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RESEARCH

GENERATION OF HYDROCARBONS HAVING ADAMANTINE STRUCTURE FROM BACTERIAL BIOMASS

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The chloroform-insoluble part of chemoorganoheterotrophic aerobic bacteria Arthrobacter sp. RV and Pseudomonas aeruginosa RM is subjected to thermolysis and catalytic thermolysis (aluminosilicate as catalyst). The thermolysis and catalytic thermolysis products are analyzed by chromatomass spectroscopy. It is noticed for the first time that the products of thermolysis of the insoluble part contain hydrocarbons with typical ions of adamantanes (m/z 135, 136, 149, 163) and diamantanes (m/z 187, 188, 201). It is shown by isomerization over aluminum bromide that these hydrocarbons are protoadamantanes and protodiamantanes, respectively. Unlike thermolysis, catalytic thermolysis of the insoluble part of the bacteria generates simultaneously protoadamantanes and adamantanes of the C_{10}–C_{13} composition and protodiamantanes and diamantanes of the C_{14}–C_{16} composition. It is suggested that one of the routes of formation of hydrocarbons having diamond-like (adamantine) structure in oils could be catalytic transformation of the bacterial biomass.

Keywords: *bacteria, thermolysis, catalytic thermolysis, protoadamantanes, protodiamantanes, adamantanes, diamantanes, generation of petroleum hydrocarbons.*

Diamond-like hydrocarbons (HC) (adamantanes, diamantanes, trimantanes, etc.) occur in oils of various ages and origins and different degrees of transformation that are generated in clayey and carbonate strata [1-6]. Diamantanes are also found in the organic matter (OM) of the crystalline basement [7]. We showed earlier that HC of the adamantane and diamantane series were generated by thermal cracking and

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transformations on an acidic catalyst of high-molecular-weight (boiling above 350°C) paraffin-naphthene fractions of various types of oil and also by thermal cracking of oil resins and asphaltenes [2, 8-10]. Furthermore, these HC were formed by high-temperature cracking of high-molecular-weight *n*-alkanes [11]. However, the only thing that is now known about the genesis of diamond-like HC is that they are absent in the initial (biosynthesized) matter.

According to the sedimentation-migration hypothesis of oil formation, the HC were considered to arise from remains of living organisms that inhabited the earth during past geological eras. Most attention was paid to eukaryotic organisms (animals, plants, fungi). As a rule, prokaryotes (bacteria and archaea) were assigned the role of transforming the eukaryote OM. Nevertheless, formation of HC (alkanes, alkenes, alkadienes, isoprenoids, arenes) by both eukaryotes and prokaryotes was reported [12-14]. However, the role of prokaryotes in HC formation with respect to oil genesis has drawn little attention, in contrast with

