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QUANTUM CHEMICAL INTERPRETATION OF THE REACTION BETWEEN L-ASCORBIC ACID AND FORMALDEHYDE AND ITS BIOLOGICAL SIGNIFICANCE

L. TRÉZL and J. PIPEK*

Department of Organic Chemical Technology
*Quantum Theory Group, Institute of Physics
Technical University of Budapest
H-1521 Budapest, Hungary

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Abstract

We have recognized a new nucleophilic addition reaction between L-ascorbic acid and formaldehyde in biological circumstances (pH 7.4, $T = 300$ K). UV spectroscopic investigation showed that the C2=C3 double bond of ascorbic acid rapidly disappears during the reaction, verifying that the addition breaks the double bond. On the other hand, ^{13}C NMR studies have proved that on atom C2 an additional C–C single bond is formed. Exact interpretation of the reaction mechanism is possible only by using quantum chemical argumentations. Ab initio calculations show that in ionized (anionic) form of L-ascorbic acid in water solution the Mulliken excess atomic charge is strongly negative on atom C2 while C1 and C3 exhibit significant positive net charges. Coulombic attraction arranges formaldehyde's C=O double bond over the C2=C3 double bond of ascorbic acid and the interaction of the two loose π-electron systems causes forming of a new σ C–C bond. The described reaction plays a protective role in biological systems, since in this way the toxic carcinogenic formaldehyde can be eliminated.

Keywords: formaldehyde, L-ascorbic acid, nucleophylic addition, quantum chemical interpretation.

Introduction

The biochemical role of the L-ascorbic acid (LAA), i. e. of the well-known Vitamin-C is multilateral and this fact has been stressed by numerous researchers since Professor SZENT-GYÖRGYI first had isolated it in 1928. Therefore Vitamin-C is being recommended as a preventive means in different doses for human organisms in the most different pathological cases.

The chemical reaction taking place between formaldehyde and L-ascorbic acid has been investigated last time in the late forties with analytical purpose (P. J. REITHEL and E. S. WEST, 1948; P.J. REITHEL and R.P. WEITHER, 1949); the authors wanted to elaborate a method for determining L-ascorbic acid in food products, in biological systems. They could not, however, publish any information about the reaction taking

place and they did not recognize the biological importance of the reaction either. FODOR et al. recognized that L-ascorbic acid enters into Michael-type additional reaction with α- and β-unsaturated aldehydes and ketones (R. ARNOLD et al., 1987).

The L-ascorbate-3-anion originating by dissociation of L-ascorbic acid can be considered as an ambient nucleophilic agent, with two possible reactive centers on O3 and on C2 atoms.

The mentioned authors, however, did not even suppose the possibility of a reaction between the L-ascorbic acid and formaldehyde. We have recognized first the reaction occurring between L-ascorbic acid and formaldehyde and its biological significance (E. TYIHÁK et al., 1980; L. TRÉZL et al., 1983, 1990) in the early eighties when we succeeded to verify that the spontaneous reaction between lysine (lysine containing proteins) and formaldehyde was inhibited by the L-ascorbic acid and therefore L-ascorbic acid can defend the proteins against the methylation effect of the toxic formaldehyde.

Experiments

We have studied L-ascorbic acid and formaldehyde reaction in a water solution of 0.2 mmole/cm^3 L-ascorbic acid and 0.5 mmole/cm^3 formaldehyde concentration at temperature $T = 298$ K and pH 7.4. We have chosen the above conditions in order to simulate biological circumstances, but we wish to mention here that this reaction can take place between pH 4 to 8, as well.

In a UV measurement (see *Fig. 1*) we have observed the characteristic absorption line of the C2=C3 double bond of the five membered ring of LAA at a maximum position of 265 nm. The UV absorption peak completely vanishes depending on time indicating that the double bond disappears and the entire quantity of L-ascorbic acid enters the reaction.

We have traced the reaction also by IR-spectroscopical test. The C=O band of LAA at 1759 cm^{-1} (W. LOHMAN et al., 1984) is shifted to the oscillation frequency 1790 cm^{-1} and its intensity has been doubled. The original C=C band at 1675 cm^{-1} almost completely disappears confirming the UV measurement findings. The results show that in the keto-enol tautomerism of the enediol group (*Fig. 2*) the reaction is shifted towards the keto form together with the appearance of an additional C=O double bond compared to the enol form.

