Acetylacetone Fullerene Derivatives Obtained from Fullerenes and Fullerene Soot, and their Biological Activity

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Abstract

Fullerene soot is used as the initial substance to synthesize fullerene derivatives. It is demonstrated that the fullerenes incorporated into the soot are more reactive towards acetylacetone than those isolated from the soot. Reaction products are characterized by means of IR, electron spectroscopy and powder X-ray structural analysis. The effect of the obtained water-soluble products on the catalytic capacity of the bacterial luciferase in bioluminescence reactions is studied.

INTRODUCTION

The products of plasmochemical synthesis of fullerenes are soot condensates (fullerene soot) containing substantial amounts of non-fullerene carbon forms. The separation of fullerenes from soot and separation into individual forms with the help of organic solvents are connected with the formation of solvates and fullerene aggregation in solutions. This can affect the reactivity of fullerenes towards the synthesis of their derivatives. Higher reactivity of the fullerenes incorporated into soot, compared with the reactivity of individual C60 and C70 fullerenes, in the reactions of oxidation with oxygen under heating was described by the authors of [1]. Because of this, it was interesting to consider the possibility to use fullerene soot as the initial reagent in the synthesis of fullerene derivatives.

In the present study we present the characterization of the products formed in the reaction systems F/S – Hacac – H_2O(I) and F – Hacac – H_2O (II) where F/S is fullerene soot, F is a mixture of fullerenes C60/C70 (70 : 30, %), Hacac is $CH_3COCH_2COCH_3$. The methods used in the study include IR, electron spectroscopy and X-ray diffraction patterns of the powder. The reactivity of fullerene soot is compared with the reactivity of fullerene mixtures isolated from soot. For water-soluble products, the data on their biological activity are obtained.

EXPERIMENTAL

Fullerene-containing soot was synthesized in a plasmochemical reactor in the carbon plasma beam in helium flow at atmospheric pressure [2]. Grafite rods were used as central electrodes. Fullerene (C60/C70) mixture was extracted by benzene from fullerene soot. The products under investigation were obtained by heating the fullerene soot and fullerene mixture in acetylacetone-water mixture. IR spectra were recorded with a Specord IR-75 spectrometer in KBr tablets. Electron absorption spectra were recorded with a Specord UV-vis. Luciferase was isolated from the recombinative stamm *Escherichia coli* in which the genes of luciferase *Photobacterium leiognathi* had been cloned [3].

The products formed in systems I and II under identical reaction conditions were isolated

in chloroform and water solutions and then, after the removal of solvents, in the solid form. The total yield of the products isolated from the solutions in chloroform, with respect to the amount of fullerene mixtures in the initial reagents, was about 50 % in system I and about 5 % in system II. The yield of solid products isolated from aqueous solutions was about 10 % and less than 2 %, respectively; in the latter case, the removal of water resulted in insoluble forms.

RESULTS AND DISCUSSION

Figure 1 shows IR spectra of the initial fullerene mixture A (curve *1*) and the products formed in reaction system I, extracted with chloroform and separated by fractional crystallization (B, C, D, curves *2–4*, respectively) and water-soluble (E and G, curves *5* and *6*). The

