

## The Radiolysis of Aqueous Propionitrile: Compounds of Interest to Chemical Evolution Studies

S. Jovanović<sup>1</sup>, S. Nešković<sup>2</sup>, V. Spirić<sup>1</sup>, Z. Draganić<sup>3</sup>, and I. Draganić<sup>3</sup>

<sup>1</sup>Boris Kidrič Institute of Nuclear Sciences, Beograd, Yugoslavia

<sup>2</sup>Faculty of Sciences, University of Beograd, Beograd, Yugoslavia

<sup>3</sup>Centro de Estudios Nucleares, Universidad Nacional Autónoma de México,  
Ciudad Universitaria, México 20, D.F. Mexico

**Summary.** Oxygen-free aqueous solution of  $\text{CH}_3\text{CH}_2\text{CN}$  (0.1 M, pH 6) were exposed to gamma rays from a  $^{60}\text{Co}$  source and the mixture of radiolytic products fractionated. The separated fractions were analysed by magnetic resonance methods (EPR, NMR), spectrophotometry (UV-VIS-IR), gas chromatography and amino acids analysis. About 70% of radiolytic products consist of non volatile material. A large variety of compounds was detected: propionaldehyde, acetaldehyde, sixteen carboxylic acids and, in the hydrolysate, eleven protein and nonprotein amino acids. About 20% of the non-volatile radiolytic products have a hydrophobic character and can be extracted with chloroform. Among them is a long-living nitroxide free radical which is stable for months at room temperature. It has been suggested that the nitroso compounds are formed as radiolytic products, and that they act as spin-traps by converting some of the short living radicals to the observed nitroxide radical. This and other experimental findings are discussed in the light of free radical reactions induced by ionizing radiation.

**Key words:** Aqueous solutions — Nitriles — Ionizing radiations — Products fractionation — Long-living nitroxide radical — Additional oligomerization — Chemical evolution

### Introduction

Propionitrile,  $\text{CH}_3\text{CH}_2\text{CN}$ , is one of interstellar molecules (Mann and Williams 1980) and very likely was one

of the primitive Earth's constituents. Previous work has shown that the action of ionizing radiation on aqueous solutions of propionitrile leads to the formation of a mixture of compounds of interest to chemical evolution studies (Draganić et al. 1976a, 1976b, 1977). Their amounts increase with radiation dose. The purpose of the present study was to fractionate the mixture of products formed at high radiation doses where up to 40% of the nitrile was decomposed, and to analyze the fractionated material. We have also considered possible pathways leading to the formation of products by taking into account our earlier results on radical-radical and radical-solute reactions in the radiolysis of aqueous propionitrile.

### Experimental

Propionitrile was a Fluka (Busch, Switzerland) product purified by distillation. The deaerated samples were prepared by the syringe technique, introducing a known amount of propionitrile into deaerated water. Absorbed doses varied between 8 and 28 Mrad, (80–280 kGy) and the dose rates between 0.2 and 0.3 Mrad.h<sup>-1</sup> (2–3 kGy.h<sup>-1</sup>). Irradiations were made at room temperature which was about 17°C. The concentration of propionitrile was 0.1 M as in our previous, detailed, investigations of this system (Draganić et al. 1976a, 1976b). From what is known about the solute concentration influence on the radiolysis of dilute solutions (Draganić and Draganić 1971). The observations made under these conditions are essentially valid also for smaller concentrations, down to one order of magnitude or more.

If not otherwise stated the materials, instruments and techniques, used in this work were the same as those described previously (Draganić et al. 1976a and 1980a).

The EPR measurements were made on a standard Varian Model E-9 spectrometer (Palo Alto Ca., U.S.A.) operated at 9050 MHz. The variable temperature accessory of Varian Associates was used for spectra recording at various temperatures.

