Preparation and high bacteriostatic action of the activated carbons possessing ultrafine silver particles

Seiichi Miyanaga, Akio Hiwara, Hajime Yasuda*

Department of Applied Chemistry, Graduate School of Engineering, Hiroshima University, 1-4-1 Higashi-Hiroshima, Hiroshi-Hiroshima 739-8527, Japan

Received 23 August 2001; revised 14 December 2001; accepted 14 December 2001

Abstract

The new carbon composites possessing ultrafine silver particles (0.001 wt%, size 1.8–2.5 nm) show high bacteriostatic action toward bacteria such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis and a yeast fungus like Candida albicans. The present carbon composite was prepared by the steam activation of a mixture of a petroleum pitch and N,N-dimethylaminomethylphenylsilver (0.012 mg/g) at 930 °C for 15–19 min. The growth inhibitory effect for Gram-negative bacteria such as E. coli and P. aeruginosa varies greatly depending on the amount of ultrafine silver particles, while the Gram-positive bacteria show little effect by the amount of silver particles used. A yeast fungus like C. albicans exhibits relatively small biostatic action as compared with bacteria, but it shows enough activity when we used the carbon material possessing 0.01 wt% of Ag particles. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Activated carbon; Bacteriostatic action; Silver particles

1. Introduction

Recently, microbial contaminations and deteriorations induced by microorganisms are spreading to various fields such as foodstuffs, plastic materials [1], building materials [2], petroleum products, electronic equipments, optical instruments, and textile materials [3]. Infection and contamination in the medical treatment became serious problems. In response to these problems, new pasteurizations and antibacterial technologies are required, and the applications of metal, especially silver, which has a strong growth inhibitory effect on bacteria are attracting considerable attention. Silver is known to show strong growth inhibitory effect for Escherichia coli and Pseudomonas aeruginosa [4–6]. Many carbon materials covered with colloidal silver are already known, but these materials are generally prepared by the soaking in AgNO₃ or AgOCOME solution followed by the reduction with some reducing agents such as formaldehyde and hydrazine or simply by heating. Therefore, the silver metal lies on the carbon surface in the cases of conventional carbon materials prepared from pitch precursors [7,8] and viscose fibers [9,10]. Although the antibacterial function of phenolic resins [11] and methyl methacrylate grafted phenolic resins containing AgNO₃ [12] or spinable isotropic petroleum pitch containing AgOCOME [13] are already known and noted briefly as only active (+) or inactive (−), more detailed studies are necessary. Antibacterial characteristics of carbon materials containing ZnO or MgO are also reported, although their activities are much lower than those containing Ag particles [14–16]. Herein we report the novel method to prepare carbon materials possessing ultrafine silver particles in the carbon (not on the carbon), and the detailed studies on the antibacterial functions of these materials by batch systems. Fortunately, most ultrafine silver particles lie on the surface of pores, and therefore the present material showed very strong inhibitory effect to various bacteria.

2. Experimental

2.1. Materials

N,N-Dimethylaminomethylphenylsilver was prepared according to the literature method [17,18]. Cobaltocene, cyclopentadienyl (1,5-cyclooctadiene)rhodium, nickelo-cene, mesityleneopper was purchased from Azumax. Co. Petroleum pitch possessing a softening point of 85 °C (elemental analysis C 93.2, H 4.7, N 1.2, O 0.4, S 0.5%) and hard pitch possessing softening point of 280 °C (elemental analysis C 94.0, H 3.5, N 1.1, O 1.0, S 0.4%)
were gifted from Osaka Gas Co. The commercial carbon material containing colloidal silver (0.01 and 0.001 wt%) was purchased from N.E. Chemcat Co.

2.2. Measurements

BET (Brunauer–Emmet–Teller) specific surface areas and pore were measured by Autosorb-6 (Quanta Chrome Co.) using nitrogen gas. XRD investigations were performed on a Rigaku RDA-1B diffractometer using Cu Kα radiation at 40 kV, 30 mA. Thermogravimetric analyses were carried out on a Seiko SSC-5100 DSC-22C apparatus. The particle induced X-ray emission analysis (PIXE) was performed on a van de Graff accelerator system in Hiroshima University using the proton radiation.