We have followed the reaction of formaldehyde and L-ascorbic acid also with the use of ^{13}C NMR testing. The reaction was implemented with paraformaldehyde dissolved in D_2O (approx. pH 4) and L-ascorbic acid

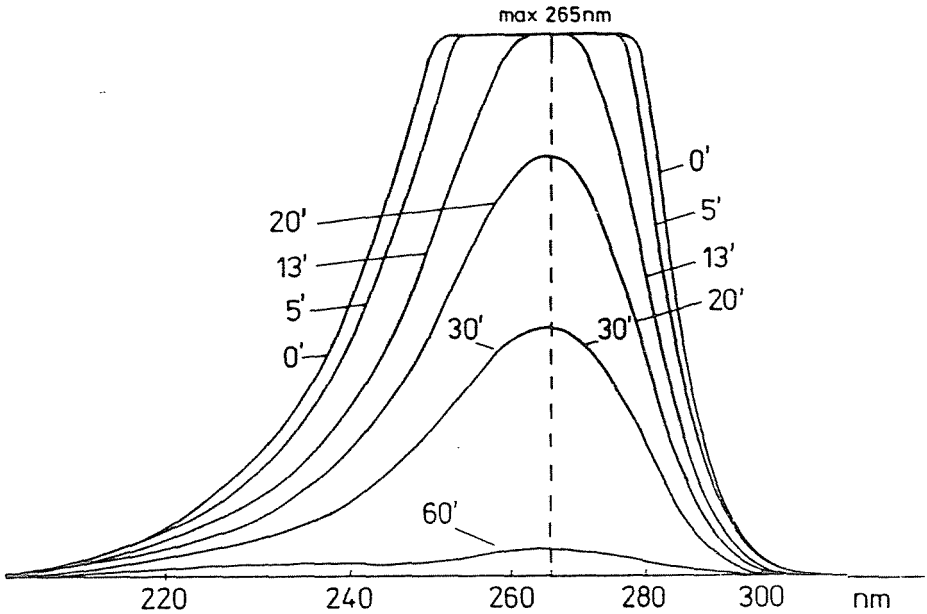
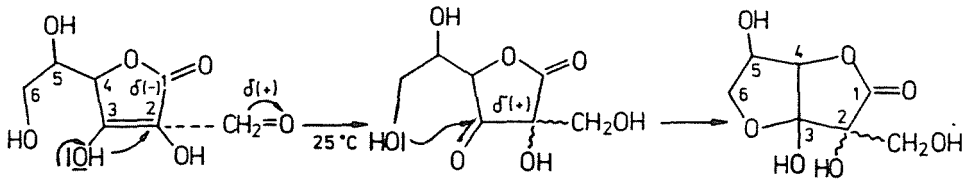


Fig. 1. UV absorption spectrum vs. time of the reacting mixture 0.2 mmole/cm^3 L-ascorbic acid and 0.5 mmole/cm^3 formaldehyde in water solution at $T = 298$ K and pH 7.4

Fig. 2. Enediol class keto-enol tautomerism in Vitamin-C

at T=298 K temperature. The measurements were made after a reaction time of 24 hours, in order to assure that the reaction had been entirely implemented. *Table 1* shows the chemical shifts of the reaction mixture of L-ascorbic acid and formaldehyde as well as the actual parameters of the reaction. The ^{13}C NMR spectroscopic tests verify unambiguously the formaldehyde addition to the double bond of L-ascorbic acid. An additional carbon peak appears in the spectrum indicating the addition of the C7 atom. The shifts of the double bond between the C2 and C3 carbon atoms have disappeared but the shift of lactone has remained and thus the reaction had not stopped at structure II, but a cyclical ketale structure III was formed instead as it is shown in *Fig. 3.* The identification of the shifts



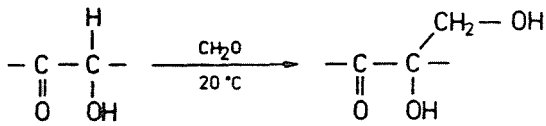


Fig. 3. Reaction of L-ascorbic acid and formaldehyde

Table 1
^{13}C NMR testing of reaction between L-ascorbic acid and formaldehyde*

	^{13}C NMR chemical shifts in D_2O (in ppm)						
	C1	C2	C3	C4	C5	C6	C7
pure LAA	174.2	118.9	156.6	77.3	70.1	63.4	—
LAA + H_2CO	177.6	79.7	107.3	96.8	75.6	73.8	63.3

*Reaction conditions: $T = 298$ K, time duration=24 hours, solvent=D_2O, LAA concentration=10^{-2} mole, paraformaldehyde concentration=$2 \cdot 10^{-2}$ mole. NMR spectrometer: JEOL FX 100 MHz

given by us has been supported by the tests implemented by FODOR et al. (1984) on the reaction between L-ascorbic acid and acrolein.

