LITERATURE CITED

1. V. A. Azimov, V. G. Granik, S. I. Grizik, et al., Khim.-farm. Zh., No. 8, 947-952 (1985).
2. Z. A. Arutyunyan, V. I. Gunar, E. P. Gracheva, and S. A. Zav'yalov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 655 (1969).
3. N. N. Bychikhina, V. A. Azimov, V. G. Granik, et al., Khim.-farm. Zh., No. 11, 1343 (1982).
4. L. V. Ershov and V. G. Granik, Khim. Heterotsikl. Soedin., No. 5, 646-649 (1985).
5. T. V. Sycheva and L. N. Yakhontov, Ibid., No. 1, 84.
6. M. Ya. Uritskaya, V. A. Loginova, and L. N. Yakhontov, Ibid., No. 10, 1311 (1973).
7. D. Atlas, M. L. Steer, and A. Levitzki, in: Strategy in Drug Research, Noordwükerhout (1981), p. 10.
8. J. J. Raldwin, E. L. Engelhardt, R. Hirchman, et al., J. Med. Chem., 23, 65 (1980).
9. A. T. S. Duggan, E. J. J. Grabowski, and W. K. Russ, Synthesis, No. 7, 573 (1980).
10. L. N. Jakhontov (Yakhontov), D. M. Krasnokutskaya, E. M. Peresleni, et al., Tetrahedron, 22, 3233 (1966).
11. W. E. Kreigbaum, W. L. Matier, R. D. Dennis, et al., J. Med. Chem., 23, 285 (1980).
12. Y. Murakami, T. Watanabe, A. Kobayashi, and Y. Yokoyama, Synthesis, No. 9, 738 (1984).

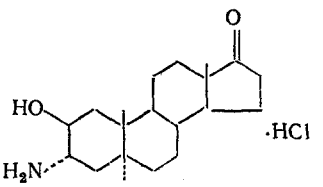
SYNTHESIS AND ANTIARRHYTHMIC ACTIVITY OF 3 α-AMINO-5-α-ANDROSTAN-2 β-OL-17-ONE

S. D. Shuvalova, N. I. Men'shova,
G. S. Grinenko, K. A. Zaitseva,
and M. D. Mashkovskii

UDC 615.22.012.1.07

There have been a few reports in the literature of the occurrence of antiarrhythmic activity in compounds of steroidal structure. For example, it has been reported [3] that the steroidal sex hormones testosterone, progesterone, and estradiol are able to decrease the maximum reproducible frequency of contraction of isolated rabbit auricle, and they also terminate fibrillation in cat auricle induced by electrical stimulation. The highest activity is shown by testosterone, with progesterone and especially estradiol being less active. Antiarrhythmic properties are also possessed by the mineralocortocoid desoxycorticosterone and the synthetic steroid spironolactone, in which quinidine-like activity has been observed experimentally [1], whereas the spironolactone antagonist aldosterone and the glucocorticoid prednisolone are devoid of these properties [5].

Reports have recently appeared in the foreign literature of the experimental study of antiarrhythmic activity in 3α-amino-5α-androstan-2 β-ol-17-one hydrochloride (VI, ORG 6001) [8, 10, 12].

