HOPANOIDS OF METHYLOTROPIC ACETOBACTERIA

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UDC 547.597+576.851.134

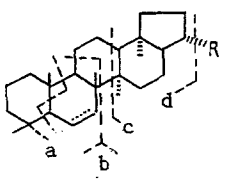
Squalene, 35-methoxyho-6-ene-32R,33R,34S-triol, hop-22(29)-ene, and hopan-22-ol have been isolated from Acetobacter sp. VSB 917. The structures of the substances isolated and of the 35-methoxyhop-6-ene-32R,33R,34S-triol triacetate obtained have been established by IR, mass, and ^1H and ^{13}C NMR spectroscopies.

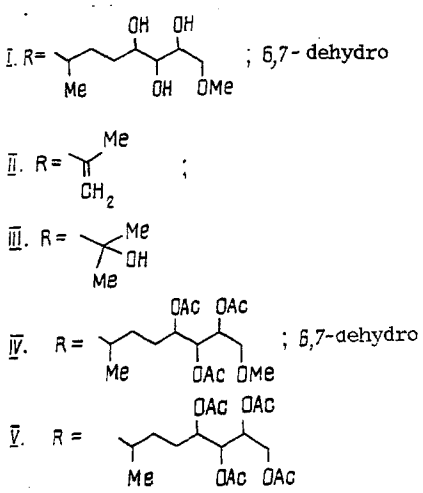
Hopane derivatives are widely distributed in prokaryotes and, in these microorganisms, they fulfill the role of the sterols of eukaryote membranes. A hypothesis exists according to which if simple hopanoids are structural elements of the membranes, more complex polyhydroxyated hopanoids also have a functional value [1]. We have previously reported the isolation from the methylotrophic bacteria Acetobacter sp. VSB 917 of the 35-methyl ether of hop-6-ene-32,33,34,35-tetrol (I) [2].

The present work was devoted to a further study of the hopanoids of methylotrophic acetobacteria.

The dry biomass of A. sp. VSB 917, obtained by the method described in [2], was extracted with acetone. When the acetone extract was concentrated, a precipitate sparingly soluble in organic solvents deposited, and its recrystallization yielded substance (II).

Chromatography on silica gel of the mother solution after the separation of (II) yielded (in order of increasing polarity of the substances), squalene, compound (III), and 35-methoxyhop-6-ene-32R,33R,34S-triol (I).





The identification of squalene, which is a biogenetic precursor of the hopanoids [1], was achieved with the aid of IR, mass, and ^1H NMR spectra and also by comparison with an authentic sample.

The mass spectra of compounds (I)-(IV) showed the peaks of the molecular ions and the peaks of the ions of fragments characteristic for the breakdown of hopanoid molecules under electron impact. These include fragments A, B, C, and D obtained on the cleavage of the cyclic skeleton by pathways b, c, and d, and also the fragments M^+ and $M^+ - CH_3$ [3, 4] (Table 1). In the spectra of (III) and (IV) the $M^+ - CH_3$ ions were not observed but the peaks of ions corresponding to them – $M^+ - H_2O - CH_3$ for (III) and $M^+ - AcOH - CH_3$ for (IV) – were. The mass spectra of (I)-(IV) each contained the peak of an ion with m/z 111 corresponding to fragment (E) formed on cleavage by pathway a. The formation of such a fragment has been observed in the mass spectra of tricyclic diterpenes with a similar structure of ring A [5].

Vitaminy Scientific–Production Association, Moscow. Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 367-372, May-August, 1992. Original article submitted September 25, 1991; revision submitted January 6, 1992.

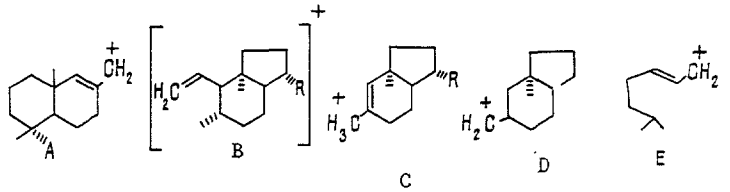
0009-3130/92/0304-0321$12.50

TABLE 1. m/z Values of Characteristic Fragments of Compounds (I-IV)

Compound	m/z for fragments					
	A	B	C	D	M+—CH_3	M+—R
I	189	368	339	149	543	367
II	191	218	189	149	395	367
III	191	236*	207	149	413	309
IV	189	—	465	149	—	367

*In the mass spectrum obtained with ionization by ammonia.

