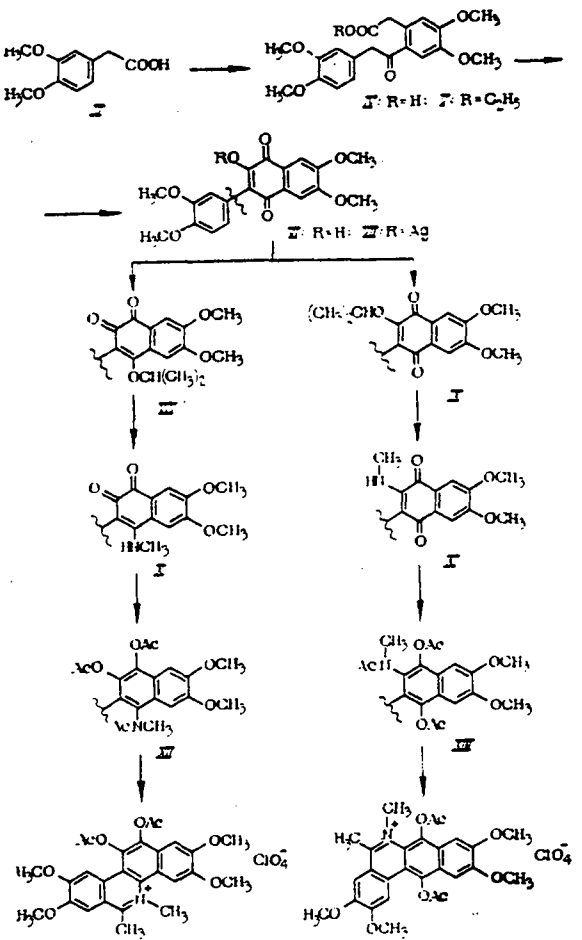
SYNTHESIS AND BIOLOGICAL ACTIVITY OF BENZO[c]- AND BENZO[b]PHENANTHRIDINIUM SALTS

V. I. Sladkov, V. A. Khokhlov
Yu. A. Ershova, V. A. Chernov,
Yu. V. Khokhlova, A. F. Nanasyuk,
and N. N. Suvorov

UDC 615.277.3:547.836.3].012.1

Benzo[c]phenanthridine alkaloids are known to possess antileukemic activity in mice against strains L-1210 and P-388 [5]. The most active of the antileukemic quaternary benzo[c]phenanthridinium salts are nitidine, fagaronine, and fagaronine methyl ether [4, 6]. Their use in medicine is however prevented by their high toxicity. For this reason, we have examined the effects of modifying fagaronine methyl ether on its antitumor activity.

We here describe the total synthesis of the perchlorate of 11,12-diacetoxy-6-methylfagaronine methyl ether (I), together with its structural isomer, 7,12-diacetoxy-5,6-dimethyl-2,3,9,10-tetramethoxybenzo[b]-phenanthridinium perchlorate (II), and report the results of test for antitumor activity, together with some data for the metabolism of human skin fibroblasts in culture.



D. I. Mendeleev Chemical Technology Institute. S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry. Scientific-Research Institute of Rheumatology, Academy of Medical Sciences of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 21, No. 6, pp. 660-663, June, 1987. Original article submitted May 21, 1986.

 0091-150X/87/2106-0394$12.50

The starting material for the synthesis of the isomeric phenanthridinium salts (I) and (II) was homoveratric acid (III), which was converted by intramolecular acylation into the bicyclic acid (IV). The ethyl ether (V) of (IV) on treatment with aqueous alkali underwent intramolecular Claisen condensation, tautomerism, and oxidation to the quinone (VI) [3].

Alkylation of the silver salt (VII) of the quinone (VI) with isopropyl iodide as described in [1] affords the isomeric alkoxynaphthoquinones 4-isopropoxy-3-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,2-naphthoquinone (VIII) and 2-isopropoxy-3-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,4-naphthoquinone (IX).

Boiling the alkoxynaphthoquinones (VIII) and (IX) with 25% aqueous methylamine in ethanol gave the enaminones (X, XI), which on reductive acylation ($NaBH_4$ and Ac_2O) gave the amidonaphthaquinones (XII, XIII). Cyclization of (XII) and (XIII) to the tetracyclic compounds (I) and (II) was effected by boiling in excess $POCl_3$.

The structures of the products were confirmed by their IR, UV, and PMR spectra. Molecular masses were found by mass spectrometry.

