Basic Bile Acids. V. On the Syntheses and Properties of Basic Bile Acids and Their Derivatives

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In the previous papers of the series,[1] the syntheses and properties of the several basic bile acids and their derivatives have been reported. The present paper will summarize the syntheses and properties of the basic bile acids, including new derivatives.

3-Amino Derivatives

In the catalytic reduction of the oxime of 3-keto bile acid, it was found that the configurations of the amino groups formed are identical with those of the hydroxyl groups formed in the case of 3-keto steroids.[2] That is, in the catalytic reduction of the oxime in an acidic medium, a 3β-amino derivative is formed, with a speedy absorption of hydrogen, as in the case of 3-keto bile acids. The configuration of this product has been determined by a comparison of its properties with those of the 3β-amino derivative prepared by Walden's inversion of 3α-tosylate with liquid ammonia. Moreover, in the catalytic reduction of the oxime in a neutral medium, a 3α-amino derivative is formed, with a slow absorption of hydrogen, contrary to that formed in the case of an acidic medium.

On the other hand, in the metallic reduction of the oxime, a 3α-amino derivative is obtained which has the same configuration as the hydroxyl group formed in the case of the reduction of 3-keto steroids.[2]

These results agree well with the facts obtained previously from the reduction of the oxime of 5β-cholestan-3-one by Shoppee et al.[3,4]

The reaction of the 3-amino derivatives with nitrous acid was also examined. It has generally been confirmed that the course of the deamination of cyclohexylamines and decalylamines is conformationally specific[5,6]: if the amino group is equatorial in the most stable conformation of the molecule, deamination gives an alcohol of the same configuration; if it is axial, elimination and inversion occur.

In basic steroids, the same result has been reported by several workers.[4,7] When the reaction is examined with respect to the synthesized 3-aminocholanic acids, the following results are obtained:

3α-Amino derivative (C_3–NH_2: e) $\rightarrow$
3α-Hydroxy derivative (C_3–OH: e)

3β-Amino derivative (C_3–NH_2: a) $\rightarrow$
3β-Hydroxy derivative (C_3–OH: a)
+ 3-Cholenic acid

These results coincide with the experiential rule described above for the deamination of alicyclic and steroidal amines.

The additive charactor of the molecular rotation has generally been proposed to exist in the steroid field.[8] When the molecular rotations of methyl 3-acetamidocholanates are calculated from that of the 3-acetamido-5β-cholestane prepared by Shoppee et al.,[3] the results are as follows:

M_D of 3α-acetamido-5β-cholestane $= +206$ (a)

M_D of 3β-acetamido-5β-cholestane $= +112$ (b)

M_D of 5β-cholestane $= +97$ (c)

(a) $-$ (c) $= (+206) - (+97) = +109$

ΔM_D of 3α-$NHCOCH_3$ in the 5β series

(b) $-$ (c) $= (+112) - (+97) = +15$

ΔM_D of 3β-$NHCOCH_3$ in the 5β series

M_D of methyl cholanate $= +80$ (d)

(d) + (ΔM_D of 3α-$NHCOCH_3$ in 5β series)
$= (+80) + (+109) = +189$

1) Y. Satoh and A. Hagitani, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **80**, 1310 (1959): Y. Satoh, ibid., **84**, 829 (1963); **85**, 54 (1964).

2) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York (1949), p. 99; W. Klyne, "The Chemistry of the Steroids," Methuen & Co. Ltd., London (1957), p. 80; D. H. R. Barton and C. H Robinson, *J. Chem. Soc.*, **1954**, 3045.

3) J. H. Pierce, C. W. Shoppee and G. H. R. Summers, ibid., **1955**, 690; J. H, Pierce, C. W. Shoppee, H. C. Richards, R. J. Stephenson and G. H. R. Summers, ibid., **1955**, 694; H. C. Richards, C. W. Shoppee, J. C. P. Sly and G. H. R. Summers, ibid., **1956**, 1054; C. W. Shoppee, D. E. Evans, H. C. Richards and G. H. R. Summers, ibid., **1956**, 1649.

4) C. W. Shoppee, D. E. Evans and G. H. R. Summers, ibid., **1957**, 97; C. W. Shoppee, R. J. W. Cremlyn, D. E. Evans and G. H. R. Summers, ibid., **1957**, 4364.

5) A. K. Bose, *Experimentia*, **9**, 256 (1953).

