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Acyl Transfer during Borohydride Reduction of Steroidal 16,17-Ketol Acetates[1]

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The occurrence of the 16-oxosteroids as the natural products[3] prompted several workers to develop the convenient synthetic routes leading to these steroids.[4] One of these methods deals with an accessible way to prepare 3β-hydroxyandrost-5-en-16-one in three steps starting from the corresponding 3β,16β-diacetoxy-17-ketone.[4c] The present paper describes some observations during the course to extend the method to estrogens from the necessity for biochemical problems.

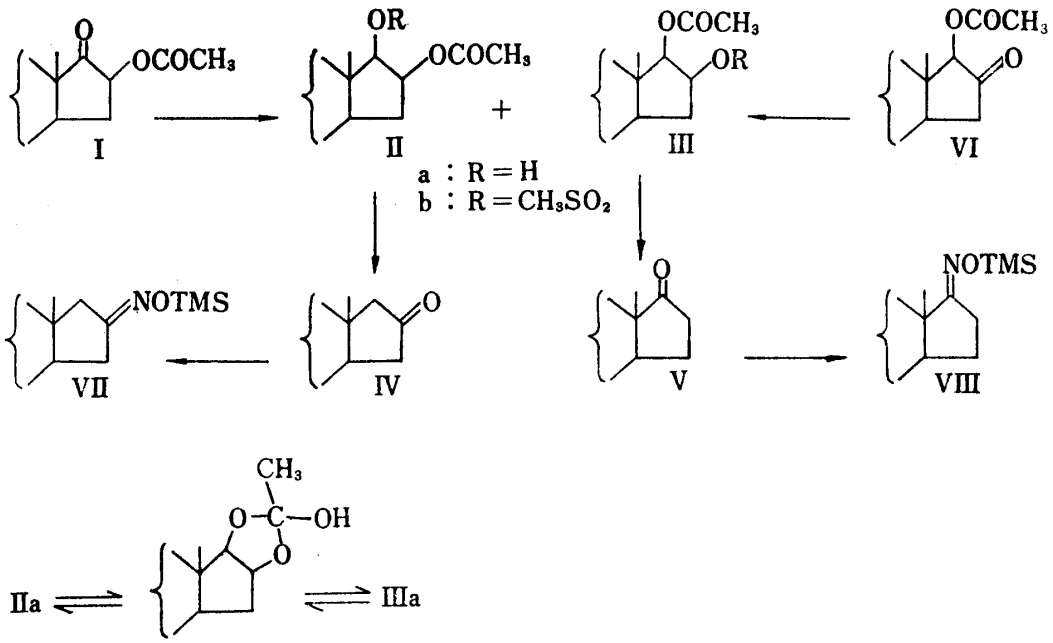

Chart 1

Treatment of 16β-hydroxyestrone diacetate (I) with sodium borohydride gave the reduction product, which showed a single spot on thin-layer chromatogram. However, inspection of the nuclear magnetic resonance (NMR) spectra revealed that the product would consist of two similar compounds exhibiting the C-18 proton signals at 0.83 and 0.93 ppm with the magnitude of *ca.* 2 to 1. On the other hand alkaline hydrolysis gave 16-epiestriol as a sole product.

1) This paper constitutes Part XXXIV of the series entitled "Analytical Chemical Studies on Steroids"; Part XXXIII: *Chem. Pharm. Bull.* (Tokyo), **18**, 474 (1970).
2) Location: *Aobayama, Sendai.*
3) M.N. Huffman and M.H. Lott, *J. Biol. Chem.*, **207**, 431 (1954); R.V. Brooks and W. Klyne, *Biochem. J.*, **65**, 663 (1957); C. Schöpf, *Experientia*, **17**, 285 (1961); G. Habermehl, *Naturwissenschaften*, **53**, 123 (1966).
4) *a*) J. Fajkoš and F. Sorm, *Collection Czech. Chem. Commun.*, **19**, 349 (1954). *b*) J.E. Bridgeman, E.R.H. Jones and G.D. Meakins, *Chem. Commun.*, **17**, 898 (1967). *c*) S. Hara and Y. Ike, *Yakugaku Zasshi*, **87**, 1573 (1967).

