Notes

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Epimerization of C-17 Substituent during Formation of Isobufadienolide[1]

Toshio Nambara, Kazutake Shimada, Shujiro Goya, and Nobuo Sakamoto

Pharmaceutical Institute, Tohoku University[2]

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It has recently been reported that 14-deoxy-14β-uzarigenin exhibits a cardiotonic activity in spite of lacking a hydroxyl group at C-14.[3] This fact evidently tells us that *cis*-fusion of C/D-ring is mainly responsible for physiological potency. The preparation of the 14,17-*cis*-isobufadienolides, therefore, appeared to be a quite attractive subject. In this paper we wish to report some findings obtained during the course of this project.

An initial attempt was made on the synthesis of the 14α,17α-isobufadienolide. For this purpose 3β-acetoxy-14α,17α-pregn-5-en-20-one (Ib) was prepared from the corresponding 14α,17β-epimer (Ia) utilizing Serini reaction.[4] The formation of the α-pyrone ring at C-17 was undertaken according to the method previously established.[5] Treatment of Ib with ethyl orthoformate in the presence of perchloric acid gave 3β-acetoxy-20-ethoxy-21-formyl-14α,17α-pregna-5,20-diene (IIb) together with a by–product in a ratio of 7 to 1. Inspection of the nuclear magnetic resonance (NMR) spectra revealed that epimerization took place at C-17 during the reaction process to yield the known 17β-epimer (IIa). The satisfactory separation of these two epimers could not be attained, and therefore the mixture was submitted to further step without purification. Condensation with malonic acid in the presence of morpholine as catalyst followed by cyclization gave rise to epimerization to furnish the thermodynamically more stable 14α,17β-isobufadienolide (III) as a single product.

The possible occurrence of epimerization during formation of the pentadienolide ring was further examined with 3β-acetoxy-16β-methyl-17β-pregn-5-en-20-one (IVa). Similar treatment with ethyl orthoformate as mentioned above again resulted in a mixture of C-17 epimeric compounds (Va and Vb), whose ratio was spectrometrically determined to be *ca.* 6 to 5. Subsequent reaction with malonic acid gave solely the thermodynamically more stable 16β-methyl-14α,17α-isobufadienolide (VI), identical with the product derived from the 16β-methyl-14α,17α-epimer (IVb) in similar fashion.

These results prompted us to reexamine the reactions involving the synthesis of 16α-methyl-14β,17β-isobufadienolide (IXa) starting from 3β-acetoxy-16α-methyl-14β,17β-pregn-5-en-20-one (VII). On treatment with ethyl orthoformate VII was led to a mixture of C-17 epimeric unsaturated aldehydes (VIIIa and VIIIc). Alkaline hydrolysis followed by condensation with malonic acid resulted in formation of the 17β- and 17α-isobufadienolides (IXa and IXb) in a ratio of 7 to 3. It is of interest that the less stable epimer forms 30% of the

1) This paper constitutes Part III of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part II: T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968).
2) Location: *Aobayama, Sendai.*
3) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **16**, 2223 (1968); T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).
4) M.B. Rubin and E.C. Blossey, *Steroids*, **1**, 453 (1963).
5) *a*) T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968); *b*) J.C. Knight, G.R. Pettit, and C.L. Herald, *Chem. Commun.*, **1967**, 445.

