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MODIFICATION OF THE SESQUITERPENE LACTONE ARTEANNUIN B AND ANTIMICROBIAL ACTIVITIES OF THE PRODUCTS OBTAINED

B. Kh. Abduazimov,[a] Kh. T. Zoirova,[a] I. D. Sham'yanov,[b] M. Kh. Nurmukhamedova,[a] M. M. Yusupov,[a] and V. M. Malikov[b]

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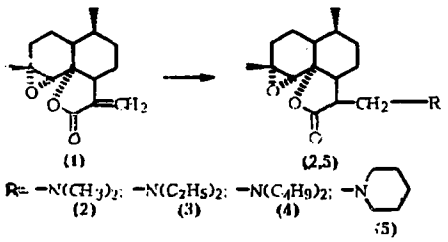
A number of amino derivatives have been synthesized from the sesquiterpene lactone arteannuin B, and quaternary salts have been obtained from them with alkyl halides. The results are given of a study of the antimicrobial activities of the modified derivatives.

It is known that the epigeal organs of sweet wormwood *Artemisia annua* L. produce sesquiterpene lactones of which the main representatives are artemisinin and arteannuin B [1, 2]. Pharmacological investigations have shown the presence of a high antimalarial activity of artemisinin and a number of its derivatives, and this has led to the introduction into medical practice of a new class of compounds as antiparasitic drugs [3, 4].

Arteannuin B (**1**) — the second lactone of sweet wormwood in terms of availability, being a waste material from the production of artemisinin — has not so far found practical use. In order to discover new physiologically active compounds, we have carried out a chemical modification of the initial (**1**) molecule.

Active reaction centers in the structure of arteannuin B (**1**) are the epoxy group and the α,β-unsaturated γ-lactone ring. In the present paper we report the addition of secondary amines at the exomethylene group of the γ-lactone ring (by a reaction of the Michael type) and the preparation of quaternary salts by their quaternization with alkyl halides, and we also present the results of a study of the antimicrobial activities of the derivatives obtained.

Amino derivatives of arteannuin B (**1**) were obtained by its interaction with dimethylamine, diethylamine, dibutylamine, and piperidine according to the following scheme



The addition of the amines was carried out by the usual procedure (in alcohol solution at 0-5°C). The yields of products were 80-95%.

Quaternary salts of the dimethylamino adduct of arteannuin B (**2**) were then obtained by treatment with various alkyl iodides according to the scheme:

a) Tashkent Pharmaceutical Institute, Tashkent-15, pr. Aibeka, 45. b) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 714-717, September-October, 1997. Original article submitted October 21, 1994; revision submitted April 21, 1997.

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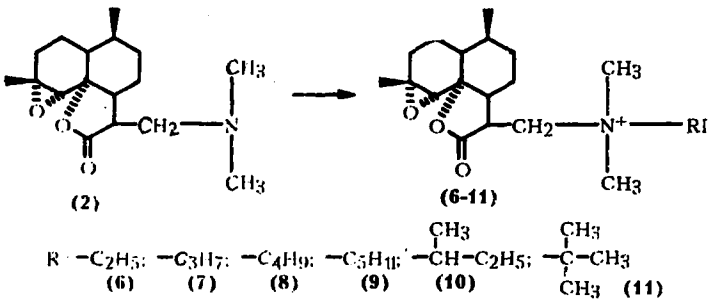

The structures of all the derivatives synthesized (**2-11**) were confirmed by spectral methods (IR, mass, PMR).

To determine the bactericidal activities of the compounds obtained we used the method of serial dilutions, as the most accurate in the quantitative respect, enabling the minimum suppressive concentration to be determined [5, 6]. The results of the experiments are given in Table 1.

As can be seen from Table 1, the substances synthesized exhibited activity in relation to such microorganisms as *E. coli, Sh. flexneri, Sh. dezentheria, S. typhimurium,* and *St. aureus*.

It must be mentioned that with an increase in the number of atoms in the amine radical (compounds **2-5**) the minimum inhibiting concentration increased in relation to *E. coli, Sh. flexneri, Sh. dezentheria, S. typhimurium,* and *St. aureus,* while these compounds (**2-5**) exhibited no activity whatever in relation to *Prot. mirabilis* and *Enterobacter aerogenes*. The opposite effect was observed for *St. aureus* in the case of the adduct of arteannuin B with the cyclic amine piperidine (**5**). The activities on *E. coli, Sh. flexneri, Sh. dezentheria,* and *S. typhimurium* of the quaternary salts obtained (**6-11**) were somewhat lower than those of the initial amines (**2-5**).