Interpretation

As experimental results suggest a quite unusual C → C nucleophilic addition of formaldehyde carbon to LAA-C2, an important question is to understand the physical backgrounds of the reaction process.

It is expected that in the first phase of the reaction, i. e. at relatively large distances of the reacting LAA and formaldehyde the high dipole moment (2.34 D) of H_2CO should play the leading role. Since both UV and IR measurement to indicate the disappearance of C2=C3 double bond of LAA during the reaction, net charges on these atoms are of primary importance. There exist semiempirical (ECKERT-MAKSIĆ et al., 1986) and

ab initio (CARLSON et al., 1976) calculations on L-ascorbic acid and its derivatives showing that both C2 and C3 are positive in the neutral form of LAA (AH_2). The corresponding Mulliken excess atomic charges are $Q_{C2} = +0.03\,e$ and $Q_{C3} = +0.11\,e$ (see *Fig. 4*) indicating that the driving electrostatic forces are repulsive for the positively charged carbon of H_2CO and thus the reaction is highly improbable.

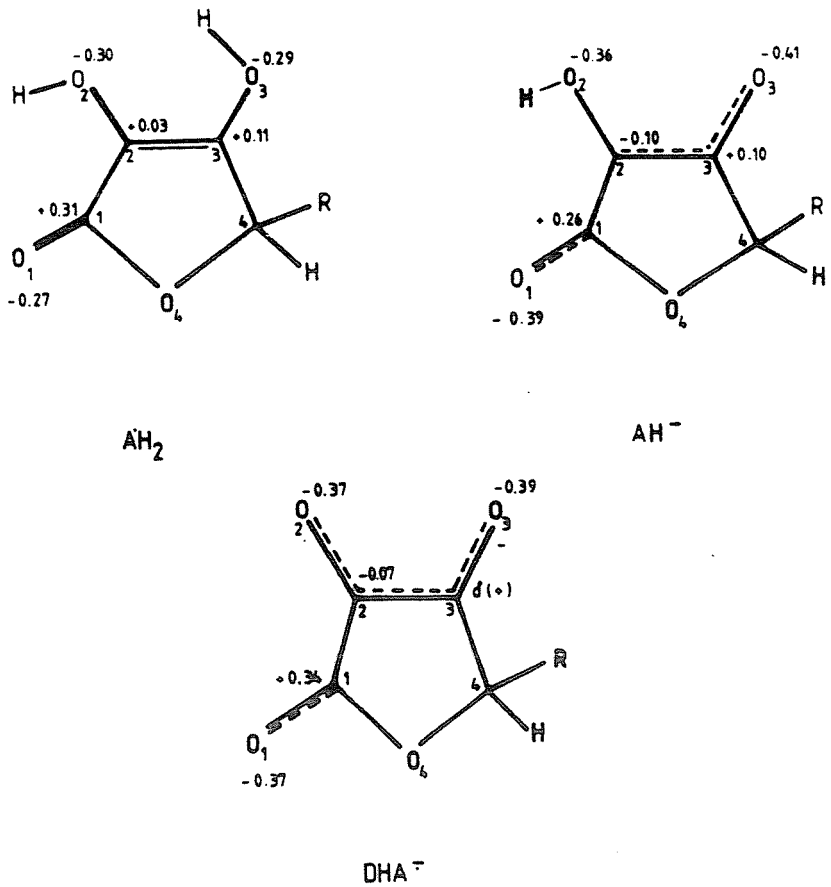


Fig. 4. Bond structure and Mulliken excess atomic charges in units of $|e|$ for neutral L-ascorbic acid (AH_2) (G. L. CARLSON et al., (1976)) and anionic forms AH^- (G. L. CARLSON et al., (1976)) and $DHA\cdot^-$ (M. ECKERT-MAKSIĆ et al., (1986)). R=CH(OH)CH_2(OH)

