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MASS SPECTRAL FRAGMENTATION OF 3β-HYDROXYCHOL-5-EN-24-OIC ACID DERIVATIVES

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Abstract

Making preparation of several deuterium-labeled methyl ester acetates of 3β-hydroxychol-5-en-24-oic acid was carried out. The mass spectra of these compounds were determined in the energy of bombarding electron (70 eV). By using the deutero-derivatives, the mass fragmentation of 3β-hydroxychol-5-en-24-oic acid derivatives was confirmed.

INTRODUCTION

Recently, the studies of analysis by the instrument combined gas chromatography and mass spectrometry have been developed[1,2]. This method might be very significant on the studies of analysis of biosynthetic meterials such as steroids. Mitropoulos et al.[3] had proposed the pathway of the formation of bile acids based on the results with incubation of cholesterol with rat liver mitochondrial and particle free supernatant fractions in vitro, which showed the formation of 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, 3α, 6β-dihydroxy-5β-cholanic acid, chenodeoxycholic acid, and α- and β-muricholic acids. Ikawa et al.[4] also reported that lithocholic acid, chenodeoxycholic acid, β-muricholic acid and 3α, 6β-dihydroxycholanic acid had been biosynthesized from 3β-hydroxychol-5-en-24-oic acid and its derivatives with a similar method. This pathway was considered important with regard to animal, but not to human. However, Sjövall et al.[5,6] found 3β-hydroxychol-5-en-24-oic acid in the urine of infants with extrahepatic biliary atresia and in the plasma of a woman with recurrent cholestasis of pregnancy and, furthermore, Back et al.[7] identified it in the meconium of prenature and nature neonants. Although 3β-hydroxychol-5-en-24-oic acid might be an abnormal metabolite, the acid may be considered an important intermediate in the conversion of cholesterol to bile acids. The confirmation of the detailed mass spectral fragmentation of the acid

seems to be helpful in the studies of the biosynthetic pathway of bile acids from cholesterol.

MATERIALS AND METHODS

1) *Preparation of 3β-Hydroxychol-5-en-24-oic Acid Derivatives.*

3β-Hydroxychol-5-en-24-oic acid derivatives used in this work were prepared from the commercial hyodeoxycholic acid (E. Merck) via methyl 3β-chlorochol-5-en-24-oate.

a) *Methyl 3β-acetoxychol-5-en-24-oate.* A mixture of methyl 3β-chlorochol-5-en-24-oate (mp 117-118°C)[8] (1.0 g), silver acetate (10 g) and acetic acid (50 ml) was refluxed on an oil bath for 3 hr. Insoluble inorganic solid was filtered off and washed with a small amount of acetic acid. The filterate was diluted with cold water and extracted with ether. The extract was washed with water and dried on anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residual solid was crystallized from ethyl acetate to give 3β-acetoxy derivative as colorless needles, mp 157-159°C (lit. mp 155-156°C[8]); yield, 0.8 g.

b) *3β-Hydroxychol-5-en-24-oic acid.* A mixture of previous acetate derivative (1.0 g), 4N-potassium hydroxide solution (10 ml) and methanol (10 ml) was refluxed on a water bath for 1 hr. After cooling, the reaction mixture was diluted with a small amount of water and acidified with 2N-hydrochloric acid. The separate solid was extracted with ether, the ethereal extract was washed with water and dried on anhydrous sodium sulfate. The solvent was evaporated and the residual solid was crystallized from methanol to give crystals as colorless needles, mp 235-236°C (lit. mp 235-236°C[8]); yield, 0.8 g.

c) *General method of methyl esterification.* The diazomethane in ether was added to the solution of previous cholenoic acid and allowed to stand at room temperature for 1 hr. The solvent was evaporated and the residual solid was crystallized from methanol to give crystals.

d) *General method of deuteromethyl esterification.* Deuteromethyl esterification was carried out by adding the diazomethane collected in dioxane containing a small amount of deuterium oxide (E. Merck, above 99%) to the solution of previous acid in dioxane, which contains a small amount of deuterium oxide. The percentage of the deuterium in the methyl ester group was 85% or more.

e) *General method of acetylation.* A mixture of methyl ester deriva-

tive and acetic anhydride was refluxed on an oil bath for 2 hr. After cooling, the reaction mixture was poured into ice-water. The separate solid was collected, washed with water and crystallized from methanol to give crystals.