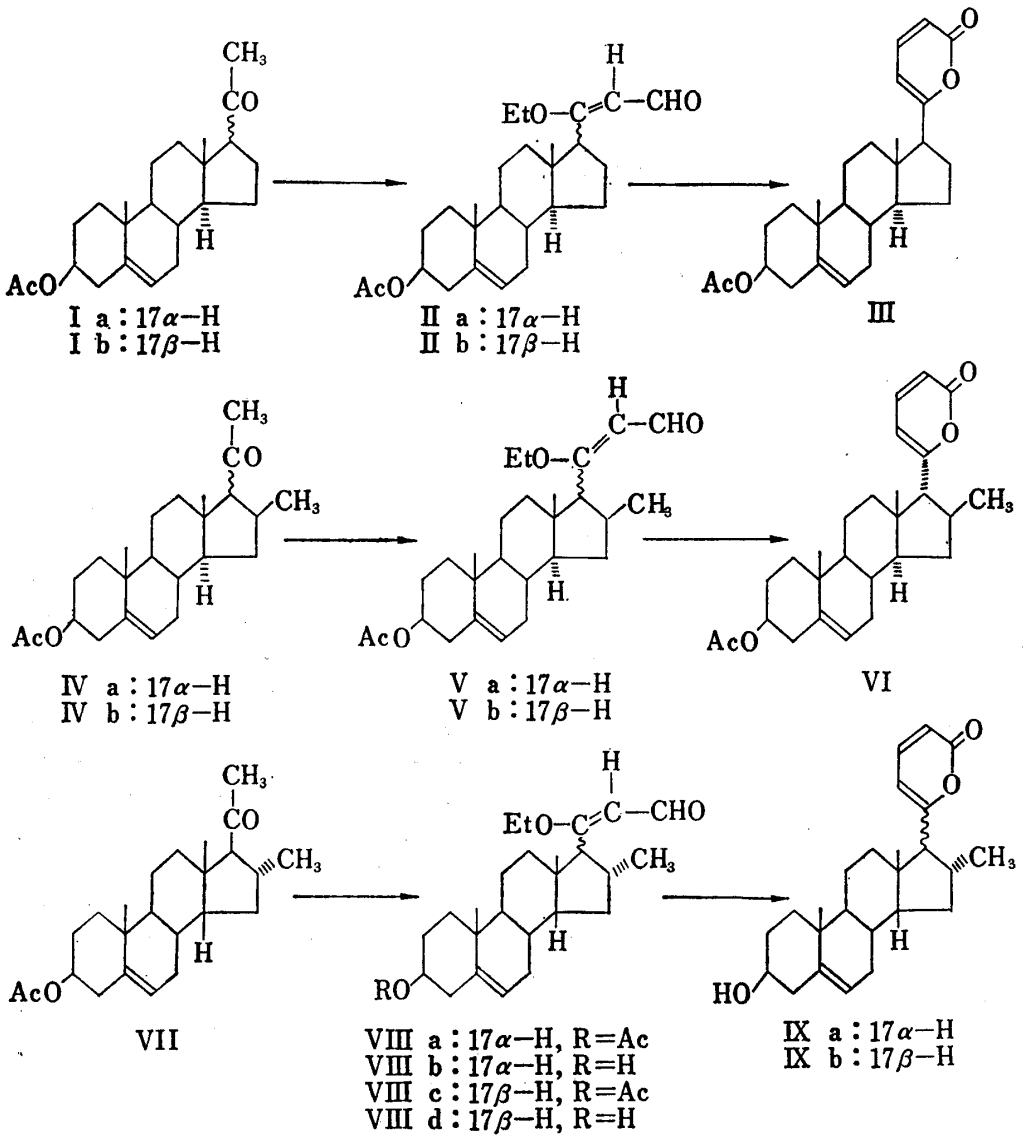

Chart 1

equilibrium mixture at the final stage in contrast to the case of the 16β-methyl-14α,17β-pregn-20-one (IVa).

Thus it has been clarified that the method for preparation of the thermodynamically less stable isobufadienolide through the above reaction sequence would be unsuccessful due to facile epimerization at C-17. Further studies on the development of an alternative way without accompanying epimerization are being in progress and the details will be reported in near future.

Experimental[6]

Reaction of 3β-Acetoxy-14α,17α-pregn-5-en-20-one (Ib) with Ethyl Orthoformate——To a solution of Ib (150 mg) in ethyl orthoformate (5 ml) was added a few drops of $HClO_4$ dropwise under ice-cooling over

6) All melting points were taken on a micro hot-stage apparatus and uncorrected. The NMR spectra were run on Hitachi H-60 spectrometer at 60 Mc: the chemical shifts are quoted as ppm downfield from $(CH_3)_4Si$ as an internal standard. Abbreviation used s=singlet, d=doublet, q=quartet and m=multiplet. Thin-layer chromatography (TLC) was carried out on silica gel G (E. Merck AG) by the following systems: TL-I=benzene–ethyl acetate (50:1); TL-II=benzene–ethyl acetate (9:1); TL-III=hexane–ethyl acetate (4:1).

a period of 10 min. After addition of several drops of pyridine to decompose the perchlorate, the resulting solution was extracted with ether. The organic layer was washed with cold 5% HCl, 5% $NaHCO_3$ and H_2O successively, and dried over anhydrous Na_2SO_4. On usual work-up an yellow oily product was obtained. TLC: TL-I 0.50 (IIb), 0.43 (IIa). NMR (4% solution in $CDCl_3$) δ: 0.67 (0.4H, s, 18-CH_3), 0.90 (2.6H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.01 (3H, s, 3β-$OCOCH_3$), 3.79 (2H, q, J=6.5 cps, -OCH_2Me), 4.55 (1H, m, 3α-H), 5.40 (2H, d, J=8 cps, $>C=C\langle^{CHO}_{H}$, 6-H), 9.80 (1H, d, J=8 cps, -CHO).

