INFLUENCE OF TRANSFORMED STEROIDS ON Na^+,K^+-DEPENDENT ATPase. II

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The relationship of the structures of a number of transformed steroids containing an open or closed 20-oxo-21a,17-tetrahydrofuran ring E and 23-oic acid derivatives with their Na^+,K^+-ATPase inhibiting activity has been studied. It has been found that steroids with branched side chains are more active than steroids with additional tetrahydropyran-4- and -2-one rings E. The most active in this series of 20-ketosteroids are those containing such polar groupings as diketone and epoxyethyl groupings. Steroids with a spirotetrahydrofuran ring at C(17) exhibit an activity greater than their pyranoside analogs.

The capacity of steroid hormones for regulating the work of Na pump of the cell by acting on Na^+,K^+-dependent ATPase is usually connected with the presence of a glycosidic chain attached to the steroid nucleus [1], the necessity for the cis linkage of rings C and D of the aglycone [2], and the presence of a $\Delta^{20(22)}$ double bond in it [3]. The role of the glycosidic chain here may consist in ensuring the necessary reaction between the steroid substrate and the receptor [4]. So far as concerns the link between the structure of the steroid itself and its capacity for affecting ATPase, the available information is not systematic and does not make it possible to draw strict conclusions.

We have investigated the influence on Na^+,K^+-ATPase of a series of transformed steroids containing modified, mainly polyhydroxylated, side chains [5], the hypothesis having been put forward that the ATPase-inhibiting effect is directly proportional to the accumulation of polar groups in one of the sections of the skeleton.

Continuing the investigation of the influence of such a systematic set of compounds, we made use of an enzymatic method of testing described previously [5] which is based on the inhibiting effect of the compounds under study of the Na^+,K^+-dependent ATPase from a highly active purified fraction of the medullary layer of porcine kidneys.

The compounds tested were combined according to their structures into two series — derivatives of 20 ketosteroids with an open or closed 21a,17-tetrahydrofuran ring E, and derivatives of the 23-oic acid, also with an open chain or one cyclized to form a 23,16-lactone.

The influence of the compounds of these series that were studied on Na^+,K^+-dependent ATPase is shown in Table 1. As we see, compound (II) with a branched 23-ethoxycarbonyl side chain is more than three times as active than its cyclic analog — the lactone (I) with an activity close to that of the $\Delta^{20(22)}$-unsaturated analog that we have described previously [5]. Of the $\Delta^{20(22)}$-ethoxycarbonyl compounds given here, the most active is that unsubstituted at C(16) and C(17), its activity being comparable with the activity of the corresponding 5,6α:16,17α-diepoxide (IV).

We have previously [5] discussed the Na^+,K^+-ATPase-inhibiting activity of steroids with a tetrahydropyran ring E having a 20-carbonyl function. The 20-ketosteroids with a branched chain shown in Table 1 (V-VIII) exhibit greater inhibition than the cyclic 20-ketones [5].

The presence of a 22,23-epoxyethyl group (VII) or of a β-diketone function (VIII) in the side chain leads to a particularly pronounced increase in inhibiting activity. These cases apparently confirm a hypothesis put forward concerning the fundamental role of polar substituents concentrated in one part of the molecule. The same effect is shown in a number of compounds with a 17-spirotetrahydrofuran ring bearing various substituents in rings A and