6) J. A. Mills, *J. Chem. Soc.*, **1953**, 260.

7) W. G. Dauben, R. C. Tweit and C. Mannerskantz, *J. Am. Chem. Soc.*, **76**, 4420 (1954).

8) D. H. R. Barton, *J. Chem. Soc.*, **1945**, 813; **1946**, 512; **1946**, 1116.

calculated value of M_D for methyl 3α-acetamidocholanate

(d) + (ΔM_D of 3β-NHCOCH$_3$ in 5β series)
= (+80) + (+15) = +95

Calculated value of M_D for methyl 3β-acetamidocholanate

Observed values:

Methyl 3α-acetamidocholanate = +177

Methyl 3β-acetamidocholanate = +95

From the good agreement between the calculated values and the observed values, the configurations previously deduced from each methyl ester acetate seem indeed to be correct; almost no effect of the side chain in C_{17} is present, and, in these substances, the additive character of the molecular rotation holds.

7-Amino Derivatives

The 7-position of cholanic acid suffers a larger steric hindrance than that in the 3-position, considered not only from the molecular model but from the following facts. In the catalytic reduction of 7-keto bile acid, it is well known that the 7α-hydroxyl group (C_7–OH: a) is mainly formed either in an acidic or in a neutral medium, in contrast to the behavior of the 3-keto bile acids. That is, in the catalytic reduction of 3α-hydroxy-7-ketocholanic acid, the main product is chenodeoxycholic acid (C_7–OH: a) rather than ursodeoxycholic acid (C_7–OH: e), which has a stable equatorial hydroxyl group in the 7-position.[9,10] Moreover, in the reduction of that acid with sodium ethoxide, the results are similar to those in the case of catalytic reduction.[9] On the basis of these data and the results of catalytic reduction in an acidic medium for decalones, their oximes[11,12] and the oxime of 3-keto 5β-steroid,[3] it seems reasonable to assume that the product obtained from the oxime of 7-ketocholanic acid by catalytic reduction in an acidic medium is 7α-aminocholanic acid.

In order to determine more precisely the configuration of this product, a reaction with nitrous acid was attempted with the methyl ester of 7-amino acid similar to that described above for the 3-amino derivatives. As has been described in the previous paper,[1] an unsaturated compound is obtained from the methyl 7-aminocholanate in this reaction. The 7-amino group formed by the catalytic reduction of the oxime, therefore, may be assigned the α-configuration (axial).

Furthermore, the unsaturated compound formed by the reaction of methyl 7α-aminocholanate with nitrous acid is assumed to be 7-cholenate. This assumption is based on the consideration that, although two axial hydrogens at the C_6- and C_8-positions, are present in this compound, elimination between C_8–H and C_7–NH_2 may be expected according to the Saytzeff rule. A similar situation has also been reported in the case of deamination for 6β-amino-5α-cholestane.[4]

In order to confirm the structure of the unsaturated product in this reaction, it was desirable to examine the propriety of the structure presumed above by the application of the additive character in molecular rotation:

ΔM_D of C_7-double bond = +119[2] (a)

M_D of methyl cholanate = +80[13] (b)

(a) + (b) = (+119) + (+80) = +199 Calculated value of M_D for methyl 7-cholenate

Observed value: +195

This good agreement between calculated and observed values gives further confirmation that the product from the reaction with nitrous acid and the 7-amino derivative is methyl 7-cholenate, and that the reduction product of the oxime in an acidic medium is 7α-aminocholanic acid.

A number of years ago attempts by several workers to prepare the 7β-hydroxy derivative from 7-keto bile acid were unsuccessful.[9,10,14] It was reported by Hoshino et al.[15] in 1954, however, that ursodeoxycholic acid could be obtained in a good yield by reduction with sodium and *n*-propanol. In order to prepare the 7β-amino derivative from the oxime of 7-ketocholanic acid, therefore, this procedure was used. The results showed that the methyl ester acetate of the product is different from the 7α-amino epimer (obtained from the oxime by catalytic reduction) in melting point, infrared spectrogram, thin-layer chromatogram and specific rotation, while their elementary analyses are the same. It might, therefore, be concluded that the product is 7β-aminocholanic acid.

