Communications to the Editor

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SIGNIFICANCE OF BILE ALCOHOL IN URINE OF A PATIENT WITH CHOLESTASIS: IDENTIFICATION OF 5ß-CHOLESTANE-3α,7α,12α,26,27-PENTOL (5ß-CYPRINOL) AND 5ß-CHOLESTANE-3α,7α,12α,26-TETROL (27-DEOXY-5ß-CYPRINOL)

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Recently, several bile alcohols have been identified in bile and urine of patients with liver disease. Their appearance may be related to the altered steroid metabolism in the liver and reflect changes in mitochondrial and microsomal functions. In the present study, in addition to the previously reported bile alcohols, i.e, 27-nor-5ß-cholestane-3α,7α,12α,24,25-pentol and 5ß-cholestane-3α,7α,12α,25,26-pentol (5ß-bufol), two bile alcohols, 5ß-cholestane-3α,7α,12α,26,27-pentol (5ß-cyprinol) and 5ß-cholestane-3α,7α,12α,26-tetrol (27-deoxy-5ß-cyprinol), were identified in urine from a patient with obstructive jaundice. The presence of the latter two bile alcohols, not hydroxylated at C-25, suggests that in cholestasis disturbance of mitochondrial 26-hydroxylation is incomplete and the 26-hydroxylated compounds are also hindered in their further degradation.

KEYWORDS —— bile alcohol; cholestasis; 5ß-cyprinol; 27-deoxy-5ß-cyprinol; mitochondrial 26-hydroxylation; microsomal 25-hydroxylation; gas chromatography-mass spectrometry

In some lower vertebrates, bile alcohols are the major components of bile.[1] However, in man, these compounds occur only in specific diseased states, such as cerebrotendinous xanthomatosis (CTX).[2] Recently, large amounts of 27-nor-5ß-cholestane-3α,7α,12α,24,25-pentol and 5ß-cholestane-3α,7α,12α,25,26-pentol (5ß-bufol) were identified in urine from patients with liver disease,[3] and two C25 and one C26 bile alcohols appeared in bile from a patient with choledochal stone.[4] Because of these discoveries the significance of these compounds have drawn considerable attention.

In our present study, two bile alcohols not previously detected in human urine or bile were identified in the urine of a patient with obstructive jaundice. The patient was a 66-year-old man suffering from jaundice for about 10 days before admission to our hospital. External cholecystostomy was performed to release the biliary tract from obstruction. A later cholangiogram revealed the obstruction to be complete and due to pancreatic cancer. After the release from the obstruction, urine was collected in an ice-box for 24 hours and part of the specimen was stored at -20°C. Three ml of urine was passed through Bond-Elut cartridge (Analytichem

International, INC., Harbor City, CA, USA.) and steroid conjugates were eluted with 90% EtOH. The eluate was evaporated and dissolved in 4 ml of 0.075 M phosphate buffer (pH 7.0), and then added to an enzyme solution containig 500 units of ß-glucuronidase from E. coli (Sigma Chemical Co., St. Louis, MO, USA.). After incubation at 37°C for 24 hours, liberated bile alcohols were extracted with three 4-ml portions of ethyl acetate. The extracts were combined, evaporated to dryness under a stream of nitrogen and converted to dimethylethylsilyl (DMES) ethers.[5] Gas chromatography (GC) was carried out using Shimadzu GC-6AM equipped with a flame ionization detector and Van den Berg's solventless injector. Gas chromatography-mass spectrometry (GC-MS) was done using Shimadzu AUTO GCMS 9020-DF equipped with a data processing system (SCAP 1123) and Van den Berg's solventless injector. The column used was WCOT, 25m x 0.35mm i.d., coated with SE-30 (LKB-Producktor, Stockholm, Sweden). Several authentic compounds were kindly donated from Prof. T. Hoshita, Hiroshima University, Hiroshima, Japan.

A gas chromatogram is shown in Fig 1. Peak A coincided with the DMES ether of ethyl cholate added as the internal standard. The retention time of peak B, C, D and E relative to the DMES ether of ethyl cholate was 1.88, 2.61, 3.50, 3.80 respectively. Peak C coincided with the DMES ether of 27-nor-5ß-cholestane-3α,7α, 12α,24,25-pentol and peak D was the DMES ether of 5ß-bufol.

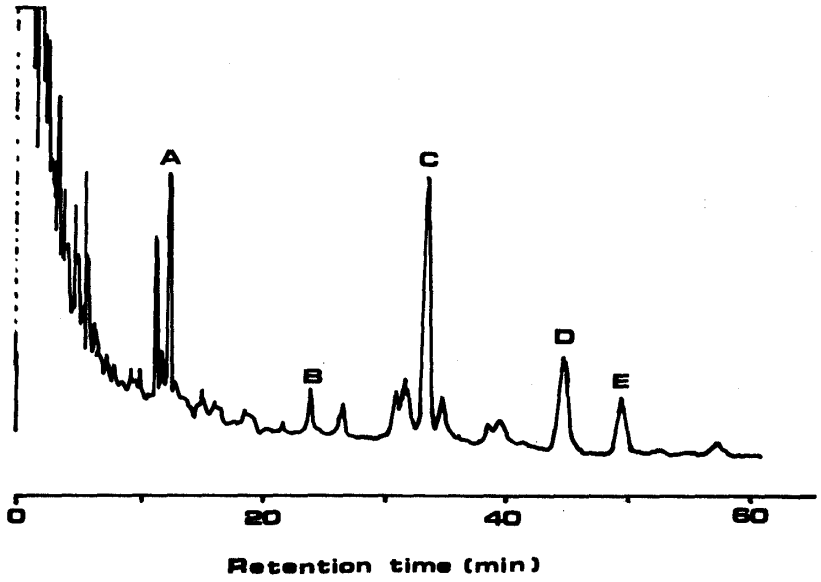

Fig 1. Gas Chromatogram

The retention time and the mass spectrum of peak B (Fig 2.) were identical to those of authentic 27-deoxy-5ß-cyprinol. Molecular ion (M;780) was not observed, and diagnostic side-chain fragmentation was poor as described for the trimethylsilyl

