2. S. F. Aripova, O. Abdilalimov, É. S. Bagdasarova, et al., Dokl. Akad. Nauk UzSSR, No. 6, 34-35 (1983).
3. S. A. Aripova, O. Abdilalimov, É. S. Bagdasarova, et al., Khim. Prir. Soedn., No. 1, 84-86 (1984).
4. A. Weisberg, Establishment of the Structure of Organic Compounds by Physical and Chemical Methods [in Russian], Book 1, Moscow (1967), pp. 89, 169, 255.
5. V. M. Vinogradov, Pharmacology of Amidine Compounds [in Russian], Kishinev (1972), pp. 106-114.
6. M. V. Korablev and P. I. Lukienko, Antihypoxic Properties [in Russian], Minsk (1976).
7. M. V. Plotnikov, T. M. Plotnikova, and T. V. Yakimova, Farmakol. Toksikol., No. 4, 38-41 (1988).
8. M. A. Polyakova, Pharmacological Approaches to the Solution of Urgent Clinical Problems [in Russian], Perm' (1980), pp. 35-36.
9. O. V. Tolstikova, A. G. Tolstikov, Estonian Republican Conference of Young Scientists: Summaries of Reports [in Russian], Tallin (1987), p. 144.
10. O. V. Tolstikova, A. G. Tolstikov, V. S. Shmakov, et al., Synthesis and Reactivity of Sulfur [in Russian], Tbilisi (1989), p. 194.
11. I. N. Yanvareva, I. D. Il'chenko, M. P. Bugrova, and V. M. Vinogradov, Farmakol. Toksikol., No. 2, 47-50 (1988).

SYNTHESIS AND ANTITUMOR ACTIVITY OF SALTS OF O-METHYLFAGARONINE AND ITS STRUCTURAL ANALOG – C-NORBENZO[C]PHENANTHRIDINE METHYL CHLORIDE

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The synthesis of the completely aromatic benzo[c]phenanthridine alkaloids is attracting wide attention due to their high antileukemic activity [8, 10, 12]. In this context, nitidine (I) and fagaronine (II) are the most widely studied [3, 6, 7, 9, 11]. Up to now, antitumor activity has been shown for the nitidine analog (III) and the fagaronine analog (IV) [4, 5] having the cyclopentane ring.

The given work describes a new synthesis and the antitumor activity of salts of O-methylfagaronine – the iodide and chloride (XI), (XII), as well as its previously unknown structural analogs, the indenoisoquinolines (XIII)-(XVI), based on 13α-hydroxyxylopinine (V) [2].

Alkylation of (V) by methyl p-toluenesulfonate afforded the mixture of the cis- and trans-quinolizidinium salts (VIa, b) [1, 2], which were converted to 3-(2-vinyl-4,5-dimethoxyphenyl)-6,7-dimethoxy-2-methylisoquinolone (VII) by the Hoffman cleavage in tert-butyl alcohol [1].

The hydroboration of (VII) with diborane in abs. THF at 20°C gave the alcohol (VIII). Its oxidation by pyridinium chlorochromate in CH_2Cl_2 led to 5-methyl-6-oxo-2,3,8,9-tetramethoxy-5,6-dihydrobenzo[c]phenanthridine (IX). The compound (IX) was reduced by $LiAlH_4$ in THF at 0°C to the 5,6-dihydrobenzo[c]phenanthridine (X), which was converted to O-methylfagaronine iodide (XI) by boiling it in an ethanolic solution of I_2 in the presence of AcONa. The exchange of the anions $I^e \rightarrow Cl^e$ was accomplished in boiling MeOH and an excess of AgCl. The structure of the chloride (XII) was confirmed by the data of the elemental analysis and the ^1H and ^{13}C NMR spectra.