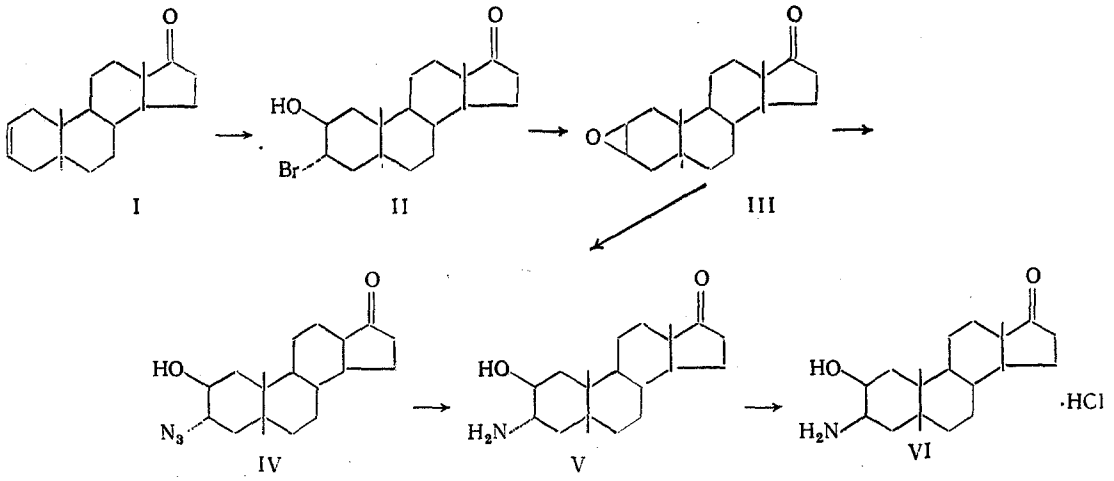


According to these workers, the compound ORG 6001 shows antiarrhythmic properties in several types of experimental arrhythmia. Chemically speaking, this compound may be regarded as an analog of testosterone.

We repeated the synthesis of VI from 5α-androst-2-en-17-one (I) as described in [2, 4]:

S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific-Research Institute, Moscow.
Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 20, No. 3, pp. 286-289, March, 1986. Original article submitted January 28, 1985.

 0091-150X/86/2003-0170$12.50 © 1987 Plenum Publishing Corporation



Treatment of I with dibromodimethylhydantoin in the presence of perchloric acid in ether gives the bromohydrin II. Dehydrobromination of this with caustic alkali gives the 2 β,3-oxide III. Two routes were used to obtain 3α-amino-5α-androstan-2 β-ol-17-one (V) from III: introduction of the amino-group by successive conversion of III into the 3α-azide (IV) followed by catalytic hydrogenation of IV over palladium/charcoal, or cleavage of the oxide III by heating with a saturated solution of ammonia in methanol at 150°C in an autoclave. For the biological studies, V was converted into its hydrochloride.

The antiarrhythmic activity of VI was examined in comparison with that of the local anesthetic lignocaine. The types of antiarrhythmic activity of the two compounds were found to be similar. An examination of the electrophysiological properties of ORG 6001, reported in [9], also confirmed that the mechanism of the antiarrhythmic activity of this compound is due to its membrane-stabilizing properties, such as are typical of local anesthetics.

EXPERIMENTAL (CHEMICAL)

PMR spectra were obtained on an XI-100 spectrometer (USA, operating frequency 100 MHz) in $CDCl_3$ solution, internal standard tetramethylsilane. IR spectra were obtained on a UR-10 spectrometer (East Germany), and $[\alpha]_D^{20}$ values were measured on an A-1-EPL polarimeter.

3α-Bromo-5α-androstan-2β-ol-one (II). A solution of 10 g (0.0367 mole) of I in 60 ml of ether was added to 20 ml of 12% perchloric acid. There was then added with stirring 5.8 g (0.02 mole) of 1,3-dibromo-5,5-dimethylhydantoin at 20°C, stirring continued for 3 h, the solid which separated filtered off, washed with ether, and then with water until neutral to give 8.68 g (64.05%) of II, mp 148-151°C [2].

2β,3-Oxido-5α-androstan-17-one (III). To a suspension of 8 g (0.0216 mole) of II in 40 ml of methanol was added a solution of 2.7 g (0.0482 mole) of potassium hydroxide in 5 ml of methanol. The mixture was stirred for 3 h at 20°C, then 90 ml of water was added to the mixture, and the solid which separated was filtered off and washed with water until neutral, to give 5.4 g (86.44%) of III, mp 117-120°C, $[\alpha]_D^{20}$ +138° (1% in methanol) [2].

