CYANO-METHYLATION REACTION BY CYANO-HYDRINE AND ITS DETERMINATION IN TOBACCO-SMOKE. (STRECKER-REACTIONS)

Viktória HORVÁTH,¹ Lajos TRÉZL¹, Tibor SZARVAS², János PIPEK³,
Csaba VIDA¹ and Krisztina BAUER⁴

¹ Department of Organic Chemical Technology,

³Department of Physics

Technical University of Budapest,

² Institute of Isotopes of the Hungarian Academy of Sciences

⁴ Plant Protection Institute, Hungarian Academy of Sciences.

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Abstract

The results of our research work [1-7] of several years aimed at the binding of the unhealthy components of tobacco smoke directed our attention to the analysis and identification of the unknown peak observed during the high sensitivity radiochromatographic analysis of tobacco smoke condensates. It was a fruitless effort to remove the formaldehyde, the acetaldehyde and hydrogen cyanide present in tobacco smoke, or even to try to eliminate them separately, during the analysis the new, unknown peak appeared again and again on the radiochromatogram. During our research work we stated, that we were facing the Strecker reaction [8-9], known since 1850, that is, with the formation of cyanohydrine of the aldehydes and hydrogen cyanide present in tobacco smoke, corresponding to the new peak. Cyanohydrines to react quickly and energetically with basic aminoacids, during which reaction cyanomethyl derivatives are being formed. This reaction is proceeding also under physiological circumstances with the basic amino groups of proteins, contributing to the development of respiratory and cardiovascular insufficiencies. By means of model reactions and using solutions of tobacco smoke gases the process of the reaction was proved in a primary way, also its circumstances having been cleared. By radiochromatographic measurements it was proved that the labelled L-lysine-6 H is being cyanomethylated by formaldehyde-cyanohydrin at an extraordinary speed and cyanomethylation is complete within a very short time (5 min). The tests carried out on formaldehyde- C, cyanide- C and inactive L-lysine led to similar results, with a yield 93.9 percent within 5 min in all these cases. It was proved also by ¹³C NMR, ¹³C NMR DEPT and ¹H NMR spectroscopic measurements that inactive L-lysine is cyanomethylated by cyanohydrine in an extraordinarily energetic and quick reaction, according to the reaction mechanism supposed by us. Our results obtained during the radiochromatographic analysis of tobacco smoke condensate agreed in all respects with the model test results. That is, the cyanohydrine formation is proceeding very quickly in the tobacco smoke too (by the reaction of aldehydes with hydrogen cyanide contained by tobacco smoke) which can be unequivocally traced back to the Strecker-reaction recognized 140 years ago. The mechanism of the reaction was described by LAPWORTH in 1903. [10]

Keywords: tobacco smoke, cyanohydrine-cyanomethylation, cyanomethyl derivatives

Introduction

The fact that the relatively large quantities of gaseous aliphatic aldehydes contained in tobacco smoke (formaldehyde, acetaldehyde, acrolein etc.) are even more unhealthy than tar containing products, is nowadays widely documented. Among the unhealthy components formaldehyde is present at 20–90 $\mu\text{g}/\text{cigarette}$, acetaldehyde at 25–110 $\mu\text{g}/\text{cigarette}$, acrolein at 25–40 $\mu\text{g}/\text{cigarette}$, hydrogen-cyanide at 30–200 $\mu\text{g}/\text{cigarette}$ [11]. It was supposed that the reaction of aldehydes with HCN both contained in tobacco smoke produces cyanohydrine. Quantum chemical computations (Pauling bond order) prove the extraordinary reactivity of cyanohydrine, [12] according to which the bond order of carbon-carbon is 0.48, that of carbon-nitrogen 3.16, that of carbon-oxygen 1.56 in cyanohydrine.