Offprint requests to: I. Draganić

## Results

### Fractionation of the Mixture of Radiolytic Products

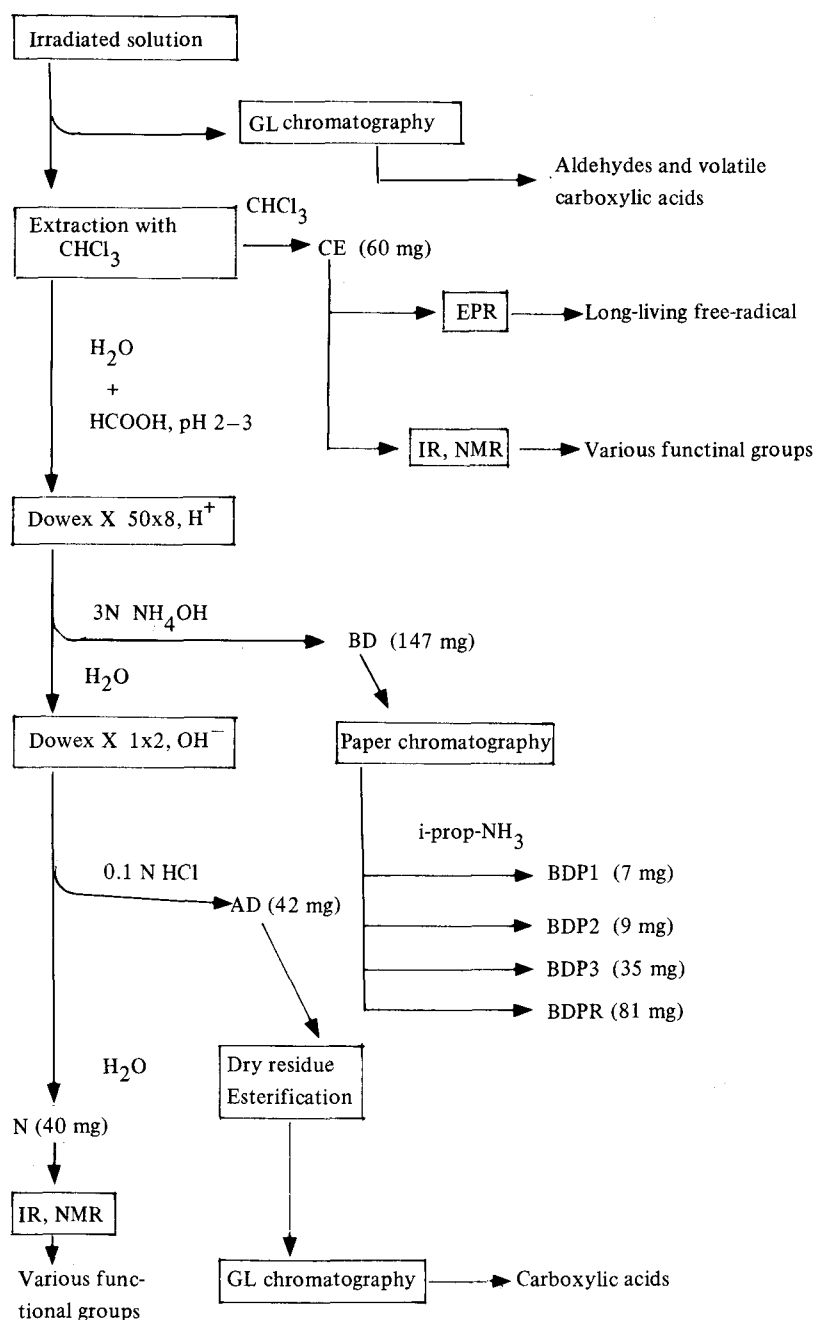
The starting material was usually 200 ml of a deaerated aqueous solution of propionitrile (0.1 M, natural pH), which has received a 18 Mrad dose of  $^{60}\text{Co}$  gamma at a  $0.25 \text{ Mrad}\cdot\text{h}^{-1}$  dose rate. Upon irradiation the solution becomes yellow-green and the hydrophobic material appears as droplets. The sample scums abundantly during evaporation in vacuum at  $45^\circ\text{C}$ . About 300 mg of non-

volatile radiolytic products appear as a dry residue. Chart 1 summarizes the procedure used for fractionation and gives the amounts of fractionated materials for a typical run.

