NEW GLYCOSIDES FROM THE HOLOTHURIAN Cucumaria japonica

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UDC 547.996:593.96

Four new compounds have been isolated from the fraction of weakly polar triterpene glycosides of the holothurian Cucumaria japonica: cucumariosides A_1-2 (I), A_0-1 (II), A_0-2 (III), and A_3-3 (IV). The structures of these substances have been established by chemical and physical methods.

A fraction of weakly polar glycosides of the holothurian Cucumaria japonica was obtained in [1] by chromatography on silica gel and did not undergo further separation by the usual methods. The desulfation of this fraction with the subsequent separation of the desulfation products on silica gel and by HPLC has led to the isolation of the individual derivatives (V), (VI), (VII), and (VIII), the amounts of which were 35.4, 4.0, 7.6, and 15.2%, respectively, on the weight of the fraction of desulfated derivatives.

A comparison of the ^{13}C NMR spectra of (V) and of the desulfated derivative of cucumarioside A_4-2 (IX) [1] permitted the conclusion that in (V) an acetate group (170.4 and 20.6 ppm) was attached to the C-6 atom of the terminal glucose residue. In fact, in (V) the C-6 signal of one of the glucose residues was shifted from 62.5 to 64.5 ppm, and the C-5 signal from 78.1 to 75.1 ppm, which is explained by the acetylation effect [2]. The assignment of these signals to the terminal glucose residue was confirmed by taking a series of partially relaxed spectra, in which slower relaxation is observed for a terminal monosaccharide residue than for the other monosaccharide units [3].

The mass spectra of (V) confirmed the proposed structure. In the $LSIMS^{(+)}$ (matrix – glycerol + NaCl) and $LSIMS^{(-)}$ (matrix – glycerol) spectra the peaks of ions with m/z 1267 $(M + Na)^+$ and 1243 $(M - H)^-$, respectively, were observed. The alternative splitting out from the latter of fragments with 132 and 204 a.m.u. (ions with m/z 1111 and 1039) showed the presence of terminal pentose and acetylhexose units in the molecule. The subsequent detachment of sugar residues led to ions with m/z 877 (1039 – 162), 745 (877 – 132), 599 (745 – 146), and 467 $(AgIO)^-$.

The structure of (V) was confirmed by the fact that when it was treated with a deacetylating agent (solution of NH_3 in 50% ethanol) it gave (IX) in quantitative yield, while the analogous treatment of the initial total mixture of glycosides did not change the amount of minor glycosides present in the initial fraction but converted the (I) into the considerably more polar cucumarioside A_4-2 (X) [1]. Thus, the structure of the aglycon, the linkage sequence of the monosaccharide residues in the carbohydrate chain, and the position of the acetate group were established by the comparative study of (V) and (IX). The position of the sulfate group became clear after (X) had been obtained as described above. It was also confirmed by the presence in the $LSIMS^{(-)}$ spectrum of (X) of a sulfur-containing anion with m/z 679 arising as the result of a single-stage or multistage elimination of four nonsulfated carbohydrate units from the $(M_{Na} - Na)^-$ ion with m/z 1281. Thus, cucumarioside A_1-2 (I) is 3β-{O-(6-O-acetyl-β-D-glucopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-[O-β-D-xylopyranosyl-(1 → 2)]-O-β-D-quinovosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy}holosta-7,25-dien-16-one. A_1-2 (I) is the first glycoside containing an acetyl group in the carbohydrate chain to have been isolated from a holothurian.

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 0009-3130/93/2902-0200$12.50