2.3. Steam activation of the precursor

Petroleum pitch (4 g) possessing a softening point of 85 °C was dissolved in THF (50 ml) and N,N-dimethylaminomethylphenylsiloxane (0.024 mg/g-pitch) was mixed with the petroleum pitch. Then the solvent was removed by flash distillation under vacuum and the product was mixed with a powdered hard pitch (4 g) whose softening point is 280 °C. Homogenization of the pitch was made by pulverizing and kneading the mixture at ambient temperature followed by heating to 360 °C every 30 min four times. Then air oxidation of the mixture was carried out at 360 °C for 40 min–1 h using a crucible (alumina > 99.5%, Nippon Kagaku Togyo) in Kantal combustion furnace (Motoyama MTKW-11-1040, 299 V, 3.7 kW). Steam activation was applied to the mixture at 930 °C for 5–19 min using a nitrogen gas saturated with steam (flow rate, 180 ml/min). The temperature was controlled by a digital program (Chino KP-1131) coupled with a thyristor unit (Sansa Elec. Co. VP1-2020) and was monitored by the sheathed thermocouple (type R; Pt/Pt–Rh). Resulting material shows the microporous carbon as shown in Table 1. Blank carbon was obtained by the same method as described earlier without addition of N,N-dimethylaminomethylphenylsiloxane. Thermogravimetric analysis of the pitch possessing a soft point of 85 °C reveals the beginning of the decomposition at ca. 200 °C, and weight loss of 60% being achieved at 700 °C after 20 min, while the pitch possessing a soft point of 280 °C begins the decomposition at ca. 290 °C and the weight loss of 14% was observed at 700 °C after 40 min.

2.4. Bacteriostatic activity

Vital cells used in this work are *E. coli* (NIHJ-IC2), *P. aeruginosa* (ATCC-27853), *S. aureus* (NIHJ-SC1), *Bacillus subtilis* (JCM-1465) and *Candida albicans* (IFO-1594). MIC (minimal inhibitory concentration) was measured using the agar dilution method recommended by Japan Society of Chemotherapy at 37 °C. The bouillon medium for bacteria is prepared by mixing of extract of beef (5–10 g), peptone (10 g), NaCl (5 g), and glucose (10 g) with 11 of water at pH 6.8–7.0, and the GYP medium for the yeast was prepared from extract of yeast (5 g), peptone (5–10 g), and glucose (10 g) in water at pH 6.3–7.2. The initial vital cell concentration was 10⁶ CFU/ml and the incubation was carried out at 37 °C for 48 h.

2.5. TEM measurement

The Ag/carbon composites (Ag, 0.001 wt%, heated to 930 and 1100 °C) were impregnated into polyester resin (Ohken Shoji, Rigolac 2004) and the resin was cut into slices using an ultramicrotome (LKB-Producer, Ultratome NOVA LKB 2188). TEM measurements were carried out on a JEM-2000FX analytical electron microscope (JEOL) operated at an electron voltage of 120 kV. The number-average particle sizes and particle size distributions were determined by measuring more than 300 nanoparticles with a diameter larger than 2 nm.

3. Results and discussion

3.1. Preparation of carbon materials possessing ultrafine silver particles

The activated carbons possessing ultrafine metal particles were prepared by the steam activation of pitches mixed with N,N-dimethylaminomethylphenylsiloxane, cobaltocene, η⁵-cyclopentadieny-η⁴-(1,5-cyclooctadiene)rhodium, nickelocene, and mesitylene copper at 930 °C for 19–30 min. Resulted carbon materials contained Ag [19], Co [20], Rh [21], a mixture of Ni [22] and NiO [23] (55/45), and CuO [24], as evidenced by the powder X-ray analyses. The mixture of pitch (1 g) and N,N-dimethylaminomethylphenylsiloxane (0.012 mg) was heated to 750–1100 °C using nitrogen gas (flow rate, 50 ml/min) saturated with steam.

| Temperature (°C) | Carbonization yield (%) | Ag content (wt%) | BET surface (m²/g) | Pore size (Å) |
|-----------------|-------------------------|-----------------|--------------------|--------------|
| 750             | 41                      | 0.0008          | 768                | 18           |
| 930             | 35                      | 0.0011          | 835                | 20           |
| 1100            | 31                      | 0.0021          | 896                | 21           |

* Heated for 20 min.

* Determined by PIXE analysis.

