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AMINODERIVATIVES OF SESQUITERPENE LACTONES FROM TANACHIN AND TAVULIN AND THEIR BIOLOGICAL ACTIVITY

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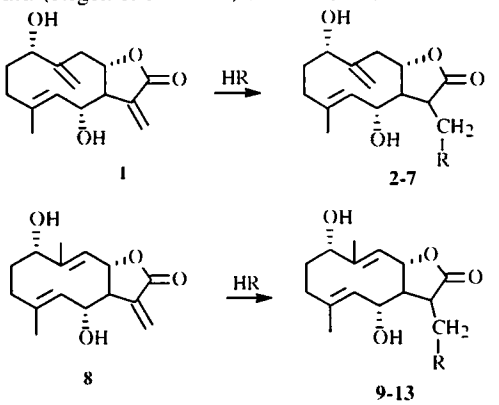
Michael reaction of sesquiterpene lactones of tanachin and tavulin yields eight new aminoderivatives, the structures of which are confirmed by spectral methods. The antimicrobial activity of the synthesized compounds against certain strains of microorganisms and the inhibition of ion-transport enzymes in biological membranes are examined.

Key words: *Tanacetopsis mucronata*, tanachin, tavulin, aminoderivatives, antimicrobial and inhibitory activity.

Sesquiterpenes are convenient starting materials for synthesizing new biologically active compounds. Therefore, the modification of sesquiterpenes and the search for applications in medicine and commerce are timely issues for modern bioorganic chemistry.

The active principle of many medicinal plants of the composite family are conformationally labile germacranolide sesquiterpene lactones [1-6]. Therefore, modification of conformationally labile germacranolides and subsequent examination of their biological activity are of interest to science.

Thus, we investigated available sesquiterpene γ-lactones with the germacrane framework, tanachin (**1**) and tavulin (**8**), which were isolated from widely distributed plants of the Compositae family in Middle Asia [7-11]. We isolated modified samples from *Tanacetopsis mucronata* (Regel. et Schmalh) S. Kovalevsk.



1 **2-7**

8 **9-13**

2, 9: R=N(CH$_3$)$_2$; **3, 10**: R=N(CH$_2$CH$_3$)$_2$; **4**: R=N(CH$_2$CH$_2$OH)$_2$
5,11: R=N(C$_4$H$_9$)$_2$; **6, 12**: R= N◯; **7, 13**: R= N◯O

We previously reported that tanachin and three aminoderivatives (**2, 6, 7**) exhibit antimicrobial activity [12]. The present article contains results for new aminoderivatives from tanachin and tavulin (Scheme 1) and their biological activity.

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TABLE 1. Minimally Inhibiting Concentration of investigated compounds, mg/l

Compound	Microbe tested			
	E. coli	Enterobacter aerogenes	St. aureus	Sh. flexneri
Total lactones	15.6	-	-	15.6
Tavulin (**8**)	250	125	-	-
Tavdma (**9**)	250	250	-	-
Levomycetin	62.5	125	125	62.5
Penicillin	125	250	62.5	125

The structures of the obtained aminoderivatives were confirmed by IR, mass, and PMR spectra, the data of which are given in the Experimental section.

The interesting mass-spectral behavior of the obtained aminoderivatives was reported previously [12]. The mass-spectral fragmentation patterns of the aminoderivatives of tanachin and tavulin also revealed that introducing an amino group into these lactones affects only the base peak, i.e., the same base peaks were observed for almost all synthesized aminoderivatives of tanachin and tavulin that have the same amine (see Experimental) although the base peaks are different for the starting materials. This is explained by the predominant loss of the fragment with the N atom in both instances.

The antimicrobial activity and inhibition of ion-transport enzymes of biological membranes were investigated in order to define the type of biological activity. Tavulin and its dimethylaminoderivative were studied for activity against certain microbes and were compared with tanachin and its aminoderivatives. The antimicrobial activity was studied by serial administrations, which enabled the minimal tolerated concentration to be determined [13].