EXPERIMENTAL CHEMICAL PART

The course of the reactions and the purities of the products were followed by TLC on Silufol UV-254 plates (Czech SSR) in the system chloroform–ethanol (20:1). IR spectra were obtained on a UR-20 instrument (East Germany) in vaseline oil, and UV spectra on a Perkin-Elmer-575 spectrophotometer (USA) in chloroform or ethanol. PMR spectra were obtained on a Bruker WM-250 spectrometer (250 MHz) (West Germany); internal standard TMS.

Mass spectra were obtained on a Varian MAT-112 (West Germany), with direct introduction of the sample into the ionization zone.

4-Methylamino-3-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,2-naphthoquinone (X). To a suspension of 0.66 g (1.6 mmole) of (VIII) in 50 ml of ethanol was added 3 ml (24 mmole) of a 25% aqueous solution of methylamine. The mixture was boiled for 2 h and kept for 12 h at 20°C. The solid which separated was filtered off and recrystallized from ethanol to give 0.5 g (76%) of (X), mp 268-270°C. Found, %: C 65.61; H 5.57; N 3.69. M^+ 383 $C_{21}H_{21}NO_6$. Calculated, %: C 65.80; H 5.48; N 3.66. M 383. IR spectrum, ν, cm^{-1}: 3315 (NH), 1665 (C=O). UV spectrum, λ_{max}, nm (log ε) ($CHCl_3$): 294 (4.13), 346 (3.75), 530 (3.12). PMR spectrum (d_6-DMSO), δ, ppm): 7.69 s (1H), 7.53 s (1H), 6.95-6.80 m (3H), 4.02 s (OCH_3), 3.98 s (OCH_3), 3.83 s (OCH_3), 3.81 s (OCH_3), 2.55 d (NCH_3, J 4.7 Hz).

2-Methylamino-3-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,4-naphthoquinone (XI). This was obtained similarly from 0.5 g (1.3 mmole) of (IX) and 2.5 ml (20 mmole) of 25% aqueous methylamine in 40 ml of ethanol. Yield of (XI) 0.42 g (84%), mp. 228-230°C. Found, %: C 65.80; H 5.48; N 3.66. M 383. IR spectrum, ν, cm^{-1}: 3370 (NH), 1660 (C=O). UV spectrum, λ_{max}, nm (log ε) ($CHCl_3$): 289 (4.56), 352 (4.09), 508 (3.02). PMR spectrum (d_6-DMSO), δ, ppm: 7.58 s (1H), 7.49 (1H), 9.95-6.81 m (3H), 4.00 s (OCH_3), 3.99 s (OCH_3), 3.91 s (OCH_3), 3.88 s (OCH_3), 2.47 d (NCH_3, J 5.9 Hz).

4-N-Acetyl-N-methylamino)-1,2-diacetoxy-3-(3,4-dimethoxyphenyl)-6,7-dimethoxynaphthylamine (XII). A mixture of 1 g 2.6 mmole) of (X) and 6 g (170 mmole) of $NaBH_4$ in 60 ml of ethanol was boiled for 15 min, and 15 ml (160 mmole) of acetic anhydride added. After 5 min, the solvent was removed under reduced pressure (25 mm), 300 ml of water added, and the solid which separated was filtered off, washed with water, and recrystallized from methanol to give 0.93 g (72%) of (XII), mp 284-286°C. Found, %: C 63.58; H 5.73; N 2.75. M^+ 511. $C_{27}H_{29}NO_9$. Calculated, %: C 63.45; H 5.68; N 2.74. M 511. IR spectrum, ν, cm^{-1}: 1785 (C=O) $OCOCH_3$), 1665 (C=O, $NCOCH_3$). UV spectrum, λ_{max}, nm (log ε) (ethanol): 202 (3.81), 250 (4.09), 286 (4.19), 335 (3.41), 373 (2.03). PMR spectrum ($CDCl_3$), δ, ppm: 7.08 s (1H), 6.94 (1H), 6.90-6.73 (3H), 4.02 s (OCH_3), 3.96 s (OCH_3), 3.92 s (OCH_3), 3.84 s (OCH_3), 3.07 s (NCH_3), 2.47 s ($NCOCH_3$), 1. 99 s ($OCOCH_3$), 1.82 s ($OCOCH_3$).