* Measured by Autosorb-6 in nitrogen.
and the carbons possessing ultrafine silver particles (0.001 wt%) were obtained in 35% carbonaceous yield when the material is heated up to 930 °C for 20 min (see Eq. (1) and Table 1).

\[
\text{Pitch} \xrightarrow{\text{Steam activation} \ 930^\circ \text{C, } 5-19 \text{ min}} \text{Carbon/Ag Composite}
\]

The average size of silver particles ranges 1.8–2.5 nm and the carbon assumes micropore structure (Table 2). This value is smaller than those, 20–40 nm, obtained from AgNO₃ [7,11]. Continuous heating of the matrix to 1100 °C resulted in the aggregation of silver particles to the bigger particles of size 18–22 nm as evidenced by TEM measurement (Fig. 1). At this temperature, we can still observe the small ultrafine silver particles of size 1.8–2.5 nm simultaneously with the newly formed large particles, while heating to 930 °C produced only the particles of size 1.5–2.5 nm. No strong interaction was observed between the carbon and the silver particles. The increase of the Ag content in carbon is known to decrease the BET specific surface area significantly [8]. Maximum antibacterial activity was observed when we used the C/Ag composite heated to 930 °C (Table 2). This result indicates that coagulation of silver particle at 1100 °C resulted in decreased biostatic activity, presumably due to the decrease in the surface area of silver particles which resulted to release less silver ions.

3.2. Bacteriostatic action of activated carbons containing ultrafine Ag particles

3.2.1. Evaluation of the biostatic action by MIC method

The growth inhibitory effects for bacteria, i.e. E. coli, P. aeruginosa, S. aureus, B. subtilis and C. albicans, were evaluated by the MIC method using the activated carbons containing 0.05 and 4.8 wt% ultrafine silver particles as well as CoO, Rh, CuO, and Ni ultrafine metal particles (Table 3). Among the samples tested here, every carbon possessing silver particles as well as silver colloids show high biostatic activity (low inhibitory concentration) for bacteria and a Eumycete Asporogenous yeast (C. albicans). Especially, these particles exhibit high efficiency for the destruction of E. coli and P. aeruginosa, even using carbon materials having only 0.001 wt% of silver particle. Surprising is the high activity of the carbon containing 0.12 wt% silver particles as compared with 100% colloidal Ag toward four bacteria as well as antifungal action toward C. albicans. This should be ascribed to the high surface of the ultrafine silver particle as compared with colloidal silver, which resulted in the enhanced solubility of the silver ion. These carbon materials are less effective to the destruction of B. subtilis and a yeast fungus, C. albicans, even using excess colloidal silver. The carbons including other metal particles such as CoO and Rh show a moderate sterilizability for all bacteria and a yeast fungus. These actions are nearly the same with that of cobalt powder. However, the carbons containing a mixture of Ni and NiO, CuO, FeO [25], Ru, [26] and TiO [27] particles (prepared from nickelocene, mesitylene,poly(vinyl ferrocene), (acenaphthylene)-Ru(CO)₃ and TiCl₃(C₂H₅)₂) showed practically no activity.
Blank carbons prepared from pitches at 930 °C also exhibit no activity.

The bacteria were found to preferably adhere to the solid support made of carbon material, indicating that the activated carbon fiber (ACF) has good biocompatibility [28]. Bacteria may breed on the ACF during the purification process for drinking water, itself becoming a pollutant [11]. In sharp contrast to these findings, carbon materials containing Ag particles show strong antibacterial activity.