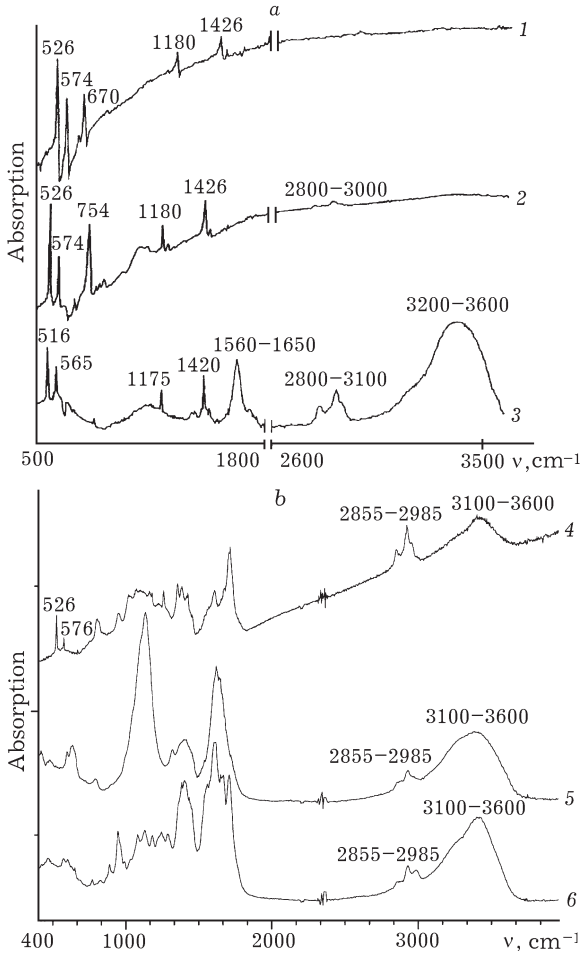

Fig. 1. IR spectra of the initial fullerene mixture (*1*) and the products formed in system I: *a* – soluble in chloroform (*2* and *3*), *b* – soluble in chloroform and acetone (*4*), water (*5*), and alcohol (*6*).

spectra A and B exhibit the bands of normal vibrations of the C60 fullerene molecule (526, 577, 1183 and 1449 cm^{-1}). The bands of C70 fullerene vibrations are present in the spectrum of the initial fullerene mixture but are not observed in the spectra of the products isolated from system I, possibly because the concentration of C70 fullerene is small in the initial fullerene condensates.

In the spectrum of the product C (curve *3*) the characteristic bands of C60 are shifted to lower frequencies (516, 565, 1173, 1420 cm^{-1}) which is the evidence of the formation of chemical bond between the reagents. A broad intensive band is observed in the spectra C, D, E and G in the region of 3200–3600 cm^{-1} which is characteristic of the stretching vibrations of OH groups involved in intra- and intermolecular hydrogen bonds, and the bands observed at 1560–1650 cm^{-1} can be attributed to the stretching vibrations of C=C and CO groups. These positions and shapes of bands are characteristic of acetylacetone attached in the enol form [4]. At the same time, the spectra of D and E products contain the bands at 1700–1720 cm^{-1} characteristic of the vibrations of C=O groups in the keto-form of acetylacetone [4]. Individual compounds will be identified in our future investigations.

The spectrum of the compound B, which was isolated in the individual form, contains the bands of C60 fullerene vibrations and a narrow intensive band with a maximum at 754 cm^{-1}; however, above 1000 cm^{-1} the background absorption is observed which complicates the analysis of the spectrum. According to the data of powder X-ray diffraction patterns (Fig. 2), this compound is a solvated form of C60 with the hexagonal lattice parameters a = 10.154 Å, and b = 10.229 Å in which a single primitive cell contains one C60 molecule. We assume that the solvating reagents are the products of acetylacetone destruction. The content of C60 solvate in reaction system I was about 40 %. Unlike system I, in system II, a similar solvate was isolated with a yield of only 5 %, and this was practically the only reaction product. We believe that this is the consequence of lower reactivity of fullerenes isolated from soot, compared to the fullerenes present in the initial soot condensate. We did not observe a similar compound with C70