When the vinyl derivative (VII) underwent cyclization in 29% HCl in abs. alcohol, we found that the compound isolated directly from the reaction mass is the protonated form of the lactam (XIII). The spectrum of the compound (XIII) contains the singlet signals of the

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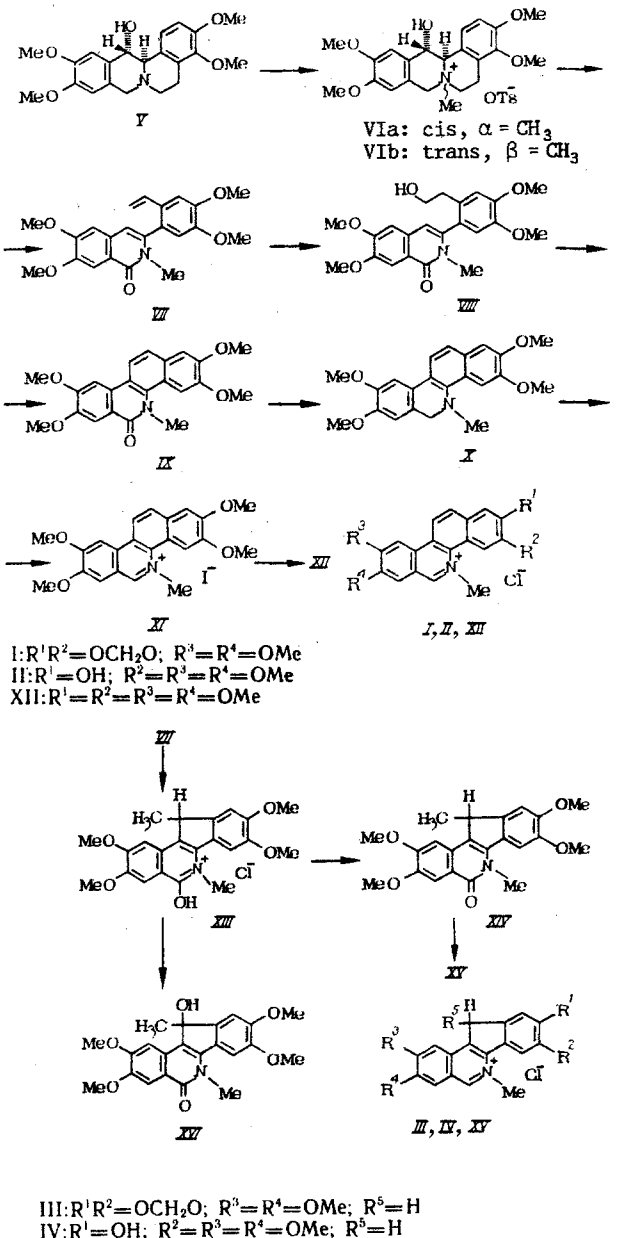
protons at C(4) with the δ 8.26 ppm and the $^{+}NCH_3$ group with the δ 4.27 ppm as well as the broad signal of the OH group at C(6) with the δ 5.04 ppm. Recrystallization of (XIII) from 2-propanol gives the neutral form of the lactam (XIV); this is confirmed by the singlet signals of the protons at C(4) with the δ 7.89 ppm and the NCH_3 group with the δ 4.13 ppm in the PMR spectrum of this compound.



Reduction of the lactam (XIV) with $LiAlH_4$ in abs. THF at 0°C in a stream of N_2 with the subsequent treatment by 10% HCl in ether gives the quaternary salt – 5,11-dimethyl-C-nor-benzo[c]phenanthridine chloride (XV) – with the yield of 50%.

The treatment of the compound (XIII) with NaH in the mixture of THF–DMF at 20°C for 3 h gives the product (XVI) with the yield of 83%.

EXPERIMENTAL (CHEMICAL)

The IR spectra were recorded using the UR-20 or "Perkin-Elmer 457" instruments with prisms of NaCl and NaF in mineral oil. The ^{1}H and ^{13}C NMR spectra were recorded on a "Bruker WP-200-SW" instrument (200 MHz); shifts were measured relative to tetramethylsilane. Mass spectra were obtained on the MAT-112 chromato-mass spectrometer; the energy of the ionizing electrons was 70 eV, and the temperature of the ionization chamber was 180°C. There was direct input of the sample at the source.

The course of the reactions and the purity of the products were monitored by the method of TLC using plates of Silufol UV 254, "Kavalier" (produced in Czechoslovakia). Spots were detected by the exposure to UV light or by the spraying of the plates with a 1% solution of ninhydrin in acetone. The values found for the elemental analyses correcpond to the calculated values.