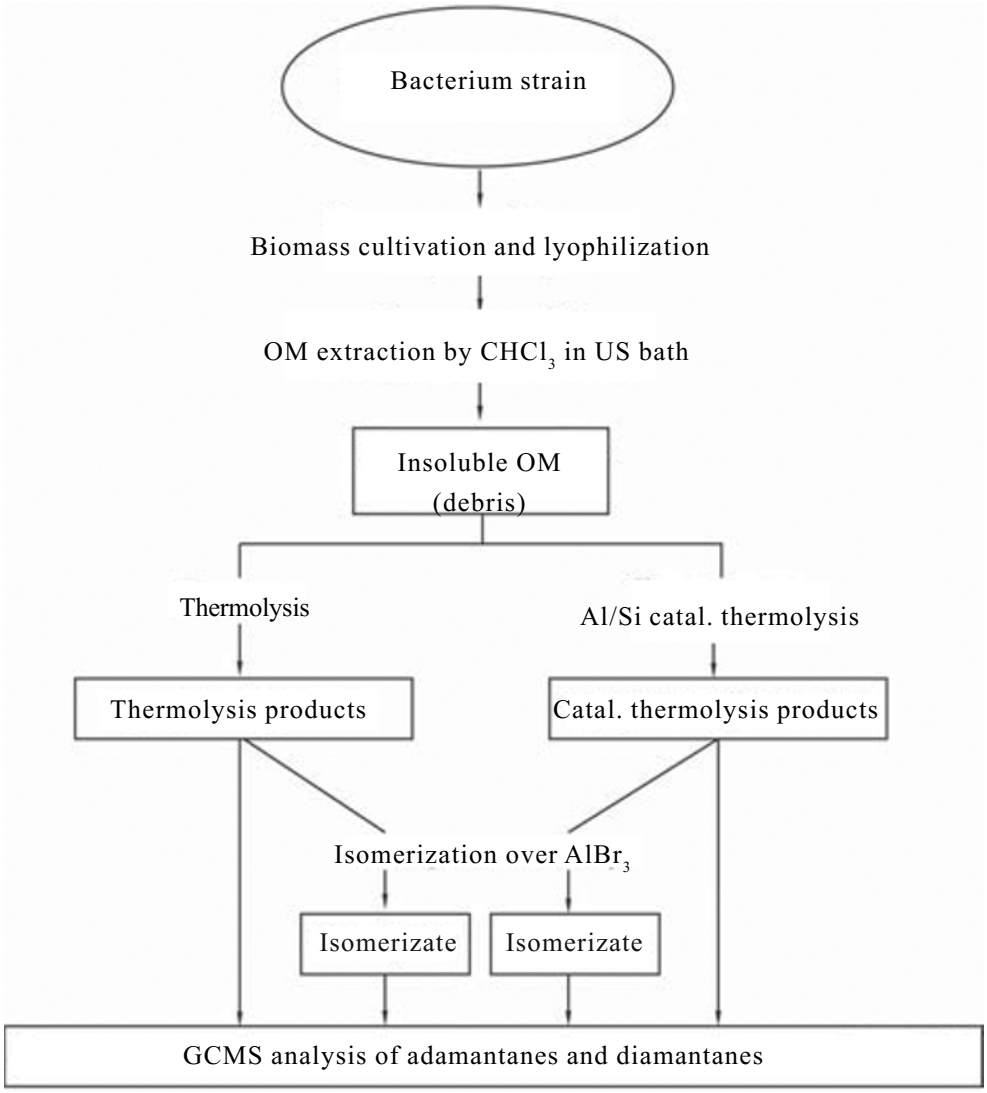

Fig. 1. Study scheme of hydrocarbons in thermolysis and catalytic thermolysis products from the insoluble part of bacterial biomass (US, ultrasound; GCMS, gas chromatography-mass spectrometry).

eukaryotes. Moreover, we showed [15-17] that relatively low-molecular-weight *n*-alkanes with an odd number of C atoms (C_7, C_9, C_{11}, C_{13}, C_{15}, C_{17}, C_{19}, C_{21}) and the corresponding unsaturated fatty *n*-acids with an even number of C atoms (C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) were primarily found in the soluble part of the native biomass of bacteria *Arthrobacter* sp. RV and *Pseudomonas aeruginosa* RM. Homologs with an even number of C atoms (C_{22}, C_{24}, C_{30}, C_{32}, C_{34}) dominated the higher-molecular-weight *n*-alkanes. Both strains were found to synthesize the unsaturated irregular isoprenane squalene (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene.

Thermolysis and catalytic thermolysis of the initial OM is currently of most interest to the sedimentation-migration theory of oil generation. It was found that various classes of HC were produced at different rates during OM ripening by kerogen. Thermolysis and catalytic thermolysis of kerogen generated the same HC that were found in oil [2]. Kerogen (insoluble OM) is known to be a complicated geobiopolymer. Therefore, it can be assumed that it could also contain the insoluble part of the bacterial biomass, in particular, of *Arthrobacter* sp. RV and *P. aeruginosa* RM.

Therefore, it seemed interesting to determine if diamond-like HC are formed by thermolysis and catalytic thermolysis of the insoluble part (debris) of prokaryotes. The present study is focused on this question.