However, in water solution at physiological pH LAA exists as the singly negative anion AH^- formed by the dissociation of $H(O_3)$. Another possible form of LAA is the radical anion $DHA\cdot^-$ (dehydroascorbic acid)

formed by removing of $H(O_3)$, $H(O_2)$ and one electron from AH_2. ECKERT-MAKSIĆ et al. (1986) argue that the radical anion is thermodynamically more stable than AH_2 itself, as it can be concluded considering the corresponding heats of formation -1164 and -1055 kJ/mole, respectively. The approximately planar five-membered rings of the above species along with their Mulliken excess charges are shown in *Fig. 4*. In contrast to AH_2 the C2 carbon of both anionic forms AH^- and $DHA\cdot^-$ becomes slightly negative, attracting the positively charged carbon of H_2CO. At the same time the strongly negative oxygen of formaldehyde approaches the positively charged C1 or C3 of the ring. Since there are strong electrostatic interactions between the two molecules originating from the above two different sources, the reaction cannot be treated as a Klopman-type (KLOPMAN, 1974) charge controlled single site attack.

Instead, we suggest the following reaction mechanism. The first step is illustrated in *Fig. 5*. Due to the mutual electrostatic attraction of C2 and H_2CO carbon as well as C3 and H_2CO oxygen, formaldehyde approaches LAA. The planes of the five-membered ring and H_2CO are parallel. Formaldehyde carbon is placed above C2 while its oxygen is above C1 or C3. Supposing that the reaction is determined by frontier-orbitals we have calculated the orbital arrangement of H_2CO by ab initio Hartree-Fock method, using 4–31G* atomic basis set (L. TRÉZL et al., 1988). As it is shown in *Fig. 6* HOMO is a non-bonding C–O orbital (n) and the next occupied orbital C–O (π) is energetically rather close to it. LUMO is the antibonding π^* of the C–O bond. The HOMO of AH^- is the bonding type loose π orbital of C2–C3 with a rather high component on C2 and a small contribution on C3. In the radical anion $DHA\cdot^-$ the highest molecular orbital is singly occupied (SOMO). It corresponds again to a loose bonding π-type orbital on C2–C3 as it is indicated in *Fig. 4*, with the orbital energy -3.2 eV (M. ECKERT-MAKSIĆ, 1986).

From this it follows that in the reaction the most favourable arrangement is when formaldehyde oxygen is placed over C3 making the appropriate overlap of frontier-orbitals possible. Since the non-bonding n-orbital of H_2CO lies in the plane of the molecule it is also clear that no interaction is expected with other π-like orbitals which are orthogonal to n. The importance of the geometrical arrangement is also underlined by the experimental fact that a similar reaction between Vitamin-C and acetaldehyde is also observable, which can be explained by the similar geometrical shapes of formaldehyde and acetaldehyde.

It seems to be obvious that the second step of the reaction is orbital controlled determined by the overlap of the C=O π-orbital of H_2CO and the C2=C3 π-orbital of LAA.

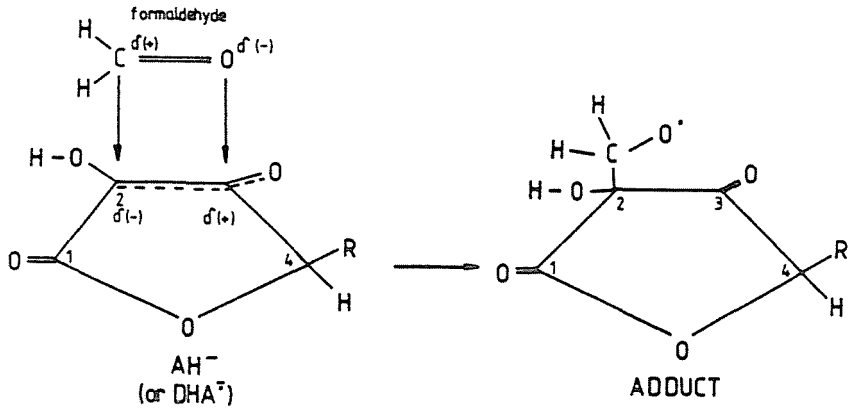


Fig. 5. Attack of formaldehyde on LAA derivatives and the intermediate reaction product

