IMPROVED METHODS FOR THE PREPARATION OF 3(α)-HYDROXY-Δ⁷,11-CHOLENIC ACID*

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Any method for the synthesis of Compound E (1) must involve the introduction of oxygen at the C₁₁ position, either as a secondary hydroxyl 

![Diagram](image)

or as a keto group. Of all the approaches to such a synthesis afforded by general methods of organic chemistry, the addition reactions of a double bond, in this case at either C₉–C₁₁ or at C₁₁–C₁₂, are the most attractive. The fact that procedures for synthesizing both of these unsaturated compounds from desoxycholic acid have been published (1-3) provides sources of starting material. However, the low yields reported in these preparations have led to attempts to modify the procedures, thereby increasing the yield of the desired products. This study deals with the preparation of the compounds containing the double bond at the C₉–C₁₁ position.

The work of Chakravorty and Wallis (1) on the preparation of 3(α)-hydroxy-Δ⁷,11-cholenic acid provided for the first time a method for introduction of the double bond at the C₁₁ position. This was accomplished in the following manner. 3(α)-Acetoxy-12-ketocholanic acid was brominated with 1.05 moles of bromine at 70° for 4 hours to give the corresponding 11-bromo derivative. This compound was dehydrobrominated to 3(α)-hydroxy-Δ⁷,11-12-ketocholenic acid by refluxing for 2 hours with 10 per cent sodium ethylate solution. The semicarbazone was

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prepared by refluxing for 2 hours with semicarbazide acetate in dilute alcohol. This was reduced by heating with sodium ethylate for 15 hours at 180° to give the desired hydroxycholenic acid of melting point 183-184°. The over-all yield, however, was poor and in this paper we wish to record certain improvements. Each of the above reactions has been restudied for the purpose of obtaining optimum conditions for maximum yields of the desired 3(α)-hydroxy-Δ²,α₁₁-cholenic acid. The results of these experiments are described below.

Preparation of the C₁₂ Ketone—The method of Schwenk and collaborators (4) for the direct oxidation of 3(α)-succinoxy-12-hydroxycholanic acid was employed. A mixture of succinic anhydride, pyridine, and desoxycholic acid was heated for 3 hours on the steam bath; the reaction was nearly quantitative and the subsequent oxidation at room temperature resulted in the preparation of the crystalline C₁₂-keto compound in excellent yields. Alkaline saponification led to pure 3(α)-hydroxy-12-ketocholanic acid, its physical properties and conversion to various derivatives indicating excellent purity.

α-Bromination of the C₁₂ Ketone—The high temperature bromination employed by Chakravorty and Wallis (1) involves the addition in one portion of 1.05 moles of bromine in acetic acid to a solution of 3(α)-acetoxy-12-ketocholanic acid in the presence of a small amount of hydrobromic acid. In a restudy of this reaction, we have found that maintaining the solution at 70° for 4 hours reduces the yield of desired product and leads to a mixture of by-products produced by halogenation reactions other than at C₁₁. Therefore, experiments were made under varying conditions with 3(α)-formoxy, 3(α)-acetoxy, and 3(α)-succinoxy compounds, and with the methyl ester of 3(α)-benzoxy-12-ketocholanic acid.

With pure crystalline 3(α)-formoxy-12-ketocholanic acid it was found that although bromination at room temperature favors the formation of halogen-substituted derivatives the compound brominates in an anomalous manner to give a crystalline tribromo derivative.

Bromination of 3(α)-succinoxy-12-ketocholanic acid was found to require a temperature of 90°. The product was a crystalline material melting at 234° with decomposition and having, on analysis, 1 bromine atom per molecule. Unfortunately, owing to difficulties in dehalogenation, the bromination of the succinate could not be used as a preparative method.

Our next experiments were conducted on the acetoxy derivative. When bromination of 3(α)-acetoxy-12-ketocholanic acid was carried out at 55° over a period of 6 hours (5), a clear colorless product was obtained, which on reesterification could be isolated in crystalline form.

Good results were also obtained by the rapid bromination of methyl
3(α)-benzooxy-12-ketochohanate at 90°. Although in this case no crystalline product was isolated, the non-crystalline monobromo compound was produced in a high yield. Thus, despite the fact that it was necessary to use the increased temperature to effect the halogenation, various side reactions observed with other derivatives did not appear to take place.

