SYNTHESIS AND PHARMACOLOGY OF 17-AMINO-5-α-ANDROSTANE-3-OL DERIVATIVES

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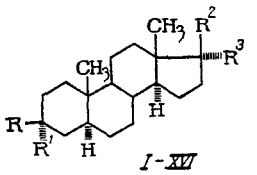
Steroid hormones acting upon the CNS are known to cause various neuroendocrine and behavioral effects, including sedation and anesthesia [9, 13, 14]. Thus, 3α,5β-tetrahydrodesoxycorticosterone (3,5-TNDOS) has exhibited anxiolytic, sedative, and anti-aggressive effects on experimental animals [6, 10]. There have been recent reports about amino steroids whose principal action affects excitation transmission in the spinal cord [8]. In particular, the compound RU 5135-3α-hydroxy-16-imino-5β-17-azaandrostane-11-one is closely related to GABA and the bicuculline bonding sectors [7, 12]. Studies have shown that some of the aminoandrostane series derivatives did not react with the GABA-BD receptor complex but did exhibit anxiolytic properties in animal experiments [2]. We therefore felt it would be of interest to study the effect that certain amino alcohol derivatives of the 5α-androstane series had on the CNS and behavioral responses in animals. We synthesized C(3) and C(17) isomer derivatives (VI-IV) of 17-amino-5α-androstane-3-ols (I-IV) obtained from the domestic plant steroid thigogenin which was isolated from the Georgian-cultivated "yucca gloriosa" (*yucca gloriosa*, fam. *Liliaceae*), and we studied its effect on the CNS.



I-XVI

R = OH (I, II, V, VI, IX—XI, XIII), H (III, IV, XV, XVI), $OCOCH_2Cl$ (VII), $OCOCH_2N^+(MeCl^-$ (VIII), OCOOMe (XII, XIV), R^1 = H (I, II, V—XIV), OH (III, IV, XV, XVI); R^2=NH_2 (I, III), H (II, IV, XIII, XIV, XVI), NHMe (V); $MeNCH_2CN$ (VI—VIII), NHCOOMe (X, XI, XII, XV), $MeNCH_2CH_2NH_2$ (IX), $MeNCH_2CH_2NHCH_2CN$ (X); R^3 = H (I, III, V—XII, XV), NH_2 (II, IV), NHCOOMe (XIII, XIV, XVI).

The amino alcohols I-IV were obtained by a previously described method [3], i.e., compound I was synthesized by the Leuckart-Wallach amination reaction, compound II was obtained by a modified Streitwieser—Schaeffer method [16], and compounds III and IV were synthesized from the formamide derivatives of the amino alcohols I and II by epimerization at C(3) employing the Mitsunobu method [11]. In addition, we synthesized 17β-methylamine (V) by reducing the Leuckart—Wallach reaction product with an excess of $LiAlH_4$ in THF [4].

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TABLE 1. Physicochemical Properties of Compounds VI-XVI

Compound	Yield, %	mp, °C (crystallization solvent)	Empirical formula	M^+ found	M^+ calculated	IR spectrum, cm^{-1}
VI	74	172—4 (diethyl ether)	$C_{22}H_{36}N_2O$	344	344,54	2250 weak 3470 singlet 3500 singlet
VII	92	185—7 (acetone)	$C_{24}H_{37}Cl_2N_2O_2$	421	421,07	1765 singlet 2250 weak
VIII	85	220—5 (decomposition)	$C_{27}H_{46}N_3O_2Cl$			1740 singlet 3450 wide
IX	81	119—21 (ethanol–hexane)	$C_{22}H_{40}N_2O$	348	348,57	1610 weak 3150 wide
X	90	125—7 (ethanol)	$C_{24}H_{41}N_3O$	387	387,61	2250 weak 3300 wide
XI	42	189—91	$C_{21}H_{35}NO_3$	349	349,51	1560 singlet 1710 singlet
XII	30	151—3	$C_{23}H_{37}NO_5$	407	407,55	1560 singlet 1710 singlet 1760 singlet 3330 singlet
XIII	39	183—5	$C_{21}H_{35}NO_3$	349	349,51	1560 singlet 1710 singlet 3320 wide
XIV	33	118—20	$C_{23}H_{37}NO_5$	407	407,55	1560 singlet 1720 singlet 1760 singlet 3330 singlet
XV	84	169—71 (diethyl ether–hexane)	$C_{21}H_{35}NO_3$	349	349,51	1560 singlet 1710 singlet 3300 wide
XVI	73	156—8 (benzene–hexane)	$C_{21}H_{35}NO_3$	349	349,51	1560 singlet 1710 singlet 3300 wide
VIa	90	230—35	$C_{22}H_{37}ClN_2O$	...	...	...
X	85	160—65 (decomposition)	$C_{22}H_{42}Cl_2N_2O$	...	...	...