3α-Azido-5α-androstan-2β-ol-17-one (IV). To a solution of 5.2 g (0.018 mole) of III in 34 ml of dimethylacetamide was added 1.4 g (0.02 mole) of sodium azide in 4.2 ml of water, and the mixture boiled for 4.5 h, cooled to 20°C, and poured into 150 ml of water. The solid separated was filtered off, and washed with water. Recrystallization of the crude product from a fivefold amount of a 1:1 mixture of methylene chloride and ether gave 4.88 g (81.74%) of IV, mp 150-152°C, $[\alpha]_D^{20}$ +140° (1% in chloroform). IR spectrum, ν, cm^{-1}: 1720 (C=O), 2080 (—N_3), 3420 (—OH) [2].

3α-Amino-5α-androstan-2β-ol-17-one (V). A. Compound IV (3.68 g, 0.01 mole) was hydrogenated in 75 ml of methanol over 0.36 g of 5% palladium on charcoal until one mole of hydrogen had been taken up. The catalyst was then filtered off an washed with methanol, the combined

TABLE 1. Acute Toxicity of 3α-Amino-5α-androstan-2β-ol-17-one Hydrochloride

Animal species	LD_{50}, mg/kg (intravenous)	Source
Mice	108 (96,3—119,7)	Our data
Rats	117,5 (102,6—132,4)	" "
Rats	147 (118—184)	[10]

Note. Error limits shown in brackets.

filtrates evaporated under reduced pressure, and the residue crystallized from ether to give 2.99 g (88.2%) of V. This material was purified by stirring a suspension of 2.99 g of V in 90 ml of water containing 4.5 ml of conc. hydrochloric acid for 1 h at 20°C. The insoluble steroid starting material was filtered off to give 0.56 g of IV. To the filtrate was added 20 ml of 10 N potassium hydroxide, and the mixture stirred for 10 min. The solid which separated was filtered off, and washed with water until neutral to give 2.24 g (66.07%) of V, mp 190-192°C, $[\alpha]_D^{20}$ +100° (1% in chloroform). IR spectrum, ν, cm^{-1}: 1590 ($-NH_2$), 1740 (C=O) [4]. PMR spectrum, δ, ppm: 0.72 (singlet, $18CH_3$), 1.22 (singlet, $19CH_3$), 4.00 (multiplet, 3CH), 4.9 (multiplet, 2CH).

B. An autoclave was charged with 6 g (0.02 mole) of III in 40 ml of methanol saturated with ammonia, and the mixture was heated for 5 h at 150°C, then cooled, transferred to a flask, and the solvent distilled off under reduced pressure. The residue was treated with 6 ml of acetic acid and 6 ml of water, and the mixture heated for 1 h at 90°C. The insoluble residue was filtered off, and the filtrate treated with 10 N potassium hydroxide until alkaline. The solid which separated was filtered off and washed with water until neutral to give 2.79 g (43.91%) of V.

3-α-Amino-5α-androstan-2β-ol-17-one Hydrochloride (VI). Compound V (2.18 g, 0.007 mole) was dissolved in 100 ml of dry acetone at the boil, then cooled to 20°C and the solution treated with 6 ml of ethanol saturated with hydrogen chloride until the pH reached 2.0. The solid which separated was filtered off and washed with cold acetone. The crude product was recrystallized from absolute ethanol to give IV in almost quantitative yield, mp 280°C, $[\alpha]_D^{20}$ +104° [4].

EXPERIMENTAL (PHARMACOLOGICAL)

The antiarrhythmic activity, local anesthetic properties, and acute toxicity of VI were examined. Antiarrhythmic activity was studied in model arrhythmias induced by aconitine in rats, calcium chloride in rats, and strophanthine G in guinea pigs. The aconitine model of arrhythmia in rats was used to examine the restorative effects of VI.

The tests were carried out using male rats weighing 120-170 g under urethane narcosis (10% solution, 1 ml per 100 g body weight intraperitoneally). Aconitine was administered intravenously in a dose of 30 μg/kg [6]. Changes in cardiac rhythm were recorded by the ECG in standard II leads. Aqueous solutions of VI were administered into the vein following onset of arrythmia, in doses of 0.1, 0.5, 1. 2.5, and 5 mg/kg (34 rats). It was found that VI has an antiarrhythmic effect, but in all cases the effect was short-lived (in most of the tests, the duration of the observed effect was 15-30 seconds, and in one only it lasted for 10 min). The ED_{50} was 1.9 mg/kg, in agreement with the literature value [10] for the compound ORG 6001.