6-(3β-Acetoxy-14α-androst-5-en-17β-yl)-2-pyrone (III)——To a solution of a mixture of IIa and IIb (150 mg) in pyridine (5 ml) were added a trace of morpholine and malonic acid (75 mg), and the resulting solution was refluxed for 4 hr. The reaction mixture was diluted with CH_2Cl_2, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using hexane–AcOEt (4:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.70) was eluted with AcOEt and recrystallization of the eluate from MeOH gave III (23 mg) as colorless needles. mp 212—215°. Mixed melting point on admixture with the sample (mp 213—216°)[5] obtained from Ia in the same manner showed no depression.

Reaction of 3β-Acetoxy-16β-methyl-17β-pregn-5-en-20-one (IVa) with Ethyl Orthoformate——IVa (300 mg) was treated with ethyl orthoformate (6 ml) and $HClO_4$ in the same manner as described in II. On similar treatment an yellow oily product was obtained. UV λ_{max}^{EtOH} mμ: 264.5. TLC: TL-I 0.36 (Va), 0.43 (Vb). The crude product was submitted to further step without purification.

6-(3β-Acetoxy-16β-methylandrost-5-en-17α-yl)-2-pyrone (VI)——A mixture of Va and Vb (110 mg) was treated with malonic acid (50 mg), pyridine (5 ml) and a trace of morpholine in the same manner as described in III. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (10:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.39) was eluted with AcOEt. Recrystallization of the eluate from acetone gave VI (30 mg) as colorless prisms. mp 257.5—259°. Mixed melting point on admixture with the sample (mp 257.5—259°)[5a] obtained from IVb in the same manner showed no depression.

Reaction of 3β-Acetoxy-16α-methyl-14β,17β-pregn-5-en-20-one (VII) with Ethyl Orthoformate——VII (280 mg) was treated with ethyl orthoformate (4 ml) and $HClO_4$ in the same manner as described in II. On similar treatment an yellow oily-roduct was obtained. UV λ_{max}^{EtOH} mμ: 264.5. TLC: TL-III 0.25 (VIIIa), 0.28 (VIIIc). The crude product was submitted to further step without purification.

6-(3β-Hydroxy-16α-methyl-14β-androst-5-en-17β-yl)-2-pyrone (IXa), 6-(3β-Hydroxy-16α-methyl-14β-androst-5-en-17α-yl)-2-pyrone (IXb)——To a solution of VIIIa and VIIIc (280 mg) in MeOH (5 ml) was added 5% K_2CO_3 (2 ml) and the resulting solution was refluxed for 1 hr. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up an yellow oily product was obtained. UV λ_{max}^{EtOH} mμ: 264.5. TLC: TL-II 0.10 (VIIIb), 0.12 (VIIId). The crude product was submitted to further step without purification. A mixture of VIIIb and VIIId (130 mg) was treated with malonic acid (80 mg), pyridine (4 ml) and a trace of morpholine in the same manner as described in III. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (9:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.12) was eluted with AcOEt. Unfortunately the eluate (20 mg) could not be crystallized, but substantially homogeneous according to TLC. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.50 (1H, m, 3α-H), 5.40 (1H, m, 6-H), 5.95 (1H, d, J=6 cps, 23-H), 6.10 (1H, d, J=9 cps, 21-H), 7.25 (1H, q, J=6, 9 cps, 22-H). The adsorbent corresponding to the spot (*Rf* 0.10) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave IXb (50 mg) as colorless plates. mp 188—190°. Mixed melting point on admixture with the authentic sample (mp 188—190°)[5a] showed no depression. NMR (4% solution in $CDCl_3$) δ: 0.95 (3H, s, 19-CH_3), 1.05 (3H, s, 18-CH_3), 3.50 (1H, m, 3α-H), 5.35 (1H, m, 6-H), 6.00 (1H, d, J=6 cps, 23-H), 6.14 (1H, d, J=9 cps, 21-H), 7.25 (1H, q, J=6, 9 cps, 22-H).

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