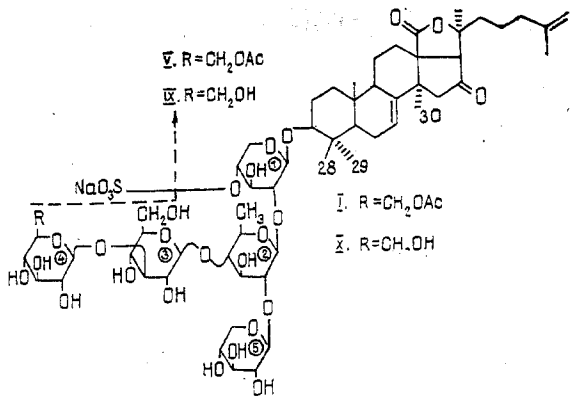

Fig. 1

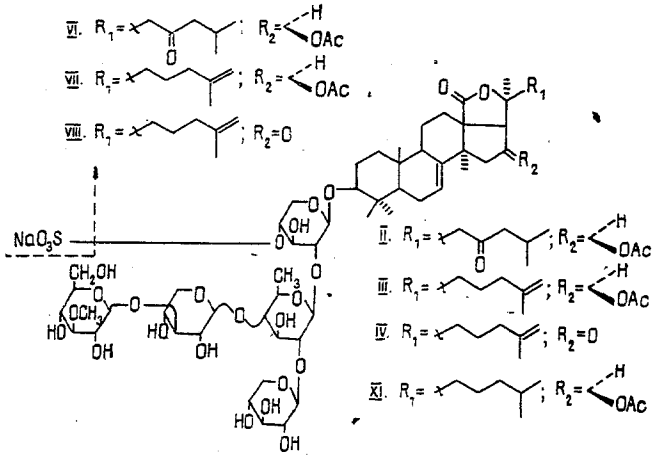

Fig. 2

The treatment of this fraction of glycosides with ammonia solution led to the separation of the glycosides containing an acetate group in the carbohydrate chain and, accordingly, to a considerable "simplification" of the residual mixture of substances, which permitted it to be separated by the HPLC method. This gave glycosides (III) and (IV) in the individual form, while (II) was obtained in the form of a fraction weighing 20 mg that, according to NMR, contained about 85% of the main substance. Analysis of spectra permitted the conclusion that the glycosides contaminating the (II) had the same carbohydrate chains and differed by the structures of the aglycons.

A comparison of (II), (III), (IV), the previously described frondoside A (XI) [4], and the desulfated derivatives (VI), (VII), and (VIII) showed that these glycosides had identical carbohydrate chains, consisting of xylose, quinovose, and 3-O-methylglucose in a ratio of 3:1:1 and differed only in the structures of their aglycons, as was confirmed by a comparison of the results of the monosaccharide analysis of the corresponding signals in the ^{13}C NMR spectra of the carbohydrate moieties of these compounds and the LSIMS$^{(+)}$ spectra of the native samples of (III) and (IV), containing the peaks of carbohydrate fragments with m/z 741, 609, and 477 that are characteristic for frondoside A [4].

A comparison of the ^{13}C NMR spectra of (III) and of frondoside A showed that (III) differed from (XI) only by the presence of an additional double bond in the side chain of the aglycon. In actual fact signals appeared at 145.9 and 110.9 ppm in the ^{13}C NMR spectra and at 4.77 ppm (2H-26) and 1.68 ppm (CH_3-27) in the ^1H NMR spectrum, which are characteristic for a terminal 25(26)-double bond. The mass number of the $(M_{Na} + Na)^+$ ion in the LSIMS$^{(+)}$ spectrum of (III) with m/z 1355 was two units less than for the analogous ion from frondoside A. The catalytic hydrogenation of (III) led to the dihydro derivative (XI), coinciding completely in its physicochemical characteristics and spectra with frondoside A.

Consequently, cucumarioside A_0-2 (III) is 16-acetoxy-3β-{O-[3-O-methyl-β-D-glucopyrano-

TABLE 1. Aglycon Parts of the ^{13}C NMR Spectra of the Desulfated Derivatives of Glycosides (V)-(VIII) (δ_{TMS} = 0, pyridine)

