SYNTHESIS AND MOLECULAR-BIOLOGICAL ACTIVITY OF THE PYRIDINE ANALOGUE OF CARDIOTONIC STEROIDS

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Abstract -- Pyridylandrostane derivative 1 was synthesized from compound 4 and shown to exert remarkably high molecular-biological activity in the Na,K-ATPase test for cardiotonic steroids.

Recently one of our groups has reported its preliminary results on the model synthesis of pyridine- and pyridone-androstane derivatives related to cardiotonic steroids.[1)] This communication has prompted Wiesner and his collaborators to present their synthesis of azabufalin[2)]. Now we wish to report the synthesis of 17β-(3'-pyridyl)-14β-androst-4-ene-3β,14-diol 1 which differs from scillarenin 2 and canarigenin 3 only in the nature of the heterocycle at the position 17β (Scheme 1), and to disclose the biological activity of the target compound 1, its natural counterparts 2 and 3, as well as the synthetic intermediates 9, 10, and 11 in the Na,K-ATPase test for cardiotonic steroids[3)] (Table 1).

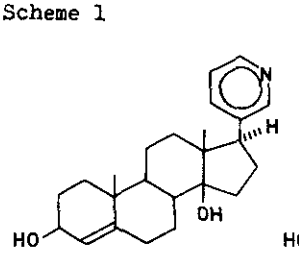
The readily accessible[4)] hydroxy-ketone 4a was converted to its tetrahydropyranyl ether 4b (dihydropyran, p-TSA in CH_2Cl_2), and the latter compound, dissolved in ether, was treated at -78°C with 3-pyridyllithium prepared[5)] from 3-bromopyridine and n-butyllithium. The addition product thus obtained was acetylated with acetic anhydride in pyridine in the presence of N,N-dimethyl-4-aminopyridine, subsequently the tetrahydropyranyl group was split off with p-TSA in acetone, and the resulting alcohol was acetylated with acetic anhydride in pyridine to afford compound[6)] 5 in 80% overall yield; ν_{max} 1735 cm^{-1}; ^1H NMR δ(ppm) 8.48 (2H, m, C_{21}- and C_{24}-H), 7.46(1H,d,J=8Hz,C_{22}-H), 7.24(1H,dd,J=8Hz,J=5Hz,C_{23}-H), 6.43 (1H,dd,J=6Hz,J=2Hz,C_{15}-H), 6.28(1H,d,J=6Hz,C_{16}-H), 5.36(1H,m,C_6-H), 4.50(1H,m, C_3-H), 2.03(3H,s,OCOCH_3), 1.96(3H,s,OCOCH_3), 1.10 and 0.98 (angular CH_3).

The diacetate 5 was rearranged to the diacetate 6 by boiling its 0.064 M solution in the xylene - acetic acid mixture (24:5) for 12 h; 70% yield[7]; m.p. 171-174°C; ^{1}H NMR δ(ppm) 6.18(1H,d,J=3Hz,C_{16}-H), 5.43(2H,m,C_6- and C_{15}-H), 1.35 and 1.15 (angular CH_3).

Selective reduction of C_{16}-C_{17} double bond in compound 6 was carried out by means of diimide[8](generated from hydrazine hydrate, propionic acid, and air) to give the product 7 in 75% yield; m.p. 195-197°C; ^{1}H NMR δ(ppm) 5.28(2H,m,C_6- and C_{15}-H), 1.03 and 0.65 (angular CH_3).

The diacetate 7 was hydrolyzed with methanolic KOH, and the dihydroxy derivative was treated first with acetic anhydride in pyridine at room temperature for 1h and then with mesyl chloride in pyridine. The 5,14-diene 8 was obtained in 73% yield; m.p. 189-192°C; ^{1}H NMR δ(ppm) 5.44(1H,m,C_6-H), 5.31(1H,br s,C_{15}-H), 1.05 and 0.62 (angular CH_3).

Scheme 1



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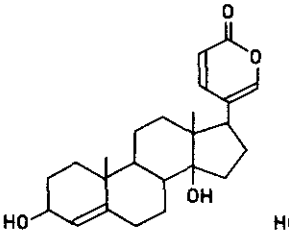

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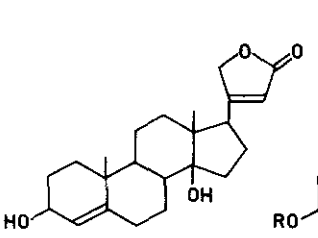

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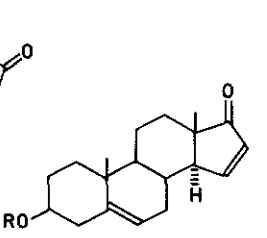
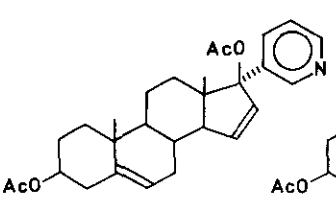


4a R = H
4b R = THP



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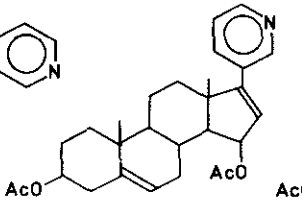