3-[2-(2-Hydroxyethyl)-4,5-dimethoxyphenyl]-6,7-dimethoxy-2-methylisoquinolone (VIII). Compound (VII) (2 g) is dissolved in 60 ml of abs. THF at 50°C. The solution is cooled to 19°C prior to the careful dropwise addition of 5.9 ml of a 1.4 M solution of diborane in THF in a stream of argon. The reaction mixture is stirred at room temperature for 2 h. The mixture is then cooled prior to the addition of 3 ml of 10% NaOH and 2 ml of H_2O_2 at 0°C; the mixture is stirred at 0°C for 1 h for complete oxidation, and is left overnight at 20°C. The solvent and the water are evaporated in vacuo to dryness. The colorless inorganic residue is washed thoroughly with $CHCl_3$ and filtered twice through a folded filter. The filtrate is concentrated in vacuo, and the white residue is triturated with abs. ether. The yield of 2 g (98%) of the compound (VIII) is obtained; it has the mp 128-130°C (from ether). The IR spectrum (ν_{max}, cm^{-1}) is as follows :3390 (OH), 1645 (CO), 1600, and 1590 (C=C). The ^1H NMR spectrum (δ, ppm) is as follows: 7.80 s, 6.89 s, 6.83 s, 6.75 s [4H, C(5)-H, C(8)-H, C(3')-H, C(6')-H], 6.37 s [1H, C(4)-H], 4.02 s, 3.98 s, 3.95 s, 3.87 s (12H, $4OCH_3$), 3.75 m (2H, OCH_2CH_2), 3.30 s (3H, NCH_3), and 2.6-2.9 m (2H, OCH_2CH_2). The [M^+] found is 399 (100%). $C_{22}H_{25}NO_6$. Calculated M 399.45.

O-Methyl-6-oxofagaronine (IX). The mixture of 1 g of the alcohol (VIII), 1 g of the Corey reagent (pyridinium chlorochromate), and 0.39 g of AcONa in 50 ml of abs. CH_2Cl_2 is stirred at 20°C for 4 h. The residue is filtered off, washed with CH_2Cl_2, and concentrated in vacuo to remove the solvent. The residue is chromatographed on a column with SiO_2 100/160 μm using $CHCl_3$ as the eluent. The yield of 0.6 g (63%) of (IX) is obtained; it has the mp 247-249°C (from $CHCl_3$). The IR spectrum (ν_{max}, cm^{-1}) is as follows: 1630 (CO), 1620, and 1590 (C=C). The ^1H NMR spectrum (δ, ppm) is as follows: 8.04 d [1H, C(11)-H, J = 8.79 Hz], 7.62 d [1H, C(12)-H, J = 8.79 Hz], 7.94 s, 7.62 s, 7.20 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 4.12 s (3H, NCH_3), 4.06 s (6H, $2OCH_3$), 4.07 s, and 4.05 s (6H, $2OCH_3$). The [M^+] found is 379 (100%). $C_{22}H_2NO_5$. Calculated: M 379.42.

5-Methyl-2,3,8,9-tetramethoxy-5,6-dihydrobenzo[c]phenanthridine (X). To the suspension of 2.2 g of (IX) in 50 ml of abs. THF at 0°C is added 0.4 g of $LiAlH_4$, and the reaction mixture is stirred at 0°C for 30 min. The cooling is then discontinued, and the mixture is stirred at 20°C. After 3 h, 3 ml of H_2O are added to the mixture at 0°C, and it is left overnight at room temperature to ensure the complete decomposition of the $LiAlH_4$. The residue is filtered off and washed with hot $CHCl_3$ (2 × 50 ml). The filtrate is evaporated in vacuo, and the residue is triturated with abs. ether. The yield of 1.8 g (85%) of compound (X) is obtained; it has the mp 210-212°C (from ether). The ^1H NMR spectrum (δ, ppm) is as follows: 7.72 d [1H, C(11)-H, J = 8.54 Hz], 7.53 d [1H, C(12)-H, J = 8.54 Hz], 7.65 s, 7.32 s, 7.13 s, 6.81 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 4.16 broad s [2H, C(6)-H_2], 4.07 s, 4.02 s, 4.00 s, 3.95 s (12H, $4OCH_3$), and 2.64 s (3H, NCH_3). Found: [M^+] 365 (80%) and 349 (100%). $C_{22}H_{23}NO_4$. Calculated M 365.43.