Thus, in spite of the absence of a direct correlation between the structures of the compounds synthesized and their activities, on the basis of the results given in Table 1 it may be concluded that a number of the substances obtained (**2-4, 10, 11**) exhibit pronounced antimicrobial activity relative to some strains of microorganisms.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in KBr tablets, PMR spectra on a Tesla BS 567 A instrument with a working frequency of 100 MHz, and mass spectra on a MKh-1310 instrument fitted with a system for the direct injection of the sample into the ion source at 100-180°C under an ionizing energy of 70 eV. The presence of peaks of the molecular ions of the quaternary salts of alkyl halides was confirmed by the method of metastable defocusing.

Preparation of Amino Derivatives (2-5) of the Lactone Arteannuin B (1). General Procedure. A solution of the initial substance (**1**) in ethanol was cooled to 0-5°C (ice bath) and the appropriate secondary amine in a ratio of 1:1.5 (g/mole [sic]) was added slowly with vigorous stirring. Stirring was continued for 2 h, and then the alcohol was distilled off and the residue was dissolved in ethyl acetate. The solution was washed with water and dried over $MgSO_4$, after which the solvent was distilled off, leaving as residue the corresponding adduct (**2-5**) of the lactone (**1**),

13-Dimethylaminoarteannuin B (2). Obtained with a yield of 92%, composition $C_{17}H_{27}O_3N$ (amorph.), M^+ 293. IR spectrum (cm^{-1}): 1772 (ν γ-lactone C=O), 1600 (δ N−H), 1460 (δ N−CH_3), 1100 (ν C−O−C). PMR spectrum ($CDCl_3$ + DMSO-d_6, ppm): 0.82 (3H, d, J=5 Hz, C_{10}−CH_3), 1.23 (3H, s, C_4−CH_3), 3.08 (6H, s, −N(CH_3)$_2$).

13-Diethylaminoarteannuin B (3). Obtained with a yield of 90%, composition $C_{19}H_{31}O_3N$ (amorph.), M^+ 321. IR spectrum (cm^{-1}): 1772 (ν γ-lactone C=O), 1460 (δ N-CH_3), 1100 (ν C−O−C). PMR spectrumm (DMSO-d_6, ppm): 0.9 (3H, d, J=5 Hz, C_{10}−CH_3), 1.18 (3H, s, C_4−CH_3), 2.25 (6H, s, −N-(CH_2)$_2$−(CH_3)$_2$).

13-Dibutylaminoarteannuin B (4). Obtained with a yield of 85%, composition $C_{25}H_{39}O_3N$ (amorph.), M^+ 377. IR spectrum (cm^{-1}): 1775 (ν γ-lactone C=O), 1455 (δ−N−CH_3), 1150 (ν C−O−C). PMR spectrum (DMSO-d_6, ppm): 0.68 (3H, d, J=5 Hz, C_{10}−CH_3), 1.18 (3H, s, C_4−CH_3), 0.75 (6H, s, (−CH_2−CH_3)$_2$).

13-Piperidinoarteannuin B (3). Obtained with a yield of 88%, composition $C_{20}H_{31}O_3N$ (amorph.), M^+ 333. IR spectrum (cm^{-1}): 1770 (ν γ-lactone C=O), 1300 (δ N−CH_2−CH_3), 1130 (ν C−O−C). PMR spectrum (DMSO-d_6, ppm): 0.82 (3H, d, J=5 Hz, C_{10}−CH_3), 1.22 (3H, s, C_4−CH_3), 1.30, 1.32, 1.34 (each 2H, 3CH_2 of the piperidine ring).