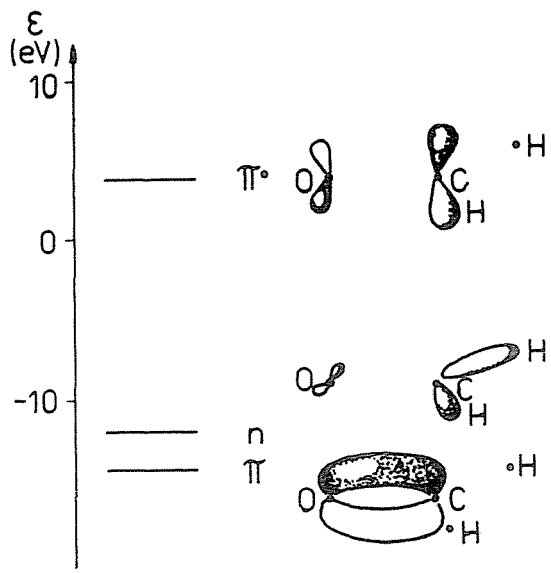


Fig. 6. Energy level diagram of formaldehyde frontier orbitals calculated by ab initio Hartree-Fock method using 4-31G* atomic basis set

In the case of DHA·⁻ the process can be easily interpreted as a donor-acceptor interaction by the transition of one π(C=O) electron of formaldehyde to the unoccupied SOMO of LAA, which is energetically really favourable. In the third phase of the reaction the emerged full double bond in the five-membered ring rearranges to the C3–O3 region, while at

the same time formaldehyde carbon C7 binds to C2 and an unpaired electron remains on formaldehyde oxygen making later the capture of a free proton possible.

In AH^- all orbitals are double occupied and interaction with H_2CO cannot be treated as a simple electron transfer from one system to another. The process is rather complex consisting of simultaneous breaking of the formaldehyde C=O bond, the binding of C7 to C2 and the rearrangement of AH^- C3=C2 double bond to the C3–O3 region. As *Fig. 5* shows there is no local symmetry in the geometrical arrangement of the formaldehyde attack and thus the symmetry conservation rules for concerted reactions (R. B. WOODWARD and R. HOFFMANN, 1971) cannot be applied here. Nevertheless, there are good reasons, why this reaction path might be also preferred. In AH^- HOMO (CARLSON et al., 1976) the contribution of C2(p_z) orbital is 0.66 while the C3 coefficient is negligible (0.07) causing the frontier-orbital interaction be localized on C2. On the other hand, we have shown in an ab initio CISD calculation (TRÉZL et al., 1988) that the ground state of formaldehyde can be described to a good approximation as

$$\Psi = 0.956\Phi_0 + 0.105\Phi(\pi^2 \rightarrow \pi^{*2}) + 0.274\Phi_C\,,$$

where Φ_0 is the Hartree-Fock ground state, $\Phi(\pi^2 \rightarrow \pi^{*2})$ is an $^1A_1$ double excited state and Φ_C is a collective state which cannot be assigned to any particular independent particle excitation. This unexpectedly high correlation between Φ_0 and $\Phi(\pi^2 \rightarrow \pi^{*2})$ leads to the conclusion that perturbing the frontier orbitals can easily cause the breaking of the double bond in formaldehyde. This indicates also that detailed numerical studies are probably hopeless without including electron correlation in the calculations.

Biological Significance

We have recognized that this new nucleophilic addition reaction discussed previously can play a crucial role in biological systems, since in liver carcinogenic formaldehyde can be liberated from various carcinogenic precursors (e. g. from the extremely carcinogenic dimethyl-nitrosamine DMN) with the help of cytochrome-P450 isoenzymes. These enzymes are inactive under anaerobic conditions and consume hydrogen peroxide (H_2O_2) during oxidation. H_2O_2 is produced by the NADPH coenzyme from O_2 occurring in liver tissues.

Details of the described process are shown in *Fig. 7*. We have illustrated here that by the in vivo metabolism of DMN in liver 1 mole formaldehyde can be liberated with oxidation from 1 mole DMN. This amount is enormous in biological scale, considering the toxic, mutagenic and carcino-

genic effect of formaldehyde. The above metabolic pathway of DMN is well known in the literature and is discussed in details by REYNOLDS and THOMSON (1987).