The results showed that tavulin and its dimethylaminoderivative (**9**) possess antimicrobial activity. Their inhibition is directed mainly against intestinal microbes. The obtained aminoderivative has weaker activity than the starting compound against *Enterobacter aerogenes*. For *E. coli*, the activities are the same. The inhibition by tavulin is weaker than that of tanachin [12] and lies in a narrow range (Table 1). Tavulin exhibits weaker inhibition toward *St. aureus* and *Sh. flexneri*, in contrast with tanachin [12]. The different behaviors of these two lactones can be explained by their conformational and configurational features. The structures of these lactones are similar. However, they differ in the position of one of the double bonds. For tanachin, C-10 has an exocyclic double bond; for tavulin, the double bond is endocyclic and located between C9 and C10. Furthermore, the 10-membered ring in tavulin has the chair—boat conformation; in tanachin, boat—boat.

We observed during the synthesis of the aminoderivatives of tavulin and tanachin that these lactones have different reactivities. Tavulin reacts with amines much more difficultly than tanachin. This may be why the antimicrobial activity of tavulin is weaker than that of tanachin. The antimicrobial activity of sesquiterpene lactones depends mainly on the presence of an exocyclic methylene that can react with the amino group of amino acids and thereby disrupt protein synthesis and stop growth of the microorganisms and on the different configuration and conformation. The antimicrobial activity and other biological activities may possibly arise due to other active centers. This is consistent with the fact that some of the lactone derivatives have higher and some lower antimicrobial activity than others.

The antimicrobial activity of the total lactones against *E. coli* is higher than for tanachin (by eight times) and tavulin (by 16 times). Apparently this results from synergism among the total lactones. Furthermore, tanachin exhibits antimicrobial activity against certain microbes, which is not observed for the total lactones. This is consistent with antagonism in the total lactones.

Thus, it can be concluded that the antimicrobial activity of the compounds depends on not only structural factors but also the individual sensitivities for each microorganism.

The effect of tanachin, tavulin, and their aminoderivatives on the activity of ion-transport enzymes of biological membranes, Na^+, K^+-ATPase and Ca^{2+}-ATPase, of rat brain at a concentration of 2×10^{-4} M was studied. Inhibition was observed only against Ca^{2+}-ATPase. Enzyme activity was inhibited by an average of 71%.

Further pharmacological studies of the synthesized compounds is continuing.

EXPERIMENTAL

The course of the reactions and the purity of the products were monitored by TLC on Silufol UV-254 plates using benzene—alcohol (2:1) and hexane-ethylacetate—diethylamine (3:1:1). The developer was 1% vanillin in conc. H_2SO_4.

PMR spectra were recorded on a Tesla BS-567A spectrometer in C_5D_5N (0 = HMDS); mass spectra, on a MX-1310 spectrometer; IR spectra, on a UR-20 (KBr pellets) spectrometer.

Preparation of Aminoderivatives of Tanachin (3-5) and Tavulin (9-13). General Method. The starting lactones were treated with ethanol until they completely dissolved. The resulting saturated solution was treated during 30 min with the appropriate amine and stirred. The final product was obtained as crystals.

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(14)-dien-13-diethylaminogermacr-8,12-olide (3). The final product was obtained after 2 h as crystals. Yield, 85%, $C_{19}H_{31}O_4N$, R_f 0.30 (benzene—alcohol, 4:1), mp 134-135° C, M^+ 337.

IR spectrum (KBr, ν, cm^{-1}): 3368 (OH), 1762 (C=O), 1672 (R_2C=CRH), 1295, 1214.

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 6.17 (2H, 1,6-OH), 5.15, 5.47 (2H, H-14, 14′), 4.0-4.41 (3H, H-1, 6, 8), 3.05 (2H, m, N–CH_2), 2.0-2.96 (H-aliph. methylenes), 1.63 (3H, s, H-15), 0.87 (6H, s, CH_3-17, 17′).

