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PREPARATION OF NEW AMINO DERIVATIVES OF THE LACTONE TANACHIN AND THEIR ANTIMICROBIAL ACTIVITY

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Several amino derivatives have been obtained from the sesquiterpene lactone tanachin by means of the Michael reaction, and their structures have been confirmed by spectral methods. The antimicrobial activities of the compounds synthesized relative to some strains of microorganisms are considered.

The active principles of many medicinal plants of the Compositae family are conformationally labile sesquiterpene lactones of the germacranolide type which are systematized configurationally into four subgroups based on the presence of double bonds at C-1,10 and C-4,5 of the cyclodecadiene skeleton [1-6]. In this connection, modification of the conformationally labile germacranolides followed by a consideration of biological activities is of scientific interest.

We have investigated the readily available sesquiterpene γ-lactone tanachin (**1**), which has been isolated from a number of species of plants of the Compositae family widely distributed in Central Asia [7-11].

There are seven reaction centers in the (**1**) molecule. The presence of an exomethylene group in the γ-lactone ring makes it possible to add nucleophilic reagents by means of the Michael reaction [12, 13]. As a result of this reaction we have obtained amino adducts of tanachin with dimethylamine (**2**), piperidine (**4**), and morpholine (**5**) (Scheme 1). The structures of

Scheme 1

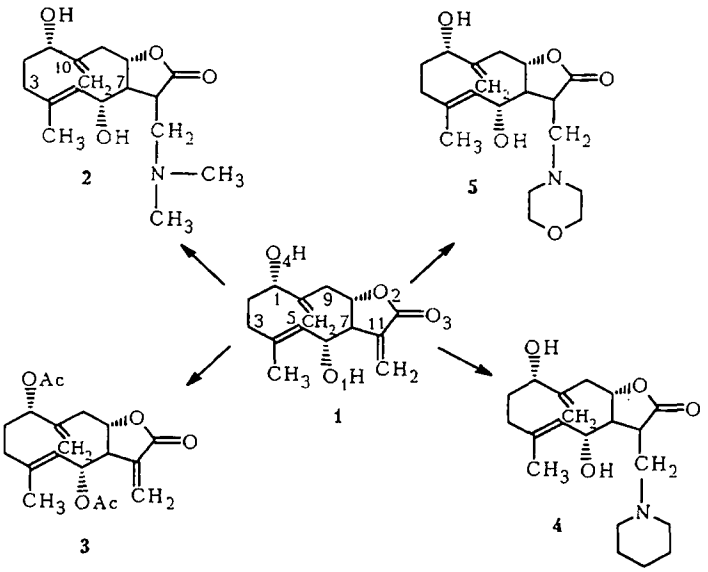

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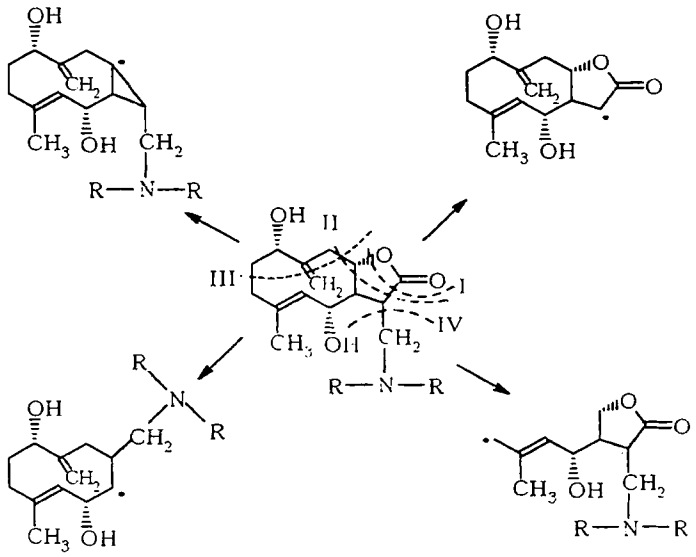
TABLE 1. Minimum Inhibiting Concentrations of the Substances Investigated (mg)

Substance	Test microbes			
	E.coli	Enterobacter aerogenes	St. aureus	Sh. flexneri
1	125	62.5	125	500
2	62.5	62.5	125	500
3	250	500	–	–
4	250	125	125	500
5	250	125	125	500
Levomycetin	62.5	125	125	62.5
Penicillin	125	250	62.5	125

the amino derivatives obtained were confirmed by IR-, mass-, and PMR-spectral methods, characteristics of which are given in the Experimental part.