2-(N-Acetyl-N-methylamino)-1,4-diacetoxy-3-(3,4-dimethoxyphenyl)-6,7-dimethoxynaphthalene (XIII). This was obtained similarly from 1 g (2.6 mmole) of (XI), 5 g (140 mmole) of $NaBH_4$, and 20 ml (210 mmole) of acetic anhydride in 145 ml of ethanol. Yield of (XIII) 0.72 g (55%), mp 272-273°C. Found, %: C 63.54; H 5.74; N 2.75. M^+ 511. $C_{27}H_{29}NO_9$. Calculated, %:

C 63.45; H 5.68; N 2.74. M 511. IR spectrum, ν, cm^{-1}: 1770 (C=O, $OCOCH_3$), 1660 (C=O, $NCOCH_3$), UV spectrum, λ_{max}, nm (log ε) (ethanol): 203 (4.01), 254 (4.69), 286 (3.42), 373 (2.03). PMR spectrum ($CDCl_3$), δ, ppm; 6.99-6.69 m (5H), 3.99 s (OCH_3), 3.98 s (OCH_3), 3.89 s (OCH_3), 3.81 s (OCH_3), 2.92 s (NCH_3), 2.42 s ($NCOCH_3$), 2.07 s ($OCOCH_3$), 1.89 s ($OCOCH_3$).

11,12--Diacetoxy-5,6-dimethyl-2,3,8,9-tetramethoxybenzo[c]phenanthridinium Perchlorate (I). To 0.5 g (1 mmole) of the acetamide (XII) was added 30 g (190 mmole) of $POCl_3$, the mixture boiled for 4 h, cooled to 45°C, poured into 200 ml of ice water containing 2 ml of conc. Hcl, and 4 ml of 57% $HClO_4$ added. After 10 h, the solid which separated was filtered off dried, and recrystallized from ethanol containing 0.05 ml of 57% $HClO_4$ to give 0.32 g (53.5%) of (I), mp 258-260°C. Found, %: C 54.54; H 4.77; N 2.23. $C_{27}H_{28}ClNO_{12}$. Calculated%: C 54.42; H 4.72; N 2.46, IR spectrum, ν, cm^{-1}: 1775 (C=O, $OCOCH_3$), 1615 (C=N). UV spectrum, λ_{max}, nm (log ε) (ethanol): 202 (4.25), 230(3.75), 280 (4.12), 3.85 pl (3.45), 440 pl (2.99). PMR spectrum (d_6-DMSO), δ, ppm: 8.46 (1H), 8.06 (1H), 7.96 (1H), 7.39 (1H), 4.66 s ($^+N-CH_3$), 4.15 s (OCH_3), 4.12 s (OCH_3), 4.07 s (OCH_3), 4.04 s (OCH_3) 4.04 s (OCH_3), 3.24 ($C-CH_3$), 2.61 s ($OCOCH_3$), 2.59 s ($OCOCH_3$).

7,12-Diacetoxy-5,6-dimethyl-2,3,9,10-tetramethoxybenzo[b]phenanthridinium Perchlorate (II). This was obtained similarly, from 0.5 g (1 mmole) of (XIII) and 20 g (127 mmole) of $POCl_3$. Yield of (II) 0.4 g (67%), mp 182-184°C Found, %: C 54.33; H 4.83; N 2.31. $C_{27}H_{28}ClNO_{12}$. Calculated %: C 54.42; H 4.72; N 2.36. IR spectrum, ν, cm^{-1}: 1775 (C=O, $OCOCH_3$), 1620 (C=N). UV spectrum, λ_{max}, nm (log ε), (ethanol): 253 pl (4.31), 258 (4.34), 279 (4.40), 324 (4.38), 412 (3.77). PMR spectrum (d_6-DMSO), δ, ppm: 8.61 s (1H), 8.04 s (1H), 7.34 s (1H), 7.27 s (1H), 4.40 s ($^+N-CH_3$), 4.20 s (OCH_3), 4.09 s (OCH_3), 4.03 s (OCH_3), 3.33 s ($C-CH_3$), 2.79 s ($OCOCH_3$), 2.71 s ($OCOCH_3$).

EXPERIMENTAL BIOLOGICAL PART

The antitumor activity of (I) and (II) was examined in mongrel white male rats weighing 110-120 g with grafted Jensen's sarcoma, and black mice strain BDF with B-16 melanoma and lymphoid leukemia L-1210, by the method used at the VNIKhFI (All-Union Scientific-Research Institute of Pharmaceutical Chemistry [2]. The toxicities of the compounds were measured when administered in single doses to mongrel white mice weighing 20-22 g. The lethal dose of (I) following a single administration to white mice was 100 mg/kg.