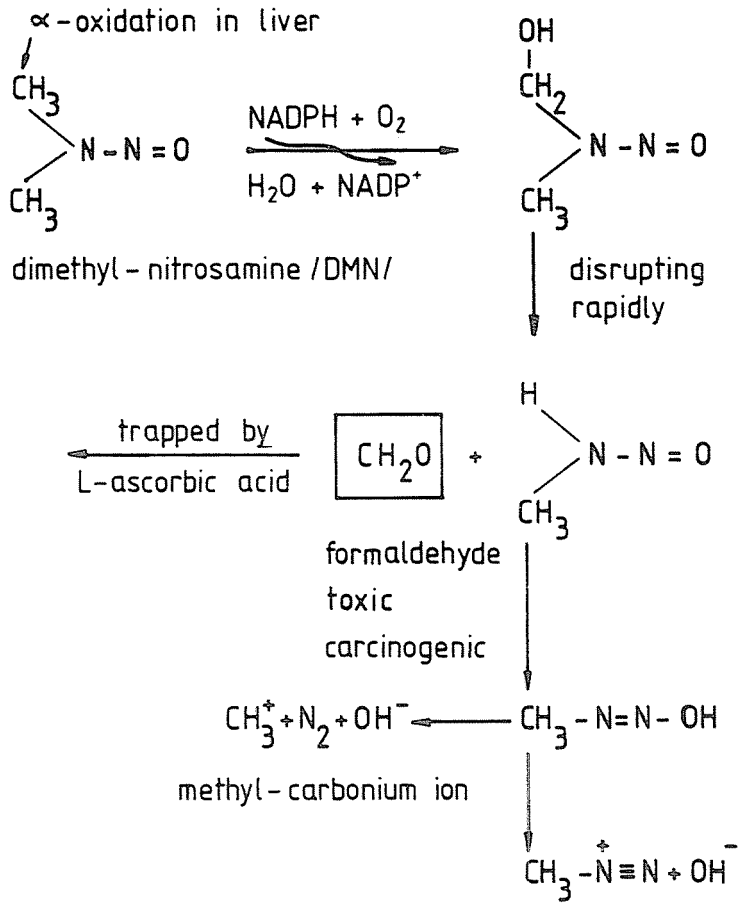


Fig. 7. In vivo metabolism of dimethyl-nitrosamine by cytochrome-P450 monooxygenase enzymes in liver

The reaction described in the previous chapter leads to the assumption that L-ascorbic acid can serve as an excellent tool for trapping the liberated formaldehyde originating from DMN. It is important to note that nobody has attempted to trap H_2CO in the above metabolic pathway by LAA, since the necessary reaction is published first by us. We have proved experimentally in liver homogenizate that the expected effect takes really place under biological circumstances. *Table 2* indicates clearly that increasing L-ascorbic acid concentration in the incubation mixture of liver homo

genizate the amount of liberated free formaldehyde is decreasing, i. e. more formaldehyde is trapped by LAA. As Vitamin-C is a biological molecule its dose can be highly increased in the human body, making in this way an effective trapping of carcinogenic formaldehyde liberating from DMN in liver in vivo possible.

Table 2
Change in the amount of formaldehyde liberating from DMN in liver homogenizate under the influence of L-ascorbic acid

Concentration of LAA in incubation mixture (M)	Released H_2CO (nmole/mg protein/30 min)	Relation to control (%)
0 (control)	31.4 ± 1.7	100
10^{-4}	29.1 ± 0.8	92.7
10^{-3}	15.9 ± 0.6	50.6
10^{-2}	8.3 ± 1.1	26.4

An important practical application of this reaction has been found as well. As it is well-known, tobacco smoke contains a great amount of toxic carcinogenic formaldehyde (approx. 40–140 mg/m^3). Animal experiments proved that formaldehyde in smoke may cause nasal squamous carcinoma. We have developed a new tobacco smoke filter containing L-ascorbic acid and other ingredients (e. g. active carbon and catalysts). The filter can trap a large amount of toxic formaldehyde from smoke, protecting in this way the health of smokers. The effectivity of the filter has been investigated by radioactive experiments. Tobacco smoke containing a considerable amount of formaldehyde was condensed into a water solution of tritiated L-lysine amino acid (L-lys-6-^{3}H).