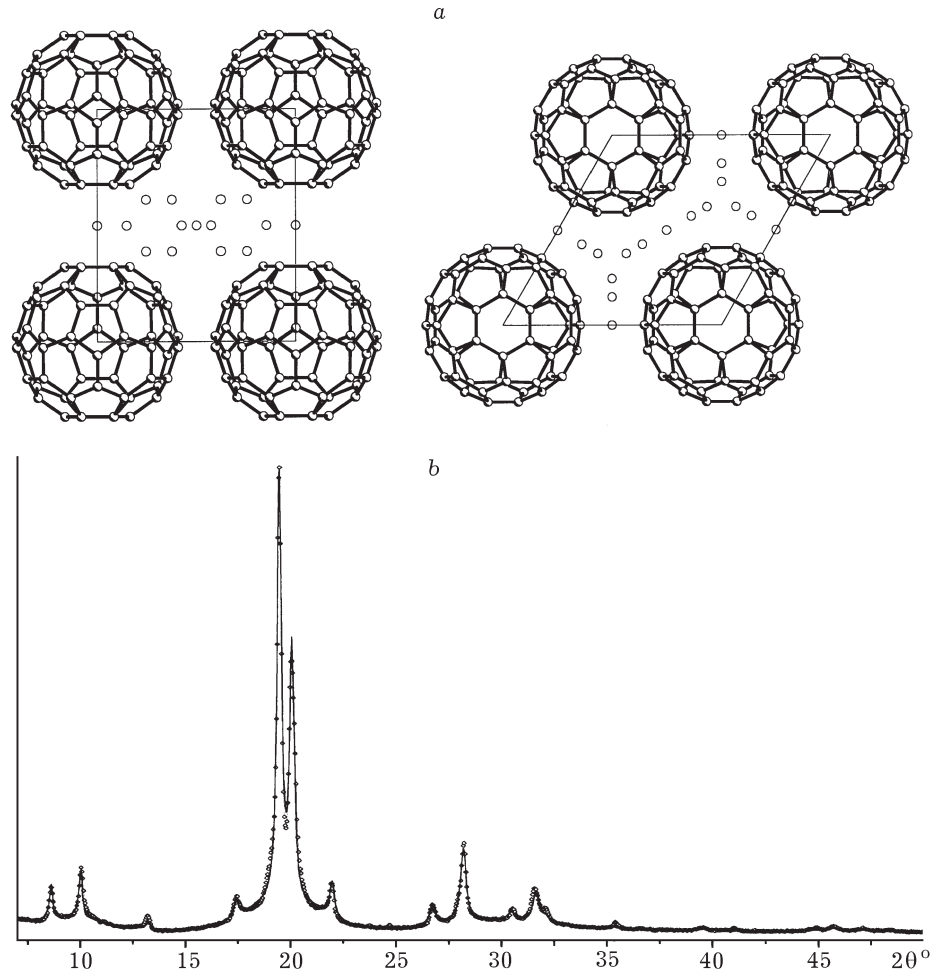

Fig. 2. Two projections of the crystal structure of the C60 solvate (*a*) and its diffraction patterns (*b*).

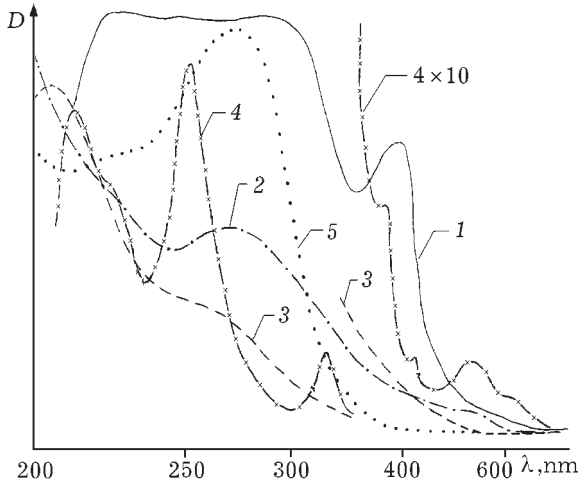

Fig. 3. Electron absorption spectra of water-soluble products formed in the systems I (*1*), II (*2*) and C60 – Hacac – H_2O (*3*), initial fullerene mixture (*4*), and aqueous solution of Hacac (*5*).

which allows us to conclude that C60 is solvated selectively.

Electron absorption spectra (Fig. 3) correspond to aqueous solutions of the products formed in the systems I, II and C60 – Hacac – H_2O. The spectrum of the product obtained on the basis of fullerene soot differs from those of the products formed when C60 fullerene and fullerene mixture are used. In the latter (curves *2* and *3*), we observe an absorption band at 275 nm, similar to the spectrum of aqueous Hacac solution (curve *5*). The maxima of bands in spectrum *1* are shifted to the red region, with respect to the Hacac bands in the C60/C70 mixture (curve *4*). We believe that the band in the visible region at 378 nm points to the presence of fullerene derivatives in the aqueous solutions under investigation. The preparation of water-soluble fullerene derivatives on the basis of

fullerene soot as the initial component excludes the stage of fullerene isolation from soot using toxic organic solvents. In our opinion, this is especially attractive in the directed synthesis of water-soluble fullerene compounds. This problem is closely connected with the medical and biological problems.

We investigated the effect of water-soluble products E and G isolated from reaction system I on the catalytic activity of the luciferase of *Photobacterium leiognathi* in the bioluminescent reaction with tetradecanal used as aldehyde substrate, and without it. The reaction was initiated by the addition of photoreduced flavin mononucleotide $FMNH_2$. The following parameters were determined: maximum intensity of luminescence I_0 (initial rate of reaction), the rate constant of bioluminescence decay (k) which determines the rate of the decomposition of the enzyme-substrate complex, and the total number of quanta Q, equal to I_0/k, which is proportional to the total number of the molecules of enzyme-substrate complex decomposed while emitting radiation. The initial concentration of compounds in solutions introduced into the reaction mixtures (C_0) was 0.05 %. The compound E was added as an aqueous solution, and the compound G as a solution in alcohol (ethanol).

