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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CERTAIN AMINO-DERIVATIVES OF 5α-STEROIDS

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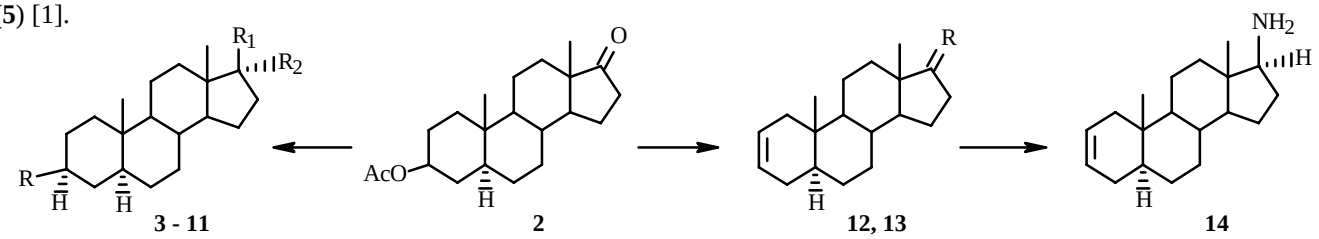
Certain 17β-aminoderivatives of 5α-steroids based on tigogenin were synthesized and their antitumor activity was studied. The structures of the synthesized compounds were confirmed by NMR and IR spectroscopy and mass spectrometry.

Key words: aminosteroids, antitumor activity.

We previously reported the synthesis and biological activity of certain derivatives of 17-amino alcohols of the 5α-androstane type and demonstrated their effect on the CNS [1] and radioprotective properties [2]. We synthesized 17β-amino-5α-androstane, a compound known to have anti-inflammatory and fungicidal activity [3]. In order to explain the antitumor activity, we synthesized certain derivatives of 17-aminosteroids.

The starting material for the synthesis of the amine derivatives of 5α-androstanes was tigogenin (**1**), which was isolated from *Yucca gloriosa* cultivated in Georgia. Tigogenin was transformed into epiandrosterone acetate (**2**) and then into the 17β-aminoderivatives (**3-9**) by the literature method [1-3]. Thus, 17β-amino-5α-androstan-3β-ol (**3**) was prepared from **2** by Leuckart—Wallach amination [4]; 17α-amino-5α-androstan-3β-ol (**4**), by a modified Streitweiser—Schaeffer method [5]. The product of the Leuckart—Wallach reaction was reduced by an excess of $LiAlH_4$ in THF to give the 17β-methylamino derivative (**5**) [1].



3 - 11 **2** **12, 13** **14**

3: R = OH, R_1 = NH_2, R_2 = H; **4:** R = OH, R_1 = H, R_2 = NH_2; **5:** R = OH, R_1 = CH_3N, R_2 = H
6: R = OH, R_1 = $NHCOOCH_3$, R_2 = H; **7:** R = $OCOOCH_3$, R_1 = $NHCOOCH_3$, R_2 = H
8: R = OH, R_1 = H, R_2 = $NHCOOCH_3$; **9:** R = $OCOOCH_3$, R_1 = H, R_2 = $NHCOOCH_3$
10: R = OH, R_1 = $CH_3NCH_2CONH_2$, R = H; **11:** R = OH, R_1 = $CH_3NCH_2CH_2NH_2$, R_2 = H
12: R = O; **13:** R = NOH

The carbamoyl derivatives **6-9** were synthesized by treatment of **3** and **4** with an excess of $Cl(CO)OCH_3$ in pyridine. The mixture formed by this of the mono- and diacylated compounds (**6**, **7**, and **8**, **9**, respectively) was separated by chromatography over a silica-gel column with elution successively by mixtures of petroleum ether and ethylacetate (20:1 and 10:1) [1]. Diaminoderivative **11** was prepared by reaction of 17β-methylamine (**5**) with chloroacetamide in the presence of Na_2CO_3 in DMF at 80-90°C with subsequent reduction of amide **10** by an excess of $LiAlH_4$ in 1,4-dioxane. We also synthesized 17β-amino-5α-androst-2-ene (**14**) according to the previously published scheme [3] from epiandrosterone acetate (**2**) by forming 5α-androst-2-en-17-one (**12**) and reduction of its oxime **13** by metallic Na in *n*-propanol.

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TABLE 1. Antitumor Activity of Certain Aminoderivatives of 5α-Steroids

Compound	NSC code*	Tumor type	Cell line	$-\text{Log}_{10}\ GI_{50}$	$-\text{Log}_{10}\ TGI_{50}$	$-\text{Log}_{10}\ LC_{50}$
7	D 737255/1	Leukemia	RPMI-8226	-5.20	-4.56	-4.00
		Breast cancer	T-47D	-5.32	-4.23	-4.00
11	D 737253/1	Leukemia	K 562	-5.58	-4.98	-4.01
			SR	-5.93	-5.23	-4.00
		Colon cancer	HT-29	-6.12	-4.58	-4.17
		CNS	SF 539	-7.05	-4.93	-4.34
		Kidney cancer	RXF 393	-6.23	-5.04	-4.38
13	D 737245/1	Melanoma	MALME 3M	-6.09	-4.68	-4.33
		Kidney cancer	RXF 393	-6.72	-4.98	-4.38
14	D 737244/1	Leukemia	HL60 (TB)	-6.17	-5.55	-4.58
			MOLT 4	-5.69	-5.34	-4.97
		Colon cancer	COLO	-5.89	-5.60	-5.30
			HCC-2998	-5.79	-5.52	-5.25
			HCT-216	-5.75	-5.50	-5.25
			HCT-15	-5.78	-5.51	-5.23
			HT-29	-5.79	-5.50	-5.21
			KM-12	-5.75	-5.49	-5.22
			SW-620	-5.79	-5.52	-5.22

*Code adopted by the National Cancer Institute, Bethesda, Maryland, USA.