Between formaldehyde and tritiated L-lysine special spontaneous methylation and formylation reactions take place, which were first described by us earlier. The gross reaction is as follows

$$2\,\text{Lys–N}^{\varepsilon}\text{H}_2 + 2\,\text{CH}_2\text{O} \rightarrow \underset{N^{\varepsilon}-methyl-L-lysine}{\text{Lys–N}^{\varepsilon}\text{H–CH}_3} + \underset{N^{\varepsilon}-formyl-L-lysine}{\text{Lys–N}^{\varepsilon}\text{H– CHO}} + \text{H}_2\text{O}.$$

These mechanisms can already take place on 25°C without any reduction compounds, e. g. $NaBH_4$.

Radiograms on chromatoplate of the smoke condensate without and with Vitamin-C filter are shown in *Figs. 8* and *9*, respectively. The Figures

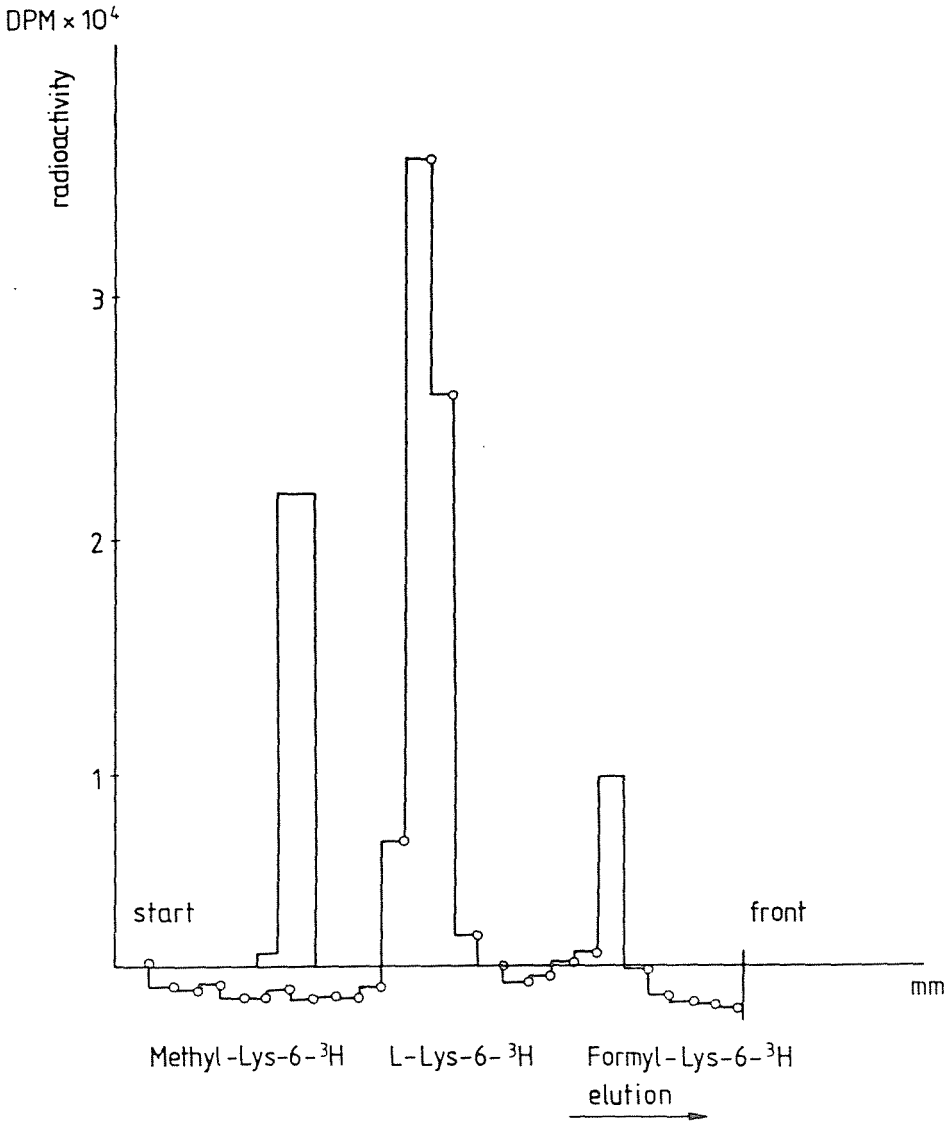


Fig. 8. Radiogram of tobacco smoke condensate of a smoke filter (containing active carbon only) with tritiated L-lysine

show uniquely that these processes can take place in smoke condensate, as well. If tobacco smoke filter contains L-ascorbic acid the amount of methylated and formylated reaction products will be considerably less than