O-Methylfagaronine Iodide (XI). The solution of 34 mg of I_2 in 30 ml of abs. ethanol is added to the boiling suspension of 100 mg of (X), 68 mg of AcONa, and 30 ml of abs. ethanol. The mixture is boiled for 2 h, cooled at 20°C, and SO_2 is passed through the reaction mass. The residue is filtered off and washed with 5 ml of ethanol and then 20 ml of ether. The yield of 120 mg (89%) of the iodide (XI) is obtained; it has the mp 310-312°C (from the mixture ethanol–ether). The ^1H NMR spectrum (δ, ppm) is as follows: 9.88 s [1H, C(6)-H], 8.91 d [1H, C(11)-H, J = 9.14 Hz], 8.33 d [1H, C(12)-H, J = 9.14 Hz], 8.38 s, 8.17 s, 7.91 s, 7.81 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 4.99 s (3H, N^+CH_3), 4.24 s, 4.08 s, 4.06 s, and 4.03 s (12H, $4OCH_3$). Found: $[M]^+$ 349 (100%) and 335 (17%). $C_{22}H_{22}NO_4I \cdot H_2O$. Calculated: M491.34.

O-Methylfagaronine Chloride (XII). The iodide (XI) (1.4 g) in 200 ml of MeOH is boiled with 1.38 g of AgCl for 2 h. The mixture is cooled and decanted. To the residue are again added 200 ml of MeOH, and the mixture is boiled for 2 h more. The methanol solution is concentrated, and the residue is triturated in abs. ether. The yield of 1 g (88%) of the chloride (XII) is obtained; it has the mp 266°C (subl.). The ^1H NMR spectrum (δ, ppm) is as follows: 9.89 s [1H, C(6)-H], 8.90 d [1H, C(11)-H, J = 8.77 Hz], 8.32 d [1H, C(12)-H, J = 8.77 Hz], 8.38 s, 8.17 s, 7.91 s, 7.80 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 5.00 s (3H, $^+NCH_3$),

TABLE 1. Antitumor and Antileukemic Activity of the Compounds Synthesized

Compound	LD_{50}, μg/kg	P-388		L-1210		Jensen's sarcoma	
		dose, mg/kg	T/C, %	dose, mg/kg	T/C %	dose, mg/kg	(C–T)/C, %
XIII	350—400	50	100	10	100	60	44
		100	100	20	100	—	—
		—	—	25	107	—	—
IX	350—400	10	100	25	105	10	16
		20	100	—	—	20	24
XI	350—400	20	126	25	100	12,5	25
		25	132	—	—	25	50
		40	116	—	—	—	—
XII	350—400	10	120	25	100	10	18
		20	100			20	20
XIII	350—400	20	122	20	115	20	60
		40	100	25	115	—	—
XIV	350—400	50	100	25	137	60	60
		100	100	—	—	—	—
XV	350—400	20	100	—	—	15	32
		40	100	—	—	—	—
XVI	350—400	20	100	10	105	10	13
		50	100	25	119	20	42

4.24 s, 4.08 s, 4.05 s, and 4.03 s (12H, $4OCH_3$). The ^{13}C NMR spectrum in DMSO-d_6 (δ, ppm) is as follows: 158.25 [C(3)], 151.43 [C(9)], 150.80 [C(6)], 150.51 [C(8)], 149.06 [C(2)], 132.14, 131.97, 130.93, 123.83, 119.44, 118.46 [C(4a), C(4b), C(6a), C(10a), C(10b), C(12a)], 118.86 [C(11)], 129.66 [C(12)], 108.64 [C(1)], 108.64 [C(4)], 107.71 [C(7)], 103.09 [C(10)], 57.18, 56.20, 55.96, 55.79 ($4OCH_3$), and 51.34 (N^+CH_3). Found: [M^+] 349 (100%). $C_{22}H_{22}NO_4Cl \cdot 3H_2O$. Calculated: M 453.93.