The structures of the known and previously undescribed compounds were confirmed by NMR and IR spectroscopy and mass spectrometry.

Seven of the synthesized steroids **4**-**14** (**5**, **7**, **9**, **10**, **11**, **13**, and **14**) were selected for screening at the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Maryland, USA. The selection criterion was the novelty of their chemical structure. Compounds were tested for antitumor activity toward 60 lines of cancer cells. The research protocol called for a determination of the vitality of the cells in a test with sulforhodamine B with proteins after 48-hours incubation with the investigated compounds. All compounds were studied as a minimum at five different concentrations (from 1×10^{-4} to 1×10^{-8} M) with a subsequent 10-fold dilution [6, 7]. The effectiveness of the compounds was determined by measuring the parameters GI_{50} (concentration of the compound that causes lowering of total protein by 50%); TGI_{50} (concentration of the compound causing 50% suppression of cell growth), and LC_{50} (concentration of the compound causing decreased protein concentration at the end of the experiment by 50% from that at its start). Four of the seven studied compounds, 3β-methoxycarbonyloxy-17β-methhoxycarbamoyl-5α-androstane (**7**), 3β-hydroxy-17β-(*N*-methyl-*N*-aminoethylamino)-5α-androstane (**11**), 17-hydroximino-5α-androst-2-ene (**13**), and 17β-amino-5α-androst-2-ene (**14**) exhibited distinct antitumor activity toward certain lines of cancer cells (Table 1).

The properties of 17β-amino-5α-androst-2-ene (**14**) turned out to be most interesting, which was singularly effective toward all studied lines of colon cancer cells.

The biological tests provided a basis for continuing the search for effective antitumor compounds among the aminoderivatives of 5α-type steroids.

EXPERIMENTAL

Melting points were measured on a Kofler block. IR spectra were recorded on a Magna-IR Spectrometer 550 instrument in KBr disks. Mass spectra were recorded on a MAT-112 GC-MS (ionizing electrons 70 eV, ionization-chamber temperature 1800°C, direct sample introduction into the source). NMR spectra were obtained on a Bruker AC 250 instrument (250 MHz working frequency for ^{1}H). The chemical shifts of protons are given on the δ scale, ppm, with tetramethylsilane (TMS) as the internal standard. NMR spectra were recorded in $CDCl_3$. Elemental analyses agreed with the empirical formulas.

The course of reactions and purity of products were monitored by TLC on Silufol UV 254 plates (Kavalier, Czech Rep.) using $CHCl_3$:CH_3OH (6:1). Spots were developed by spraying with phosphomolybdic acid (10%) in ethanol with subsequent heating.

3β-Hydroxy-17β-[*N*-methyl-*N*-(carbamoylmethyl)-amino]-5α-androstane (10). A mixture of **5** (1.5 g, 4.9 mmol) and Na_2CO_3 (1.5 g, 17.85 mmol) in DMF (50 mL) at 40°C was treated over 20 min with a solution of chloroacetamide (0.93 g, 100 mmol) in DMF (20 mL), stirred at 80-90°C for 10 h, cooled to 20°C, poured into water (100 mL), filtered, and washed with water. Recrystallization from ethanol afforded **10** (1.54 g, 80%), mp 242-244°C.

IR spectrum (ν, cm^{-1}): 3450-3150 (NH_2, OH), 1680, 1620 ($CONH_2$). Mass spectrum (*m*/*z*, I_{rel}, %): 362 (35) $[M]^+$, 347 (50), 304 (25). PMR spectrum (δ, ppm): 0.78 (3H, s, CH_3-18), 0.80 (3H, s, CH_3-19), 2.27 (3H, s, CH_3-21), 3.03 (2H, s, CH_2), 3.59 (1H, m, C-3), 5.36 (1H, s, NH), 7.3 (1H, s, NH).

3β-Hydroxy-17β-[*N*-methyl-*N*-(aminoethyl)amino]-5α-androstane (11). A suspension of $LiAlH_4$ (1.1 g, 32.35 mmol) in 1,4-dioxane (50 mL) was treated over 30 min with a solution of **10** (1.1 g, 3.03 mmol) in 1,4-dioxane (10 mL) and boiled for 24 h. The excess of reductant was destroyed by adding H_2O (1.1 mL), aqueous NaOH (1.1 mL, 15%), and H_2O (3.3 mL). The reaction mixture was filtered. The precipitate was washed with 1,4-dioxane (2 × 30 mL) and diethylether (2 × 30 mL). The filtrate was evaporated in vacuo to afford product (1.1 g), crystallization of which from ethanol:hexane (1:4) isolated **11** (0.9 g, 86%), mp 119-121°C. The IR and PMR spectrum in addition to the mass spectrum of **11** agreed with those obtained by us earlier during the synthesis of **11** by the literature method [1].

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