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Epimerization of Halogen during *in Vivo* Transformation of 16α-Chloroestrone Methyl Ether

In the previous paper we reported isolation and characterization of the urinary metabolites in the rabbit administered with 16α-chloroestrone methyl ether (Ia), which is widely used as a lipid-shifting drug.[1,2] Of the principal metabolites formation of 16β-chloro-17α-estradiol (IIb) is of particular interest indicating the occurrence of epimerization at C-16 during the process of transformation in the living animals. However, whether epimerization occurred enzymatically or not still remians unsolved, since the steroid was orally given. It is sufficiently substantiated that in the C/D-*trans* steroids the 16α-halo-17-ketone is readily epimerized to the thermodynamically more stable 16β-epimer.[3] We now wish to present further evidences for *in vivo* epimerization of halogen at C-16 during the metabolic course of this drug.

The desired substrate for this purpose, 16α-chloroestrone-6,7-^{3}H methyl ether, was prepared from estrone-6,7-^{3}H through the Δ^{16}-enol acetate according to the method developed by Muller, *et al.*[4] The product was diluted with a carrier and crystallized repeatedly until the constant specific activity was attained. Homogeneity of the substrate was further checked by leading to the 16,17-chlorohydrins upon lithium aluminum hydride reduction. There could be seen a pair of C-17 epimeric 16α-chloro-17-ols, but not any detectable amounts of 16β-chloroestradiol 3-methyl ether on thin-layer chromatography (TLC).

A single dose of a suspension of the labeled steroid (0.25 mg, 1.2 μCi) in corn oil was injected intravenously to the male rabbit and urine was collected for following 48 hr. The pooled urine (recovered radioactivity 24.7% of the dose) was percolated through a column packed with Amberlite XAD-2 resin.[5] After thorough washing with distilled water the conjugated metabolites were eluted with methanol. The eluate was redissolved in water, adjusted to pH 4.5 with acetate buffer, and incubated with beef-liver β-glucuronidase. Usual work-up afforded a mixture of the steroidal aglycones, which in turn was submitted to TLC after addition of nonlabeled 16β-chloro-17α-estradiol as a carrier. Developments with benzene–ethyl acetate (5:1) and then with benzene on multiple runs, followed by elution of the corresponding spot with ethyl acetate gave the desired substance representing 9.3% of the radioactivity of the urinary metabolites. The structure of 16β-chloro-17α-estradiol was definitely characterized by obtaining the constant specific activity upon repeated crystallization as listed in Table I. Thus, *in vivo* transformation of 16α-chloroestrone methyl ether into the 16β-chloro-17α-ol, that is epimerization of halogen at C-16, has been unambiguously demonstrated.

a : R_1=Cl, R_2=H b : R_1=H, R_2=Cl

Chart 1

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TABLE I. Identification of 16β-Chloro-17α-estradiol Formed from 16α-Chloroestrone-6,7-^{3}H Methyl Ether by Isotope Dilution Analysis

No.	Crystallization from	Specific activity dpm/mg
1	$CHCl_3$	18300
2	$CHCl_3$	18200
3	$CHCl_3$	18500

The present finding prompted us to examine possible epimerization of the 16β-chloro-17-ketone during *in vivo* transformation. The desired substrate was prepared from the 16α-epimer by brief contact with basic alumina, followed by fractional crystallization from methanol. A suspension of 16β-chloroestrone methyl ether (Ib), mp 135—136°, $[\alpha]_D^{23}$ +181.8° (c=0.12, $CHCl_3$), in corn oil was injected intraperitoneally to the rabbit and the collected urine was processed in the manner as described above. A mixture of the steroidal aglycones obtained was submitted to TLC in benzene–ethyl acetate–chloroform (7 : 3 : 5), by which four isomeric 16-chloro-17-ols could be readily differentiated. Formation of 16α-chloro-17α-estradiol (IIa) together with its 16β-epimer and 17α-estradiol was definitely confirmed by comparison with the authentic samples, respectively.

It is evident from these data that in the rabbit the 16-halo-17-ketosteroids are transformed along a novel metabolic pathway, in which a certain enzyme system capable of epimerizing halogen may probably be involved. To the best of our knowledges this is the first record on *in vivo* epimerization of halogen in the metabolism of the foreign substances. Further studies on these problems are being conducted in this laboratory and the details will be reported in the near future.

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