### Search for Volatile and Gaseous Radiolytic Products

An aliquot of irradiated solution was introduced directly in the GL chromatograph under conditions usual for identification of aldehydes and volatile carboxylic acids.

**Chart 1.** Fractionation of the radiolytic products. Analyzed sample: 200 ml of aqueous propionitrile (0.1 M, natural pH). Absorbed dose 18 Mrad



By coinjection of corresponding standards the acetaldehyde, propionaldehyde, propionic, 2-methylbutyric, butyric and valeric acids were tentatively identified. The concentrations estimated for an 18 Mrad dose vary between  $10^{-3}$  M and  $10^{-5}$  M, from propionic acid being the most abundant (1.8 mM) to valeric acid which appears at the lowest concentration (0.03 mM). Both aldehydes appear in millimolar concentration, 1.4 and 0.5 for propionaldehyde and acetaldehyde respectively, in agreement with our earlier observation of the total aldehyde yield and its dependence on absorbed dose. Besides aldehydes, earlier measurements also show the presence of other simple molecules:  $H_2O_2$ ,  $H_2$ ,  $NH_3$ ,  $CO_2$ ,  $CH_3CH_3$  (Draganić et al. 1973, 1976a) and  $C_2H_5NH_2$  (Draganić et al. 1977).

#### Fraction CE and the Long-living Free-radical

The chloroform extract is a greenish-yellow transparent liquid which on concentration by evaporation in vacuum remains transparent but becomes oily and brownish-yellow in color. The identification of functional groups was made by IR,  $NMR^1H$  and  $NMR^{13}C$ . They offer the supporting evidence for the presence of  $-CH_3$ ,  $>CH_2$  and  $\geq CH$  groups as hydrophobic sites and explain the hydrophobic character of fraction CE. The presence of  $>CH-CN$  and  $>CHC(=O)OR$  groups was also found. Their presence explains fairly low solubility of CE in nonpolar solvents.

Acid hydrolysis followed by amino acids analysis shows that amino acids represent 1.8% of the total weight of fraction CE.

The chloroform extract (CE) is a composite material where heterogeneous oligomers with hydrophobic characteristics are the main constituents. One finding of particular interest is the presence of a long-lived radical among the constituents of fraction CE, which is stable at room temperature for several months.

The EPR spectra were recorded in chloroform solution at various temperatures between 213 K and 353 K. Prior to the measurements the organic extract was evaporated to dryness in vacuum at room temperature ( $17^{\circ}$ – $19^{\circ}C$ ). Trace amounts of propionitrile, that might be present in the sample, were removed at the same time. Figure 1 shows the spectrum at 333 K. The EPR signal almost disappears at 353 K presumably due to a faster spin exchange between the observed radical and a radical-like structure (Ayscough 1976). A reversible process was observed when the sample was restored to lower temperatures: the EPR signal returned to its earlier values. The signal could be irreversibly attenuated by addition of  $CuCl_2$  in methanol, as one would expect from the redox reaction between cupric ions and paramagnetic species. Presence of oxygen had no visible influence on the EPR signal.

The results of measurements at various temperatures and in different solvents suggest that the EPR signal

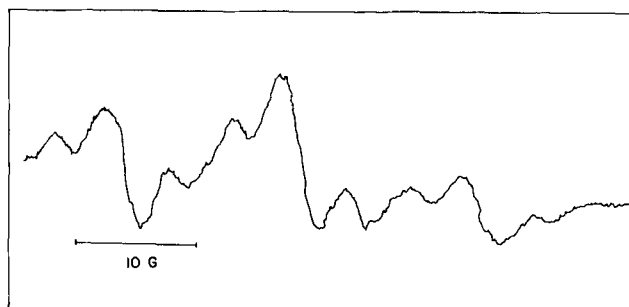


Fig. 1. Stable free-radical produced radiolytically in aqueous propionitrile. ESR spectra recorded on chloroform extract at 333K, and 160mW microwave power

should be assigned to a nitroxide radical species with  $a_N = 14.0$  G and  $a_{\beta-H} = 4.125$  G, assuming an interaction with two equivalent  $\beta$ -protons. The supporting evidence for the above interpretation is obtained by a numerical Monte Carlo technique for simulating the absorption function and EPR line shape (V. Beltrán-López 1981).