Experiments showed that in the case when tetradecanal is used as an aldehyde substrate, the inhibition of bioluminescent reaction is observed in the whole investigated range of the concentrations of the added substances. The dependences of the initial luminescence intensity and luciferase quantum yield on the concentrations of the added substances E and G are of threshold behaviour (Fig. 4). The solution of G in ethanol is a stronger denaturant than the aqueous solution of E because a 50 % loss of the enzymatic activity ($C50$) occurs at the concentration of G ten times less than that of E. Figure 4, *b* shows that the denaturating component of the solution of G in ethanol at low concentrations (till 7 % of C_0) is the product G because within this concentration range ethanol activates the intensity and quantum yield of luciferase by about 50 %.

For "non-aldehyde" luminescence, the dependences of I and Q on the concentrations of ethanol and the solution of G in ethanol are bell-

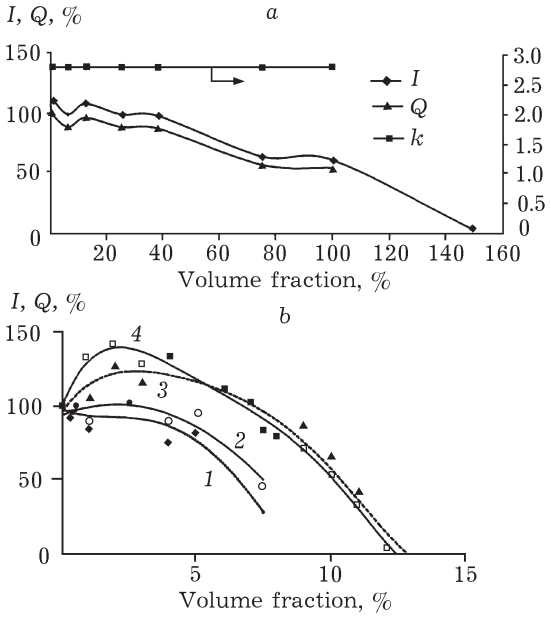

Fig. 4. The dependence of I (*1* and *4*) and Q (*2* and *3*) on the concentration of E (*a*), G (*b*) and ethanol.

like curves (Fig. 5). The inhibiting action of ethanol is approximately the same as that of the product G since their $C50$ values are close to each other and are about 11 %. At the same time, the solution of G in ethanol is a stronger activator of the intensity of "non-aldehyde" luminescence: the initial reaction rate increases by a factor of 3.5 and the yield of the reaction product by a factor of 5. A substantial increase of I and Q that accompanies the addition of the solution of G in ethanol was observed in a very narrow range of activating concentrations

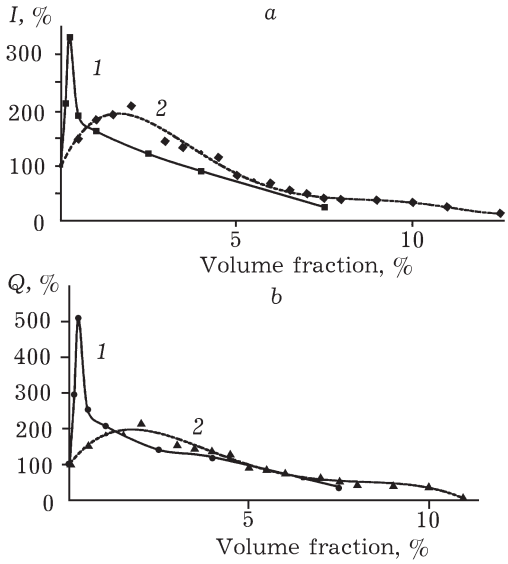

Fig. 5. The dependence of I (*a*) and Q (*b*) on the concentration of G (*1*) and ethanol (*2*) for the case of "non-aldehyde" luminescence.