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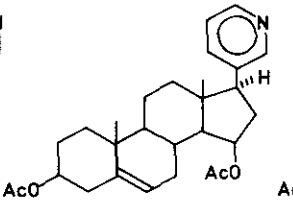

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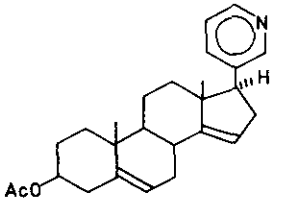

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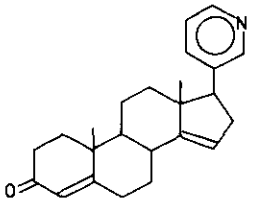

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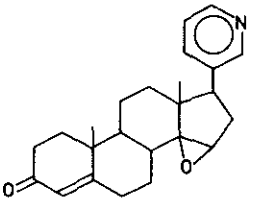

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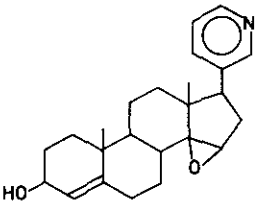

11

Hydrolysis of ester group in 8 (KOH-MeOH) followed by Oppenauer oxidation (aluminum isopropoxide, cyclohexanone, toluene) furnished the α,β-unsaturated ketone 9; 86% yield; m.p. 170-173°C; ν_{max} 1680 cm^{-1}; ^{1}H NMR δ(ppm) 5.76(1H,s,C_4-H), 5.35(1H,m,C_{15}-H), 1.23 and 0.65 (angular CH_3).

The compound 9 was reacted with N-bromoacetamide in the presence of perchloric acid in aqueous dioxane[9] for 1.5 h. The reaction mixture was made alkaline with 10% KOH which resulted in the transformation of intermediate bromohydrin to epoxide 10; m.p. 213-215°C; ν_{max} 1675 cm^{-1}; ^{1}H NMR δ(ppm) 5.70(1H,s,C_4-H), 3.50(1H,s,C_{15}-H), 1.18 and 0.52 (angular CH_3).

The carbonyl group in compound 10 was selectively and stereospecifically reduced with lithium tri(tert-butoxy)aluminum hydride[10] to give 3β-hydroxy compound 11 in 95% yield; m.p. 202-204°C; ^{1}H NMR δ(ppm) 5.31(1H,s,C_4-H), 4.15(1H,m,C_3-H), 3.48(1H,s,C_{15}-H), 1.05 and 0.59 (angular CH_3).

Finally, the epoxide ring in compound 11 was reduced with $LiAlH_4$ in boiling THF to give the diol 1 in 92% yield; ν_{max} 3600 cm^{-1}; ^{1}H NMR δ(ppm) 8.49(1H,br s,C_{21}-H), 8.37(1H,d,J=5Hz,C_{24}-H), 7.72(1H,d,J=8Hz,C_{22}-H), 7.16(1H,dd,J=8Hz,J=5Hz,C_{23}-H), 5.29(1H,s,C_4-H), 4.14(1H,m,C_3-H), 2.80(1H,m,C_{17}-H), 0.98 and 0.51 (angular CH_3).

The molecular-biological activities of the compounds examined are compiled in the Table 1. These are expressed in terms of the concentration producing, in the equilibrium state, half-maximum inhibition of the enzyme activity, I_{50} values.

Table 1

Molecular-biological activity as characterized by the concentrations required to affect half-maximum inhibition of the activity of Na,K-ATPase from cardiac muscles of guinea-pig and man.

Compound	Guinea-pig enzyme, I_{50} μM	Human enzyme, I_{50} μM
1	1.6	0.13
2	0.22	not determined
3	2.5	not determined
9	not determined	46
10	13	6.4
11	15	1.8

As can be seen from Table 1, biological activity is weakened after formal replacement of the pentadienolide- by the pyridine-substituent (2→1) but at least maintained after formal replacement of butenolide- by pyridine-substituent (3→1). The observed gradation of biological activity of pyridine derivatives 1>11>>9 is similar to that of corresponding butenolide derivatives, namely 3β-acetoxy-14-hydroxy-5β,14β-card-20(22)-enolide (I_{50}=1.2μM), 3β-acetoxy-14,15β-epoxy-5β,14β-card-20(22)-enolide (I_{50}=11μM), and 3β-acetoxy-5β-card-14,20(22)-dienolide (I_{50}>>100μM). Apparently, compounds in the two compared series interact with the same binding site area of Na,K-ATPase, which implies that the pyridine androstane derivative 1 is a true analogue of natural cardiotonics 2 and 3. It is of relevance that 17β-(3'-furyl)-steroid derived from digitoxygenin[11], likewise devoid of carbonyl group in the side substituent, exhibit cardiotonic activity comparable to the natural cardenolides.[12]

REFERENCES AND FOOTNOTES

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6) All new compounds had the expected spectral data and satisfactory analyses for C,H,N (crystals) or high-resolution mass spectra (oils).

7) This product was accompanied by the corresponding 5,14,16-triene, m.p. 160°C.

8) For review see: C.E.Miller, J.Chem.Educ., 1965, 42, 254.

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12) We are indebted to the referee for bringing up to our attention this reference.

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