The general laws of fragmentation in the electron-impact mass spectra permitted compounds (I)-(IV) to be assigned to the hopane series.



The compounds (II) and (III) that had been isolated were, according to their melting points, specific rotations, and their IR, mass, and NMR spectra, hop-22(29)-ene and hopan-22-ol, respectively [4-9].

By the acetylation with acetic anhydride in pyridine of the 35-methoxyhop-6-ene-32R,33R,-34S-triol (I) isolated previously [2] we obtained its more stable triacetate (IV). Completeness of acetylation was confirmed by the absence of the characteristic absorption bands of hydroxy groups in its IR spectrum. The ^1H NMR spectrum of the triacetate (IV) showed, in comparison with the spectrum of the triol (I) [2], downfield shifts of the signals of the protons at C-32, C-33, and C-34 ($\Delta\delta^{IV-I}$ 1.30, 1.74, and 1.21 ppm. Characteristic for the ^1H NMR spectra of compounds (I) and (IV) were downfield shifts of the signals of the protons of the angular methyl groups at C-8 and C-10 and of the β-methyl group at C-4 in comparison with the signals of the analogous protons in the tetraacetate (V) and its stereoisomers at C-32, C-33, and C-34, which were obtained by Rohmer et al. [11, 12], i.e., compounds with side chains of similar structure but not containing a double bond in the cyclic skeleton ($\Delta\delta$ 0.12, 0.27, and 0.03 ppm, respectively). Such shifts in the spectra of 6,7-didehydrohopanes in comparison with the corresponding hopanes can be observed in other cases, as well [13]. Thus, the shifts of the signals of the protons of the methyl groups at C-8, C-10, and C-4 (β-Me) may serve as one of the criteria of the presence of a double bond in position 6 of the skeleton of a hopane compound.

The ^{13}C NMR spectra of compounds (I) and (IV) contained the signals of methine carbons at a double bond ($\delta^{I,IV}$ 125.8 and 134.4 ppm). As compared with the ^{13}C NMR spectrum of the tetraacetoxyhopane (V) [12], in the spectra of (I) and (IV) one of the signals of the carbons of methylene groups at 18.6 ppm corresponding to C-2 and C-6 in (V) had disappeared and so had one of the signals in the 33-34 ppm region belonging to C-7 and C-15 in (V). Since vicinal carbon atoms participate in the formation of a double bond, the facts under consideration indicated that in each of compounds (I) and (IV) the double bond could only be between C-6 and C-7, which agreed with the mass and ^1H NMR spectra.

Both in the spectrum of the triol (I) and in the spectrum of the triacetate (IV), signals of the carbon atoms of the CH_3 and CH_2 groups of the (C-35)H_2OCH_3 fragment of the side chain were observed ($\delta^{I,IV}_{OCH_3}$ 59.3 ppm, $\delta^{I,IV}_{OCH_2}$ 75.4, 71.9 ppm) The chemical shifts of the carbon atoms of the acetate groups in the signals of the C- and C-33 methine carbon atoms in the acetate groups of compound (IV) practically coincide with those given in the literature [12] for (32R,33R,34S)-tetraacetoxybacteriohopane (V) ($\Delta\delta_{max}$ 0.2 ppm). For the (C-34)H, $\Delta\delta$ amounted to 0.5 ppm, which can be explained by the different effects of a terminal acetate group (in the methoxytriacetate, compound (IV)). In the ^{13}C spectrum of the methoxytriol (I), the signals of the C-32, C-33, and C-34 methine groups were located in the 69.5-74.4 ppm region.