Mass spectrum *m/z* (I_{rel}, %): 337 (M^+, $C_{19}H_{31}O_4N$, 7), 322 (M^+ - 15, 27), 308 (M^+ - 29, 10), 304 (M^+ - 15, -18, 3), 290 (M^+ - 29, -18, 12), 266 (M^+ - 71, 2), 254 (37), 170 (12), 87 (41), 86 (100), 73 (14), 72 (32), 69 (13).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(14)-dien-13-diethanolaminogermacr-8,12-olide (4). The final product was obtained after 24 h as crystals. Yield, 90%, $C_{19}H_{31}O_6N$, R_f 0.16 (benzene—alcohol, 4:1), R_f 0.36 (ethylacetate—alcohol, 1:3), mp 143-144° C, M^+ 369.

IR spectrum (KBr, ν, cm^{-1}): 3420 (OH), 1766 (C=O).

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 6.22 (4H, 1, 6, 17, 17′, OH), 5.12, 5.20 (2H, H-14, 14′), 4.1-4.40 (3H, H-1, 6, 8), 3.08 (2H, m, N–CH_2), 2.05-2.98 (H-aliph. methylenes), 1.58 (3H, s, H-15).

Mass spectrum, *m/z* (I_{rel}, %): 369 (M^+, $C_{19}H_{31}O_6N$, 7), 354 (M^+ - 15, 23), 338 (M^+ - 31, 13), 307 (M^+ - 31, -31, 24), 170 (16), 87 (47), 86 (100), 73 (11), 72 (38), 69 (17).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(14)-dien-13-dibutylaminogermacr-8,12-olide (5). The final product was obtained after 48 h as crystals. Yield, 90%, $C_{23}H_{39}O_4N$, R_f 0.28 (benzene—alcohol, 4:1), mp 106-107° C, M^+ 393.

IR spectrum (KBr, ν, cm^{-1}): 3342 (OH), 1765 (C=O), 1671 (R_2C=CRH), 1648, 1471, 1456, 1217, 1171, 1139.

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 5.15, 5.62 (2H, H-14, 14′), 3.95-4.44 (3H, H-1, 6, 8), 1.9-2.78 (H-aliph. methylenes), 1.52 (3H, s, H-15), 0.73 (6H, s, CH_3-19, 19′).

Mass spectrum, *m/z* (I_{rel}, %): 393 (M^+, $C_{23}H_{39}O_4N$, 10), 392 (8), 378 (M^+ - 15, 10), 375 (M^+ - 18, 10), 360 (M^+ - 18, -15, 7), 357 (M^+ - 36, 5), 350 (M^+ - 43, 52), 336 (M^+ - 57, 10), 307 (M^+ - 43, -43, 5), 265 (M^+ - 128, 15), 264 (M^+ - 129, 24), 256 (13), 227 (30), 202 (70), 200 (10, 55), 184 (28), 149 (75), 143 (40), 142 (45), 129 (45), 101 (25), 100 (35), 99 (45), 95 (100), 87 (75), 86 (70), 85 (55), 84 (55), 83 (60), 82 (65), 81 (55), 69 (76), 67 (75).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(9)-dien-13-dimethylaminogermacr-8,12-olide (9). The product was obtained as white needlelike crystals in 90% yield, $C_{17}H_{27}O_4N$, R_f 0.34 (benzene—alcohol, 4:1), mp 209-210° C, M^+ 309.

IR spectrum (KBr, ν, cm^{-1}): 3340 (OH), 1770 (C=O), 1190, 1220 (R_3–N), 2940 (=C=CH_2), 1680 (R_2C=CRH).

Mass spectrum, *m/z* (I_{rel}, %): 309 (M^+, $C_{17}H_{27}O_4N$, 3), 292 (1), 280 (M^+ - CHO, 1.2), 58 (100).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(9)-dien-13-diethylaminogermacr-8,12-olide (10). The final product was obtained after 24 h as crystals. Yield, 85%, $C_{19}H_{31}O_4N$, R_f 0.33 (benzene—alcohol, 4:1), mp 162-163° C, M^+ 337.

IR spectrum (KBr, cm^{-1}): 3468 (OH), 1772 (C=O), 1676 (R_2C=CRH), 1265, 1211.