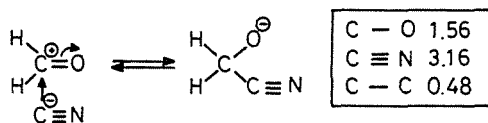


Fig. 1. Bond order of cyanohydrine

These cyanomethylated products (derivatives) formed in tobacco smoke are extremely dangerous, because they may destruct lung proteins and paralyse MAO [13] enzymes. According to measurements carried out by Japanese criminal research people, when tobacco was mixed with metamphetamine as a vitalizing drug (increasing the performance of sportsmen) N-cyanomethylated amphetamine derivatives have been identified during the burning of the tobacco, by means of gas chromatographic and mass spectroscopic techniques [14] According to our opinion also in this case it is the well-known Strecker-reaction which takes place as follows:

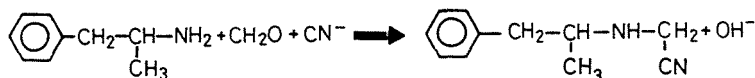


Fig. 2. Formation of N-cyanomethyl amphetamine

It has been well known for a long time the works of Hungarian researchers [15–17], that under the effect of formaldehyde itself a spontaneous formylation and methylation reaction is proceeds with the L-lysine. Nevertheless

these reactions are much slower than the reactions of cyanohydrine with L-lysine. Researchers in Canada, P.H. YU and his coworkers [18-19] detected cyanomethylated derivatives in tobacco smoke condensate, without mentioning any cyanohydrine formation, neither did they deal with the reaction of formaldehyde with lysine in itself (they rejected it) although we also detected formylated, methylated and cyanomethylated lysine derivatives.

Materials and Methods

The parameters of model reactions are: for ^1H NMR measurements were as follows:

Materials	mole ratio	time	reaction temp.	Solvent D_2O
Formaldehyde L-lysine	1 mM 0.6 mM	120 min	298 °K	0.6 cm^3
Formaldehyde sodium cyanide	4 mM 3 mM	5 min	298 °K	0.6 cm^3
Formaldehyde sodium cyanide L-lysine	4 mM 3 mM 0.6 mM	120 min	298 °K	0.6 cm^3

Parameters for model reactions for ^{13}C NMR measurements:

L-lysine	1 mM	120 min	298 °K	0.6 cm^3
Formaldehyde sodium cyanide L-lysine	4 mM 3 mM 0.6 mM	120 min	298 °K	0.6 cm^3

For ^{13}C NMR DEPT measurements:

L-lysine	1 mM	120 min	298 °K	0.6 cm^3
Formaldehyde sodium cyanide L-lysine	4 mM 3 mM 0.6 mM	120 min	298 °K	0.6 cm^3

NMR spectra were recorded by a BRUKER AC-80 apparatus. In the DEPT connection mode the carbon atoms containing paired and odd hydrogen atoms are separated. The signals of carbon atoms containing odd-order hydrogen atoms are showing upwards from the ground line on our ^{13}C NMR DEPT spectra.

Radiometric tests

Materials applied :

L-lysine/ $6\text{-}^3\text{H}$ /, specific radioactivity: 34 Mbq/mM

Sodium cyanide- ^{14}C , specific radioactivity: 14.6 Mbq/mM

Formaldehyde- ^{14}C , specific radioactivity: 74 Mbq/mM

Phosphate buffer: 0.02 M, pH=7.4, Reaction time: 5 min

Temperature: 307°K. Chromatographic method: thin layer chromatography, layer: Kieselgel 60 HF

Running solution: CH₃-OH : NH₄OH 25 : 75

Measuring technology :KLB-Rack Beta, fluid scintillation spectrometer. Scintillator: Clinisol^R

Preparation of Cigarette Smoke Solution

We have 25 filter cigarettes exhausted for each measurement lot (tar content max 15 mg) cigarette, nicotine contents max: 0.8 mg/cigarette) using a Filtron-300 type exhauster according to the standard KORESZA of the International Tobacco Convention. Exhaustion was carried out by means of Cambridge filters through 50 ml distilled water, after which we soaked the Cambridge filter in 50 ml distilled water, washed it thoroughly and united it with the 50 ml smoke condensate. This 100 ml emulsion contains the entire smoke condensate of 25 cigarettes.

Results

Testing of the cyanomethylation reaction by radiometric analysis and by radiochromatographic measurements: we allowed 0.1 mM L-lysine/ $6\text{-}^3\text{H}$ / in 2 ml phosphate buffer react at a temperature of 37 °C with a solution of 0.2 mM formaldehyde and 0.2 mM of NaCN, previously reacted and allowed to stand for 10 min. We continued the reaction for 5 and 30 minutes, respectively after which we made a chromatographic test on 20 μl . The results are shown by histograms 1 and 2.