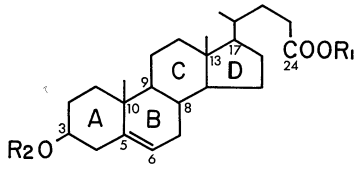
f) *General method of deuteroacetylation.* Deuteroacetic anhydride (E. Merck, above 99%) instead of acetic anhydride was used and the acetylation was carried out as described in the above "e".

2) *Purification.* The purification of these compounds used in this work was carried out by column chromatography on Silica Gel (E. Merck, Kieselgel type 60, 230 mesh) in refreshed chloroform. The check of purity was made by gas chromatography, using a Shimadzu gas chromatograph model 4BPTF (column: 0.75% SE-52, 4 mm×2 m, carrier gas: N_2 (flow rate: 88 ml/min), temperature: 230°C) and TLC on Silica Gel (E. Merck, Kieselgel H, 0.25 mm thickness) employing isooctane, ethyl acetate and acetic acid (20 : 40 : 1, by vol.) as developer.

3) *Measurement of the mass spectra.* The mass spectra of the compounds used in this work were measured with a Hitachi mass spectrometer model RMU-6MG, using the direct insertion probe, electron impact energy, 70 eV; temperature, 220°C.

RESULTS

The structures of chol-5-en-24-oic acid derivatives used in this work are shown in Fig. 1. These derivatives were once purified with



I	R_1=CH_3	R_2=H
II	R_1=CH_3	R_2=$COCH_3$
III	R_1=CH_3	R_2=$COCD_3$
IV	R_1=CD_3	R_2=H
V	R_1=CD_3	R_2=$COCH_3$
VI	R_1=CD_3	R_2=$COCD_3$

Fig. 1. The chemical structures of 3-oxygenated chol-5-en-24-oic acid derivatives used in this work.

Silica Gel column chromatography and applied to the measurement of the mass spectrometry. The mass spectra of I and II observed in the condition are shown in Fig. 2A and 2B, illustrating in line diagram above m/e 200. In the mass spectral patterns of I-VI, IV was very similar to I, and those of III, V and VI were also similar to that of II. The molecular ion of I presented at m/e 388 and that of IV shifted to higher

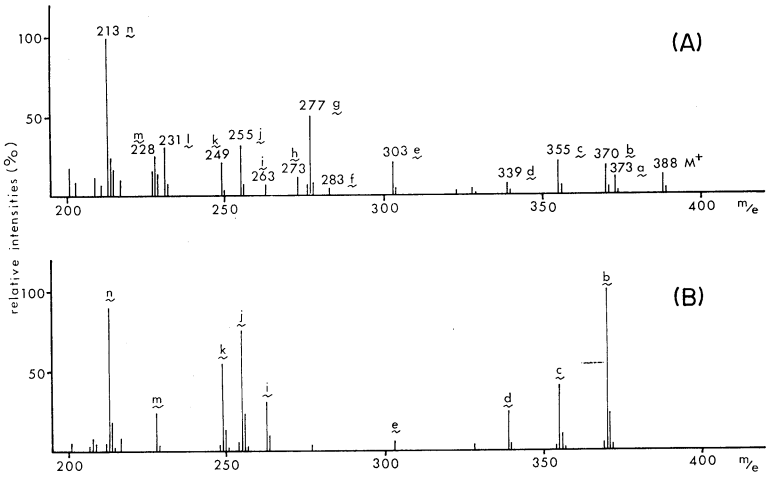


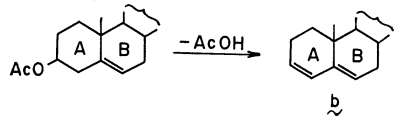
Fig. 2. The mass spectra of methyl 3β-hydroxychol-5-en-24-oate (I): A and methyl 3β-acetoxychol-5-en-24-oate (II): B, measuring with a Hitachi mass spectrometer model RMU-6MG (direct insertion probe, electron impact energy: 70 eV, temperature: 220°C).

mass field at m/e 391. The other fragment ions of I exhibited at m/e 373, 370, 355, 339, 303, 283, 277, 263, 255, 249, 231, 228, and 213, and among them the ion peaks at m/e 373, 370, 355, 303, 277, 263 and 249 in the spectrum of IV showed the shift to higher mass field. Therefore, these fragment ions must have possessed the deuteromethyl ester group at C_{24}-position. All of the mass spectral patterns of acetate and deuteroacetate derivatives (II, III, V and VI) resembled one another. Moreover, their molecular ions were not detectable. The mass fragmentation patterns of these compounds containing the hydroxyl or acetoxyl group at C_3-position were very close with the exception of the fragment ions of acetate at m/e 277 and 273 which can be distinctly observed in the spectrum of I. The fragment ions of II and III at m/e 370, 355, 263 and 249 shifted in the spectra of V and VI, yielding the same pattern as observed in I and IV.