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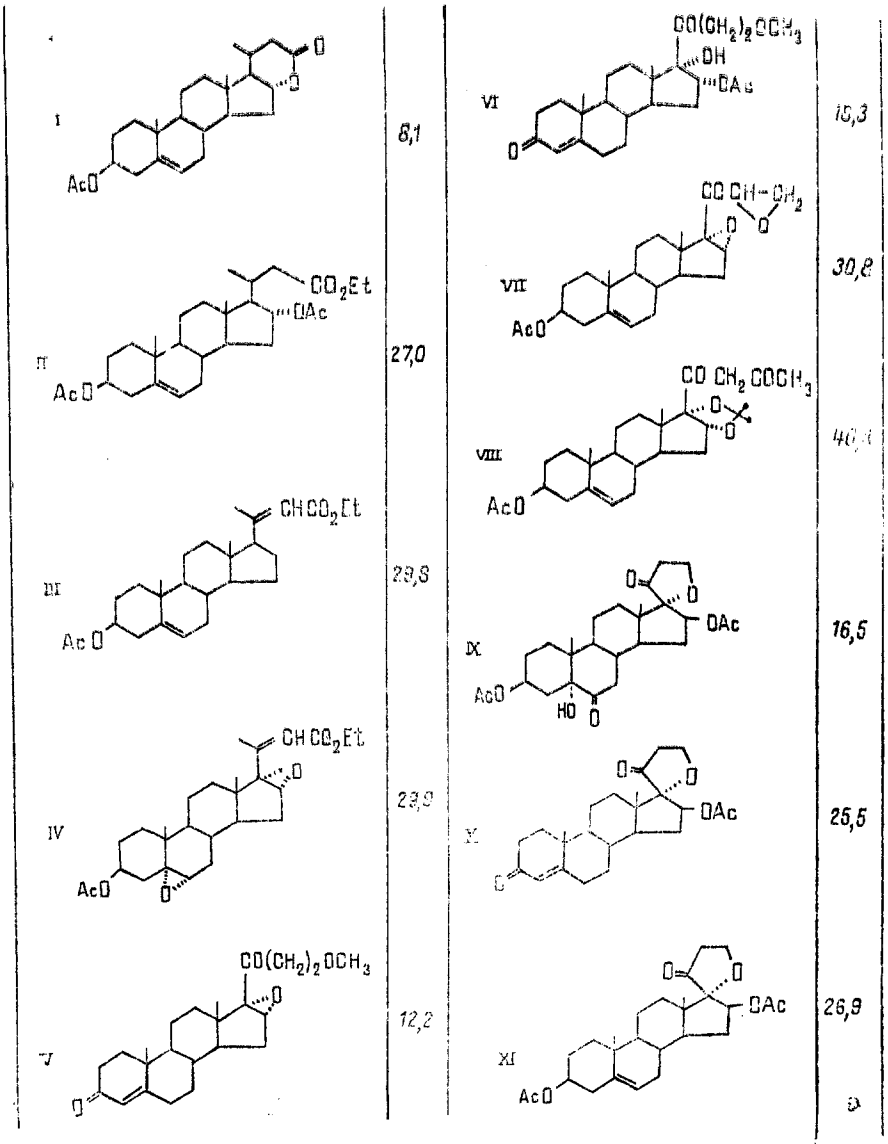
TABLE 1. Dependence of the Percentage Inhibition of Na^+,K^+-ATPase on the Structures of Steroids (inhibiting activity in a concentration of $1\cdot10^{-5}$ M)



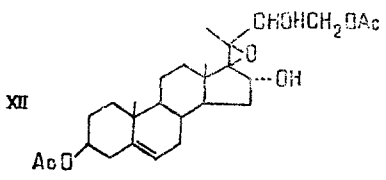
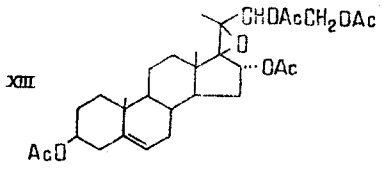
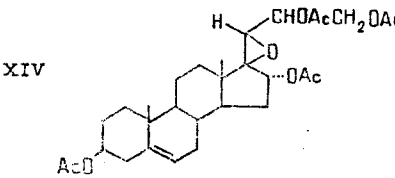
B (IX-XI). On the whole, these compounds exhibit a considerably greater ATPase-inhibiting activity than the pyranosidic steroids [5], the inhibition increasing in the following sequence:

	IX	<	*X*	<	*XI*
10^{-5}	16,5		25,5		26,9
10^{-6}	14,7		15,4		20,5
10^{-7}	1,8		2,8		18,6

It is curious to note that, according to Repke [2] a well-known antimineralocorticoid — spirolactone — inhibits ATPase to the extent of 25% in a concentration of $100\cdot10^{-6}$ M, i.e., its activity is two orders lower than that of the spirosteroids (IX-XI).