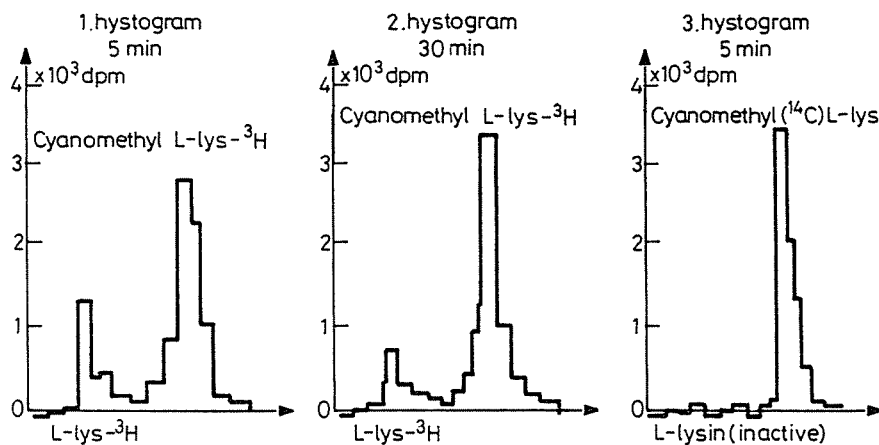


Fig. 3. Radiochromatographic histograms of tobacco smoke condensates.

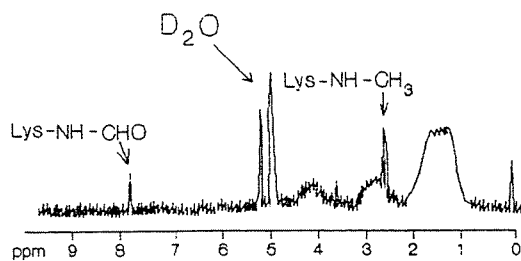


Fig. 4. ^1H NMR spectra of model reaction mixtures

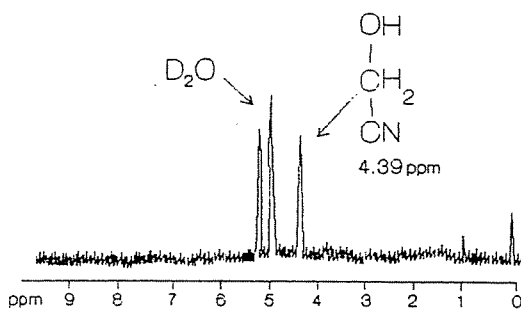


Fig. 5. ^1H NMR spectra of model reaction mixtures

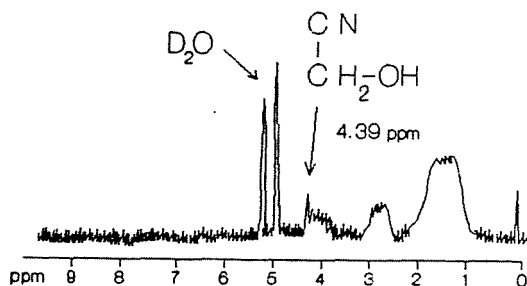


Fig. 6. ^1H NMR spectra of model reaction mixtures

It can thus be stated, that L-lysine/ $6\text{-}^3\text{H}$ / was cyanomethylated at a high speed within 5 min by the cyanohydrine developed in the meantime. During this time (5 min) the reaction proceeds to an 84%, and completely within 30 min. The experiments carried out with formaldehyde- ^{14}C and inactive potassium cyanide and inactive L-lysine, further potassium cyanide- ^{14}C and inactive L-lysine and inactive formaldehyde led to completely similar results (using reaction parameters agreeing with the previous ones) as shown by histogram no. 3. Cyanomethylation reached a degree of 89.9% within 5 min in these cases too.

^1H NMR and ^{13}C NMR Spectrometric Tests

To 1 cm of 20% -formaldehyde solution prepared from para-formaldehyde dissolved in D_2O and boiled for 5 h 100 mg ($6 \cdot 10^{-4}$ mole) lysine dissolved in $0.6 \text{ cm}^3 \text{ D}_2\text{O}$ was added and the ^1H NMR spectrum of the reaction mixture prepared in this way was recorded several times. The measurement results are shown in Fig. 4. The signal of the formyl group is shown at 8 ppm, that of the methyl group at 2.6 ppm. The ^1H NMR spectrum of the formaldehyde-sodium cyanide system was recorded using increasing sodium cyanide concentration. The spectrum of the paraformaldehyde (4 mM) diluted in $0.6 \text{ cm}^3 \text{ D}_2\text{O} + \text{NaCN}$ (3 mM) system is shown in Fig. 5. Under the effect of NaCN a new peak appeared at 4.39 ppm compared with the formaldehyde. Its intensity increased strongly with increasing the NaCN concentration, which made probable that it was due to the methylene group of cyanohydrine. When adding to the paraformaldehyde (4mM) diluted in $0.6 \text{ cm}^3 \text{ D}_2\text{O} + \text{NaCN}$ (3mM) + L-lysine (0.3mM) it is shown by the ^1H NMR spectrum of the system (Fig. 6) that under the effect of L-lysine the