3.2.2. Evaluation of biostatic activity for C/Ag composites by the batch system

The bacteriostatic action of carbon materials possessing ultrafine silver particles was finally evaluated by a batch culture method. After an addition of the C/Ag composite to the aqueous culture solution, the number of colonies of the va till cell was measured at regular interval using agar substrates. For example, the number of E. coli colonies reduced significantly by using the activated carbon possessing 0.001 wt% of ultrafine Ag obtained at 930 °C. When we used the commercial granular carbons covered with 0.001 wt% of colloidal silver at 30 °C, we could not recognize any inhibitory effect (Fig. 2(a)), while the commercial carbon containing 0.1 wt% of colloidal silver (Ag/C, Ag size ca. 52 nm) (c) shows the nearly the same activity as that of Ag/C (0.001 wt%) composites (b). Thus, the present carbon/Ag composite shows much higher bacteriostatic activity than the commercially available carbon powder covered with colloidal silver. This should be originated from the homogeneous dispersion of ultrafine silver particle, which allows the high elution of silver ions from the silver particles. Then, this in turn may cause a shortened lifetime of this formulation, which should become a drawback in the circumstance of practical application. However, observed lifetime (0.5 day) is longer than 0.3 day of the carbon materials covered with colloidal silver due to the matrix formation of silver particles with surrounded carbon opposed to our expectation. Thus, silver ion oozes out from the surface of carbon materials effectively. The C/Ag composites prepared at 750 °C shows again high biostatic action, while the C/Ag composite synthesized at 1100 °C (Ag size, 20 nm) significantly reduced its biostatic action (Table 2). The Ag particle itself is known to be inactive for bacteria but Ag⁺ shows high bacteriostatic action [29]. The elution of silver ion from the carbon material possessing 0.045 wt% of Ag (prepared at 930 °C) is 40 ppb as determined by Japan Food Analytical Center using diphenylthiocarbazone at 25 °C for 24 h (7.5 mg of sample in 200 ml water), the value being smaller than the value (‘Safe Drinking Water Action’, 50 ppb/h at 25 °C) for the drinking water regulated by US Public Health Center. Therefore, we can use the present C/Ag composite for destruction of E. coli in the tap water or waste water. We could wash away 83 wt% of the Ag particle from the composite by treating with conc. HNO₃ for 5 min followed by washing with neutral water, as evidenced by PIXE analysis. This result indicates the location of ultrafine silver around the pore, not in the carbon. In sharp contrast to the present carbon composite, the carbon covered

| Composites     | Minimum inhibitory concentration (%) |
|----------------|---------------------------------------|
|                | E. coli | P. aeruginosa | S. aureus | B. subtilis | C. albicans |
| Ag/C (0.12 wt%)| 0.05    | 0.05         | 0.30      | 0.30        | 0.60        |
| Ag/C (0.001 wt%)| 0.60    | 1.25         | 2.50      | 2.50        | 2.50        |
| Ag colloid     | 0.12    | 0.12         | 0.25      | 0.50        | 0.50        |
| CoO/C (0.08 wt%)| 1.25    | 2.50         | 0.63      | 1.25        | 1.25        |
| Rh/C (0.15 wt%)| 1.25    | 1.25         | 1.25      | 1.25        | 1.25        |
| Ni/C (0.1 wt%) | >2.50   | >2.50        | >2.50     | >2.50       | >2.50       |
| CuO/C (0.14 wt%)| >2.50  | >2.50        | >2.50     | >2.50       | >2.50       |
| Blank carbon   | >2.50   | >2.50        | >2.50     | >2.50       | >2.50       |

Table 3
The MIC values of various metal–carbon composites for bacteria and a yeast fungus (10⁴ CFU/ml, glucose/bouillon agar (pH 7.0), 37 °C for 48 h)

![Fig. 2. Bacteriostatic action of C/Ag composite toward E. coli.](image-url)

(a) Commercially available C/Ag composite covered with 0.001 wt% Ag, (b) carbon composite obtained by steam activation of pitch/N,N-dimethylanilino- methylphenylsilver at 930 °C for 20 min exhibiting 0.001 wt% of Ag, (c) commercially available C/Ag composite covered with 0.1 wt% of Ag.
with 0.001 wt% of colloidal silver shows less elution (13 ppb, 24 h at 25 °C).

The bacteriostatic action toward *P. aeruginosa* was also examined using the same C/Ag composite. The C/Ag composite bearing 0.001 wt% of ultrafine silver particle exhibits much higher activity than the commercial carbon covered with colloidal silver (0.001 wt%). Destruction of *P. aeruginosa* completed in 2 h by using the Ag/C composite (Fig. 3(b)), while it takes 24 h to destruct the *P. aeruginosa* using the carbon covered with colloidal silver (a). Thus, the growth inhibitory effect for gram-negative bacteria such as *E. coli* and *P. aeruginosa* were found to vary seriously depending on the amount of used silver and the size of particles.