The assignments of the other ^{13}C signals of the methyl, methylene, and methine groups and also of quaternary ^{13}C atoms were made by comparing the spectra of compounds (I) and (IV) with the spectrum of bacteriohopanetetrol tetraacetate (V) [12]. For the majority of signals the difference in the values of the chemical shifts did not exceed 0.8 ppm, and their assignment caused no difficulties. The value of $\Delta\delta_{(C\text{-}31)H_2}$ observed in the spectrum of the triol (I) as compared with that of (V) amounted to 5.5 ppm, which was obviously due to the difference in the influence of the hydroxy [compound (I)] and acetoxy [compound (V)] groups at C-32. For both compounds (I) and (IV) a considerable downfield shift of the C-8 signal was observed ($\Delta\delta^{I,IV\text{-}V}$ 4.8-4.4 ppm), which was due to the influence of the 6(7)-double bond in compounds (I) and (IV). The influence of the 6(7)-double bond on the conformation of the skeleton of the methoxytriol (I) and its triacetate (IV) is apparently the reason for the considerable change in the chemical shifts of the methyl groups at C-8, C-10, and C-14, ($\Delta\delta^{I,IV\text{-}V}$ 2.9, 1.7 and 1.2 ppm, respectively) and the chemical shift of the $(CH\text{-}1)H_2$ group ($\Delta\delta^{I,IV\text{-}V}$ 2.2 ppm) in the ^{13}C NMR spectra. The absolute configuration of the side chain (I) and, consequently, of the triacetate (IV) was assigned by analogy with that established for the side chain of natural bacteriohopanetetrol [12].

EXPERIMENTAL

Melting points were determined on a Yanaco heated stage (Japan). Specific rotations were measured on a JASCO ORD/UV-5 polarimeter. IR spectra were recorded on a Perkin-Elmer 180 instrument (USA). Mass spectra were obtained on a Finnigan MAT 4615 and Hitachi 80A instruments with electron-imapct ionization or chemical ionization by ammonia. ^1H NMR spectra were obtained on a AM-400 spectrometer (Bruker, Germany) with a working frequency for protons of 400.13 MHz. The computer memory used was 32K/400 Hz. The ^{13}C NMR spectra were obtained on a WP-200 spectrometer (Bruker, Germany) with a working frequency for ^{13}C nuclei of 50.32 MHz. The computer memory used was 16K/10,000 Hz. The internal standard was tetramethylsilane (TMS). The assignment to the carbon-13 atoms of the methyl, methylene, and methine groups and also to quaternary carbon atoms was made with the aid of pulses in the DEPT sequence and of spectra with broad-band suppression of protons. For the assignment of the methyl groups of the acetate substituents of compounds (II) to carbon atoms, we used selective $^{13}C[^1H]$ resonance.

Isolation of Terpenoids from Acetobacter sp. VSB 917. The acetobacteria were grown as previously by the continuous method, the culture mass was centrifuged, and the biomass was dried in a spray-dryer [2].

The extraction of 500 g of the dry biomass was carried out in 1200 ml of acetone for 2 h and, after filtration, the meal was reextracted with 800 ml of acetone for 0.5 h. The combined extracts were evaporated in vacuum to a volume of 100 ml, this residue was kept at 4-5°C for 16 h, and the precipitate that had deposited was filtered off and was washed with cold acetone. Recrystallization of the precipitate from hexane yielded hop-22(29)-ene (II), $C_{30}H_{50}$, mp 205.8-213°C (changes in the crystal form at 166 and 179°C), $[\alpha]_D$ + 56.08°, $[\alpha]_{546}$ + 67.69° (c 0.63; chloroform); literature figures: mp 211-212°, $[\alpha]_D$ + 60.2° [7]. IR spectrum: λ_{max}^{KBr} 2930, 2860, 1655, 16455, 1460, 1375 885 cm^{-1}. Mass spectrum, m/z (%): 410 (M^+, 9), 395 ($M^+ - CH_3$, 4), 382 ($M^+ - CH_2CH_2$, 2) 367 (M^+ − side chain, 2), 342(2), 328(1), 327(1), 299 (E, 7), 285(1), 273(1), 259(1), 245(1), 231(4), 218(17), 204(16), 191 (A, 100), 189 (B, 93), 177(10), 161(19), 149(Г, 16), 137(21), 135(21), 133(16), 123(25), 121(29), 119(21), 111(5), 95(51), 81(47), 69(40), 67(30), 55(23). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.72 (3H, s, 18α-CH_3), 0.80 (3H, s, 4β-CH_3), 0.82 (3H, 10β-CH_3), 0.85 (3H, s, 4α-CH_3), 0.94 (3H, s, 14α-CH_3, 0.96 (3H, s, 8β-CH_3), 1.75 (3H, s, 22-CH_3), 2.69 (1H, q, J = 7.3 Hz, H-21), 4.78 [2H, br. s. (=CH_2)-29].