Protonated Form of the Lactam (XIII). The mixture of 0.5 g of compound (VII) and 5 ml of ethanol, saturated with HCl, is boiled for 30 min. The reaction mixture is cooled, and the residue is filtered off and washed with abs. ethanol and ether. The yield of 0.44 g (88%) of the protonated form of the lactam (XIII) is obtained; it has the mp 193°C (with decomposition, from the mixture ethanol–HCl–ethanol). The ^1H NMR spectrum (δ, ppm) is as follows: 8.26 s [1H, C(4)-H], 7.40 s, 7.17 s, 7.11 s, [3H, C(1)-H, C(7)-H, C(10)-H], 5.04 broad s (1H, OH), 4.27 s (3H, $^+NCH_3$), 4.13 s, 4.02 s, 4.00 s, 3.96 s (12H, $4OCH_3$), 3.90 d [1H, C(11)-H, J = 7.08 Hz], and 1.57 d [1H, C(11)-CH_3, J = 7.08 Hz]. Found: [M^+] 381 (100%). $C_{22}H_{23}NO_5 \cdot HCl$. Calculated: M 417.88.

5,11-Dimethyl-2,3,8,9-tetramethoxy-11H-indeno[1,2-c]isoquinolone (XIV). The recrystallization of (XIII) from 2-propanol affords the neutral form of the lactam (XIV); it has the mp 196-198°C (from 2-propanol). The IR spectrum (ν_{max}, cm^{-1}) is as follows: 1640 (CO). The ^1H NMR spectrum in $CDCl_3$ (δ, ppm) is as follows: 7.89 s, 7.44 s, 7.10 s, 6.99 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 4.13 s (3H, NCH_3), 4.06 s, 4.03 s, 3.99 s (12H, $4OCH_3$), 3.90 d (1H, J = 7.08 Hz), and 1.56 d [1H, C(11)-CH_3, J = 7.08 Hz]. Found: [M^+] 381 (100%). $C_{22}H_{23}NO_5$. Calculated: M 381.42.

5,11-Dimethyl-2,3,8,9-tetramethoxy-11H-indeno[1,2-c]isoquinolinium Chloride (XV). To the suspension of 1 g of (XIV) in 50 ml of abs. THF in an atmosphere of N_2 at 0°C is added 0.3 g of $LiAlH_4$, and the mixture is stirred for 8 h prior to the addition of 1 ml of 15% NaOH and 2 ml of water; the temperature of the reaction mixture should not exceed 10°C. The residue is filtered off and washed with $CHCl_3$, and the filtrate is evaporated in vacuo. The residue is treated with a 10% solution of HCl in diethyl ether. The yield of 0.53 g (50%) of the compound (XV) is obtained; it has the mp 180°C (with decomposition from the mixture ethanol–ether). The ^1H NMR spectrum (δ, ppm) is as follows: 10.45 s [1H, C(6)-H], 7.70 s, 7.53 s, 7.49 s [4H, C(1)-H, C(4)-H, C(10)-H], 4.77 s (3H, ^+NCH), 4.29 s, 4.05 s, 4.01 s, 3.92 s (12H, 4OCH), and 1.62 d [3H, C(11)-CH, J = 5.85 Hz]. $C_{22}H_{24}NO_4Cl$.

11-Hydroxy-5,11-dimethyl-2,3,8,9-tetramethoxy-11H-indeno[1,2-c]isoquinolone (XVI). To the solution of 0.5 g of compound (XIII) in 30 ml of THF and 20 ml of DMF are added 200 mg of NaH, and the reaction mixture is stirred at 20°C for 3 h. The mixture is then poured into water prior to the extraction with $CHCl_3$, the washing with water (2·100 ml), and drying with Na_2SO_4. The solvent is evaporated in vacuo, and the residue is triturated in abs. ether. The yield of 0.39 g (83%) of (XVI) is obtained; it has the mp 279.5°C (from MeOH). The IR spectrum (ν_{max}, cm^{-1}) is as follows: 3360 (OH), 1645 (CO), 1620, and 1580 (C=C). The