TABLE 1. Minimum Inhibiting Concentrations of the Compounds Investigated, mg/liter

Compound	Test microbes							
	E. coli	Sh.flexneri	Sh.dezentheria	S.typhimurium	St.aureus	Enterobacter aerogenes	Kleb.pneumoniae	Prot.mirabilis
1	62.5	31.5	31.5	31.5	+	31.2	+	31.2
2	3.9	31..2	31..2	31.2	+	+	+	+
3	3.9	15.6	15.6	15.6	+	+	+	+
4	15.6	31.2	31.2	15.6	+	+	+	+
5	-	+	+	+	15.6	+	+	+
6	250	+	+	15.6	31.2	+	+	+
7	125	+	+	31.2	62.5	+	+	+
8	31.5	31.5	31.2	62.5	+	+	-	+
9	+	+	+	125	125	+	+	+
10	15.6	31.2	31.2	31.2	+	-	+	+
11	15.6	62.5	62.5	31.2	62.5	+	+	+
Penicillin	12.5	62.5	62.5	125	62.5	125	62.5	62.5
Chloramphenicol	62.5	62.5	62.5	125	125	62.5	125	125

Preparation of the Quaternary Salts (6-11). General Procedure. An equimolar amount of the appropriate alkyl iodide was added at 5°C to a solution of 13-dimethylaminoarteannuin B (**2**) in ethyl acetate. After a day, the crystalline precipitate was filtered off, washed with ethyl acetate, and dried, to give the corresponding quaternary salt.

13-Dimethylethylammonioarteannuin B Iodide (6). Obtained with a yield of 83%, composition $C_{19}H_{31}O_3NI$, mp 168-170°C, M^+ 449. IR spectrum (cm^{-1}): 1770 (ν γ-lactone C=O), 1460 (δ N−CH_3), 1160 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.83 (3H, d, J=5 Hz, C_{10}−CH_3), 1.25 (3H, s, C_4−CH_3), 1.23 (3H, s, N−CH_2−CH_3), 3.0 (6H, s, N−$(CH_3)_2$).

13-Dimethylpropylammonioarteannuin B Iodide (7). Obtained with a yield of 60%, composition $C_{20}H_{34}O_9NI$, mp 77-79°C, M^+ 463. IR spectrum (cm^{-1}): 1771 (ν γ-lactone C=O), 1465 (δ N−CH_3), 1010 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.84 (3H, d, J=5 Hz, C_{10}−CH_3); 1.05 (3H, s, N−CH_2−CH_3), 1.28 (3H, s, C_4−CH_3), 3.06 (6H, s, N−$(CH_3)_2$).

13-Dimethylbutylammonioarteannuin B Iodide (8). Obtained with a yield of 65%, composition $C_{21}H_{36}O_3NI$, mp 108-111°C, M^+ 477. IR spectrum (cm^{-1}): 1760 (ν γ-lactone C=O), 1430 (δ N−CH_3), 1100 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.86 (3H, d, J=5 Hz, C_{10}−CH_3), 1.28 (3H, s, C_4−CH_3), 3.03 (6H, s, N−$(CH_3)_2$).

13-Dimethylamylammonioarteannuin B Iodide (9). Obtained with a yield of 70%, composition $C_{22}H_{38}O_3NI$, mp 178-180°C, M^+ 491. IR spectrum (cm^{-1}): 1770 (ν γ-lactone C=O), 1490 (δ N−CH_3), 1135 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.85 (3H, d, J=5 Hz, C_{10}−CH_3), 0.86 (3H, s, N−$(CH_2)_4$−CH_3), 1.28 (3H, s, C_4−CH_3), 3.06 (6H, s, N−$(CH_3)_2$).

13-Dimethyl-*sec*-butylammonioarteannuin B Iodide (10). Obtained with a yield of 72%, composition $C_{21}H_{36}O_3NI$, mp 98-99°C, M^+ 477. IR spectrum (cm^{-1}): 1775 (ν γ-lactone C=O), 1460 (δ N−CH_3), 1070 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.83 (3H, d, J=5 Hz, C_{10}−CH_3), 1.27 (3H, s, C_4−CH_3), 3.0 (6H, s, N−$(CH_3)_2$).

13-Dimethylisobutyl*ammonioarteannuin B Iodide (11). Obtained with a yield of 72%, composition $C_{21}H_{36}O_3NI$, mp 96-98°C, M^+ 477. IR spectrum (cm^{-1}): 1775 (ν γ-lactone C=O), 1455 (δ N−CH_3), 1110 (ν C−O−C). PMR spectrum (D_2O, ppm): 0.83 (3H, d, J=5 Hz, C_{10}−CH_3), 0.86 (9H, s, C-$(CH_3)_3$), 1.25 (3H, s, C_4−CH_3), 3.06 (CH, s, N−$(CH_3)_2$).

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*Sic. The formula for (**11**) shows *tert*-butyl — Translator.

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