#### Basic Fraction (BD)

It represents almost half of the total weight of the mixture of products. During concentration in vacuum at  $45^{\circ}C$  it scums very strongly. After lyophilization the residue is a yellow powder. The determination of amino acids upon acid hydrolysis shows that they represent 2.5% of the total weight of fraction BD. Paper chromatography was used for some further purification of the fraction BD (Chart 1). This fractionated material was hydrolysed and analysed by amino acid analyzer. The results are presented in Table 1. Fraction BDP3 represents 55% of the starting material and contains only 1.3% of amino acids in its hydrolysate. However, the other three fractions give on hydrolysis amino acids

Table 1. Amino acid composition of the basic fraction (BD) and its constituents in  $\mu$ moles per gram of the material

Amino acid	BD	BDP1	BDP2	BDP3
Aspartic acid	15.4	20.1	22.0	12.0
Threonine	4.1	6.1	7.5	6.7
Serine	17.1	28.3	20.0	26.6
Glutamic acid	13.0	13.6	19.8	11.1
Glycine	20.0	43.0	32.7	24.9
Alanine	186.1	43.7	347.2	149.1
$\alpha$ - $NH_2$ -Butyric	35.0	—	—	36.5
Valine	2.2	—	a	a
Isoleucine	3.1	5.0	4.0	5.0
$\beta$ -Alanine	77.2	117.4	48.3	30.8
Lysine	2.9	8.7	6.3	1.8
Total	376.3	285.9	507.8	304.6

<sup>a</sup>Trace amounts

which vary significantly both in chemical form and amount. The largest amino acids content was in BDP2 (4% of fraction weight) and in this case alanine represents 76% of the total amino acids. The  $\beta$ -alanine represents 41% of total amino acids in BDP1. About 70% of the amino acids detected in BDP3 are alanine,  $\beta$ -alanine and  $\alpha$ -NH<sub>2</sub>-butyric acid.

The assignment of the amino acids in present work was made only taking into account the retention times of standards in amino acid analyzer determinations. The supporting evidence for the presence of alanine,  $\beta$ -alanine and  $\alpha$ -NH<sub>2</sub>-butyric acids was provided by GC-MS in a previous work (Draganić et al. 1977). Therefore, the identification of aspartic acid, serine, glutamic acid, glycine and lysine remains tentative. At variance with previous work, we have used up to 100 times more concentrated material for the analysis and some new peaks appeared. These were tentatively assigned to threonine, valine and isoleucine.

The biuret reaction was positive in all fractions of BD but the intensities were higher than expected from the amino acid contents. This suggests that peptide bonded fragments are scarce and that oligomeric materials consist predominantly of heteropolyamides. Also, a significant amount of tertiary nitrogen was present. A comparison of IR spectra of the BD fraction and of its constituents BDP1, BDP2 and BDP3, shows practically the same characteristic bands that differ only in intensity. The infrared spectra suggest the presence of hydrophobic groups ( $-\text{CH}_3$ ,  $> \text{CH}_2$ ,  $\geq \text{CH}$ ) as well as  $-\text{NH}_2$ ,  $> \text{NH}$ ,  $-\text{OH}$  and  $> \text{CH-CN}$ .

#### Acid Fraction (AD)

This is a yellow, oily, material upon lyophilization, which represents about 14% of the total weight of the mixture. The amino acids in the hydrolysate represent only 1.5% of total weight of AD.