It is interesting to note the mass-spectral behavior of the resulting amino derivatives of tanachin: the introduction of an amino group into the γ-lactone ring intensifies the fragmentation of this ring $(M^+ - CHO)^+$, $(M^+ - CO_2)^+$, $(M^+ - C_2HO_2)^+$ and the cleavage of the germacranolide at the C8-C9 and C2-C3 bonds, which was confirmed by the presence of an ion peak with *m/z* $(M^+ - C_5H_8O)^+$ that was observed for all the amino derivatives obtained. The mass spectra of the amino adducts did not include peaks of the $(M^+ - H_2O)^+$ and $(M^+ - 2H_2O)^+$ ions that are found in the mass spectrum of tanachin. In addition, the mass spectra of all the amines showed clear cleavage at the allyl bond (Scheme 2).

Scheme 2



For a comparative study of antimicrobial activities, we also obtained tanachin diacetate (**3**) by a procedure described previously [9].

To determine bactericidal activities we used the serial dilution method which enables the minimum suppressing concentration of the substance under investigation to be determined.

As can be seen from Table 1, on the addition of dimethylamine to the exocyclic bond (**2**) the activity against *E. coli,* a causative agent of intestinal infections, doubled in comparison with that of tanachin (**1**) but was unchanged in relation to the microbes *St. aureus* (125), *Sh. flexneri* (500), and *Enterobacter aerogenes* (62.5). The increase in activity against *E. coli* is possibly due to the formation of a hydrogen bond between the hydroxyl at the C-6 carbon atom and the nitrogen of the amino group, and this was confirmed by a shift in the stretching vibrations of the hydroxy group by 50 cm^{-1} in compound (**2**) relative to those of tanachin. (We have previously reported intermolecular hydrogen bonds in (**1**) on the basis of an x-ray study [15].)

According to Table 1, the morpholine (**5**) and piperidine (**4**) amino derivatives of tanachin (**1**) showed a decrease in bactericidal activity against *E. coli* and *Enterobacter aerogenes* and a retention of activity against *St. aureus* (125) and *Sh. flexneri* (500) which is due, in all probability, to the absence of intramolecular hydrogen bonds between the hydroxyl at C-6 and the nitrogen in the molecules of the amino derivatives.

When tanachin was acylated the activity against *St. aureus* and *Sh. flexneri* disappeared completely, probably because of the absence of intermolecular hydrogen bonds as a result of the blocking of the hydroxy groups.

Thus, the manifestation of bactericidal activity is possibly connected with the presence of hydroxy groups in the compounds investigated.

EXPERIMENTAL

The course of the reactions and the purity of the compounds obtained were monitored by TLC on Silufol UV-254 plates in the benzene–alcohol (2:1) and hexane–ethyl acetate–diethylamine (3:1:1) systems. The revealing agent was a 1% solution of vanillin in concentrated H_2SO_4. PMR spectra were taken on a Tesla BS-567A MHz [sic] spectrometer in C_5D_5N (0 — HMDS), mass spectra on a MKh-1310 spectrometer, and IR spectra on a UR-20 spectrometer (KBr tablets).

Preparation of 1,6-Dihydroxy-13-dimethylamino-7α,11α(H),1β,6β,8β(H)-germacra-4,10(14)-dien-8,12-olide (2). Over 30 min, 0.0016 g/mole of dimethylamine (33% aqueous solution) was added to a saturated ethanolic solution of 0.0025 g/mole of tanachin, and the reaction mixture was stirred for 2 h. The final product was obtained in the form of white acicular crystals with a yield of 90%.

Composition: $C_{17}H_{27}O_4N$, R_f 0.55, mp 197-198°C, M^+ 309.

IR spectrum (KBr, ν, cm^{-1}): 3340(OH), 1170(C=O), 1170, 1190, 1220(R_3-N), 2940(=C=CH_2), 1680(R_2C=CRH), 2880(C–CH_3), 2840(CH_3-N-CH_3).

PMR spectrum (100 MHz, $CDCl_3$, ppm, J, Hz): 4.29(1H, dd, J = 8, J = 2.5, H-1), 5.125(1H, d, J = 10, H-5), 4.238(1H, d, J = 10, H-5), 4.238(1H, t, J = 9, J = 10, H-6), 3.975(1H, sex, H-8), 4.925(1H, br.s, H-14), 5.037(1H, br.s, H-14).