The acetone solution after separation of the precipitate containing the hop-22(29)-ene was evaporated in vacuum to dryness and chromatographed on a column of silica gel. The fractions obtained by elution with hexane were subjected to preparative TLC on silica gel in hexane, which led to the isolation of squalene, $C_{30}H_{50}$. IR spectrum λ_{max}^{film}: 2965, 2920, 2850, 1660, 1435, 1370 cm^{-1}. ^1H NMR spectrum (δ, ppm): 1.58 (18H, br. s, CH_3–C=C), 1.69 (6H, br.s., trans-CH_3), 2.04 (20H, m, CH_2–C), 5.13 (6H, m, HC=C). Mass spectrum, m/z (%): 428 (M^+, 5), 177(12), 175(11), 163(14), 161(10), 149(36), 137(60), 123(44), 121(47), 109(37), 107(23), 95(47), 93(27), 83(27), 81(98), 69(100).

On further elution of the column with hexane containing a rising gradient of acetone, a fraction was obtained from which, by rechromatography on silica gel (hexane with a rising gradient of ether) and recrystallization from acetone, we isolated hopan-22-ol (III), $C_{30}H_{52}O$, mp 250-255.9°C (change in the crystal form at 221-250°C), $[\alpha]_D$ + 49° (c 0.6; chloroform); literature figures: mp 254-255°, $[\alpha]_D$ + 49° [4]. IR spectrum, $\lambda_{max}^{CCl_4}$ 3625 (OH), 1165 (tertiary OH), 1128 and 940 cm^{-1}. Mass spectrum, m/z (%): (M^+, 7), 413 (M^+-CH_3, 3), 410 (M^+-H_2O, 3), 395 ($M^+-CH_3-H_2O$, 6), 370(55), 369 (M^+ – side chain, 3). 367(3), 355(3), 231(3), 217(3), 207 (c, 22), 191 (A, 92), 189 (C–H_2O, 100), 177(5), 175(6), 163(13), 161(9), 149 (D, 95), 137(18), 135(11), 123(18), 121(17), 119(11), 111(4), 109(26), 107(23), 95(44), 93(22), 83(15), 82(16), 81(12), 69(43), 67(31), 59(44).

Mass spectrum (chemical ionization with ammonia) m/z (%): 429 [$(M + H)^+$, 3], 428 (M^+, 18), 411 [$(M-H_2O + H)^+$, 77], 395 ($M^+-CH_3-H_2O$, 2), 370(12), 369 (M^+ – side chain, 20), 367(11), 355(1.6), 341(3), 317(1), 313(2), 299(5), 287(4), 273(4), 259(7), 245(5), 236 (B, 3), 231(19), 219(60), 207(46), 191(100), 189(55), 177(20, 163(20), 149(55), 137(23), 123(17), 109(22), ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.76 (3H, s, 18α-CH_3), 0.79 (3H, s, 4β-CH_3), 0.81 (3H, 10β-CH_3), 0.84 (3H, s, 4α-CH_3), 0.96 (6H, s, 8β-CH_3 and 14α-CH_3), 1.18 and 1.21 (6H, s, 22-CH_3).

By the action of a mixture of acetic anhydride and pyridine at room temperature for 18 h we acetylated the 35-methoxyhop-6-ene-32,33,34-triol(I) that we had isolated previously [2] from the polar fractions obtained in the chromatography of the extract. The reaction mixture was diluted with water and the resulting precipitate was filtered off, chromatographed on silica gel in the hexane–acetone (1:10) system, and recrystallized from acetone–hexane, to give 35-methoxyhop-6-ene-32,33,34-triol triacetate (IV), mp 168.7-170°C. IR spectrum, λ_{max}: 1750, 1635, 1375, 1230, and 1050 cm^{-1}.