The prophylactic antiarrhythmic activity of VI was examined in the calcium chloride model arrhythmia in rats [7]. Tests were carried out on 27 male rats weighing 160-180 g, under urethane narcosis. Arrhythmia was induced by the intravenous administration of calcium chloride in a dose of 350 mg/kg (duration of administration, 30 sec). The ECG was recorded in II leads. Administration of calcium chloride to 15 control animals caused the deaths of eight of the animals in the first 1-2 min as a result of ventricular fibrillation. Marked bradycardia was seen in the survivors.

When VI was administered in doses of 2.5 and 5 mg/kg 5 minutes before administration of calcium chloride, no fibrillation was observed, and the percentage survival of the animals increased, only three rats out of 12 dying, the rest surviving.

The effects of VI on arrhythmia in guinea pigs induced by strophanthine G were examined as described in [11]. Female guinea pigs (15 animals) under urethane narcosis were treated intravenously with strophanthine G in isotonic NaCl solution. The amount of strophanthine required to induce extrasystole, ventricular fibrillation, and the deaths of the animals in the controls and following intravenous administration of VI five minutes before treatment with strophanthine was determined. In doses of 1 to 10 mg/kg, VI had no effect on arrhythmia induced by strophanthine.

Local anesthetic activity was assessed in rabbits by Reginer's method. In its ability to induce local anesthesia, VI was approximately four times as active as novocaine, but only one fifth as active as dicaine. The anesthetic index for the 0.5% solution was 600. The acute toxicity of (VI) was determined in white mice by the intravenous route (Table 1).

This study of the pharmacological properties of 3α-amino-5α-androstan-2β-ol-17-one hydrochloride has shown that in its type of antiarrhythmic effect and its toxicity (LD_{50}), it is similar to the drug ORG 6001. An examination of our own results and those reported in the literature leads to the conclusion that, despite the occurrence of antiarrhythmic activity, this drug has no advantages over the antiarrhythmic drugs in clinical use. Nevertheless, the presence of antiarrhythmic properties in (VI) provides the basis for a study of its analogs in order to obtain more active antiarrhythmic drugs.

LITERATURE CITED

1. A. H. Briggs and W. C. Holland, Am. J. Physiol., 197, 1161-1164 (1959).
2. M. M. Campbell, R. C. Craig, A. C. Boyd, et al., J. Chem. Soc., Perkin I, 2235-2247 (1979).
3. A. L. Gimeno, W. C. Vang, and M. F. Gimeno, in: Experimental Cardiac Arrhythmias and Antiarrhythmic Drugs, Budapest (1971), p. 352
4. C. L. Hewett, and D. C. Savage, West German Pat. No. 2 335 827; Chem. Abstr., 80, 108754q (1974).
5. T. F. Huang, Arch. Int. Pharmacodyn., 141, 239-253 (1963).
6. E. Hueber and D. Lehr, Arch. Exp. Path. Pharm., 189, 25-44 (1938).
7. M. R. Malinow, F. F. Batlle, and B. Malamud, Am. J. Physiol., 175, 8 (1953).
8. R. J. Marschall and J. R. Parratt, Brit. J. Pharmacol., 55, 359-368 (1975).
9. L. A. Salako, E. M. Vaughan Williams, and J. H. Wittig, ibid., 57, 251-262 (1976).
10. B. B. Vargaftig, M. F. Sugrue, W. R. Buckett, and H. -J. Van Riezen, Pharm. Pharmacol., 27, 697-703 (1975).
11. E. M. Vaughan Williams and A. Sekiya, Lancet, 1, 420-421 (1963).
12. P. D. Verdouw, H. C. Schamhardt, W. J. Remme, and J. W. De Jong, J. Pharmacol. Exp. Ther., 204, 634-644 (1978).