ON THE COMPONENTS OF BULL-FROG BILE (RANA CATESBIANA SHAW)

VI. CHEMICAL STRUCTURE OF α- AND β-TRIHYDROXY-HOMOCHOLENE

By MASAKIYO KURODA

(*From the Department of Biochemistry, Okayama Nutrist College, Okayama*)

Previously the author (*1*, *2*) isolated from bile of bull-frog three kinds of trihydroxyhomocholenes $C_{25}H_{42}O_3$. One of them melted at 177° and, upon catalytic hydrogenation, after absorbing 1 *M* of hydrogen it was converted to trihydroxyhomocholane of m.p. 185-6°. The latter was identified as 3 (α), 7 (α), 12 (α)-trihydroxyhomocholane, since the same substance could be obtained from cholic acid by Kolbe's electrolysis (*3*). Therefore this trihydroxyhomocholene was named α-trihydroxy-homocholene. One the other hand, at the catalytic hydrogenation of trihydroxyhomocholene of m.p. 238°, trihydroxyhomocholane of m.p. 199-201° was obtained after absorbing 1 *M* of hydrogen. It was oxidized with chromic acid, and triketohomocholane of m.p. 246-7° was obtained. The same triketohomocholane could be obtained from 3 (α), 7 (α), 12 (α)-trihydroxyhomocholane by chromic acid oxidation, and thus the triketohomocholene melting at 238° was named β-trihydroxyhomocholene.

The triketohomocholane was reduced by Huang-Minlon's method (*4*), and homocholane melting at 75° was obtained.

In this report the author describes on the position of the double bond and the steric configuration of three hydroxyl groups of α- and β-trihydroxyhomocholenes.

EXPERIMENTAL

Triketohomocholene—2 g. of α-trihydroxyhomocholene of m.p. 177° was dissolved in 50 ml. of glacial acetic acid, and 40 ml. of 5 per cent

chromic acid solution in glacial acetic acid was added dropwise in 30 minutes. After 1 hour the excess chromic acid was reduced with hyposulfite, and the solution was put into a bulk of water. The precipitate, after standing over night, was filtered, washed, dried, and crystallized from acetic acid. 1 g. of needle-shaped crystals melting at 242° was obtained, which absorbed bromine promptly and reduced permanganate solution.

$C_{25}H_{36}O_3$ calcd. C 78.08%, H 9.33%
found C 77.64%, H 8.95%

Two g. of β-trihydroxyhomocholene was similarly treated, and 1.2 g. of needle-shaped crystals melting at 240-2° were obtained, which absorbed bromine promptly, reduced permanganate solution and showed no depression of the melting point on admixture with the above described ketoderivative from α-trihydroxyhomocholene.

$C_{25}H_{36}O_3$ calcd. C 78.08%, H 9.33%
found C 77.54%, H 9.71%

Trihydroxyhomocholene Triacetate—0.5 g. of β-trihydroxyhomocholene was dissolved in 15 ml. of pyridine and 7.5 ml. of freshly distilled acetic anhydride were added. The solution was heated on a boiling water both for 24 hours and it was then put into a bulk of water. The precipitate was filtered, washed with water, dried, and crystallized from dilute methanol. 0.35 g. of flake-shaped crystals melting at 218° was obtained.

$C_{31}H_{48}O_6 1/2H_2O$ calcd. C 70.82%, H 9.39%
found C 70.87%, H 10.08%

3,7,12-Trihydroxy-14-keto-14 : 15-homocholanic Acid-15—0.3 g. of trihydroxyhomocholene triacetate of m.p. 218° was dissolved in 30 ml. of acetone and 10 per cent permanganate solution in acetone was added dropwise, until the color of permanganate no more discolored (about 20 ml. were necessary). The solution was then heated at 90° for 30 minutes, cooled and the excess permanganate was neutralized with hyposulfite. Water was added and the solution was concentrated to remove acetone. A small amount of precipitate (unchanged neutral substance) was filtered, and the filtrate was acidified with dilute hydrochloric acid. The precipitate was filtered, washed with water and dried.