Methyl chloroformate is known to acylate selectively equatorial steroid secondary alcohols in which case the axial alcohol groups are not affected [5]. By treating compounds III and IV with an excess of methyl chlorofomate in pyridine we obtained the monoacylated products XV and XVI respectively. From I and II we obtained a mixture of mono- and diacylated compounds (XI and XII; XIII and XIV respectively) which were separated by column chromatography. By reacting 17β-methyl amine V with chloroacetonitrile we synthesized VI whose reduction by $LiAlH_4$ and subsequent treatment with chloroacetonitrile yielded compound XI. Biological studies were made of compounds VI, VIII, X-XVI. The amino steroids VI and X were converted to hydrochlorides by dissolving them in anhydrous ethanol and treatment with an HCl solution in anhydrous ethanol up to pH 2.0-3.0.* Because compound VIa is insoluble in water we synthesized its soluble analog VIII by acylating VI with chloroacetyl chloride and by reacting VII with Me_3N.

The structure of compounds VI-XVI agreed well with element analysis, IR- and mass spectrum data (see Table 1).

EXPERIMENTAL (CHEMICAL)

IR-spectra were recorded on a UR-20 (GDR) instrument in petroleum jelly. NMR^{-1} spectra were recorded on a Bruker WP-200 (200 MHz) instrument in $CDCl_3$. Mass spectra were obtained on a MAT-112 chromatomass spectrometer.

*Hydrochlorides are designated by the same figures as the corresponding bases with index "a."

The reaction and product purity were controlled by TLC on UV-254 Silufol plates (Czechoslovakia) in 2:1, 3:1, 15:1, and 20:1 benzene—acetone systems and in 4:1:1 1-butanol—AcOH—water. Silica gel 100/250 μ (Czechoslovakia) was used for column chromatography. Spots were detected by spraying a 10% solution of phosphomolybdic acid in ethanol and subsequent heating for 3 min at 100-120°C.

3β-Methoxycarbonyloxy-17β-methoxy-carbamoyl-5α-androstane (XII), 3β-hydroxy-17β-methoxycarbamoyl-5α-androstane (XI). A 10 ml (130.14 mmole) portion of ClCOOMe was added to a solution of 3.65 g (12.52 mmole) of amine I in 50 ml of freshly distilled pyridine at 20°C for 30 min. The solution was then stirred for 1 h and decanted into 500 ml of ice water and acidified with 35% HCl to pH 7.0. The product was extracted with diethyl ether (4 × 50 ml). The combined ether extracts were washed with water until neutral, dried with Na_2SO_4 after which the solvent was vacuum-distilled to yield 3.6 g of the reaction product mix. This mixture was then separated on a silica gel chromatographic column with successive elutions with benzene and a 20:1 and 10:1 mixture of benzene—acetone. A yield of 1.5 g of XII was obtained from corresponding portions of the eluate by vacuum-evaporation of the solvent. PMR-spectrum, δ, ppm: 0.65 s (3H, 18-CH_3), 0.8 s (3H, 19-CH_3), 3.65 s (3H, NH—CO—OCH_3), 3.76 s (3H, H_3COCOO), 4.49 broad d. (J ≈ 8 Hz, 1H, NHCO) and 1.8 g XI, PMR-spectrum, δ, ppm: 0.65 s (3H, 18-CH_3), 0.80 s (3H, 19-CH_3), 3.59 m (1H, C^3-αH), 3.65 d (3H, $NHCOOCH_3$), 4.52 broad d. (J ≈ 8 Hz, 1H, NHCO).