In contrast, gram-positive bacteria such as *S. aureus* and *B. subtilis* show less responsibility to the amount of silver used. For example, Fig. 4 shows the growth inhibitory effect for *S. aureus*. This bacterium shows strong halotolerant properties and multiplies even in sea water. Only a little difference is observed between the biostatic action by C/Ag composite possessing 0.001 wt% of ultrafine Ag (b) and that containing 0.0005 wt% of silver (a). The activity of the commercially available carbons coated with colloidal silver (0.1 wt%) also showed the similar behavior (c). Complete destruction was realized in 24 h after soaking the C/Ag composite into the aqueous solution of *S. aureus*.

*B. subtilis* is readily destructed by a little environmental change. Fig. 5 shows the growth inhibitory effect for the activated carbon possessing 0.001 wt% ultrafine silver particles (a), and that for the commercially available carbon material covered with colloidal silver (0.001 wt%) (b). Both C/Ag composites reveal strong growth inhibitory effect, independent of the amount of used silver particles. Thus, the gram-positive bacteria generally show the strong bacteriostatic action which is independent of the amount of used silver. The following two considerations may account for earlier noted difference. One is that the gram-positive...
bacteria have a heavy cell wall (peptidoglycan layer, 20–80 nm thickness) while the gram-negative bacteria has a thin (2–3 nm) wall [30]. Therefore, the latter becomes more sensitive to the amount of silver. Another explanation is as follows. The outer side cell wall consists of telchoic acid and abounds in anion, which makes the greater affinity of the bacterium with the Ag particle. Therefore, once a gram-positive bacterium clings to the Ag particle, it is rather hard to leave the site, so it becomes ignorant of the total Ag-amount. On the other hand, gram-negative bacteria whose outer membrane is coated with lipopolysaccharide have relatively small affinity for the Ag silver [31]. Hence, a gram-negative bacterium migrates from a particle to another particle, and is seriously influenced by the Ag-amount.

We measured also the growth inhibitory effect for C. albicans, belonging to the Eumycetes (not a bacterium in an exact meaning). High resistivity was observed for disinfectants or chemotherapy. The C/Ag composites containing 0.001 wt% of silver (Fig. 6(d)) and commercially available carbon materials possessing 0.001 wt% colloidal silver (c) showed no activity, while the activated carbon possessing ultrafine Ag particles (0.01 wt%) demonstrates the nearly the same activity (b) with the commercially available carbon containing colloidal silver (0.01 wt%) (a). Of course, the addition of carbon possessing 0.1 wt% of silver particles shows higher activity. Thus, we can use the present carbon material for purification of tap water or waste water.

4. Conclusions

The new carbon composite prepared by the steam activa-

tion of a mixture of a petroleum pitch and N,N-dimethylaminomethylphenylsilveryl at 930 °C for 20 min contains ultrafine silver particles dispersed homogeneously and it shows high bacteriostatic action toward E. coli, P. aerugi-
nosa, S. aureus, and B. subtilis. The observed activity is higher than the carbon composite covered with silver particles which was generated by soaking the carbon with aqueous AgNO₃ followed by reduction.