Trials to crystallize it being failed, it was hydrolyzed with 10 per cent alcoholic caustic soda. The solution was diluted with water and evaporated to remove alcohol. Dilute hydrochloric acid was added, and the precipitate was filtered, washed with water, dried and crystallized from dilute alcohol. One-tenth g. of needle-shaped crystals melting at 99–101° was obtained.

$C_{25}H_{42}O_6$ calcd. C 68.45%, H 9.19%
found C 68.66%, H 8.53%

3,7,12-Trihydroxy-14-keto-14:15-homocholanic Acid-15 Monoxime—An alcoholic solution of 50 mg. of 3,7,12-trihydroxy-14-keto-14:15-homocholanic acid-15 was heated on a water bath with 20 mg. of hydroxylamine hydrochloride and 30 mg. of sodium acetate for 3 hours. The solution was put into a bulk of water, and the precipitate was filtered, washed with water, dried and crystallized from dilute alcohol. 20 mg. of needle-shaped crystals decomposing at 133° were obtained.

$C_{25}H_{43}O_6N$ calcd. N 3.08%
found N 3.19%

Trihydroxyhomocholene 3,7-Diacetate—200 mg. of α-trihydroxyhomocholene were dissolved in 10 ml. of glacial acetic acid, and 2 ml. of acetyl chloride were added. The mixture was shaken at room temperature for 2 days, and was put into a bulk of ice water. The precipitate was filtered, washed with water, dried and crystallized from dilute alcohol. 120 mg. of needle-shaped crystals of m.p. 180° were obtained.

$C_{29}H_{46}O_5$ calcd. C 73.37%, H 9.76%
found C 72.92%, H 9.89%

Similarly from 200 mg. of β-trihydroxyhomocholene, 100 mg. of plate-shaped crystals melting at 94° were obtained.

$C_{29}H_{46}O_5$ calcd. C 73.37%, H 9.76%
found C 73.67%, H 10.02%

3,7-Dihydroxy-12-ketohomocholene—100 mg. of trihydroxyhomocholene 3,7-diacetate melting at 94° were dissolved in 3 ml. of glacial acetic acid, and 1 ml. of 5 per cent chromic acid solution in glacial acetic acid was added drop by drop during 30 minutes. After the solution was stood

for 1 hour it was neutralized with hyposulfite and was put into a bulk of water. After standing over night, the precipitate was filtered, washed with water and dried. As the trial to crystallize it was failed, it was hydrolyzed with 10 per cent alcoholic caustic soda for 3 hours, diluted with water and concentrated to remove alcohol. Precipitate was formed, which was filtered, washed with water, dried and crystallized from dilute alcohol. 20 mg. of needle-shaped crystals of m.p. 197-8° were obtained.

$C_{25}H_{40}O_3.H_2O$ calcd. C 74.46%, H 10.62%
found C 73.84%, H 10.41%

Trihydroxyhomocholene 3,7-diacetate of m.p. 180° was similarly treated, but no crystalline product was obtained.

3 (α), 12 (α),-Dihydroxy-7-ketohomocholane——50 mg. of 3 (α), 7 (α), 12 (α)-trihydroxyhomocholane, which had been prepared from α-trihydroxyhomocholene and melted at 185-6° were dissolved in 5 ml. of alcohol and put into a three necked flask. A small amount of caustic soda solution and of bicarbonate solution was added, and 0.1 ml. of bromine solution, which had been prepared by dissolving 2 g. of bromine in 1 ml. of chloroform, was added drop by drop during 2 hours at 5-0° with stirring. The mixture was stirred for another 2 hours, then kept for 3 hours at room temperature, and neutralized with dilute hydrochloric acid. After being concentrated under reduced pressure, it was put into a bulk of water. The precipitate was filtered, washed with water, dried and crystallized from dilute alcohol. 20 mg. of needle-shaped crystals of m.p. 172° were obtained.

$C_{25}H_{42}O_3$ calcd. C 76.87%, H 10.83%
found C 76.64%, H 10.96%

Trihydroxyhomocholane of m.p. 199-201°, which had been obtained from β-trihydroxyhomocholene by catalytic hydrogenation, was similarly treated.