Mass spectrum, m/z (%): 684 (M^+–AcOH, 2), 609 (M^+–AcOH–CH_3,3), 504 (M^+–3AcOH, 0.3), 465 (C, 9), 407(4), 367 (M^+ – side chain, 3), 317 (side hain, 1), 189 (A, 89), 149 (D, 17), 119(100), 95(33). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.670 (3H, s, 18α-CH_3), 0.814 (3H, s, 4β-CH_3); 0.819 (3H, s, 4α-CH_3), 0.880 (3H, s, 10β-CH_3), 0.899 (3H, d, J_{22,CH_3} = 9.0 Hz, 22R–CH_3), 0.942 (3H, s, 14α-CH_3), 0.880 (3H, s, 8β-CH_3), 0.899 (3H, d, J_{22,CH_3} = 9.0 Hz, 22R–CH_3), 0.942 (3H, s, 4α-CH_3), 1.056 (3H, s, 8β-CH_3); 1.627 (1H, dd, $^3J_{56}$ = 3.0 Hz, $^4J_{5,7}$ = 1.7 Hz, 5H), 2.027, 2.077, 2.113 (9H, sss, $OCOCH_3$), 3.317 (3H, s, 35-OCH_3), 3.405 (2H, d, $^3J_{34,35a,b}$ = 5.7 Hz, 35-$H_{a,b}$), 5.018 (1H, ddd, $^3J_{32,31a}$ = 8.6 Hz, $^3J_{32,33}$ = 7.0 Hz; $^3J_{32,31b}$ = 3.0 Hz, 32-H), 5.225 (1H, dd, $^3J_{33,32}$ = 7.0 Hz, $^3J_{33,34}$ = 3.5 Hz, 33-H), 5.268 (1H, td, $^3J_{34,35a,b}$ = 5,7 Hz, $^3J_{34,33}$ = 3.5 Hz, 34-H), 5.474 (1H, dd, $^3J_{6,7}$ = 10.4 Hz, $^3J_{5,6}$ = 3.0 Hz, 6H), 5.597 (1H, dd, $^3J_{6,7}$ = 10.4 Hz, $^4J_{5,7}$ = 1.7 Hz). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 15.3 (1C, 18-CH_3); 17.5, 17.6, 19.2 (3C, 10β-, 14α-, 8β-CH_3); 18.9 (1C, C^2H_2); 19.9 (1C, 22R-CH_3); 20.1 (1C, $C^{11}H_2$); 20.7, 20.8, 20.9 (3C, $OCOCH_3$); 21.5 (1C, 4β-CH_3); 22.9 (1C, $C^{16}H_2$); 24.9 (1C, $C^{12}H_2$); 26.8 (1C, $C^{31}H_2$); 27.4 (1C, $C^{20}H_2$); 30.6 (1C, $C^{30}H_2$); 32.8 (2C, 4α-CH_3, C^4); 34.0 (1C, $C^{15}H_2$); 36.2 (1C, $C^{22}H$); 36.6 (1C, C^{10}); 37.9 (1C, C^1H_2); 41.3, 41.5 (2C, $C^{19}H_2$, C^3H_2); 42.0 (1C, C^{14}); 44.7 (1C, C^{18}); 46.0, 46.2 (2C, C^8, $C^{21}H$); 49.9 (1C, $C^{13}H$); 50.5 (1C, C^9H); 54.8 (1C, $C^{17}H$); 55.4 (1C, C^5H); 59.0 (1C, 35-OCH_3); 69.7 (1C, $C^{32}H$); 71.4 (1C, $C^{33}H$); 72.4 (1C; C^{34}); 71.9 (1C, $C^{35}H_2$); 125.8, 134.4 (2C, C^6, C^7); 169.9, 170.1, 170.2 (3C, $OCOCH_3$).