The carboxylic acids seem to be more significant constituents of the acid fraction. When the dry residue is esterified and introduced into the GL chromatograph, about twenty peaks appear. Some of them were tentatively identified by coinjection of standard compounds: methylmalonic, malonic, methyl succinic, 1,2-dimethylsuccinic, succinic, methylfumaric (mesaconic), itaconic, hydroxylmalonic (tartronic), adipic, methyltricarballic, tricarballic and citric acid. Their concentrations in a sample which has received 18 Mrad dose, vary between 30 and 1 micromole. The total amount of carboxylic acids in the acid fraction (AD) was estimated to be up to 10% of the total fraction weight. More work seems to be desirable for their further identification and determination.

#### Neutral Fraction (N)

This is a white, hygroscopic, powder which represents about 14% of the total weight of the mixture. The IR spectra of the neutral fraction show the presence of characteristic bands for the following groups:  $-\text{OH}$ ,  $> \text{C=O}$ ,  $\geq \text{C-O}$ ; the NMR<sup>1</sup>H measurements also support  $-\text{CH}_3$ ,  $> \text{CH}_2$ , and  $\geq \text{CH}$ . The amino acids which were identified in the hydrolysate represent 2.5% of the total weight of the fraction.

#### Discussion

Fractionation and characterization of the radiolytic products in the aqueous solutions of propionitrile shows that most of the fractions are still complex mixtures. Nevertheless, the results obtained yield a reliable over-all picture of the chemical action of ionizing radiation on aqueous propionitrile and some insight into the chemical nature of the compounds formed.

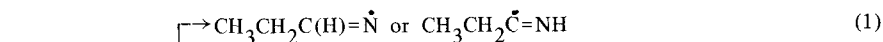
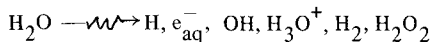
#### Free-radicals and Product Formation

When an 0.1 M solution of propionitrile is irradiated by <sup>60</sup>Co gammas their energy is deposited largely in the water, causing the formation of chemically reactive species: hydrated electrons ( $e^-_{aq}$ ), hydroxyl radicals (OH) and hydrogen atoms (H). These react among themselves and with propionitrile molecules, and lead to the formation of secondary free-radical intermediates. Scheme 1 summarizes some of these reactions in O<sub>2</sub> free aqueous solution of propionitrile.

The reactions in Scheme 1 take place on a sub-second time scale and were observed by fast kinetic spectrophotometry in pulsed beam experiments (Draganić et al. 1976b). The rate constants of these reactions were obtained by competitive kinetics (Draganić et al. 1973) or estimated by taking into account some experimental findings. The values of  $k_6$  and  $k_7$  were derived by taking the measured value for  $k_{\text{OH}, \text{tot}}$  and the known efficiency of hydrogen abstraction by H atoms. Here we have assumed that the abstraction by OH radicals is two times more efficient than the one by hydrogen atoms. The values for  $k_3$  and  $k_8$  are the estimates made by assuming that they contribute only 1% to the corresponding  $k_{\text{tot}}$ .

The free-radical intermediates, produced by reactions 1–8, disappear reacting among themselves and producing small molecules. Also, they disappear in reactions with propionitrile molecules producing various oligomers. Scheme 2 gives a survey of these reactions. It might be worth noticing that some of the radicals produced by reactions 2, 3, 7 and 8 may disappear also

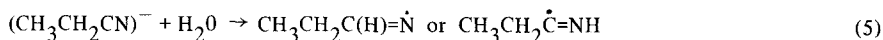
Scheme 1. Formation of intermediates in irradiated aqueous solutions of propionitrile



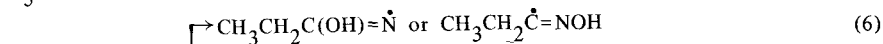
$$k_1 = 1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}; k_2 = 9 \times 10^5 \text{ M}^{-1}\text{s}^{-1}; k_3 \leq 10^5 \text{ M}^{-1}\text{s}^{-1}$$



$$k_4 = 1 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$$



$k_5$  very fast



$$k_{\text{OHtot}} = 7.3 \times 10^7 \text{ M}^{-1}\text{s}^{-1}; k_6 = 6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}; k_7 \cong 1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}; k_8 < 10^6 \text{ M}^{-1}\text{s}^{-1}$$

by hydrolysis, in competition with their recombination reactions.