The polyhydroxysteroids (XII-XIV) having no carbonyl group in the side chain, stand out somewhat from the series of compounds discussed here (Table 2). As can be seen from Table 2, the activities of the polyacetates (XIII) and (XII) are comparable with the activities of the products of the ring-opening of lactones, the replacement of acetate groups by hydroxy

TABLE 2. Dependence of the Percentage Inhibition of Na^+,K^+-ATPase on the Structures of Steroids (inhibiting activity in a concentration of $1 \cdot 10^{-5}$ M).

XII		15,4
XIII		28,7
XIV		+29,6

groups at C(16) and C(22) lowering the inhibiting activity. It is interesting that the tetraacetate (XIV), differing from (XIII) only by the absence of a 21-CH_3 group, exhibits the opposite action — it activates ATPase to an appreciable extent.

For compounds (VII) and (VIII), showing the greatest activity, the dependence of this activity on the concentration was traced. In a study of the ATPase-inhibiting activity one must mention its appreciable change with a rise in the concentration of the added substance and its lagging behind by an order of magnitude from the activity of a well-known cardiotonic, digitoxigenin.

	Conc. $1 \cdot 10^{-4}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-7}$
VII	80,5	30,8	25,4	9,5
VIII	60,0	40,8	30,5	20,4
Digitoxigenin		80,0	37,0	12,0

EXPERIMENTAL

The source of ATPase was a highly active purified fraction from the medullary layer of porcine kidneys with a specific activity of 1500 μmole of inorganic P/mg of protein/1 h [6]. To reveal their effect, the steroids were dissolved in a concentration of $1 \cdot 10^{-2}$ M in DMSO and the solution was diluted to the subsequent concentrations with double-distilled water. The modified steroids were preincubated with Na^+,K^+-ATPase preparations at 20°C for 15 min. Incubation was carried out at 37°C in a medium containing 130 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris-HCl — $pH_{37°C}$ 7.5.

The reaction was begun by the addition to the sample of 1 mM ATP and was stopped after 10 min by the addition of 3% $HClO_4$. Phosphorus was determined by a modification of Delsal and Manhouri's method [7]. Protein was determined by a modified Lowry method [8]. The steroids investigated were synthesized by methods described in [5].

SUMMARY

On analyzing the connection of the structure of the steroids studied with their Na^+,K^+-ATPase-inhibiting activity, the following conclusions can be drawn:

1. Steroids with branched side chains are more active than steroids with additional tetrahydropyran-4-one and -2-one rings E. The most active in this series are 20-ketosteroids containing in the side chain such polar groupings as a β-diketonic or an epoxyethyl grouping.

2. Steroids with a spirotetrahydrofuran ring at C(17) exhibit activities greater than those of their pyranoside analogs.

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OXIMES AND NITRILES OF CARDENOLIDES

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The influence of the oximation of cardenolides and the production of 10-cyanocardenolides on their biological activity has been studied. The prefered conformation of strophanthidin 19-aldoxime has been established; it has chelate fragment as the result of the orientation in the same direction of the nitrogen atom of the oxime group and the hydroxy groups at C-3 and C-5.

We have previously [1] established that strophanthidin 19-aldoxime has a biological activity three times greater than that of the natural cardiac aglycone. In attempting to investigate the influence of oximation and some other types of transformation of cardenolides on the change in their biological activity more widely, we have synthesized oximes both of aglycones (II, IV, VI) and also of glycosides (VIII, X, XII), and also 10-cyanocardenolides (XIII, XIV). For oximation we used substances each containing an angular aldehyde group, so obtaining the corresponding 19-aldoximes. Digitoxigenin 3-ketoxime (VI) was also synthesized.

Treatment of the oximes with dehydrating reagents and, in particular, heating with them in a mixture of acetic anhydride and pyridine, yielded the 10-cyanocardenolides (XIII) and (XIV). The nitrile derivatives of some cardiac glycosides were known previously [2, 3], but information on their biological activity was lacking.

The structures of the compounds obtained were confirmed by the results of elementary analysis and IR and PMR spectroscopy. The IR spectra of the oximes have bands in the 1610-1630 cm^{-1} region due to the C=N bond, which are usually accompanied by the absorption bands of double C=C bonds of the butenolide ring, increasing their intensity. In the PMR spectra there are signals with a chemical shift of 7.65 ppm belonging to the proton of the $-CH=N-$

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