These findings together indicated that the compounds would be isomeric and differ in only the position of acetyl group attached to the 16,17-*cis*-glycol. Gas chromatographic separation of these positional isomers, however, could not be attained, even when they were converted into the trimethylsilyl derivatives. In order to confirm the structures and composition transformation of the glycol monoacetates (IIa, IIIa) into the corresponding ketones through their mesylates was undertaken. In usual manner the mixture of the isomeric monoacetates was quantitatively converted into the acetate–mesylates (IIb, IIIb). The NMR spectra again supported the composition of the mixture of two isomers, but chromatographic separation was still difficult. The acetate–mesylates were then led to the ketosteroids (IV, V) by treatment with base. The authors have previously reported that gas chromatographic separation of the isomeric 16- and 17-oxosteroids is readily accomplished, when converted into the O-trimethylsilyloxime derivatives to accentuate the slightly existing difference in position.[5] In fact the product could distinctly be resolved into two peaks on SE-30 as well as on OV-17, where the percentage composition of the mixture was established as 76% of the 16-ketone and 24% of the 17-ketone. The steroid number differences between the parent compounds and their O-trimethylsilyloxime derivatives (VII, VIII) were found to be 2.1 and 1.9, respectively, which were in good agreement with the values previously established.[5] These results unequivocally clarified that the acetyl transfer did take place during the borohydride reduction to furnish two isomeric monoacetates of 16,17-*cis*-glycol.

Examinations were further extended to the reduction of the isomeric 17β-acetoxy-16-ketone (VI). The NMR spectrum of the crude product was indicative of the simultaneous formation of both the 16- and 17-monoacetates of *cis*-glycol with a ratio of *ca.* 2 to 1. Indeed the similar elaboration as mentioned above, namely mesylation followed by treatment with base, proved that during the borohydride reduction acyl transfer took a reverse course contrary to the isomeric 16β-acetoxy-17-ketone. It is to be noted that the ratio of two isomeric acetates of 16,17-glycol produced from either of the α-ketol acetates was found almost equal. As formulated in Chart 1, the acetyl group may undergo transfer either from the C-16 to the C-17 hydroxyl groups or along the reverse course through the otrthoacetate intermediate yielding the glycol monoacetates. As to the isomeric 16,17-ketol acetates in the androst-5-ene series borohydride reduction also proceeded in similar fashion as the estratriene series.

Several papers have already been published dealing with the acyl migration during the process of the chemical modification of the acylated glycol.[6] The present results evidently disclosed that careful consideration should be required for the borohydride reduction of the acylated 16,17-ketols which involve the important metabolites in both estrogens and androgens.

Experimental[7]

Reduction of 16,17-Ketol Acetates with Sodium Borohydride——i) To a solution of 3,16β-dihydroxyestra-1,3,5 (10)-trien-17-one diacetate (I) (520 mg) in DMF (3 ml)–MeOH (12 ml) was added dropwise a solution of $NaBH_4$ (70 mg) in 50% aq. MeOH (1 ml), and the resulting solution was kept at -10——$-13°$ for

5) T. Nambara, T. Kudo, and H. Ikeda, *J. Chromatog.*, **34**, 526 (1968).

6) D.K. Fukushima, N.S. Leeds, H.L. Bradlow, T.H. Kritchevsky, M.V. Stokem, and T.F. Gallagher, *J. Biol. Chem.*, **212**, 449 (1955); P. Wieland, K. Heusler, and A. Wettstein, *Helv. Chim. Acta*, **41**, 1657 (1958); D. Taub, R.D. Hoffsommer, and N.L. Wendler, *J. Am. Chem. Soc.*, **81**, 3291 (1959); L.L. Smith, J.J. Garbarini, J.J. Goodman, M. Marx, and H. Mendelsohn, *J. Am. Chem. Soc.*, **82**, 1437 (1960); R. Gardi, R. Vitali, and A. Ercoli, *Tetrahedron Letters*, **13**, 448 (1961); C.H. Kuo, D. Taub, and N.L. Wendler, *J. Org. Chem.*, **28**, 1619 (1963).

7) All melting points were taken on a micro hot-stage apparatus and uncorrected. NMR spectra were run on Hitachi Model H-60 spectrometer at 60 Mcps using $(CH_3)_4Si$ as an internal standard. For thin-layer chromatography (TLC) silica gel G (E. Merck AG) was used as adsorbent. The apparatus used for gas chromatography was Shimadzu Model GC-1C gas chromatograph equipped with hydrogen flame ionization detector and U-shaped stainless steel column (1.875 m$\times$3 mm inside diameter). The column was packed with either 1.5% SE-30 on a support of Chromosorb W (60—80 mesh) or 2% OV-17 on Shimalite W (60—80 mesh).