3β-Methoxycarbonyloxy-17α-methoxycarbamoyl-5α-androstane (XIV), 3β-hydroxy-17α-methoxycarbamoyl-5α-androstane (XIII). In a similar manner as in the synthesis of compounds XI and XII, compound XIV was obtained from 1.3 g (4.45 mmole) of II and 4.2 ml ClCOOCMe in 35 ml of pyridine to yield 0.6 g of XIV. PMR spectrum, δ, ppm: 0.77 s (3H, 18-CH_3), 0.81 s (3H, 19-CH_3), 3.70 s (3H, $NHCOOCH_3$), 3.76 s (3H, H_3COCOO), 4.56 broad. d. (J ≈ 8 Hz, 1H, NHCO), and 0.6 g of XIII, PMR spectrum, δ, ppm: 0.77 s (3H, 18-CH_3), 0.80 s (3H, 19-CH_3), 3.59 m (1H, C^3-αH), 3.64 s (3H, $NHCOOCH_3$), 4.54 broad. d. (J ≈ 8 Hz, 1H, NHCO).

3α-Hydroxy-17β-methoxycarbamoyl-5α-androstane (XV). A 3 ml (31.5 mmole) portion of ClCOOMe was added to a solution of 0.6 g (2.05 mmole) of amine III in 25 ml of freshly distilled pyridine at 20°C over a period of 30 min. The reaction mixture was then stirred for 1 h and decanted into 100 ml of ice water. The yield was 0.7 g of technical grade product which was crystallized from a 1:3 diethyl ether—hexane mixture. The yield of XV was 0.6 g. PMR-spectrum, δ, ppm: 0.65 s (3H, 18-CH_3), 0.77 s (3H, 19-CH_3), 3.65 s (3H, $NHCOOCH_3$), 4.04 m (1H, C^3-βH), 4.50 broad. d. (J ≈ 8 Hz, 1H).

3α-Hydroxy-17α-methoxycarbamoyl-5α-androstane (XVI). In a manner similar to the synthesis of XV, a technical grade product was obtained from 1.5 g (5.14 mmole) of IV and 4.8 ml (50.44 mmole) of ClCOOMe in 40 ml of pyridine. This crude product was crystallized from 1:3 benzene—hexane mixture to yield 1.3 g of XVI. PMR spectrum, δ, ppm: 0.78 s (6H, 18-CH_3 and 19-CH_3, 3.65 s (3H, $NHCOOCH_3$), 4.04 m (1H, C^3-βH), 4.53 broad. d. (1H, NHCO).

3β-Hydroxy-17β-(N-methyl-N-cyanomethylamino)-5α-androstane (VI). A 1.03 ml (16.36 mmole) portion of chloroacetonitrile was added to a mixture of 1 g (3.27 mmole) of amine V and 1 g (11.9 mmole) of $NaHCO_3$ in 50 ml of DMFA over a period of 30 min. The mixture was then stirred at 60-65°C for 3 h, cooled to 20°C, and decanted into 100 ml of water. After standing for 12 h the resultant precipitate was filtered off, washed with water until neutral. The resultant 0.9 g of the reaction product was then crystallized from diethyl ether to yield 0.83 g of nitrile VI. PMR-spectrum, δ, ppm: 0.77 s (3H, 18-CH_3), 0.82 s (3H, 19-CH_3), 2.32 s (3H, NCH_3), 3.59 m (1H, C^3-αH), 3.59 s (2H, NCH_2CN).