DISCUSSION

With the exception of 3β-acetoxy-5-en-steroids, generally, the unsaturated steroids exhibit an ion corresponding to the molecular ion[9]. Therefore, for 3β-acetoxyl and deuteroacetoxyl derivatives (II, III, V and VI), the ions at the highest mass field correspond to those after loss of acetic acid and deuteroacetic acid from the molecule and the peaks are

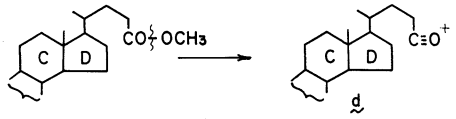
observed at m/e 370 and 373, respectively. But in the case of I and IV containing the hydroxyl group at C_3-position, the ions containing the C_3-oxygen are observed at m/e 388 and 391, respectively, and they are the molecular ions of those derivatives. The structures of these ions eliminated acetic acid from the molecule are deducible as 3, 5-diene type because of the presence of the double bond between C_5 and C_6 on the original molecule (Fig. 3). The ions exhibiting the mass shifts in the



b

Fig. 3. The ion structure (b) resulting from the original molecule.

spectra of compounds I and IV (in Fig. 1, peaks a, b, c, e, g, i, k) are apparently considered as the ions containing the side chain at C_{17}-position. In these ion peaks a (m/e 373) and c (m/e 355) in the spectra might be produced by the loss of tertiary methyl group at C_{10} or C_{13} from the peak M (m/e 388) and the peak b (m/e 370), respectively. In the acetate derivative (Fig. 2B) the peak corresponding to the peak a in the spectrum of I is not detectable, but the peak c is observed resulting from the peak b. The peak d at m/e 339 does not contain the deuteromethly group in every spectra, exhibiting no mass shifts. Therefore, the peak d is produced by the loss of methoxyl group (31 mass units) from the peak b (Fig. 4), which is a situation similar to that often observed in the spectra of the methyl ester of fatty acids[10]. The peak



d

Fig. 4. The elimination of methoxyl group from the methyl ester and the production of the ion d.

e at m/e 303 is observed in the mass shifts in the spectra of deuteromethyl esters (IV, V and VI). In addition, the mass reduction from the peak M or b amounts to 85 or 67 mass units, respectively, which is postulated to result from the decomposition of the nucleus (B-ring) of the molecule. The ion is further fragmented by the elimination of methyl acetylene ion and by the loss of hydrogen (loss of 40 mass units) to

give the peak i (m/e 263), giving rise to the mass shift in the spectra of the deuteromethyl esters (Fig. 5). The peak f at m/e 283 in the

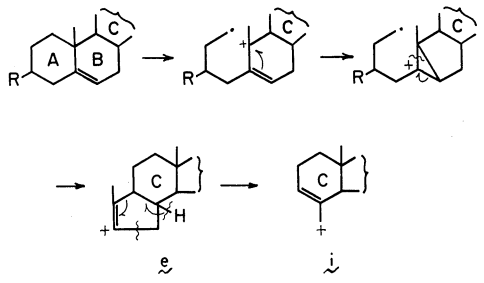


Fig. 5. The mechanism of the production of the ions e and i.

spectra of every compounds is faintly observable but there occurs no mass shift. This peak might have resulted from the peaks m, b, or d by the γ-cleavage of the side chain. However, the fragment ions considered to be produced by α- and β-cleavage from the ions can scarcely be observed (Fig. 6). In the fatty acids McLafferty rearrangement is

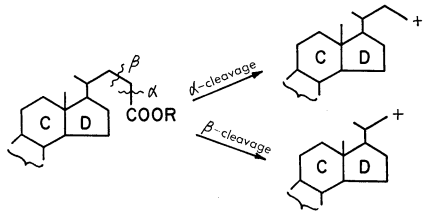


Fig. 6. The possible ions arising from the α- and β-cleavage.