Atom	V	VI	VII	VIII
C-1	36.1	36.2	36.4	36.1
C-2	27.2	27.2	27.1	27.2
C-3	89.1	89.1	89.4	89.1
C-4	39.7	39.6	39.9	39.7
C-5	48.7	48.2	48.6	48.7
C-6	23.4	23.4	23.7	23.4
C-7	121.7	120.4	120.5	121.7
C-8	144.0	145 5	145.5	144.0
C-9	47.2	47.2	47.5	47.2
C-10	35.9	35.7	36·0	35.9
C-11	22.6	22.6	22.9	22.5
C-12	29.9	31.3	31.8	29.8
C-13	56.7	57.9	59.6	59.8
C-14	45,8	47.7	47.7	45,8
C-15	52,0	43.8	44.0	52.1
C-16	212,4	76,3	75.3	212.5
C-17	63,8	55.4	55.2	63,8
C-18	178.2	179.0	179.4	178,3
C-19	24.1	24.0	24.3	24.1
C-20	83.2	82.1	85.1	83.1
C-21	26,4	28.9	28,5	26.4
C-22	38.4	52.8	38.7	38.5
C-23	22.3	207.4	22.4	22.3
C-24	38.1	51,8	38.8	38,1
C-25	145.4	24.5	145.9	145.4
C-26	110.4	22.5	110.9	110.4
C-27	22.6	22.6	23.5	22.5
C-28	17.5	17.5	17.8	17.5
C-29	28.9	29.7	29.1	22.9
C-30	31.9	32.2	32.5	31.9
O—Ac	—	168.8	169.8	—
	—	21.3	21.5	—

syl]-(1 → 3)-O-β-D-xylopyranosyl-(1 → 4)-[O-β-D-xylopyranosyl-(1 → 2)]-O-β-D-quinovopyranosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy}holosta-7,25-diene.

A study of the ^{13}C NMR spectra of cucumarioside A_0-3 (IV) and its desulfated derivative (VIII) showed that the aglycon of (IV) was 3β-hydroxyholosta-7,25-dien-16-one, which has been identified earlier in the glycosides of Cucumaria japonica [1]. The results of monosaccharide analysis and the coincidence of the signals of the carbohydrate moieties of (IV) and (VIII) in the ^{13}C NMR spectra with the corresponding spectra of (III) and (VII) indicated their identity.

Thus, A_0-3 (IV) is 3β-{O-(3-O-methyl-β-D-glucopyranosyl)-(1 → 3)-O-β-D-xylopyranosyl-(1 → 4)-[O-β-D-xylopyranosyl-(1 → 2)]-O-β-D-quinovopyranosyl-(1 → 2)]-4-O-(sodium sulfato)-β-D-xylopyranosyloxy}holosta-7,25-dien-16-ol.

Details of the LSIMS mass spectra of the substances of group A_0 are given in Table 3. Samples of A_0-2 and A_0-3 were studied in the native ((III), (IV)) and desulfated ((VII), (VIII)) forms. The $LSIMS^+$ spectra are relatively uninformative and, in the case of the desulfated derivatives (VII) and (VIII), reveal only the molecular mass and the $AglOH^+$ and Agl^+ ions, while for the native compounds (III) and (IV) they show the presence of, in addition to the above-mentioned cations from the carbohydrate chain, desulfated fragments with m/z 1253 (III) and 1209 (IV) and the products of the elimination of the terminal carbohydrate units from them.

The most representative are the $LSIMS^{(-)}$ spectra of the desulfated compounds, including the spectrum of (VI). They contain the peaks of the $(M - H)^-$ ions and the products of the subsequent elimination of all the carbohydrate units, which leads to the $AglO^-$ ions. The complete analogy in the behaviors of these fragments in the spectra of (VI)-(VIII) confirmed the identity of their carbohydrate chains, and the difference in the mass numbers of the corresponding fragments confirmed the differences in the structure of ring D and of the substituent at C-20 found from the ^{13}C NMR spectra.

A comparison of the ^{13}C NMR of the desulfated cucumariosides A_0-1 (VI) and A_0-2 (VII) showed that these compounds had identical carbohydrate chains and the same polycyclic fragment of the but differed in the structure of the side chains.

TABLE 2. Carbohydrate Parts of the ^{13}C NMR Spectra of Glycosides (II)-(IV) and Their Derivatives (δ_{TMS} = 0, pyridine)