12-Amino Derivative

The 12-position of cholanic acid suffers a larger steric hindrance than the 3- or 7-position. This can easily be understood from the fact that the acetylation and oxidation of the 7α-hydroxyl group are more easily accomplished

9) S. Miyaji, *Z. physiol. Chem.*, **250**, 31 (1932).
10) T. Iwasaki, ibid., **244**, 191 (1936).
11) H. D. Orhloff, *Chem. Revs.*, **54**, 347 (1954).
12) D. H. R. Barton, *J. Chem. Soc.*, **1954**, 1027.
13) M. Z. Nazer and C. H. Issidorides, *J. Org. Chem.*, **26**, 839 (1961).
14) M. Tsukamoto, *Biochem.*, **32**, 461 (1940).
15) T. Kanazawa, A. Shimazaki, T. Satoh and T. Hoshino, *Proc. Japan Acad.*, **30**, 391 (1954).

than in the case of the 12α-hydroxyl group (C_{12}–OH : a).[16] Moreover, in the reductions of 12-ketocholanic acid[17] and 12-hydroxyiminocholan,[18] 12α-epimers with a thermodynamically-unstable bond are predominantly formed as a result of the steric hindrances, while under the same conditions, unhindered ketone gives an equatorial hydroxyl group. Also, in the reduction of the respective oxime it has been found in the present work that the reaction rates have the same relationship.

When the hydroxyimino group at such a highly-hindered position was reduced with sodium and *n*-propanol, as has been described in the case of the 7-position, the product obtained was the same as that of the catalytic reduction in an acidic medium. Since this position has such a large steric hindrance compared with the 7-position, it seems reasonable to assume, from the results, that the factor in determining the direction of the reaction is concerned rather with the steric approach control than with the product development control.

The configuration of the amino group in the basic bile acid prepared from the 12-hydroxyimino derivative by catalytic reduction is presumed to the axial by deduction from the cases of the 3- and 7-derivatives. When this substance is allowed to react with nitrous acid, a product which gives a positive tetranitromethane test and which has an absorption band at 1635 cm^{-1} in its infrared spectrum is obtained. From these results, it may be inferred that elimination has occurred in this reaction. On the basis of these data, it was concluded that the basic derivative obtained from the oxime is 12α-aminocholanic acid (C_{12}–NH_2 : a).

Methyl 3α-Acetoxy-12α-acetamidocholanate

The molecular rotation of this substance is as follows:

M_D of methyl 12α-acetamidocholanate $= +397$ (a)

(a) $-$ (M_D of methyl cholanate)
$= (+397) - (+80) = +317$

ΔM_D of 12α-$NHCOCH_3$ in 5β series (b)

ΔM_D of 3α-$OCOCH_3$ in 5β series $= +114$ (c)

Calculated value of M_D for methyl 3α-acetoxy-12α-acetamidocholanate
$= (M_D$ of methyl cholanate$) + (b) + (c)$
$= (+80) + (+317) + (+114) = +511$

Observed value : $+547$

The observed value is in fairly good agreement with the calculated value. The small difference may come from the molecule's being subject to a little strain due to the α-orientated two groups.

On the Leuckart-Wallach Reaction of Methyl Ketocholanate

This reaction had already been attempted by Hadáček et al. for methyl 7-keto-3α, 12α-dihydroxycholanate and methyl 3, 7, 12-triketocholanate,[19] but, as their results seemed to be inadequate for a determination of the structure, it was reinvestigated. They reported that the configuration of the formamido groups in the products is equatorial. In view of the following points, however, the determination of this configuration seems to contain an error.

1) The configuration of the formamido group formed was determined by Hadáček et al. by means of the formation of a free amino group by hydrolysis. That is, the configuration was determined only on the basis that the melting point of this amino derivative was virtually identical with that of the 7-amino-3α, 12α-dihydroxycholanic acid prepared from the 7-hydroxyimino derivative with sodium and isoamyl alcohol by Webb et al.[20] According to Barton, the reduction of a ketoxime with sodium and alcohol gives an equatorial amino group predominantly.[12] Then, on the basis of the Barton report, Hadáček et al. determined that the configuration of the amino group in the product formed by Webb et al. has a 7β-orientation (C_7–NH_2 : e), and, since the melting points of both this 7β-amino derivative and their product derived from formamido derivative by hydrolysis were almost the same, they concluded that the product of the Leuckart-Wallach reaction of methyl 7-keto-3α, 12α-dihydroxycholanate might be methyl 7β-amino-3α, 12α-dihydroxycholanate. However, Barton's theory seems inadequate to determine the configuration of the 7-position in bile acids, because the reaction of this position gives a result different from that of the 3-position, as has been mentioned above for the catalytic reduction of the 7-ketone.