References

[1] D. Kubota, H. Sawai, M. Takeuchi, F. Kofushwaki, J. Antibact. Anti-
fung. Agents 26 (1998) 301.
[2] H. Takahashi, K. Kuroda, A. Yamaguchi, Y. Inoue, Inhibitory effects of quaternary ammonium compounds against wood-rotting fungi, J. Antibact. Antifung. Agents 21 (1993) 195–200.
[3] Y. Tsunoda, H. Egawa, H. Yuge, J. Antibact. Antifung. Agents 20 (1992) 571–575.
[4] J.F.A. Esmarch, Hygienische Rundschau 11 (1901) 461.
[5] A. Oflofs, C. Crosse-Siestrup, S. Bisson, M. Rinck, R. Rudolph, U. Gross, Biocompatibility of silver-coated polyurethane catheters and silver-coated Dacron material, Biomaterials 15 (1994) 753–758.
[6] A. Singh, G.A. McFeters, Survival and virulence of copper- and chlorine-stressed Yersinia enterocolitica in experimentally infected mice, Appl. Environ. Microbiol. 53 (1987) 1768–1774.
[7] C.Y. Li, Y.Z. Wan, J. Wang, Y.L. Wang, X.Q. Jiang, L.M. Han, Antibacterial pitch-based activated carbon fiber supporting silver, Carbon 36 (1998) 61–65.
[8] S.K. Ryu, S.Y. Kim, Z.J. Li, M. Jarontec, Characterization of silver-containing pitch-based activated carbon fibers, J. Colloid Interface Sci. 220 (1999) 157–162.
[9] Y.L. Wang, Y.Z. Wan, X.H. Dong, G.X. Cheng, H.M. Tao, T.Y. Wen, Preparation and characterization of antibacterial viscos-based activated carbon fiber supporting silver, Carbon 36 (1998) 1567–1571.
[10] Y.Z. Wan, Y.L. Wang, T.Y. Wen, Effect of specific surface area and silver content on bacterial adsorption onto ACF(Ag), Carbon 37 (1999) 351–353.
[11] A. Oya, S. Yoshida, Y. Abe, T. Iizuka, N. Makiyama, Antibacterial activated carbon fiber derived from phenolic resin containing silver nitrate, Carbon 31 (1993) 71–73.
[12] A. Oya, M. Kimura, T. Sugo, A. Katakai, Y. Abe, T. Iizuka, N. Makiyama, A. Linares-Solano, C. Salinas-Martinez de Lecea, Antibacterial activated carbon fiber derived from methyl methacrylate-grafted phenolic resin fiber, Carbon 32 (1994) 107–110.
[13] A. Oya, T. Wakahara, S. Yoshida, Preparation of pitch-based anti-
bacterial activated carbon fiber, Carbon 31 (1993) 1243–1247.
[14] O. Yamamoto, J. Sawai, T. Sasamoto, H. Nakagawa, K. Miura, Antibacterial characteristic of spherical carbon containing ZnO, Tanus (1999) 176–178.
[15] J. Sawai, H. Igarashi, A. Hashimoto, T. Kokugan, M. Shimizu, Evaluation of growth inhibitory effect of ceramics powder slurry on bacteria by conductance method, J. Chem. Engng Jpn 28 (1995) 288–293.
[16] J. Sawai, H. Igarashi, A. Hashimoto, T. Kokugan, M. Shimizu, Effect of ceramic powder slurry on spores of Bacillus subtilis, J. Chem. Engng Jpn 28 (1995) 556–561.
[17] A.J. Leusink, G. Van Koten, J.G. Noltes, Stable arylsilver compounds containing dimethylamino, (dimethylamino)methyl or methoxy groups at the aryl nucleus, J. Organomet. Chem. 56 (1973) 379–390.
[18] A.J. Leusink, G. Van Koten, J.W. Marsman, J.G. Noltes, Bis[2-
(dimethylamino)methyl]phenylsilverlithium, a tetranuclear organo-
metallic compound with bridging aryl groups between silver and lithium, J. Organomet. Chem. 55 (1973) 419–425.
[19] Powdered Diffraction File, International Center for Diffraction Data, New York, 1957, pp. 4–783.
[20] Powdered Diffraction File, International Center for Diffraction Data, New York, 1967, pp. 9–418.
[21] Powdered Diffraction File, International Center for Diffraction Data, New York, 1957, pp. 5–685.
[22] Powdered Diffraction File, International Center for Diffraction Data, New York, 1957, pp. 4–850.
[23] Powdered Diffraction File, International Center for Diffraction Data, New York, 1980, pp. 22–1189.
[24] Powdered Diffraction File, International Center for Diffraction Data, New York, 1986, pp. 28–775.
[25] Powdered Diffraction File, International Center for Diffraction Data, New York, 1980, pp. 21–920.
[26] Powdered Diffraction File, International Center for Diffraction Data, New York, 1990, pp. 40–1290.
[27] Powdered Diffraction File, International Center for Diffraction Data, New York, 1980, pp. 21–1272.
[28] M. Kuroda, M. Yuzawa, Y. Sakakibara, M. Okamura, Methanogenic bacteria adhered to solid supports, Water Res. 22 (1988) 653–656.
[29] F.A. Bell Jr., Review of effects of silver-impregnated carbon filters on microbial water quality, J. Am. Water Works Assoc. 83 (1991) 74–76.
[30] Biology Handbook, Gihousha, Japan, 1957, p. 125.
[31] A.A. Tolstopyatova, T.N. Filatova, E.F. Korytnyi, A.A. Balandin, Izv. Akad. Nauk. SSSR., Ser. Khim. 1439 (1969).