Mass spectrum (EI, 70 eV, *m/z* I_{rel}, %): 309($M^+-C_{17}H_{27}O_4N$) (2), 292(0.7), 280(M^+–CHO, 0.8) 252(M^+-C_2HO, 0.5), 226($M^+-C_5H_7O$, 5.4), 212(1.4), 185(0.7), 142(2.7), 117(2), 105(2), 95(3.4), 91(5.2), 84(3.5), 79(4), 69(4), 59(8), 58(100), 55(7).

Preparation of 1,6-Dihydroxy-13-piperidino-7α,11α(H),1β,6β,8β(H)-germacra-4,10(14)-dien-8,12-olide (4). As for the preparation of (**2**), 0.00065 g/mole of tanachin and 0.0013 g/mole of piperidine gave white crystals with a yield of 85%.

Composition $C_{20}H_{31}O_4N$, M^+ 349, R_f 0.48, mp 198-199°C.

IR spectrum (KBr, ν, cm^{-1}): 3380(OH), 1765(C=O), 1163, 1180, 1230(R_3-N), 2940(=C=CH_2), 1675(R_2C=CRH), 2872(C=CH_3).

PMR spectrum (100 MHz, d-Py, ppm, J, Hz): 4.05(1H, m, H-1), 5.49(1H, d, J = 11, H-5), 4.48(1H, t, J = 9, J = 10, H-6), 3.79(1H, m, H-8), 2.125(2H, m, H-13), 5.15(1H, br.s, H-14), 5.625(1H, br.s, H-14), 1.562(3H, s, H-15).

Mass spectrum (EI, 70 eV, *m/z*, I_{rel}, %): 349($M^+-C_{20}H_{31}O_4N$, 7.5), 320(M^+–CHO, 6.7%), 266($M^+-C_{15}H_{22}O_4$, 5.4), 254(M^+–95.5.6), 169(10.3), 164(7.8), 161(5.0), 160(6.8), 149(5), 134(8.3), 132(9.9), 120(9.5), 119(8.2), 118(7), 117(5), 99(10), 98(100), 94(11), 91(15), 87(15.8), 85(6.7), 84(12), 83(5), 73(9.5), 65(9.5), 55(9.5), 51(4.1), 45(24.5), 44(8.2).

Preparation of 1,6-Dihydroxy-13-morpholino-7α,11α(H),1β,6β,8β(H)-germacra-4,10(14)-dien-8,12-olide (5). As for the preparation of (**2**), 0.0008 g/mole of tanachin and 0.0016 mole of morpholine gave white crystals with a yield of 90%.

Composition $C_{19}H_{29}O_5N$, M^+ 351, R_f 0.52, mp 229-230°C.

IR spectrum (KBr, ν, cm^{-1}): 3400(OH), 1765(C=O), 1145, 1180, 1218(R_3-N), 2935, 1675(R_2C=CRH), 2865.

PMR spectrum (100 MHz, d-Py, ppm, J, Hz): 4.04(1H, m, H-1), 5.45(1H, d, J = 10, H-5), 4.47(1H, t, J = 9, J = 10, H-6), 4.04(1H, m, H-8), 2.3(2H, m, H-13), 5.13(1H, br.s, H-14), 5.6(1H, br.s, H-14), 1.525(3H, s, H-15).

Mass spectrum (EI, 70 eV, *m/z*, I_{rel}, %): 351($M^+-C_{19}H_{29}O_5N$, 2.6%), 322(M^+–CHO, 1.3), 283(7.9), 282(22.4), 281(100), 280(11.2), 269(8.4), 267(M^+–CHO, 7.6), 248(M^+–18, –85, $C_{15}H_{20}O_3$, 6.3), 236(7), 235(28), 234(13.6), 219(9.2), 205(7.2), 191(9.6), 190(45.8), 176(10.5), 175(13.6), 162(6.6), 161(26.3), 160(18.4), 149(9.2), 146(9.3), 144(18), 129(25), 119(31.6), 106(76.9), 105(25), 102(27.6), 100(34.2), 92(26.3), 91(96.5), 85(51.3), 84(14), 83(75.0), 77(52.6), 71(31.6), 69(57.9).

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