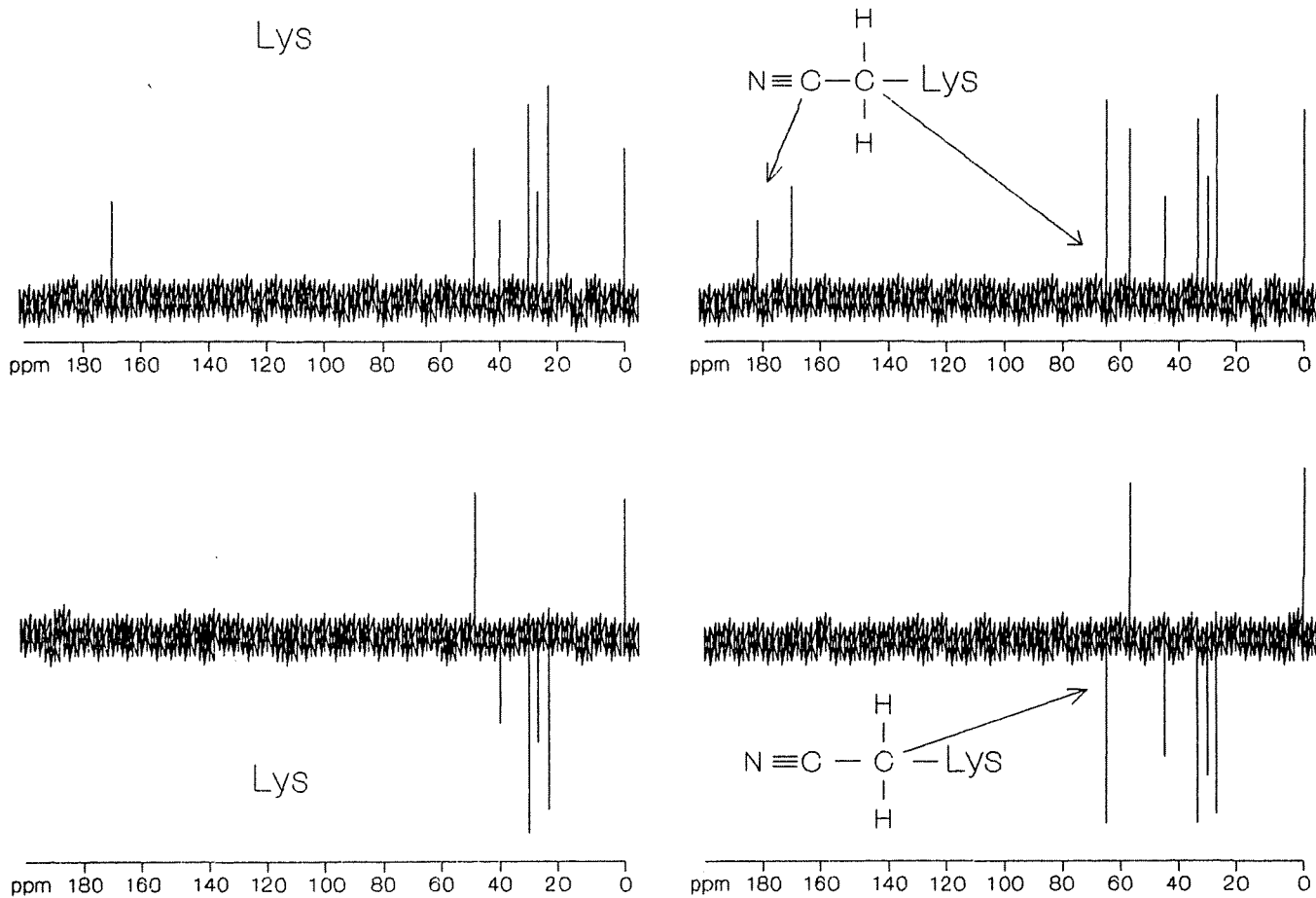


Fig. 7. ^{13}C NMR spectra of model reactions

signal appearing at 4.39 ppm decreases strongly, indicating the reaction of L-lysine with cyanohydrine.