On repeated administration in a dose of 30 mg/kg, (II) displayed a toxic effect (death of the animals commenced after 4-5 injections). The lethal dose of this compound following a single dose was 80 mg/kg.

The compounds were administered in a 10% polyvinylpyrrolidone solution with the addition of Tween-80 (up to 0.4%), intraperitoneally once daily for eight days. Treatment was commenced 3-4 days following transplantation of the tumor (solid forms), and on the day following transplantation of the ascitic tumor L-1210.

Antitumor activity was expressed as the index of retardation of tumor growth:

$$T = \frac{B_C - B_O}{B_C} \cdot 100,$$

where B_C and B_O are the masses of the tumors in the control and experimental groups respectively. In the animals with L-1210 leukemia, the lifespan was measured in both groups.

These tests for antitumor activity showed that in a dose of 25 mg/kg, (I) had no antitumor effect on Jensen sarcoma and L-1210 leukemia, but it retarded the growth of melanoma B-16 by 35%. In a dose of 12.5 mg/kg, (II) retarded the growth of Jensen sarcoma by 40%, and in a dose of 25 mg/kg it increased the lifespan of mice with L-1210 leukemia by 40%.

These findings encourage a further search for drugs for the treatment of malignant neoplasms among both benzo[c]phenanthridinium and the isomeric benzo[b]-phenanthridinium salts.

The effects of (I) and (II) on proliferative activity were examined in cultures of fibroblasts from healthy human skin. Compounds (I) and (II), dissolved in DMSO, were added to the growth medium, and cells were cultured in their presence for five days. The effects of different concentrations of (I) and (II) on the proliferation of the fibroblasts was estimated from the numbers of cells in the culture, and in the case of (II) also by the

incorporation of ^{3}H-thymidine into DNA. The effects of (II) on the protein synthesizing apparatus of the fibroblasts was estimated by the inclusion of ^{14}C-uridine into RNA.

In concentrations of 50 μg/ml, (I) and (II) were toxic, causing the deaths of all the cells in the culture. In concentrations of 10 μg/ml or less, neither compound directly killed the cells, only suppression of the proliferative activity of the fibroblasts being seen. In concentrations of 0.1, 1, 5, and 10 μg/ml, (I) reduced the ability of the cells to multiply by factors of 1.3, 1.5, 1.4, and 2.3 respectively. Compound (II) was less effective, suppressing fibroblast proliferation only at concentrations of 5 and 10 μg/ml (by factors of 1.3 and 1.5 respectively), while in concentrations of 0.1 and 1 μg/ml there was no difference from the controls. It was, however, found that (II) in concentrations from 10 to 0.5 μg/ml reduced the incorporation of the tag into DNA in the culture by an average of 57%, and from 0.1 to 0.05 μg/ml the synthesis of DNA increased by a average of 46%. In doses of from 0.5 to 10 μg/ml, (II) suppressed incorporation of the tag into RNA by an average of 62%. In concentrations of 0.1 μg/ml, RNA synthesis was increased by 16%, and in a dose of 0.05 μg/ml by 27%.

Hence, the effects of (I) and (II) on proliferative activity are dose-dependent. Depending on their concentrations, these compounds may be used as modifiers of cell multiplication, or in the case of (II), as a modifier of protein synthesis.

LITERATURE CITED

1. V. A. Khokhlov, V. I. Sladkov, L. N. Kurkovskaya, et al., Org. Khim., 21, No. 5, 1072-1079 (1985).
2. V. A. Chernov, Methods of Experimental Chemotherapy [in Russian],, G. N. Pershin (editor),, Moscow (1971), pp. 357-388.
3. H. R. Bentley, W. Dawson, and F. S. Apring, J. Chem., Soc., No. 5, 1763-1769 (1952).
4. W. M. Messmer, M. Tin-Wa, H. H. S. Fong, et al., J. Pharm. Sci., 61, No. 11, 1858-1859 (1972).
5. F. R. Stermitz, J. P. Gillespie, L. G. Amoros, et al., J. Med. Chem., 18, No. 7, 708-713 (1975).
6. K. V. Zee-Cheng, K. V. Robert, and C. C. Cheng, J. Med Chem., No. 1, 66-67.