often observed, if the rearrangement occurs in these samples analysed, the ion peak at m/e 296, for example, should be observable. In these samples analysed, such peaks cannot be observed. The peak g at m/e 277 which exhibits the mass shifts is apparently observed in the spectra of I and IV but scarcely in those of acetates. The ion peak might be considered to result from the mechanism illustrated in Fig. 7. The peak h at m/e 273 is observed in the spectra of I and IV, exhibiting the mass shifts, but not observed in those of acetates. This ion peak (h) might be produced by the elimination of the side chain at C_{17}-position, containing oxgen group at C_3-position. From this fragment ion, the loss of water gives rise to the peak j (m/e 255), which has also resulted from

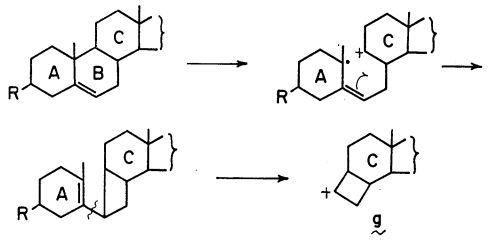


Fig. 7. The mechanism of the production of the ion g.

the peak b by the elimination of the side chain and the further cleavage of D-ring of skeleton gives rise to the peak l (m/e 231). The peak j (m/e 255) is characteristic in the type of dioxgenated cholanoic or monooxygenated cholenoic acids. The peak m at m/e 228 is produced from the peak j by the cleavage of D-ring. The peak n at m/e 213 has resulted from the peaks l and m, and it is due to the ready elimination of acetic acid from the original molecule not to be observed at the peak (m) in the spectra of acetates. The peak k at m/e 249, showing the mass shifts, might have resulted from the retro-Diels Alder reaction on the B-ring and from the loss of hydrogen at C_8-position. The sequential mechanism of such phenomena is as described in Fig. 8. From the sequential mechanism of each ion peak, the mode of the fragmentation

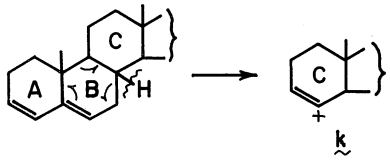


Fig. 8. The ion k resulting from the retro-Diels Alder type reaction on the B-ring and from the loss of hydrogen.

on methyl 3β-hydroxychol-5-en-24-oate might be demonstrated as in Fig. 9.

The mass spectral pattern differed slightly from the conditions observed by the following: electron impact energy, temperature and so on. The principal ion peaks, however, can always be observed under any conditions. Thus, the fragmentation pattern of 3-oxygenated chol-5-en-24-oic acid derivatives was confirmed by using deuterium-labeled methyl ester acetates.

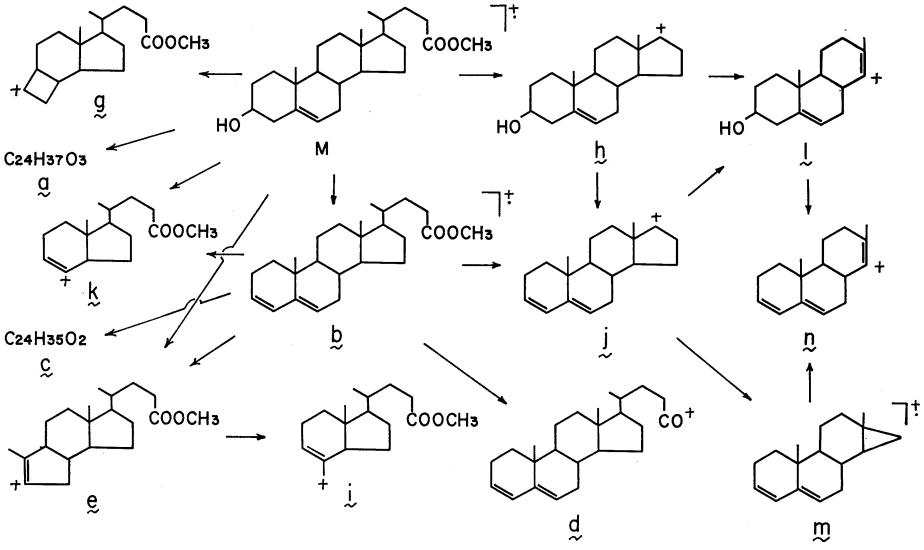


Fig. 9. The mode of the fragmentation of methyl 3β-hydroxy-chol-5-en-24-oate (I).

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