3β-Chloroacetoxy-17β-(N-methyl-N-cyanomethylamino)-5α-androstane (VII). A 3.5 ml (44.01 mmole) portion of $ClCH_2COCl$ was added to a mixture of 1.5 g (3.56 mmole) of VI and 6 ml (44.11 mmole) of Et_3N in 50 ml of DMFA at 20°C over a 30 min period. After 24 h the reaction mixture was decanted into 250 ml of ice water, filtered, washed with water until neutral, and crystallized from acetone to yield 1.68 g of VII. PMR spectrum, δ, ppm: 0.75 s (3H, 18-CH_3), 0.82 s (3H, 19-CH_3), 2.36 s (3H, NCH_3), 3.57 s (2H, NCH_2CH), 4.02 s (2H, $COCH_2Cl$), 4.70 m (1H, C^3-αH).

3β-Trimethylaminoacetyloxy-17β-(N-methyl-N-cyanomethylamino)-5α-androstane HCl (VIII). Me_3N gas was passed through a solution of 1.45 g (3.44 mmole) of VII in 70 ml of DMFA for 1.5 h. The resultant precipitate was filtered and washed with diethylether to yield 1.4 g of VIII. PMR-spectrum, CD_3CN, δ, ppm: 0.74 s (3H, 18-CH_3), 0.84 s (3H, 19-CH_3), 2.28 s (3H, NCH_3), 3.25 s (9H, $N^+(CH_3)_3$), 3.59 a (2H, CH_2CN), 4.20 s (2H, $COCH_2$), 4.82 m (1H, C^3-αH).

3β-Hydroxy-17β-(N-methyl-N-aminoethylamino)-5α-androstane (IX). A solution of 1.1 g (3.19 mmole) of VI in 39 ml of THF was added to a suspension of 1.1 g (32.35 mmole) of $LiAlH_4$ in 25 ml of THF over a period of 30 min after which the mixture was boiled for 1.5 h. The excess reducing agent was decomposed by adding 1.1 ml of water, 1.1 ml of 15% NaOH, and 3.3 ml of water. The reaction mass was then filtered and the precipitate was washed with THF (2 × 15 ml) and diethyl ether (4 × 15 ml). The filtrate was vacuum-evaporated to yield 1.1 g of reaction product. Crystallization from a 1:4 ethanol-hexane mixture yielded 0.9 g of IX. PMR-spectrum, δ, ppm: 0.78 s (3H, 18-CH_3), 0.80 s (3H, 19-CH_3), 2.18 s (3H, NCH_3), 2.55 m (2H, NCH_2CH_2), 2.75 m (2H, CH_2CH_2NH), 3.58 m (1H, C^3-αH).

3β-Hydroxy-17β-(N-methyl-N-(2-cyanomethylaminoethyl)amino)-5α-androstane (X). In the same manner as in the synthesis of VI, 1.7 g of technical grade product was obtained from 1.6 g (4.59 mmole) of diamine IX, 1.4 ml (22.2 mmole) of $ClCH_2CN$, and 1.6 g (19.04 mmole) of $NaHCO_3$ in 30 ml of DMFA. Crystallization from ethanol yielded 1.6 g of the X nitrile. PMR-spectrum, δ, ppm: 0.75 s (3H, 18-CH_3), 0.8 s (3H, 19-CH_3), 2.19 s (3H, NCH_3), 2.55 m (2H, NCH_2CH_2), 2.75 m (2H, CH_2CH_2NH), 3.58 m (1H, C^3-αH), 3.63 s (2H, $NHCH_2CN$).

EXPERIMENTAL (PHARMACOLOGICAL)

Tests were made of groups of 10 to 12 male mice weighing 18-20 g. Overall action was judged by the state and behavior of the animals and the compounds' effect on spontaneous motor activity as recorded by an Opto-Varimex (USA) instrument. Readings were taken every 10 min over a 2 h period [17].