Needle-shaped crystals of m.p. 172° were obtained, which showed no depression of the melting point when mixed with the sample obtained from trihydroxyhomocholane of m.p. 186°.

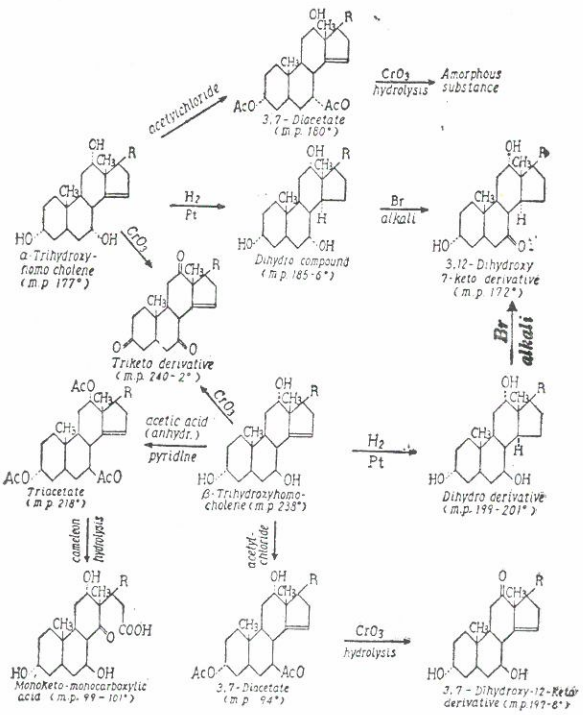
$C_{25}H_{42}O_3$ calcd. C 76.87%, H 10.83%
found C 76.95%, H 10.76%

3 (α), 12 (α)-Dihydroxy-7-ketohomocholane Monoxime—An alcoholic solution of 20 mg. of 3 (α), 12 (α)-Dihydroxy-7-ketohomocholane was heated on a water bath with 8 mg. of hydroxylamine hydrochloride and 12 mg. of sodium acetate for 3 hours, and then but into a bulk of water, dried and crystallized from dilute alcohol. 10 mg. of needle-shaped crystals decomposing at 195° were obtained.

$C_{25}H_{42}O_3N$ calcd. N 3.45%
found N 3.23%

RESULTS

The results obtained in the present experiment can be summarized as follows.



$$[R = \overset{CH_3}{\underset{|}{CH}}-CH_2-CH_2-CH_2-CH_3,\ Ac = CH_3CO-]$$

DISCUSSION

Two trihydroxyhomocholenes, which had been obtained by hydrolyzing two different sulfates of m.p. 188° and 199° and named α- and β-respectively, yielded upon oxidation with chromic acid the same unsaturated ketone of m.p. 240-2°. Previously the author have reported, that either of α- and β-trihydroxyhomocholenes have one double bond at the same position and their C_3-hydroxyl groups are probably in α-configuration. Hence it can be said that α- and β-trihydroxyhomocholenes differ each other only in the steric configurations of the C_7- and C_{12}-hydroxyl groups.

Regarding the position of the double bond of β-trihydroxyhomocholene from the author's report V and from that of bufotalin (*5*), it may be either at C_8 or at C_{14}. While the tertiary hydroxyl group is most likely to be at C_{14}, the double bond between C_8 and C_{14} is known to be resistant against hydrogenation. So it must be at C_{14-15} or C_{15-16} by migration.

The double bond of C_{14-15} should after oxidation give a monoketo monocarboxylic acid and that of C_{15-16} should give a dicarboxylic acid. β-Trihydroxyhomocholene was acetylated with pyridine and acetic anhydride to yield triacetate, which was oxidized and then hydrolyzed. 3, 7,12-Trihydroxy-14-keto-14 : 15-homocholanic acid, which gave monoxime decomposing at 133°, was thus obtained. So the position of the double bond of β-trihydroxyhomocholene as well as its α-homologue must be at C_{14-15}. C_{14-15}-Double bond should yield by catalytic hydrogenation two cis-trans isomers concerning C_{14}-hydrogen against C_{13}-methyl group. By catalytic hydrogenation of dihydroxycholenic acid, R. K. Callow (*6*) obtained only desoxycholic acid alone, and the author (*1*, *2*) obtained trihydroxyhomocholane of m.p. 199-201° in 80 per cent yield and homocholane melting at 75° from the latter. Therefore C_{14}-hydrogen of both trihydroxyhomocholane may probably have the same transposition to C_{13}-methyl group as the usual bile acids.