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 6.17 (2H, 1,6-OH), 3.9-4.4 (3H, H-1, 6, 8), 3.05 (2H, m, N–CH_2), 2.0-2.8 (H-aliph. methylenes), 1.81 (3H, s, H-14), 1.63 (3H, s, H-15), 0.89 (6H, s, CH_3-17, 17′).

Mass spectrum, *m/z* (I_{rel}, %): 337 (M^+, $C_{19}H_{31}O_4N$, 11), 322 (M^+ - 10, 82), 308 (M^+ - 29, 12), 304 (M^+ - 15, -18, 9), 290 (M^+ - 29, -18, 10), 266 (M^+ - 71, 3), 254 (29), 170 (16), 87 (34), 86 (100), 73 (11), 72 (30), 69 (14).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(9)-dien-13-dibutylaminogermacr-8,12-olide (11). The product was obtained after 48 h as crystals. Yield, 85%, $C_{23}H_{39}O_4N$, R_f 0.24 (benzene—alcohol, 4:1), mp 159-160° C, M^+ 393.

IR spectrum (KBr, ν, cm^{-1}): 3362 (OH), 1763 (C=O), 1672, 1648.

Mass spectrum, *m/z* (I_{rel}, %): 393 (M^+, $C_{23}H_{39}O_4N$, 13), 378 (M^+ - 15, 14), 265 (M^+ - 128, 15), 264 (M^+ - 129, 28), 227 (26), 202 (63), 149 (77), 143 (32), 142 (39), 129 (38), 100 (39), 99 (34), 95 (100), 87 (66), 86 (74), 69 (78), 67 (67).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(9)-dien-13-piperidinogermacr-8,12-olide (12). This was obtained

analogously to (**11**) as while needlelike crystals. Yield, 80%, $C_{20}H_{31}O_4N$, R_f 0.45 (benzene—alcohol, 4:1), mp 205-206° C, M^+ 349.

IR spectrum (KBr, ν, cm^{-1}): 3490 (OH), 1748 (C=O), 1205, 1179.

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 6.37 (2H, 1,6-OH), 5.17 (2H, H-5, 9), 4.45-4.85 (3H, H-1, 6, 8), 3.08 (2H, m, N–CH_2), 2.25-2.96 (H-aliph. methylenes), 1.83 (3H, s, H-14), 1.65 (3H, s, H-15).

Mass spectrum, *m/z* (I_{rel}, %): 349 (M^+, $C_{20}H_{31}O_4N$, 2.4), 348 (M^+ - 1, 17), 331 (M^+ - 18, 1), 313 (M^+ - $2H_2O$, 1), 266 (M^+, $C_{15}H_{22}O_4$, 83), 265 (M^+ - 84, 1.2), 246 (M^+ - 85, -18, 3.1), 228 (M^+ - 85, -$2H_2O$, 2.3), 84 (57), 98 (100).

1,6-Dihydroxy-7α,11α(H),1β,6β,8β(H)-4(5),10(9)-dien-13-morpholinogermacr-8,12-olide (13). This was obtained analogously to (**11**) as white crystals in 70% yield. $C_{19}H_{29}O_5N$, M^+ 351, R_f 0.47 (benzene—alcohol, 4:1), mp 212-213 C.

IR spectrum (KBr, ν, cm^{-1}): 3465 (OH), 1763 (C=O), 1445, 1378, 1312.

PMR spectrum (100 MHz, Py-d_5, ppm, *J*, Hz): 6.23 (2H, 1,6-OH), 5.1-5.22 (2H, H-5, 9), 4.4-4.6 (3H, H-1, 6, 8), 3.07 (2H, m, N–CH_2–), 2.5-2.9 (H-aliph. methylenes), 1.82 (3H, s, H-14), 1.63 (3H, s, H-15).

Mass spectrum, *m/z* (I_{rel}, %): 351 (M^+, $C_{19}H_{29}O_5N$, 8), 266 (M^+, $C_{15}H_{22}O_4$, 1), 149 (14), 101 (30), 100 (100), 98 (12), 87 (12), 86 (13), 84 (9), 83 (9), 79 (9), 71 (11), 69 (12).

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