The ^1H NMR spectra of triol (I) has been given in [2]. ^{13}C NMR spectrum of triol (I) ($CDCl_3$, δ, ppm): 15.5 (1C, 18α-CH_3); 17.5, 17.7, 19.3 (3C, 10β-, 14α-, 8β-CH_3); 18.8(1C, C^2H_2); 20.2 (2C, 22R-CH_3, $C^{11}H_2$); 21.5 (1C, 4B-CH_3), 23.0 (1C, $C^{16}H_2$); 25.0 (1C, $C^{12}H_2$); 27.7 (1C, $C^{20}H_2$); 30.1 (1C, $C^{30}H_2$); 31.7 (1C, $C^{31}H_2$); 32.8 (2C, 4α-CH_3, C^4); 34.1 (1C, $C^{15}H_2$); 36.5 (1C, $C^{22}H$); 36.7 (1C, C^{10}); 38.0 (1C, C^1H_2); 41.4, 41.6 (2C, $C^{19}H_2$, C^3H_2); 42.2 (1C, C^{14}); 44.8 (1C, C^{18}); 46.3, 46.4 (2C, C^8, $C^{21}H$); 49.6 (1C, $C^{13}H$); 50.2 s(1C, C^9H); 55.1 (1C, $C^{17}H$); 55.8 (1C, C^5H); 59.3 (1C, 35-OCH_3); 69.5, 73.8, 74.4 (3C, $C^{32-34}H$); 75.4 (1C, $C^{35}H_2$); 125.8, 134.4 (2C, C^6, C^7).

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C-10 AMINO ACID DERIVATIVES OF COLCHICINE

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UDC 547.944.6

The synthesis has been effected of C-10 N-acylated amino acid derivatives of colchicine by condensing (β-aminoethylamino)colchicide with N-acylated amino acids. The structures of the compounds obtained have been confirmed by their UV and PMR spectra and by thin-layer chromatography.

The modification of the chemical structure sof biologically active compounds permits their physiochemical and biological properties to be changed in a desired direction. Recently, in order to lower toxicity, ever-increasing use has been made of the method of conjugating known substances having a high antitumoral activity with such natural compounds as proteins, amino acids, and carbohydrates [1, 2]. As has been shown previously, on the condensation of deacetyl vinblastine with amino acid esters vinblastine derivatives possessing antitumoral activity and less toxic than the initial alkaloid are obtained [3]. Amino acid derivatives of ellipticine have also proved to be more effective than the initial alkaloid [4].

The study of the biological action of dipeptide derivatives of colchicine that we had synthesized previously on a culture of X63A 8-653 cells has shown that on the conjugation of colchicine with various amino acids their cytoxicity decreases [5, 6]. A dependence of the cytotoxicity on the nature of the amino acid residue is observed [6]. In view of this, the aim of the present work was to synthesize several N-acylamino acid derivatives of colchicine.

To obtain the amino acid derivatives of the alkaloid we first synthesized (β-aminoethylamino)colchicide by the nucleophilic replacement of the C-10 methoxy group of the alkaloid by an ethylenediamine group. This led to two compounds, with R_f 0.33 (system B) (IIa) and R_f 0.10 (system B) (IIb). Compounds (IIa) and (IIb) were isolated by chromatography on a column of ALugram silica gel (Macherey—Nagel) in a stepwise concentration gradient of ethanol in chloroform. The UV spectra of each compound, like the spectrum of colchicidylglycine and aminocolchicide, had adsorption maxima at 252, 355, and 408 nm (Table 1). Treatment of a chromatogram with a 0.2% alcoholic solution of ninhydrin revealed that compound (IIb) (E_f = −0.52 relative to picric acid) confirmed the presence of a free amino group in its structure. The NMR spectra of these compounds contained all the signals characteristic for the protons of colchicine apart from the C-10 methoxy group at 4.08 ppm. The results obtained permitted compound (IIa) to be identified as N,N'-dicolchicidylethylenediamine, and compound (IIb) as (β-aminoethylamino)colchicide. The N-actylated amino acid derivatives of colchicine were obtained by the scheme shown on the following page.

To study the influence of the nature of the amino acid residue on the biological properties of the alkaloid we used the following N-substituted amino acids (where F represents a formyl group and Ac an acetyl group); F-glycine (1), F-L-leucine (2), F-L-tryptophan (3),

M. A. Aidkhozhin Institute of Molecular Biology and Biochemistry, Kazakhstan Academy of Sciences, Alma-Ata. V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow. Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 372-375, May-August, 1992. Original article submitted August, 17, 1990; revision submitted October 22, 1991.

0009-3130/92/0304-0325$12.50