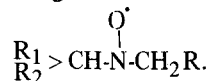
#### Formation and Fates of Unstable Radiolytic Products

The disproportionations of secondary radicals, produced by reactions 1, 5 and 6, lead to the formation of unstable radiolytic products by reactions 9 and 10. Some of them react with water (eqs. 11, 12 and 13) by producing propionaldehyde, propionic acid, ethylamine,  $\text{NH}_3$  and  $\text{CO}_2$ . They are formed in low yields (Draganić et al. 1973, 1976a, 1977). Cyanate is also unstable and splits into two radical species (eq. 14); the resulting  $\dot{\text{O}}\text{CN}$  radical reacts with water leading to the formation of  $\text{HCHO}$  or  $\text{HCO}$  and  $\text{HNO}$  or  $\dot{\text{N}}\text{O}$ . The ethyl radicals abstract hydrogen from propionitrile or some of the oligomers and produce ethane (eq. 15) or disappear in some reactions with other free-radicals which produce propionaldehyde, propionic acid, 2-methylbutyric acid and valeric acid (eqs. 16, 17, 18, 20). The origin of the acetaldehyde is very likely in some secondary reactions.

One should note that the yields of aldehydes, ammonia and carbon dioxide at 18 Mrad dose are very low. As observed also earlier (Draganić et al. 1976a) their yields decrease rapidly with increasing dose due to reactions that are not well defined. Carbon dioxide also reacts with the hydrated electron (eq. 19).

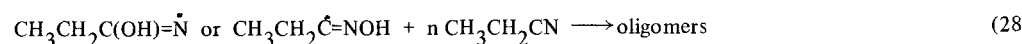
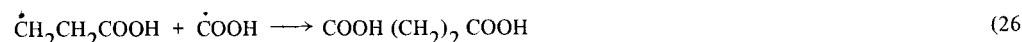
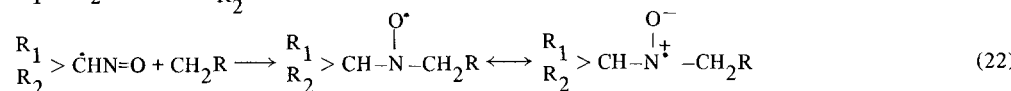
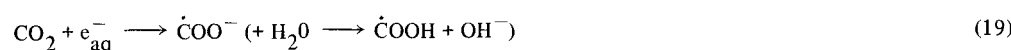
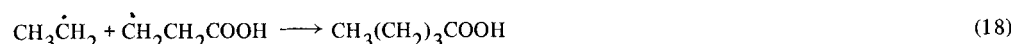
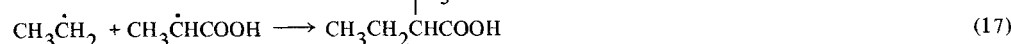
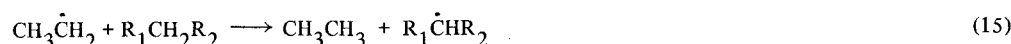
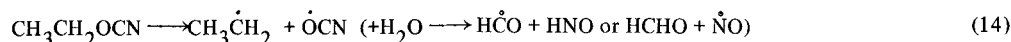
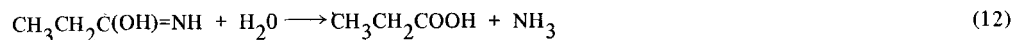
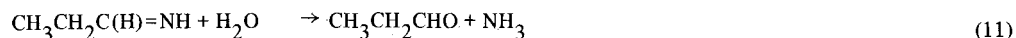
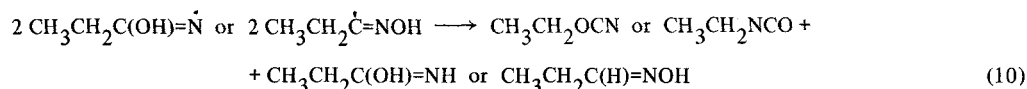
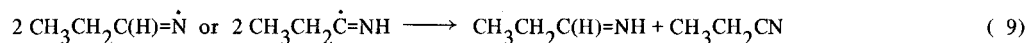
#### Nitroso Compounds and the Long-living Free-radical