Atom	V	IX	X	II	VI	III	VII	IV	VIII
C_1^1	105.0	105.1	104.8[a]	104.4[a]	105.1	104.7[a]	105.3	104.8[a]	105.1
C_2^1	82.8	82.8	81.6	81.6	83.0	81.4	83.2[a]	81.3	83.1
C_3^1	77.8	77.8	76.2	76.4	77.8	76.3	78.0	76.3	77.8
C_4^1	70.3	70.4	75.7	75.3	70.3	75.4	70.6	75.3[b]	70.4
C_5^1	66.5	66.5	64.4	64.1	66.5[a]	64.4	66.7[b]	64.3	66.5[a]
C_1^2	103.0	103.0	102.2	102.1	103.0	102.1	103.2	102.0	103.0
C_2^2	83.1	83.2	82.1	82.5	83.0	82.3	83.3[a]	82.3	83.1
C_3^2	75.8	75.8	74.7	74.6[b]	75.5	74.7[b]	75.8	74.8[c]	75.5
C_4^2	86.6	86.7	86.7	85.2	85.3	85.5	85.6	85.5	85.3
C_5^2	71.1	71.2	71.0	71.1	71.2	71.1	71.5	71.0	71.3
C_6^2	18.0	18.1	18.1	18.0	18.0	18.0	18.2	18.0	18.1
C_1^3	104.6	104.6	104.5[a]	104.3[a]	104.9	104.5[a]	105.1	104.6[a]	104.9
C_2^3	73.5	73.6	73.7	73.4	73.3	73.5	73.5	73.4	73.3
C_3^3	88.7	88.4	88.2	86.9[c]	87.6[b]	87.5[c]	87.8[c]	87.5[d]	87.6[b]
C_4^3	69.3	69.8	69.7	68.9	69.0	69.0	69.3	69.0	69.1
C_5^3	77.8	77.8	77.7	65.9	66.5	66.2[d]	66.7	66.3	66.5
C_6^3	62.1	62.2	62.1	—	—	—	—	—	—
C_1^4	105.5	105.8[b]	105.5[b]	105.0	105.3	105.2	105.6	105.2	105.3
C_2^4	75.2[a]	75.3[a]	75.3	74.5[b]	75.0	74.6[b]	75.2	74.7[c]	75.0
C_3^4	77.8	78.5	78.3	86.5[c]	87.8[b]	87.0[c]	88.1[c]	87.0[d]	87.8[b]
C_4^4	71.5	71.7	71.6	70.4[d]	70.5[c]	70.7[e]	71.0[d]	70.7[e]	70.6[c]
C_5^4	75.1[a]	78.1	78.0	77.5	78.2	77.9	78.4	77.9	78.2
C_6^4	64.5	62.5	62.5	62.0	62.3	62.2	62.6	62.1	62.4
O—CH_3	—	—	—	60.5	60.6	60.6	60.8	60.5	60.6
O—Ac	170.4	—	—	—		—	—	—	—
	20.6	—	—	—	—	—	—	—	—
C_1^5	105.8	105.9[b]	105.2[b]	104.5[a]	105.9	104.8[a]	106.1	104.[a]	105.8
C_2^5	75.5	75.5[a]	75.7	75.3	75.5	75.4	75.8	75.7[b]	75.5
C_3^5	77.0	77.0	76.2	76.4	77.1	76.3	77.4	76.3	77.2
C_4^5	70.5	70.5	70.5	70.1[d]	70.8[c]	70.3[e]	70.8[d]	70.3[e]	70.8[c]
C_5^5	66.9	66.9	66.2	66.3	66.9[a]	66.3[d]	67.2[b]	66.3	66.9[a]

a, b, c, d, e — assignment of the signals ambiguous.

On the other hand, the signals of the carbon atoms of the side chain of (VI) were close to or coincided with the corresponding signals described previously for the side chain of synaptogenin [5], with a keto group at C-23. These facts were confirmed by the ^1H NMR spectra, in which the signals of the protons at C-22 were observed in the form of two doublets at 3.15 and 3.95 ppm with a spin-spin coupling constant of 1.86 Hz, and the signals of two CH_3 groups at 0.88 ppm (d) and 0.89 ppm (d) (J = 6.0 Hz). Consequently, the aglycon of (VI) is 16β-acetoxy-3β-hydroxyholosta-7-en-23-one.

Since cucumarioside A_0-1 (II) is a minor component and its amount in the given fraction was about 4%, it was impossible to isolate it in the individual form.