To know the steric configuration of C_3 and C_7-hydroxyl groups of α- and β-trihydroxyhomocholenes by making their 12-ketoderivatives, they were acetylated with acetylchloride, and 3,7-diacetates melting at

180° and 94° respectively were obtained, which were oxidized with chromic acid and successively hydrolyzed. From β-trihydroxyhomocholene diacetate 12-ketoderivative was obtained in crystalline form, on the other hand from α-isomer no crystalline derivative was obtained.

3 (α), 7 (α), 12 (α)-Trihydroxyhomocholane and 3 (α), 7 (?), 12 (?)-trihydroxyhomocholane, which had been obtained by catalytic hydrogenation of α- and β-trihydroxyhomocholenes respectively, were oxidized with bromine at −5-0° according to W. M. Hocker's method (7). From either the same 7-ketoderivative was obtained.

Accordingly the starting two trihydroxyhomocholenes are steric isomers concerning their C_7-hydroxyl group. Therefore α-trihydroxyhomocholene may plausibly be 3 (α), 7 (α), 12 (α)-trihydroxyhomocholene and β-isomer be 3 (α), 7 (β), 12 (α)-trihydroxyhomocholene, and either of them has a double bond at C_{14-15}.

SUMMARY

1. From α- and β-trihydroxyhomocholenes, same keto-derivative was obtained, which means that either of trihydroxyhomocholenes has a double bond at the same position. From β-trihydroxyhomocholene, after permanganate oxidation, 3,7,12-trihydroxy-14-keto-14:15-homocholanic acid-15 was obtained, which means, as indicated in the previous reports (1), (2), that the double bond is between C_{14} and C_{15}.

2. To know the steric configuration of β-hydroxyl group of α- and β-trihydroxyhomocholenes, they were hydrogenated to yield 3 (α), 7 (α), 12 (α)-trihydroxyhomocholane and 3 (α), 7 (?), 12 (?)-trihydroxyhomocholane respectively, which upon oxidation with bromine gave same 6-ketoderivative of m.p. 172°. Therefore three hydroxyl groups of α-trihydroxyhomocholene are all in α-configuration and, among these three hydroxyl groups of β-isomer, that of C_7 has β-configuration.

α-Trihydroxyhomocholene is 3 (α), 7 (α), 12 (α)-trihydroxyhomocholene and β-trihydroxyhomocholene is 3 (α), 7 (β), 12 (α)-trihydroxyhomocholene.

The author is greatly indebted to President Dr. Toda for his encouragement and guidance, and to Dr. Shimizu, the President of Okayama University, for his permittance to use his laboratory at case. The author also thanks for the economical assistance of the Supporters's Association of our College.

REFERENCES

(1) Kuroda, M., *J. Biochem.*, **40**, 169 (1953)
(2) Kuroda, M., *J. Japan Biochem. Soc.*, **27**, 36 (1955)
(3) Kazuno, T.. Moori, A., Sasaki, K., Kuroda, M., and Mizuguchi, M., *Proc. Japan Acad.*, **28**, 416 (1952)
(4) Minlon, H., *J. Am. Chem. Soc.*, **71**, 3301 (1949)
(5) Wieland, H., and Weil, F. J., *Ber. dtsch. chem. Ges.*, **46**, 3315 (1913)
(6) Callow, R. K., *J. Chem. Soc.*, 462 (1936)
(7) Willand, Hocken, M., *J. Am. Chem. Soc.*, **67**, 312 (1945)

REPRINTED FROM
THE JOURNAL OF BIOCHEMISTRY
Vol. 43, No. 3
(May, 1956)