We studied the chemoorganoheterotrophic aerobic bacteria *Arthrobacter* sp. RV and *P. aeruginosa* RM, which are capable of aerobic and anaerobic growth during denitrification. Figure 1 shows the scheme that was used to study HC in the thermolysis and catalytic thermolysis products of the bacterial biomass.

The HC-oxidizing strains *Arthrobacter* sp. RV and *P. aeruginosa* RM were cultivated in liquids. The latter was grown in mineral-organic medium containing (g/L) $NaNO_3$ (2), KH_2PO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.25), $CaCl_2 \cdot 2H_2O$ (0.01), yeast extract (2), glucose (20), and distilled H_2O at pH 7 [18]. The former was grown in "rich" liquid medium containing (g/L) peptone (2), yeast extract (1), casein hydrolysate (1), glucose (1), chalk (2), glycerin (10 mL/L), and tap water at pH 6.7-7.2 [19]. The strains were cultivated in flasks on a rocker at 28°C for 24 h (*P. aeruginosa* RM) and 96 h (*Arthrobacter* sp. RV). Biomass of the entrifugation (6000rpm).The biomass was lyophilized at 25°C and $1 \cdot 10^{-3}$ kPa for 24 h.

The lyophilized bacterial biomass was extracted by $CHCl_3$ in an ultrasonic (US) bath for 16-18 h at room temperature. The $CHCl_3$ was a good solvent for polar and non-polar components. The $CHCl_3$ was purified beforehand by fractional distillation.

Bacterial biomass that was insoluble in $CHCl_3$ underwent thermolysis and catalytic thermolysis at 340°C and 280°C, respectively. Activated (450°C, 4 h) aluminosilicate was used as the catalyst. It was selected because catalysis in nature occurs in clayey strata and clay is a natural aluminosilicate. The soluble parts of the thermolysis and catalytic thermolysis products were isomerized by $AlBr_3$, which is an active acidic catalyst and can isomerize protoadamantanes into adamantanes. The products were analyzed by capillary GC and GCMS. Diamond-like HC received the most attention.

GCMS was carried out on an Agilent 6890N/5975C instrument. HC were separated over an HP-1MS capillary column (25 m $\times$ 0.25 mm $\times$ 0.5 μm). The temperature was programmed from 70 to 290°C at 4°C/min. The carrier gas was He. All mass spectra were obtained at ionization energy 70 eV. The ionization chamber temperature was 250°C. Compounds were identified by adding presumed standards to the studied samples and by using the NIST mass-spectra library.

Thermolysis and catalytic thermolysis of the insoluble parts of the biomass of both strains formed HC with characteristic C_{10}–C_{13} adamantane ions with *m*/*z* 135, 136, 149, and 163 and C_{14}–C_{16} diamantane