The  $\text{HNO}$  and  $\text{NO}$  species, produced by reaction 14, can react with various intermediates during the formation of lyophobic oligomers and produce a nitroso compound as in reaction 21. The nitroso compounds are known as efficient scavengers for short-lived radicals and their conversion to long-living free-radicals (Janzen 1971). In our case the formation of nitroso compounds, i.e., the spin-traps, occurs during the irradiation and they react as given by eq. 22. Long-lived nitroxide radicals are formed here as in other cases of spin trapping by nitroso compounds. When recorded in different solvents and solvents mixtures, the EPR spectra followed the changes expected for nitroxide radicals. It can be inferred from the anisotropic spectrum that several interacting nuclei contribute to hyperfine splitting and that presumably the oligomers have different  $\text{R}_1$  and  $\text{R}_2$  in



Nothing can be said about the factors influencing the rates of reactions of radiation-produced nitroso compounds with various radicals in the irradiated solutions. Also the decay mechanism of the nitroxide radical from irradiated aqueous propionitrile was not studied. It seems very likely that the trapping of the  $\dot{\text{C}}\text{H}_2\text{R}$  species takes place with those having different R.

Scheme 2. Reactions of intermediates in irradiated aqueous solutions of propionitrile



### Carboxylic Acids

Propionic acid is formed by hydrolysis of unstable compounds, from reaction 10, as given by eq. 12 and by combination of radicals as given by eq. 20. The combinations of radicals formed in reactions 2, 3, 7, 8, 14 and 19 lead to the formation of the following acids: 2-methyl butyric, valeric, methylmalonic, 1,2-dimethylsuccinic, succinic and adipic acid, as given by reactions 17, 18, 20, 23, 24, 25, 26. The origin of other carboxylic acids,

which were observed in the irradiated solutions, must be in some secondary reactions.

### Oligomers

About 70% of the radiolytic products appear as non-volatile material, mainly composed of oligomers. This suggests the importance of reactions 27 and 28.

At least two types of oligomers appear in irradiated solutions of propionitrile. One has a hydrophobic

character, in fraction CE, with the following functional groups:  $-\text{CH}_3$ ,  $>\text{CH}_2$ ,  $\geq\text{CH}$ ,  $>\text{CHCHN}$ ,  $>\text{CHC}(=\text{O})\text{OR}$ . The other one with the characteristics of a heteropolyamide, appearing in fractions BD and AD, besides the above functional groups contains also  $-\text{NH}_2$ ,  $>\text{NH}$ , and  $\geq\text{C-OH}$  groups.

It seems that the oligomers produced by radiolysis of aqueous propionitrile differ considerably from those in irradiated aqueous acetonitrile or ammonium cyanide (Draganić et al. 1980a, 1980b). The presence of alkyl-nitrile here is certain and the hydrolysis of the  $-\text{CN}$  group is incomplete.

#### *Oligomers with Peptidic Fragment*

The presence of various amino acids in the hydrolysate shows, nevertheless, that the formation of complex side groups of amino acids takes place during irradiation. Compared with our findings for acetonitrile, the total amino acid yield here is larger. It is also more specific, since the most abundant amino acid is alanine; other important constituents are  $\beta$ -alanine and  $\alpha$ - $\text{NH}_2$ -butyric acid.