This mixture contained glycosides with the same carbohydrate chain, which, by a comparison the ^{13}C NMR spectra of this fraction and of (VII), permitted us to establish the structure of the carbohydrate chain of native A_0-1 and to confirm the position of the sulfate group at C-3 of the first xylose residue, attached to the aglycon.

Thus, on the basis all that has been said above it follows that native A_0-1 (II) is 16β-acetoxy-3β-{O-(3-O-methyl-β-glucopyranosyl)-(1 → 3)-O-β-D-xylopyranosyl-(1 → 4)-[O-β-D

TABLE 3. Mass Numbers and Origins of the Ions in the LSIMS Spectra of (III), (IV), and (VI)–(VIII)

Compound	Charge of the ions, matrix	m/z and origin of the ions
VI	+ Glycerol + NaCl	1269 $(M+Na)^+$; 529 $(AgIOH_2)^+$; 511 $(AgI)^+$
	– Triethanolamine (TEA)	1245 $(M-H)^-$; 1113 (1245–132); 1069 (1245–176); 937 (1069–132); 805 (937–132); 659 805–146); 527($AgIO^-$)
III	+ Glycerol	1355 $(M_{Na}+Na)^+$; 1253 (1355–SO_3Na+H)$^+$; 1121 (1253–132); 1077 (1253–176); 945 (1077–132); 741; 609, 477
VII	+ Glycerol	1231 $(M+H)^+$; 513 $(AgIOH_2)^+$; 495 $(AgI)^+$
	– TEA	1229 $(M-H)^-$; 1037 (1229–132); 1053 (1229–176); (1053–132); 789 (921–132); 643 (789–146); 511 $(AgIO)^-$
IV	+ Glycerol	1311 $(M_{Na}+Na)^+$; 1209 (1311–SO_3Na+H)$^+$; 1077; 1033; 901; 741; 609; 477; 451 $(AgI)^+$
VIII	– TEA	1185 $(M-H)^-$; 1053 (1185–132); 1009 (1185–176); 877 (1009–132); 745 (877–132); 599 (745–146); 467 $(AgIO)^-$

xylopyranosyl-(1 → 2)]-O-β-D-xylopyranosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyl-oxy}holosta-7-en-16-one.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. NMR spectra were taken on a Bruker WM-250 spectrometer. Specific rotations were measured on a Perkin–Elmer 141 polarimeter at room temperature. Mass spectra were obtained on an MKH 1310 instrument fitted with an OKB Spektron system for recording negative ions and with a source of secondary ions from the Institute of Analytical Instrument Construction of the Russian Academy of Sciences (St. Petersburg), working on a cesium gun with an energy of the primary Cs^+ ions of 7 keV. GLC analysis was conducted on a Tsvet-110 chromatograph using 0.3 × 150 cm glass columns with 3% of QF-1 on Chromaton N-HMDS and the carrier gas argon (60 ml/min) at column temperatures of 150-225°C, 5°C/min. HPLC was conducted on a Du Pont 8800 chromatograph with a Silasorb C-18 column, 10 × 150 mm.

The animals were collected in September, 1981, by means of an industrial bottom trawl from a depth of 30-130 m in Posyet gulf (Peter the Great bay).

Isolation of the Glycoside Fraction. The comminuted holothurians were extracted twice with hot 70% ethanol. The extracts were concentrated in vacuum and were then lyophilized. The lyophilisate was extracted with a boiling mixture of chloroform and ethanol (1:1), and the extract was evaporated and was filtered through silica gel in the chloroform–ethanol–water (100:100:17) system. The filtrate was evaporated to dryness, dissolved in water, and chromatographed on a column with the polytetrafluoroethylene powder Polikhrom-1 (water → 50% ethanol).

The total mixture of glycosides so obtained was chromatographed repeatedly on silica gel in the chloroform–ethanol–water (100:50:4) system, with the collection of appropriate fractions.