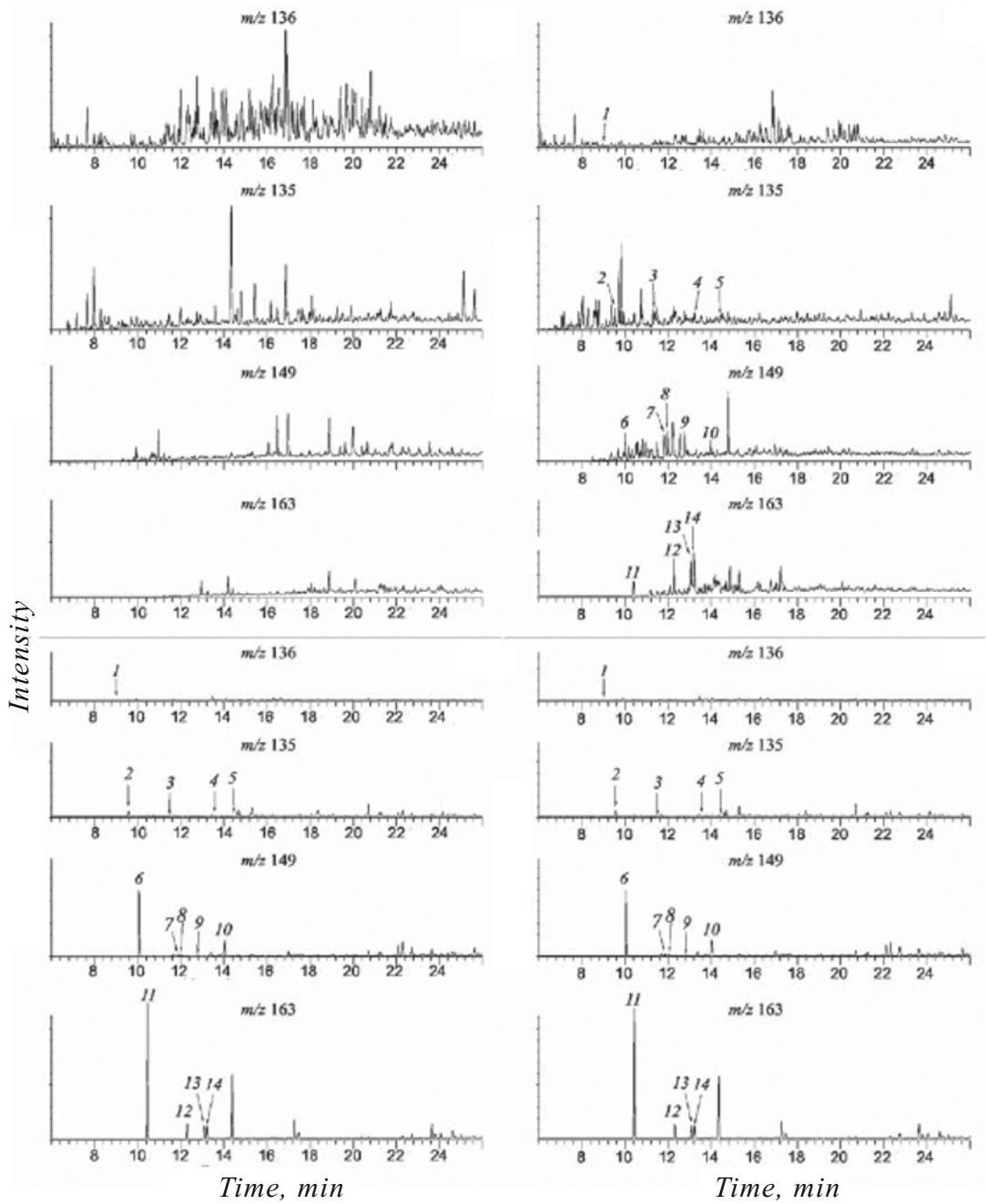

Fig. 2. GCMS of C_{10}-C_{13} protoadamantanes and adamantanes in thermolysis (*a*) and catalytic thermolysis (*b*) products of the insoluble part of *Arthrobacter* sp. RV biomass and in isomerization products of the thermolysis (*c*) and catalytic thermolysis (*d*) products. Numbers denote: adamantane (1), 1-methyladamantane (2), 2-methyladamantane (3),1-ethyladamantane (4), 2-ethyladamantane (5), 1,3-dimethyladamantane (6), *cis*- and *trans*-1,4-dimethyladamantanes (7 and 8), 1,2-dimethyladamantane (9), 1-ethyl-3-methyladamantane (10), 1,3,5-trimethyladamantane (11), 1,3,6-trimethyladamantane (12), *cis*- and *trans*-1,3,4-trimethyladamantanes (13 and 14).