Central depressive activity was evaluated by the compounds' influence on the hypnotic effect of sodium thiopental (30 mg/kg) administered iv [19]. Anxiolytic activity was evaluated by the number of penalty crossovers in the "four plates" test [15]. Anticonvulsant effects were evaluated by the compounds' influence on tremors induced by maximum electric shock (50 mA, 100 pulses/s, 0.2 s) and corazole (145 mg/kg subcutaneously). The percent of perished animals with tremors was also calculated [18].

The mice experiments also included studies of the compounds' acute toxicity when administered orally. LD_{50} was calculated by the Kerber method [1]. The compound was administered orally to the animals in the form of a suspension in a 1% CMC solution to which 0.2-0.3 ml of Tween-80 was added. The test doses were 1/10 of the LD_{50}.

Our test results showed that of all the tested compounds only the hydrochloride VIa and the dihydrochloride Xa at doses of 200 and 100 mg/kg orally caused any changes in the condition and behavior of the animals. Thus, compound VIa, starting at a dose 100 mg/kg tranquilized the mice. This effect was most pronounced at a dose of 200 mg/kg. This compound at those doses also reduced orientation-search activity, decreased the number of interactions between the animals in the group, induced disassociation, and a disturbance in the rhythmic cycles of activity and rest. Spontaneous motor activity was 50% (470 ± 35 cond. units of the counter) in comparison to the control level (920 ± 47 cond. units of the counter in a 10 min period) 20 min after oral administration. The effect lasted for more than 2 h.

In contrast to VIa, compound Xa at a dose of 100 mg/kg orally induced corazole type tremors in the mice. The convulsions were noted 2 to 3 min after administration in the form of slight clonic manifestations which over a period of 10 to 15 min developed into a generalized clonic-tonic seizure which culminated in 100% death of the animals.

When the remaining compounds were administered orally at a dose of 1/10 of the LD_{50} there was practically no change in the general condition or behavior of the animals. Further increases in the doses of the test compounds (up to 200 and 300 mg/kg) did not result in any significant changes in the animals' general condition or behavior.

Our study of the compounds' overall action showed that they (with the exception of VIa) did not exhibit sedative or hypnotic properties.

Our study of the compounds' effect on the hypnotic effect of sodium thiopental showed that only compound VIa was capable of potentiating that hypnotic effect. Thus, at a dose of 100 mg/kg compound VIa extended the duration of lateral positioning in the mice up to 15 ± 2.4 min (3.6 ± 0.3 min in the control).

Compounds VIa also exhibited moderate anxiolytic activity in the "four plates" test. Within the dose range of 100-200 mg/kg 1 h after the administration, compound VIa increased the number of penalty crossovers of the animals from one plate to the next in a dose-dependent manner within a period of 1 min from 3.7 ± 0.55 to 8.1 m ± 0.66.

Compound VIa also exhibited a slight effect on tremors induced by corazole and maximum electric shock (MES). Thus, in the corazole experiments compounds VIa at a dose of 200 mg/kg reduced by 20% the number of animals with convulsions and did not effect their death. In the experiments on tremors induced by MES, compound

VIa at the indicated dose reduced the number of animals with tonic-extensor responses by 15% and reduced the number of deaths by 30%.

The acute toxicity of compound VIa for mice was 750 mg/kg, and 80 and 500 mg/kg for compounds Xa and VIII respectively. The acute toxicity for the remaining compounds was over 1,000 mg/kg upon oral administration.

Thus, our pharmacological study of the 9 derivatives of 5α-androstane demonstrated that most of the compounds do not affect the CNS of animals with respect to central depressive anxiolytic, and antitremor activity.

It seems apparent that structural modifications of the aminoandrostanes by addition in position 3 of the trimethylaminoacyl group (compound VIII — the soluble analog of compound VI) or by acylation in position 3 (compounds XII an XIV) did not amplify effects on the CNS. At the same time, the addition of an aminomethylamino-cyanomethylamino chain to the N-methyl group in position 17 resulted in the synthesis of a compound with a high level of toxicity had pronounced anticonvulsive activity.

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