Desulfation of the Initial Glycoside Fraction. A solution of 500 mg of the glycoside fraction in 50 ml of pyridine–dioxane (1:1) was boiled for 40 min. Then the reaction mixture was evaporated to dryness, and the residue was chromatographed on silica gel in the chloroform–methanol (5:1) system (system A). This gave 152 mg of the A_0 fraction (containing derivatives (VI)-(VIII)), and 243 mg of the A_1 fraction (containing derivative (V)). The A_0 and A_1 fractions were separated by the HPLC method: mobile phases 55 and 60% ethanol, respectively; 3 ml/min. This gave 16 mg of (VI), mp 229-231°C, $[\alpha]_{578}$ –34° (c 0.1; pyridine); 30 mg of (VII), mp 204-206°C, $[\alpha]_{578}$ –55° (c 0.1; pyridien); 60 mg of (VIII), mp 196-198°C, $[\alpha]_{578}$ –78° (c 0.1; pyridine); and 140 mg of (V), mp 201-203°C $[\alpha]_{578}$ –96° (c 0.1; pyridine).

Desulfation of (III) and (IV). The desulfation of 25 mg of each of these glycosides was carried out in 10 ml of pyridine–dioxane (1:1) as described above. The residues were filtered through silica gel in the chloroform–methanol–water (65:25:4) system (system B), giving derivative (VII), mp 203-205°C, $[\alpha]_{578}$ −55° (c 0.1; pyridine) and derivative (VIII), mp 196-198°C, $[\alpha]_{578}$ −77° (c 0.1; pyridine).

Deacetylation. A solution of 500 mg of the glycoside fraction in 100 ml of a 0.1% solution of NH_3 in 50% ethanol was left for 20 h. Then the reaction mixture was evaporated to dryness and chromatographed on silica gel in system B. This gave 189 mg of a fraction containing glycosides of the A_0 group and 210 mg of a fraction containing A_4-2.

When derivative (V) was treated by the method described above, derivative (IX) was obtained.

Isolation of the Individual Glycosides (II), (III), (IV), and (X). The glycoside fractions obtained after the treatment with the deacetylating agent were separated by the HPLC method (mobile phase – 45% ethanol, 3 ml/min). This gave 20 mg of a fraction containing (II); 27 mg of pure cucumarioside A_3-2 (III), mp 231-233°C, $[\alpha]_{578}$ −44° (c 0.1; pyridine); 59 mg of cucumarioside A_3-3 (IV), mp 202°C (decomp.), $[\alpha]_{578}$ −100° (c 0.1; pyridine); and 100 mg of cucumarioside A_4-2 (X), mp 205-207°C, $[\alpha]_{578}$ −110° (c 0.1; pyridine).

Acid Hydrolysis of (IV) and (X). A solution of 10 mg of one of these glycosides in 1.5 ml of 2 N HCl was heated on the boiling water bath for 2 h and was then cooled and diluted with water (4.5 ml). The precipitate was extracted with chloroform, and the aqueous layer was neutralized with Dowex anion-exchange resin (HCO_3^-). The resin was filtered off, and the aqueous layer was evaporated to dryness. The residue was treated with 1 ml of pyridine and 5 mg of hydroxylamine hydrochloride, and the mixture was heated at 100°C for 1 h; then 1 ml of acetic anhydride was added, and heating at 100°C was continued for another 1 h. The reaction mixture was evaporated and the aldononitrile peracetates obtained were analyzed by GLC-MS.

Hydrogenation of (III). A solution of 10 mg of the glycoside in 10 ml of 50% ethanol was added to a suspension of 1 mg of Adams hydrogenation catalyst in 30 ml of 50% ethanol, and hydrogenation was carried out with vigorous stirring for 24 h. The solution was filtered to remove the catalyst and was evaporated to dryness and chromatographed on silica gel in system B. This gave 8.5 mg of (XI), mp 236°C (decomp.), $[\alpha]_{578}$ −31° (c 0.1; pyridine).

LITERATURE CITED

1. S. A. Avilov, V. A. Stonik, and A. I. Kalinovskii, Khim. Prir. Soedin., No. 6, 787 (1990).
2. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
3. A. I. Kalinovskii, Khim. Prir. Soedin., No. 4, 605 (1988).
4. M. Girard, J. Belanger, J. W. ApSimon, F.-X. Garneau, C. Harvey, and J.-R. Brisson, Can. J. Chem., 68, 11 (1990).
5. T. A. Kuznetsov, N. I. Kalinovskaya, A. I. Kalinovskii, and G. B. Elyakov, Khim. Prir. Soedin., No. 5, 667 (1985).