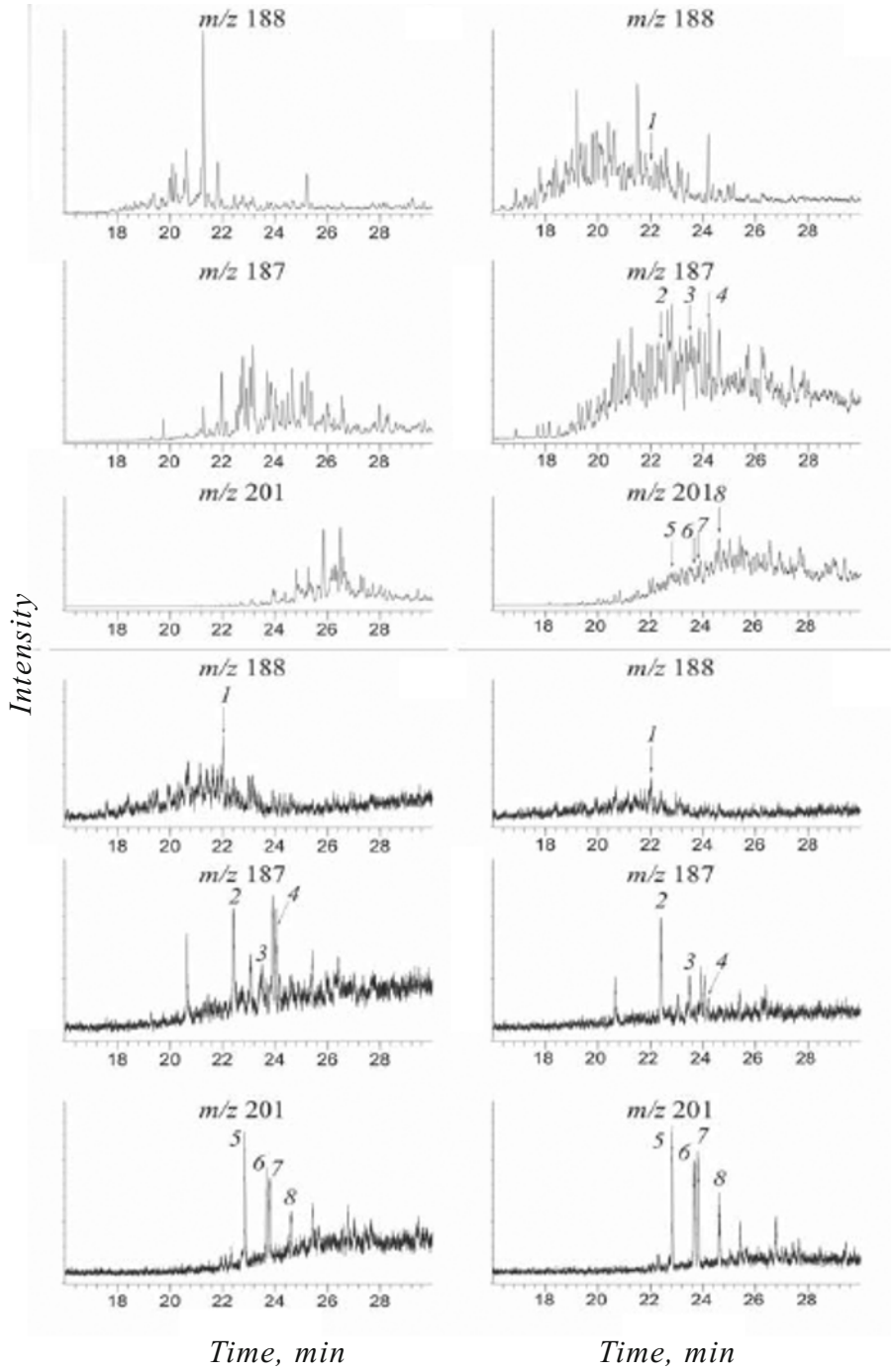

Fig. 3. GCMS of C_{14}-C_{16} protodiamantanes and diamantanes in thermolysis (*a*) and catalytic thermolysis (*b*) products of the insoluble part of *Arthrobacter* sp. RV biomass and in isomerization products of the thermolysis (*c*) and catalytic thermolysis (*d*) products. Numbers denote: diamantane (1), 4-methyldiamantane (2), 1-methyldiamantane (3), 3-methyldiamantane (4), 4,9-dimethyldiamantane (5), 1,4- and 2,4-dimethyldiamantanes (6), 4,8-dimethyldiamantane (7), 3,4-dimethyldiamantane (8).

