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Sub Title	
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Publisher	共立薬科大学
Publication year	1978
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.23 (1978.) ,p.117- 119
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000023-0117

No.23 (1978)

Disulphates of 16-Oxygenated Ketonic C_{19} Steroids as Biliary Metabolites of Androsterone Sulphate in Female Rats *

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It is well established that the 16β-hydroxy-17-oxosteroid is readily rearranged to the stable epimer, the 17β-hydroxy-16-oxosteroid, by means of alkali or acid, and by isolation procedures from urine. In a paper dealing with biliary metabolites of androsterone conjugates in female rats, we reported that androsterone sulphate was metabolized to the disulphate of 3α, 17β-dihydroxy-5α-androstan-16-one. But, we did not preclude the artifact formation by rearrangement of 16β-hydroxyandrosterone to 3α, 17β-dihydroxy-5α-androstan-16-one during the isolation procedure, because 3β, 16β-dihydroxy-5-androsten-17-one, a model compound, was entirely converted into 3β, 17β-dihyroxy-5-androsten-16-one by t.l.c. on silica gel plate. Very recently, Mattox *el al.* purified synthetic 3β, 16β-dihydroxy-5-androsten-17-one by partition chromatography on Celite 545. These studies prompted us to investigate the occurrence of 16β-hydroxyandrosterone in the 16-oxygenated ketonic steroid metabolites of androsterone sulphate by employing the partition chromatography to the separation of the solvolyzed metabolites.

In this report, we describe the synthesis of 16β-hydroxyandrosterone and the identification of 16β-hydroxyandrosterone disulphate as a biliary metabolite of androsterone sulphate in female rats.

The diacetate of 16β-hydroxyandrosterone was prepared by lead tetraacetate oxidation of 5α-androst-16-ene-3α, 17-diol diacetate. Hydrolysis of the acetate group was done by formation of a C-17 semicarbazone, alkaline hydrolysis of the acetate group, and removal of the semicarbazone group. The configuration of the 16-hydroxy group was established by comparison of the sodium borohydride reduction product with 5α-androstane-3α, 16β, 17β-triol.

GC analyses of the TMS and MO-TMS derivatives of 16α-hydroxyandrosterone, its 16β-epimer, and 3α, 17β-dihydroxy-5α-androstan-16-one demonstrated that the TMS ether of the 16β-hydroxysteroid was not separated from that of the 16-oxosteroid, while the MO-TMS derivatives provided good separation. Mass spectra of the MO-TMS derivatives showed the molecular ion at *m/e* 479, characteristic of the dihydroxymonooxo C_{19} saturated steroid. The 16α-and 16β-hydroxysteroids gave base peak at *m/e* 448, whereas the 16-oxosteroid afforded base peak at *m/e* 158. Furthermore, sev-

*本報告は Sreroids, 31, 219 (1978) に発表。

eral ion abundance differences were observed among these spectra. The ions at *m/e* 433 and 343 were more abundant in the 16α-hydroxysteroid than in the other steroids, while the fragments at *m/e* 142 and *m/e* 133 were characteristic of the 16β-hydroxysteroid and the 16-oxosteroid, respectively.

The occurrence of epimerization of the 16β-hydroxy-17-oxosteroid to the 17β-hydroxy-16-oxosteroid is of crucial problem in the analysis of the 16β-hydroxy-17-oxosteroid. To check to what extent artifact formation occurred during the isolation procedure, 16β-hydroxyandrosterone was subjected to incubation in solvolytic medium, followed by partition chromatography. GC analysis revealed that only a few % of 16β-hydroxyandrosterone was epimerized to 3α, 17β-dihydroxy-5α-androstan-16-one.

Following intraperitoneal injection of [^{3}H]-androsterone sulphate into female rats with bile fistulas, the excretion of the radioactivity in the bile was about 76% during 24 hr. The biliary radioactivity consisted of monosulphate (65%) and diconjugate (35%) fractions. By solvolysis of the diconjugate fraction, 58% of the radioactivity appeared in the liberated steroid fraction. These figures were similar to those described in a previous paper. Partition chromatography of the liberated steroid fraction yielded 79, 2, and 4% of the radioactivity in 5α-androstane-3α, 17β-diol, 3α, 17β-dihydroxy-5α-androstan-16-one, and 16β-hydroxyandrosterone fractions, respectively. The production of large amounts of 5α-androstane-3α, 17β-diol was a pronounced feature in this study. One explanation for this may be that we administered the increased amounts of androsterone sulphate into rats in this study. Eriksson *et al.* reported the change of metabolic patterns with different concentrations of progesterone in perfused rat liver. The each fraction obtained by partition chromatography was analyzed as the TMS and MO-TMS derivatives by GC and GC-MS. The identified metabolites provided relative retention times and mass spectra identical with those of the respective reference steroids. Details of the identification of 5α-androstane-3α, 17β-diol were not given here, because it was already done previously. 16β-Hydroxyandrosterone and 3α, 17β-dihydroxy-5α-androstan-16-one were identified in the respective fractions, though the 16β-hydroxyandrosterone fraction was contaminated with small amounts of 3α, 17β-dihydroxy-5α-androstan-16-one. No other steroid metabolites (16α-hydroxyandrosterone, etc.) were identified in these fractions. Although our results do not preclude the occurrence of the disulphate of 3α, 17β-dihydroxy-5α-androstan-16-one, the production of 16β-hydroxyandrosterone disulphate as a biliary metabolite of androsterone sulphate in female rats is firmly established. To our knowledge, this is the first identification of 16β-hydroxyandrosterone as a disulphate in rat bile. Thus, the major portion of 3α, 17β-dihydroxy-5α-androstan-16-one identified in the previous study must be the artifact formed from 16β-hydroxyandrosterone.

Several 17β-hydroxy-16-oxosteroids were isolated from the incubation of C_{19} steroids with rat liver or human foetal liver, and from rat bile. The present study suggests

the occurrence of the 16β-hydroxy-17-oxosteroids in these 16-oxygenated steroid metabolites. Recently, 16β-hydroxylating enzymes active on steroid sulphates were found in human foetal liver. Thus, androsterone sulphate must undergo direct metabolism by liver microsomal 16β-hydroxylase to 16β-hydroxyandrosterone 3-sulphate, which must be consequently conjugated with sulphuric acid to 16β-hydroxyandrosterone disulphate by the sulphotransferase located in the soluble fraction of the liver cell.