Based on the ^{13}C NMR spectra (*Fig. 7.*) it can be stated that in the spectra of L-lysine (*Fig. 7/a, 7/b*) in the 25 ppm region the signal of the L-lysine β, δ, γ methylene groups, at 40 ppm the signal of the ϵ methylene group, at 50 ppm the signal of the -CH- group can be found. In the spectrum ^{13}C NMR spectrum (*Fig. 7/a*) at 172 ppm the signal of the carboxyl lysine group appears. This is proved by the fact, that in a DEPT connection (*Fig. 7/b*) the signal of the carboxyl group disappears from the spectrum, as the carbon atom is not linked with a hydrogen atom, thus in this connection mode the carbon atom does not give any signal. In the spectra of the reaction mixture (*Fig. 7/c, 7/d*) the new, unknown peak appearing at 64 ppm is the signal of the methylene group linked with the cyanide group of the developed cyanomethyl-lysine. In spectrum ^{13}C NMR spectrum (*Fig. 7/c*) the signal of the cyanide group of cyanomethyl-lysine appears above 180 ppm. In the spectrum recorded in DEPT connection mode (*Fig. 7/d*) the signal to be found at 64 ppm is showing downwards from the base line, as in this mode the signals of the carbon atoms containing paired hydrogen atoms are showing in this direction. Even the signal characteristic of the cyanide group is disappearing from the picture.

Discussion

It is commonly known nowadays that the aldehydes contained by tobacco smoke in a large quantity have a genotoxic and carcinogenic effect [20]. Among the aldehydes one of the most reactive ones is formaldehyde, due to a spontaneous methylation reaction with proteins and nucleic bases. The methylated proteins thus developed can induce to cell division in normal tissues [17]. Based on the results of our research work of several years aimed at the absorption (binding) of the unhealthy components of tobacco smoke (development of new tobacco filtering compositions [1-7]) during the radiochromatographic analysis of tobacco smoke condensates a new (unknown) peak appeared. We succeeded in identifying this new peak by means of radiochromatographic and NMR measurements, and also in discovering the course and mechanism of the reaction. We proved by radiochromatographic and NMR measurements that the known for more than 140 years STRECKER reaction is taking place also with the basic amino groups of proteins. The aldehydes contained in tobacco smoke and the cyanohydrine developing in a reaction with HCN are very reactive and by a powerful, quick reaction cyanomethyl derivatives are generated. We could prove that the aldehydes contained in tobacco smoke and the cyanohydrine

developing in the HCN reaction are highly reactive, attacking the basic amino-acids of the proteins, to produce cyanomethyl derivatives. We proved by radiochromatographic, ^1H NMR, ^{13}C NMR, ^{13}C NMR DEPT measurements the development of cyanomethylated L-lysine in model reactions and in tobacco smoke condensates. (Strecker-reactions) The Strecker-reaction is taking place with the basic amino groups of proteins under physiologic conditions too, according to the following mechanism:

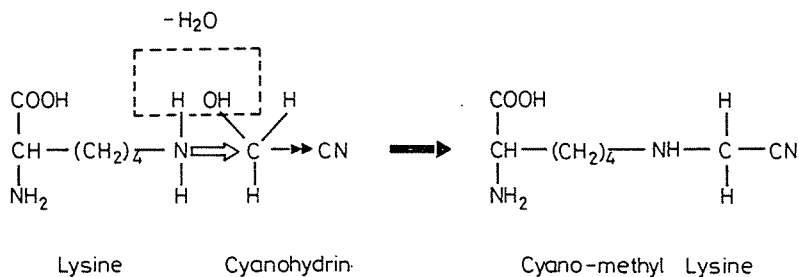


Fig. 8. Mechanism of cyanomethylation

These reactions have important biological effect, because they proceed with free amino and other extrafunctional groups contained in protein chains, paralysing the enzyme system of the human organism. Reactions of this type take place not only in tobacco smoke, thus endangering the health of smokers, but also in the large cities, at traffic junctions, where similar polluting products develop from the combustion products (exhaust gases of motor vehicles), potentially endangering the mankind.

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