ions with *m*/*z* 187, 188, and 201 (Fig. 2, *a* and 3, *a*). As shown earlier, the situation was similar for adamantanes, diamantanes, trimantanes, and tetramantanes during a study of oil primarily of marine genesis (e.g., oil of the Salym deposit in the Bazhenov oil field) [2, 4, 5, 20]. These studies established that side peaks in the GCMS with *m*/*z* 135, 136, 149, and 163 and with *m*/*z* 187, 188, and 201 belonged to protoadamantane and protodiamantane HC, respectively.

An analogous scenario was also observed in our instance. We isomerized the thermolysis and catalytic thermolysis products over $AlBr_3$ in order to confirm that the side peaks in the GCMS with characteristic adamantane ions were protoadamantanes; diamantanes, protodiamantanes. Many peaks with ions characteristic of C_{10}–C_{13} adamantanes (*m*/*z* 135, 136, 149, 163) and C_{14}–C_{16} diamantanes (*m*/*z* 187, 188, 201), the retention times of which did not agree with those of adamantanes and diamantanes, were visible in the GCMS of the thermolysis products (Fig. 2, *a* and 3, *a*). However, Fig. 2, *b* and 3, *b* (catalytic thermolysis products) showed that peaks of adamantanes and diamantanes (shown as numbers over peaks) were observed in addition to the aforementioned peaks. GCMS of the isomerization products showed that all HC isomerized into thermodynamically more stable adamantanes and diamantanes (Fig. 2, *c*; 2, *d* and 3, *c*; 3, *d* contain only peaks of adamantanes and diamantanes, respectively). Thus, it was concluded that many peaks in GCMS with the same characteristic ions as adamantanes and diamantanes belonged to protoadamantanes and protodiamantanes, like the oils. We obtained similar results for thermolysis and catalytic thermolysis of the insoluble part of *P. aeruginosa* RM biomass.

The equilibrium concentrations of thermodynamically more stable C_{11}–C_{13} adamantane isomers are known to be similar (92.5-98%) [21, 22]. However, it was shown earlier that the relative concentrations of thermodynamically more stable adamantane isomers were far from the equilibrium values in oils and decreased with increasing molecular weight [2].

An analogous pattern was observed in our instance. Table 1 presents the distribution of adamantanes in the thermolysis and isomerization products. It can be seen that adamantanes were also formed in ratios close to those in oils as a result of catalytic thermolysis of the insoluble part of the bacterial biomass. Thus, the relative content of 1-methyladamantane was 59.3 and 71.4% for *Arthrobacter* sp. RV and *P. aeruginosa* RM, respectively; the relative content of 1,3-dimethyladamantane – 22.2 and 22%; of 1,3,5-trimethyladamantane – 11 and 8.6%.

As expected, the relative distribution of C_{11}–C_{13} adamantanes in the isomerization products was close to the equilibrium value (Table 1). However, the relative concentrations of the more stable C_{11}–C_{13} methyladamantane isomers also decreased with increasing molecular weight. Apparently, this was due to the fact that di- and trimethyl-substituted adamantanes reached the equilibrium concentrations more difficultly than the mono-methyl derivatives.

Table 2 compares the characteristics of the thermolysis and catalytic thermolysis products of the insoluble parts of both strains for C_{10}–C_{13} adamantanes. It can be seen that the adamantane ratios were similar in both instances. Thus, the C_{11}/C_{13} and C_{12}/C_{13} ratios in the catalytic thermolysis products were 0.23 and 0.89 for *Arthrobacter* sp. RV and 0.11 and 0.75 for *P. aeruginosa* RM. These ratios in the isomerization products were 0.03 and 0.36-0.38 and 0.02-0.04 and 0.33-0.38, respectively.

It is noteworthy that the relative content of adamantane itself (C_{10}) and monomethyladamantanes (C_{11}) in both the catalytic thermolysis and isomerization products were significantly less than the relative content of di- and trisubstituted adamantanes (Table 2).