16) C. W. Shoppee, "Chemistry of the Steroids," Butterworths Sci. Publ., London (1958), p. 83.
17) J. W. Huffman, D. M. Alabran and T. W. Bethea, *J. Org. Chem.*, **27**, 3382 (1962); J. W. Huffman, D. M. Alabran, T. W. Bethea and A. C. Ruggles, ibid., **29**, 2963 (1964).
18) M. Alauddin and M. Martin-Smith, ibid., **28**, 886 (1963).
19) J. Hadáček, and B. Čajánek, *Publs. Fac. Sci. Univ. Masaryk*, **395**, 259 (1958).
20) S. P. James, F. Smith and M. Webb, *J. Chem. Soc.*, **1946**, 665; A. S. Jones, M. Webb and F. Smith, ibid., **1946**, 2164.

2) They carried out an elementary analysis and measured the melting point of this substance, but they did not report its optical rotatory power.

Hadáček et al. determined, as has been described above, that the product of the Leuckart-Wallach reaction of methyl 7-keto-3α, 12α-dihydroxycholanate is the 7β-amino derivative, with an equatorial amino group, they did this on the agreement of the basis of the melting point with the literature.[20] They then deduced that the reaction gave an equatorial amino group in all other positions, also. They concluded, therefore, that the product of this reaction of 3, 7, 12-triketocholanic acid is 3α, 7β, 12β-triaminocholanic acid (C_3–, C_7– and C_{12}–NH_2: e). For the reasons pointed out above, however, the present author cannot accept this conclusion. From the experimental results[1] and the consideration of them in the present work, it may be firmly concluded that the configuration of the amino group formed from the ketone of bile acid in this reaction is axial, and, at the same time, that the presumed configuration proposed by Hadáček et al.[19] should be corrected.

Thin-layer Chromatography of Methyl Acetamidocholanates

This chromatography was carried out using the technique of Hara[21] for the methyl esters of bile acids; results are listed in Table I.

On the relationship between the configuration of substituted groups and adsorbability, it has been reported that axial epimers are generally adsorbed more weakly than equatorial epimers.[22]

It would be interesting to compare this general rule with the reults obtained in the present work for acetamido derivatives. In the 3-substituted compounds, the adsorbability of axial-bonded derivatives (β) is larger than that of the equatorial-bonded derivative (α), while in the 7-substituted compounds that relation is reversed (e>a). Although this relation seems to be inconsistent with the general rule, this inconsistency may be accounted for by considering the overall shape of the molecule. The 3α(e)- and 7α(a)-bonds are oriented almost parallel to the axis of the steroidal nucleus; in other words, these bonds correspond to the axial bonds in the cyclohexane ring. Therefore, the adsorbability of 3α- and 7α-derivatives is smaller than that of 3β- and 7β-derivatives, and, consequently, the former moves more quickly than the later in thin-layer chromatography. In the case of the thin-layer chromatography of both epimer of methyl 3-acetoxycholanate, the same relations are also observed (Solvent, benzene/pet.-ether: 3α-OAc (e), R_f 0.74; 3β-OAc (a), R_f 0.65).

TABLE I. R_f VALUES OF METHYL ACETAMIDOCHOLANATES IN THIN-LAYER CHROMATOGRAPHY

Compound	Configuration of acetamido group	R_f Value
3α-Derivative(m)	e	0.36
3β-Derivative(c)	a	0.26
3β-Derivative(w)	a	0.26
3β-Derivative(f)	a	0.26
7α-Derivative(c)	a	0.62
7α-Derivative(f)	a	0.62
7β-Derivative(m)	e	0.47
12α-Derivative(c)	a	0.81
12α-Derivative(f)	a	0.81
12α-Derivative(m)	a	0.81

m, metallic reduction; c, catalytic reduction; w, Walden's inversion of the tosylate; f, Leuckart-Wallach's reaction

Adsorbent, Wako-gel B-5; thickness of layer, 0.5 mm.; activation temperature, 130°C/1 hr.; solvent system, benzene/ether (8/2, v/v); indicator, concentrated sulfuric acid

21) S. Hara, *Kagaku-no-Ryoiki*, 17, 196 (1963).