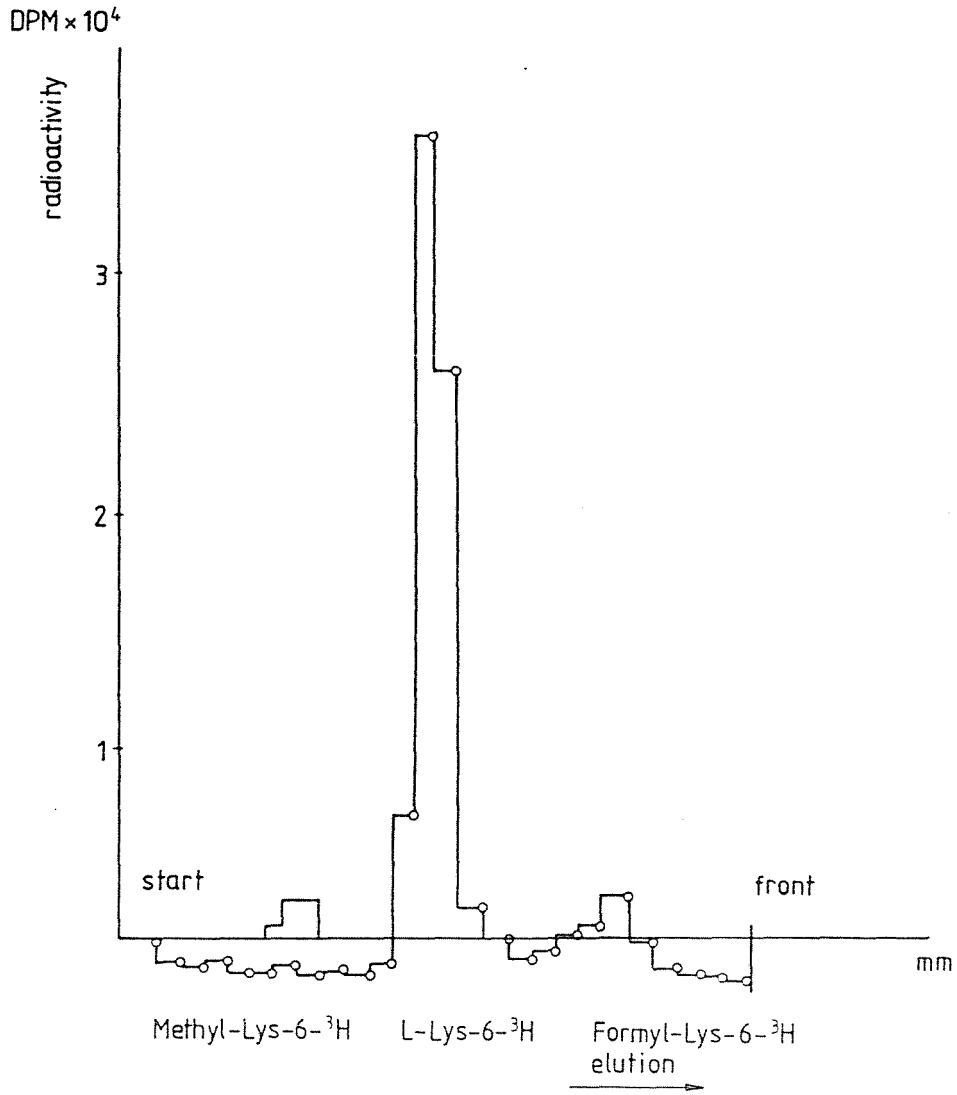


Fig. 9. Radiogram of tobacco smoke condensate of a smoke filter (containing a composition of Vitamin C) with tritiated L-lysine

in the control case, since formaldehyde was trapped by L-ascorbic acid, inhibiting the methylation and formylation reactions.

The above procedure has already been patented in Hungary, in the USA and in numerous other countries as well.

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Addresses:

Lajos TRÉZL
Department of Organic Chemical Technology,
Technical University of Budapest
1521 Hungary

János PIPEK
Quantum Theory Group
Institute of Physics
Technical University of Budapest
1521 Hungary