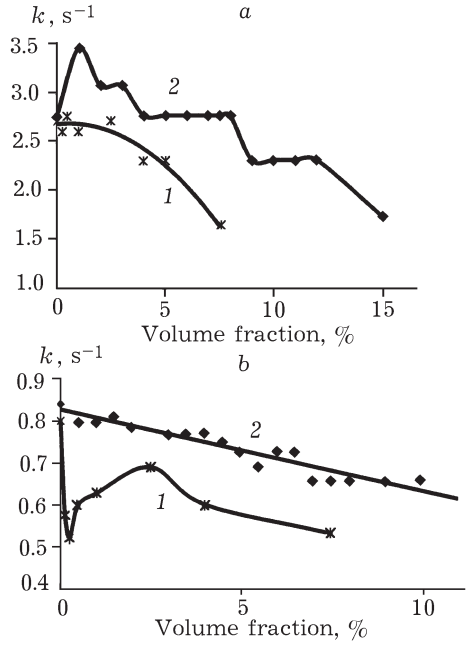

Fig. 6. The dependence of luminescence decay rate constant (k) on the concentration of G (*1*) and ethanol (*2*): *a* – reaction with tetradecanal; *b* – "non-aldehyde" luminescence.

(0–1 % of C_0), in comparison with ethanol (0–4.5 %). For the case of "non-aldehyde" luminescence, the active centre of luciferase is likely to be accessible for the activator which does not compete for the site of binding at the enzyme with the exogenic tetradecanal.

The rate constant of bioluminescence decay decreases with the increase in the concentration of the ethanol solution of G both for the "non-aldehyde" luminescence and for the case when tetradecanal is used (Fig. 6). For the "non-aldehyde" luminescence, a sharp decrease of the constant from 0.8 to 0.5 s^{-1} occurs simultaneously with the maximum increase of the initial reaction rate and quantum yield (Fig. 7). This means that a 25 % solution not only increases substantially the intensity of luminescence and the number of quanta emitted but also slows down the release of the light-induced reaction product and increases the lifetime of the intermediate product. The solutions with concentrations from 0.25 to 2.5 % of the initial one still activate the intensity of luminescence; the decay rate constant increases to 0.69 s^{-1} thus increasing the rate of substrate and product detachment. Inhibiting concentrations of this compound decrease k. So, the solution of G in ethanol slows down the decomposition of the enzyme-substrate complex and increases the lifetime of the long-lived intermediate.

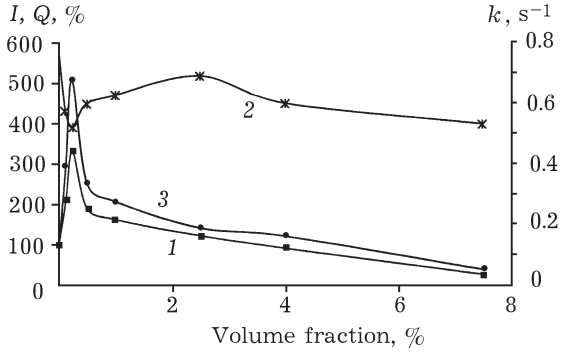

Fig. 7. The dependence of I (*1*), k (*2*) and Q (*3*) on the concentration of G for the "non-aldehyde" luminescence.

For the "non-aldehyde" luminescence, the decrease of k with increasing ethanol concentration in the reaction mixture is linear. When tetradecanal is used, k increases by 16 % in 1 % ethanol, decreases till the initial value in 4 % ethanol and remains unchanged till ethanol concentration of 8 %. The concentrations above 8 % cause a decrease of k and the rate of the enzyme-substrate complex decomposition. Figure 6 shows that for both the "non-aldehyde" luminescence and tetradecanal, increasing concentrations of the added solution of G in ethanol provide a stronger decrease of k than ethanol alone. The concentration of E in the reaction mixture of the bacterial luciferase does not affect the rate of bioluminescence decay (see Fig. 4, *a*). This means that the product E does not cause any change of the enzyme-substrate complex decomposition rate and the lifetime of the long-lived intermediate.

CONCLUSIONS

Experimental results demonstrated that the fullerenes incorporated into soot condensates exhibit higher reactivity than the fullerenes isolated from these condensates with the help of organic solvents. Using fullerene soot as the initial reagent in the reaction with acetylacetone we obtained fullerene derivatives containing active OH groups. The effect of the synthesized water-soluble compounds on the kinetic parameters of bioluminescent reactions with the

participation of luciferase was investigated. These compounds are biologically active; the activation and inhibition of the luciferase activity are determined by the type and concentration of the compounds used.

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