Eleven protein and nonprotein amino acids appear in the hydrolysates (Table 1) and suggest a common origin in the additional oligomerisation and formation of peptidebonded fragments. Similarly to the oligomers in ammonium cyanide, these fragments are the constituents of heteropolyamide oligomers; by contrast with ammonium cyanide, they represent here a very small fraction of the oligomer and are difficult to examine. Fractionated materials are not significantly enriched in amino acids: at best 2.5% for BD fraction and 4% for its constituent BDP2. Glycine represents only 4%–15% of the total amino acids in the fractions, while the enrichments of  $\beta$ -alanine vary from 2%–40% and of alanine between 15% and 76%.

#### **Concluding Remarks**

The present results confirm some of our earlier findings on the radiolysis of aqueous solutions of  $\text{NH}_4\text{CN}$  and  $\text{CH}_3\text{CN}$ , where the characterizations were also performed on fractionated materials. They show the versatility of the carbon-nitrogen triple bond in the radiolysis of simple cyano compounds, and the importance of radiation induced free-radical reactions for the formation of products of interest to chemical evolution studies. The amounts of chemical change observed, however, depend on the substituent group in cyano compounds and decrease in the following order:  $\text{NH}_4\text{CN} > \text{CH}_3\text{CH}_2\text{CN} > \text{CH}_3\text{CN}$ .

There are some other specific aspects of irradiated aqueous propionitrile. Here, the most abundant amino acids are alanine,  $\beta$ -alanine and  $\alpha$ - $\text{NH}_2$ -butyric acid, while glycine is only a minor constituent. Also the oligomeric material contains the  $>\text{CHCN}$  group which shows that, in the presence of  $-\text{CH}_2\text{CH}_3$  as the substituent group on the nitrile, the hydrolysis of  $-\text{CN}$  is incomplete.

Of particular interest is the hydrophobic character of a fraction that contains about 20% of the total amount of radiolytic products, which is dispersed as oil droplets in irradiated solution. This fraction contains also a long-lived nitroxide free-radical which merits more attention. Its presence suggests the radiation-induced formation of nitroso compounds as spin-traps.

*Acknowledgement.* The authors are grateful to Dr. M. Ptak for measurement and help in the interpretation of NMR spectra. Discussions with Dr. V. Beltrán-López concerning the nitroxide radical were also greatly appreciated.

#### **References**

- Ayscough PB (1976) *Electron spin resonance in chemistry*. Methuen, London, Chapter 8
- Beltrán-López V (1981) Private communication
- Draganić IG, Draganić ZD (1971) *The radiation chemistry of water*. Academic Press, New York, pp 127–129
- Draganić I, Draganić Z, Petković LJ, Nikolić A (1973) *The radiation chemistry of aqueous solutions of simple RCN compounds*. *J Am Chem Soc* 95:7193–7199
- Draganić IG, Draganić ZD, Shushtarian MJ (1976a) *The radiation chemistry of aqueous solutions of acetonitrile and propionitrile in megarad dose range*. *Radiat Res* 66:54–65
- Draganić IG, Draganić ZD, Marković VM (1976b) *The pulse radiolysis of aqueous solutions of simple RCN compounds*. *Int J Radiat Phys Chem* 8:339–342
- Draganić I, Draganić Z, Shimoyama A, Ponnampereuma C (1977) *Evidence of amino acids in hydrolysates of compounds formed by ionizing radiations II. Aqueous solutions of  $\text{CH}_3\text{CN}$  and  $\text{C}_2\text{H}_5\text{CN}$* . *Orig Life* 8:377–382
- Draganić IG, Jovanović S, Niketić V, Draganić ZD (1980a) *The radiolysis of aqueous acetonitrile: Compounds of interest to chemical evolution studies*. *J Mol Evol* 15:261–275
- Draganić ZD, Niketić V, Jovanović S, Draganić IG (1980b) *The radiolysis of aqueous ammonium cyanide: Compounds of interest to chemical evolution studies*. *J Mol Evol* 15:239–260
- Janzen EG (1971) *Spin trapping*. *Acc Chem Res* 4:31–40
- Mann APC, Williams DA (1980) *A list of interstellar molecules*. *Nature* 283:721–725

Received September 1, 1981/Revised March 3, 1982