ether of this compound.[6] One series of fragments at m/z 572, 469 and 365 was formed by the consecutive loss of two to four molecules of dimethylethylsilanol (DMESOH), 104 amu (M-104 x n or M-104 x n + H). The other series of fragments at m/z 461, 357 and 253 was derived from the loss of an entire side-chain and one to three DMESOH (M -215-104 x n).

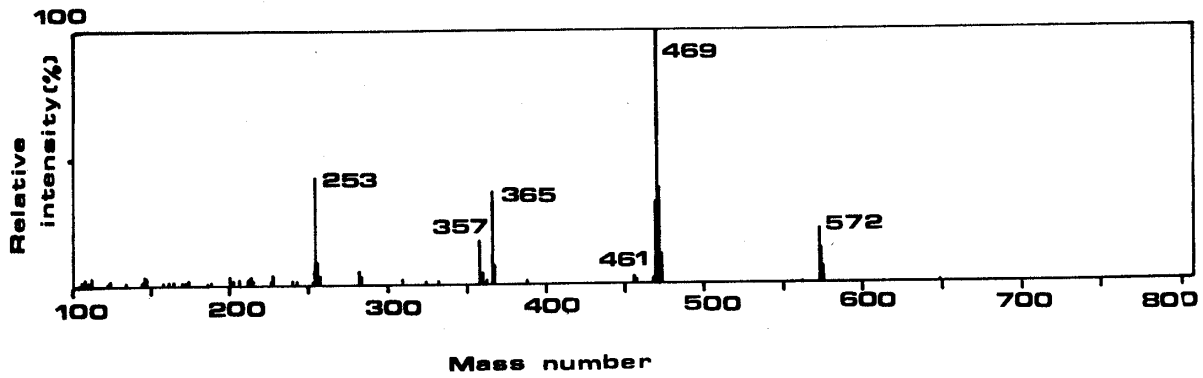

Fig 2. Mass Spectrum of Peak B (27-Deoxy-5ß-Cyprinol)

The mass spectrum of peak E is shown in Fig 3. Although molecular ion (M; 882) was not observed, two series of fragments appeared, one at m/z 778, 674, 571, 467 and 363, and the other at m/z 853, 749, 645 and 541. The former series was resulted from the consecutive loss of one to five molecules of DMESOH (M-104 x n or M-104 x n + H). The latter series was the result of the loss of the ethyl group and the consecutive loss of zero to three molecules of DMESOH (M-29-104 x n). The fragments seen at m/z 357 and 253 were derived from the loss of an entire side-chain and two or three DMESOH in the steroid nucleus (M-317-104 x n). The latter was detected as the base peak. The mass spectrum and the retention time of this compound were identical to those of authentic 5ß-cyprinol.

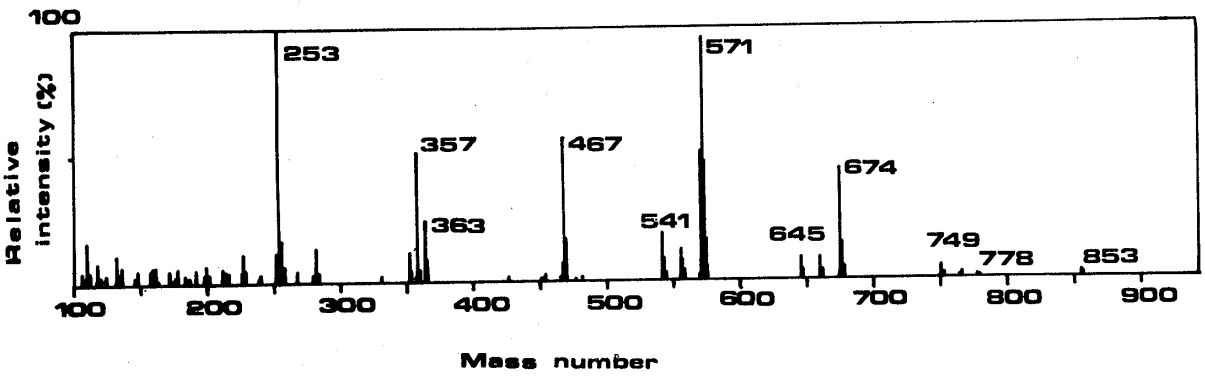

Fig 3. Mass Spectrum of Peak E (5ß-Cyprinol)

Side-chain cleavage is usually attributed to the mitochondrial 26-hydroxylation,[7] but compensatory microsomal 25-hydroxylation takes place under certain circumstances.[8] The two bile alcohols found in the present study were hydroxylated at C-26 but not at C-25. The presence of not only 25- but 26-hydroxylated alcohols in cholestatic urine indicates that both hydroxylation are operable under these circumstances and hindrance of further degradation of the latter may also exist.

Further study of the urinary bile alcohols may reveal disturbance in subcellular functions of the liver and altered metabolism in the liver.

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