On the Infrared Spectrography of Mathyl Acetamidocholanates

Some of the characteristic bands of individual methyl acetamidocholanates are listed, with their wave numbers, in Table II.

In an associated acyl amide derivative, the stretching vibration of C=O has been observed to shift to a lower wave number, the bending vibration of N–H has been observed to shift to a higher wave number, and the stretching vibration of N–H and unknown vibration appear above 3000 cm^{-1}.[23]

Each absorption band in a chloroform solution of these methyl acetamidocholanates, as is shown in Table II-I, is almost identical with the standard value for the non-associated second-amide type, while the values in the cases of the KBr disk method are observed to shift to longer wavelengths than those values for ν_{N-H} and $\nu_{C=O}$ and to shorter wavelength than those values for δ_{N-H}. In view of the above facts, the conclusion may be drawn that these compounds may exist in the non-associated state in such a solution and in the associated state in the KBr disk.

Moreover, with reference to Table II-Ia, in the 7α-acetamido derivative only one absorption band above 3000 cm^{-1} was observed, while

22) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publ. Corp., New York (1959), p. 14.

23) K. Nakanishi, "IR Absorption Spectroscopy: Practical," Nanko-do, Tokyo (1960), p. 51.

TABLE II. SOME OF THE CHARACTERISTIC ABSORPTION BANDS OF METHYL ACETAMIDOCHOLANATES IN IR SPECTRA (wave number)

Compound	ν_{N-H}	Unknown vibration	$\nu_{C=O}$	$\nu_{C=O}$	δ_{N-H}
Ia KBr disk method*1					
3α-Derivative	3238	3075	1725	1638	1558
3β-Derivative	3382 3262	3078	1726	1620	1570
7α-Derivative	3373	—	1735	1639	1539
7β-Derivative	3260	3076	1745	1638	1557
12α-Derivative	3265	3075	1735	1635	1553
Ib KBr disk method*2					
3α-Derivative	3279 3280*3	3075 3076*3	1735 1736*3	1634 1636*3	1554 1556*3
3β-Derivative	3382	—	1743	1641	1536
7α-Derivative	3378	—	1738	1640	1539
7β-Derivative	3260	3080	1745	1634	1558
12α-Derivative	3273 3275*3	3080 3079*3	1737 1740*3	1637 1638*3	1557 1554*3
II Chloroform solution					
3α-Derivative	3410	—	1717	1653	1512
3β-Derivative	3410	—	1717	1652	1514
7α-Derivative	3440	—	1719	1652	1517
7β-Derivative	3435	—	1720	1653	1518
12α-Derivative	3440	—	1725	1657	1506

*1 The sample was prepared by drying at 25°C/760 mmHg/24 hr.
*2 The sample was prepared by drying at 105—110°C/1 mmHg/6 hr.
*3 The sample was prepared by drying at 160—165°C/1 mmHg/8 hr.

the other derivatives show two bands. However, one of the two bands of the 3β-acetamido derivative above 3000 cm^{-1} disappeared for the sample in Table II-Ib. In a chloroform solution the absorption bands above 3000 cm^{-1} of all derivatives are shifted to about 3400 cm^{-1}, where the values are identical with those of the stretching vibration of N–H for the non-associated secondary amide type, as has been described above. A band at 3070 cm^{-1}, for which the assignment has not yet been distinctly made, was not observed for the 3β-derivative when it was dried at 105—110°C under 1 mmHg; on the other hand, in the 3α- and 12α-acetamido derivatives, the band does not disappear even at 160—165°C under 1 mmHg. Moreover, these compounds with an absorption band at about 3070 cm^{-1} (e. g., methyl esters of 3α-, 7β- and 12α-acetamidocholanic acids) also possess an absorption band near 720 cm^{-1} with a broad shape. (This band is called an amide V band and has been reported to possess only a secondary associated-amide type.[24]) Though the absorption bands of these derivatives are considered to shift as a result of their crystalline structure, the shift may be smaller than that arising from association. On the basis of the facts that, in Table II-Ib, considerable shifts of the absorption bands of ν_{N-H}, $\nu_{C=O}$ and δ_{N-H} are observed and that the absorption band near 720 cm^{-1} is presented, it seems reasonable to assume that the 3α-, 7β- and 12α-acetamido derivatives exist in the associated states in the KBr disk.

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24) C. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London (1958), p. 205.