As shown above, thermolysis and catalytic thermolysis of the insoluble part of biomass from both *Arthrobacter* sp. RV and *P. aeruginosa* RM formed protoadamantanes and adamantanes in addition

Table 1

Hydrocarbon	Relative hydrocarbon content, %						Equilibrium concentration at 300 K, %
	Arthrobacter sp. RV			*Pseudomonas aeruginosa* RM.			
	Catalytic thermolysis	Isomerization over $AlBr_3$ of products from		Catalytic thermolysis	Isomerization over $AlBr_3$ of products from		
		thermolysis	catalytic thermolysis		thermolysis	catalytic thermolysis	
			Adamantanes C_{11}				
1-MA	59.3	93.5	96.2	71.4	94.4	95.5	98.0
2-MA	40.7	6.5	3.8	28.6	5.6	4.5	2.0
			Adamantanes C_{12}				
1-EA	5.1	0.3	0.2	6.3	1.2	0.9	0.1
2-EA	7.7	0.3	0.2	5.2	1.2	0.5	-
1,3-dMA	22.2	85.0	90.9	22.0	89.7	90.6	92.5
1,4-dMA, *cis*	21.2	5.6	3.5	20.9	3.1	3.3	3.0
1,4-dMA, *trans*	22.2	5.4	3.3	23.1	3.1	2.8	3.0
1,2-dMA	21.6	3.4	1.9	22.5	1.7	1.9	1.4
			Adamantanes C_{13}				
1,3,5-tMA	11.0	66.6	68.9	8.6	64.4	65.0	92.5
1,3,6-tMA	26.5	9.2	9.1	28.1	9.2	1.1	3.0
1,3,4-tMA, *cis*	23.1	7.8	7.3	23.4	9.2	11.9	1.5
1,3,4-tMA, *trans*	27.4	7.8	7.3	26.2	9.2	11.5	1.5
1-Э-3-MA	12.0	8.6	7.4	13.7	8.0	10.5	1.5

Note. A–adamantane; M–methyl; E–ethyl; d–di-; t– tri-.

Table 2

Ratio	*Arthrobacter* sp. RV			*Pseudomonas aeruginosa* RM.		
	Catalytic thermolysis	Isomerization over $AlBr_3$ of products from		Catalytic thermolysis	Isomerization over $AlBr_3$ of products from	
		thermolysis	catalytic thermolysis		thermolysis	catalytic thermolysis
C_{10} :C_{11}: C_{12}: C_{13}	0.8:10.8:41.5:46.9	0.1:2.1:26.2:71.6	0.1:2.3:26.8:70.8	0.8:5.8:39.9:53.4	0.1:1.5:24.6:73.8	0.3:2.8:26.7:70.2
C_{11}/C_{13}	0.23	0.03	0.03	0.11	0.02	0.04
C_{12}/C_{13}	0.89	0.36	0.38	0.75	0.33	0.38

to C_{14}– C_{16} protodiamantanes and diamantanes (characteristic ions with *m*/*z* 187, 188, 201) (Fig. 3*a* and 3*b*). However, Fig. 3 shows that the relative content of the C_{14}–C_{16} diamantanes (with the exception of the isomerization products, Fig. 3, *c* and 3, *d*) was difficult to calculate because of the presence of a large quantity of protodiamantane HC.

Thus, the ability to form C_{10}–C_{13} protoadamantanes, protodiamantanes, adamantanes and C_{14}–C_{16} diamantanes via thermolysis and catalytic thermolysis of the insoluble parts of the chemoorganoheterotrophic aerobic bacteria *Arthrobacter* sp. RV and *P. aeruginosa* RM was demonstrated for the first time. It was found that the relative distribution of protoadamantanes, protodiamantanes, adamantanes, and diamantanes was analogous to that in marine oils. It could be assumed that an important formation pathway for diamond-like HC in oils could be catalytic transformation of bacterial biomass.

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