5 hr. The reaction mixture was poured into ice–water and the precipitate was filtered, washed and then dried. A mixture of 16-epiestriol 3,16- and 3,17-diacetates (IIa, IIIa) (350 mg) was thus obtained as amorphous product. mp 130—138°. The product showed a single spot on thin-layer chromatogram. *Rf* 0.31 (benzene:ether=7:3), 0.49 (hexane:AcOEt=7:6). NMR (5% solution in $CDCl_3$) δ: 0.83 (2H, s, 18-CH_3), 0.93 (1H, s, 18-CH_3), 2.06 (2H, s, 16β-$OCOCH_3$), 2.13 (1H, s, 17β-$OCOCH_3$), 2.24 (3H, s, 3-$OCOCH_3$), 3.60 (0.7H, d, J=7 cps, 17-CHOH), 4.35 (0.3H, m, 16-CHOH), 5.10 (1H, m, 16-, 17-CHOCOMe). Retention times relative to cholestane were observed as follows:

	1.5% SE-30	2% OV-17
Free (IIa, IIIa)	0.96	2.00
TMS deriv.	1.94	2.88
Cholestane	1.00 (5.5 min)	1.00 (10.3 min)

temperature: column 230°; detector 260°; injection chamber 260°
carrier gas: N_2 at a flow rate of 55 ml/min

Hydrolysis of the mixture of IIa and IIIa (150 mg) with 0.04N methanolic NaOH (10 ml) in usual manner and subsequent recrystallization from aq. MeOH gave 16-epiestriol (90 mg) as colorless plates. mp 289—291°.

ii) 3,17β-Dihydroxyestra-1,3,5(10)-trien-16-one diacetate (VI) (700 mg) was treated with $NaBH_4$ (90 mg) in the same manner as described in i). On usual work-up a mixture of IIa and IIIa (500 mg) was similarly obtained. mp 129—135°. Hydrolysis of the mixture with 0.04N methanolic NaOH gave 16-epiestriol as a sole product.

Transformation of 16,17-Glycol Monoacetates into 16- and 17-Oxosteroids——To a stirred solution of the mixture (IIa,IIIa) (110 mg) in pyridine (2 ml) was added dropwise $MeSO_2Cl$ (0.1 ml), and the resulting solution was stirred at 0° for 1 hr. The reaction mixture was extracted with $CHCl_3$, washed with 5% HCl, 5% $NaHCO_3$ and H_2O successively and dried over anhydrous Na_2SO_4. On usual work-up a mixture of the 16,17-acetate-mesylates (IIb,IIIb) (100 mg) was obtained as amorphous product. TLC: *Rf* 0.53 (hexane:AcOEt=3:2), 0.61 (benzene:ether=8:2). NMR (3% solution in $CDCl_3$) δ: 0.97 (3H, s, 18-CH_3), 2.07 (2.1H, s, 16β-$OCOCH_3$), 2.13 (0.9H, s, 17β-$OCOCH_3$), 2.26 (3H, s, 3-$OCOCH_3$), 2.91 (0.9H, s, 16β-OSO_2CH_3), 2.99 (2.1H, s, 17β-OSO_2CH_3), 4.41 (1H, d, J=8 cps, 17-CHOSO$_2$Me), 5.30 (1H, m, 16-, 17-CHOCOMe). A solution of the mixture (IIb,IIIb) (25 mg) dissolved in 1N methanolic KOH (8 ml) was refluxed for 1 hr. The reaction mixture was neutralized with 5% HCl, extracted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up a mixture of estrone-16 and estrone (IV,V) (15 mg) was obtained as amorphous product. TLC: *Rf* 0.64 (benzene:ether=7:3), 0.52 (hexane:AcOEt=7:3). To a solution of the mixture (IV,V) (*ca.* 1 mg) in pyridine (0.5 ml) was added $NH_2OH \cdot HCl$ (1.5 mg), and the resulting solution was heated at 70—80° for 1 hr. The reaction mixture was further treated with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) according to the procedure by Sweeley, *et al.*[8] After evaporation of solvent the residue was extracted with hexane (0.5 ml), centrifuged and the supernatant was injected to gas chromatograph. Retention times relative to cholestane were observed as follows:

	Free		TMS deriv.	=NOTMS deriv.[a]	
	1.5% SE-30	2% OV-17	1.5% SE-30	1.5% SE-30	2% OV-17
Estrone-16 (IV)	0.51	1.43	0.55	0.92	1.19
Estrone (V)	0.51	1.43	0.55	0.84	1.04
Androstane	0.12	0.12			
Cholestane	1.00 (13.2 min)	1.00 (10.8 min)			

temperature: column 220°; detector 280°; injection chamber 250°
carrier gas: N_2 at a flowrate of 70 ml/min
a) trimethylsilylated product of the oxime

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8) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).