^{1}H NMR spectrum (δ, ppm) is as follows: 7.52 s, 7.28 s, 6.99 s, 6.78 s [4H, C(1)-H, C(4)-H, C(7)-H, C(10)-H], 4.61 broad s (1H, OH), 4.11 s (3H, NCH_3), 4.05 s, 3.95 s, 3.63 s, 4.42 s (12H, $4OCH_3$), and 1.71 s [3H, C(11)-CH_3]. Found: [M^+] 397 (3%) and 379 (100%). $C_{22}H_{23}NO_6$. Calculated: M 397.43.

EXPERIMENTAL (BIOLOGICAL)

The antitumor and antileukemic activity of the compounds (VIII), (IX), and (XI)-(XVI) were studied using hybrid white rats of mass 110-420 g with the transplanted Jensen's sarcoma and BDF_1 mice of mass 20-22 g with the lymphoid leukemia P-388 and L-1210.

The tested substances at the doses of 10-100 mg/kg were injected ip in a 10% solution of polyvinylpyrrolidone with added Tween-80 to the concentration of 0.4% daily in the course of 8 days.

The treatment was commenced 3-4 days after the transplantation of the Jensen's sarcoma and 2 days after the transplant of the lymphoid leukemia L-1210 and P-388. The antiblastic effect was evaluated from the index of inhibition of tumor growth (for the Jensen's sarcoma) and the increase in the life duration of the mice in the experimental group (in percentages relative to the control).

The toxicity of the compounds (T/C) was studied using hydrid white mice of the mass 20-22 g. The LD_{50} and MID were determined for the single application of the compounds. It was shown that the LD_{50} of the investigated compounds is 350-400 mg/kg, and the MID is 200-300 mg/kg.

The compounds studied exhibited weak antitumor and antileukemic activity. Data on the antitumor and antileukemic activity of the compounds synthesized for the application in optimal doses are presented in Table 1.

The compounds most active toward Jensen's sarcoma were (XI), (XIII), (XIV), and (XVI); they inhibited the growth of the tumor by up to 60%.

Moderate antileukemic activity toward the leukemia P-388 was shown by the compound (XI) which increased the life duration of the mice by 32% at the dose of 25 mg/kg.

The greatest effect in mice with the lymphoid leukemia L-1210 was shown by the compound (XIV). Its application at the dose of 25 mg/kg increased the life duration of the mice by 37%.

LITERATURE CITED

1. N. M. Sazonova, V. I. Sladkov, and N. N. Suvorov, Zh. Org. Khim., 25, No. 6, 1298-1301 (1989).
2. V. I. Sladkov, N. M. Sazonova, L. N. Kuleshova, et al., Zh. Org. Khim., No. 4, 854-862 (1989).
3. M. A. Caolo and F. R. Stermitz, Heterocycles, 12, No. 1, 11-15 (1979).
4. M. Cushman, P. Mohan, and E. C. K. Smith, J. Med. Chem., 27, 544-547 (1984).
5. M. Cushman and P. Mohan, J. Med. Chem., 28, 1031-1036 (1985).
6. J. L. Hartwell, Cancer Chemother. Rep., 10, 19 (1960).
7. H. Ishii, Y.-I. Ichikawa, E. Kawanabe, et al., Chem. Pharm. Bull., 33, No. 10, 4139-4151 (1985).
8. W. M. Messmer, M. Tin-Wa, H. H. S. Fong, et al., J. Pharm. Sci., 61, 1858-1860 (1972).
9. F. R. Stermitz, K. A. Larson, and D. K. Kim, J. Med. Chem., 16, 939 (1973).
10. F. R. Stermitz, J. P. Gillespie, L. G. Amors, et al., J. Med. Chem., 18, 708-712 (1975).
11. D. Walterova, J. Virickova, V. Preininger, et al., J. Med. Chem., 24, No. 9, 1100-1103 (1981).
12. R. K.-Y. Zee-Cheng and C. C. Cheng, J. Med. Chem., 18, 66-71 (1975).