Ethylate Dehydrohalogenation of C11-Bromo-12-ketochohanic Acid Derivatives—In their studies on the reaction of sodium ethylate on 3(α)-acetoxy-11-bromo-12-ketochohanic acid the time of refluxing used by Chakravorty and Wallis (1) was 2 hours. Because of the low yield obtained by this method, it was suspected that since α,β-unsaturated ketones are often unstable in alkali, the refluxing time used by these workers resulted in some decomposition in Ring C of the steroid nucleus. Therefore experiments were carried out with shorter periods of dehydrohalogenation, the time of refluxing varying from 5 to 30 minutes. The results obtained indicate that, although in every case the reaction approaches completion, it is necessary to reflux with ethylate only 15 minutes to render the per cent of unchanged bromo compound negligible; consequently shorter periods of dehydrohalogenation were employed.

The detailed results obtained are described in the experimental part of this paper. However, it is pertinent to summarize briefly certain of these observations. Dehydrohalogenation of the bromination product of 3(α)-formoxy-12-ketochohanic acid resulted in a mixture which for the most part appeared to be composed of the unsaturated cholenic acid and 3(α)-hydroxy-12-ketochohanic acid. Only a small amount of the pure 3(α)-hydroxy-Δ9.11-12-ketochohanic acid could be obtained by crystallization because of its similarity in solubility to the saturated compound. Under the same conditions it was found that the brominated succinoxy derivative gave very unsatisfactory results; the ethylate reduction of this compound proceeded violently, and the product was no more pure than in the case of the formoxy derivative.

In sharp contrast to these first two derivatives, which give unsatisfactory dehydrohalogenation products even under the milder conditions developed, stands the observation that the 3(α)-benzooxy and 3(α)-acetoxy compounds yield products of the highest purity. In these two cases after a single recrystallization, pure 3(α)-hydroxy-Δ9.11-12-ketochohanic acid is obtained in 25 per cent yield, and sufficient material is obtained in further crops to increase the yield to 40 per cent. Further increased yields of the desired Δ9.11-unsaturated 12-keto compound were observed when the mother liquors from the above crystallizations were dissolved in ether and treated with gaseous hydrogen bromide. The addition product so obtained was insoluble in ether and easily separated. On solution in warm acetic acid the compound lost hydrogen bromide and on cooling gave 3(α)-hydroxy-
Δ⁹,¹¹-12-ketocholelic acid. In this manner the over-all yield based upon 3(α)-hydroxy-12-ketocholelic acid was increased to 65 per cent.

In addition it was found that formic acid treatment of the mother liquors from these two reactions enables the recovery of about 35 per cent of the saturated C₁₂-keto compound. By this method, therefore, the yield of 3(α)-hydroxy-Δ⁹,¹¹-12-ketocholelic acid was greatly increased. Its high degree of purity is indicated by its extinction coefficient in absorption spectra measurements.

Wolf-Kishner Reduction of the α,β-Unsaturated Ketone—Preparation of the semicarbazone of 3(α)-hydroxy-Δ⁹,¹¹-12-ketocholelic acid (II) prior to ethylate reduction was effected simply by refluxing a solution of the ketone and semicarbazide in alcohol. It was found that increasing the time of heating of the reaction mixture from the 2 hour period used by Chakravorty and Wallis (1) to the more generally employed 8 hour period results in a quantitative reaction. The crystalline product melts with decomposition at 218°.

It should be noted in conclusion that the original authors (1) had effected the Wolf-Kishner reduction of the above semicarbazone in fair yields by reaction in a sealed tube at 180° for 16 hours. In our hands these yields, as well as the purity of the resulting compound, improved by decreasing...
the time of heating in the autoclave to 8 hours. Under such conditions
a 70 per cent yield of crude product melting at 176° can be obtained as
needles. Two recrystallizations from acetone yielded a highly pure
product of melting point 184° (III). Acetylation and methylation resulted
in crystalline plates of methyl 3(α)-acetoxy-Δ9,11-cholenate (IV) melting
at 135°; [α]D = +63°.

It can thus be seen that the preparation of pure 3(α)-hydroxy-Δ9,11-
cholenic acid from desoxycholic acid may be accomplished in yields varying
from 30 to 50 per cent.

EXPERIMENTAL

3(α)-SuCCinoxy-12-hydroxychoLanic Acid—This compound was prepared
by the method of Schwenk, Riegel, Moffett, and Stahl (4). To 500 cc.
of dry pyridine were added 200 gm. of pure desoxycholic acid and 200 gm.
of succinic anhydride. After being refluxed for 6 hours, during which
time the solution turned black, the contents of the flask were poured into
water. The tan-colored suspension was extracted with ether, washed
four times with hydrochloric acid (1:5), twice with water, and dried.
Upon removal of the ether by distillation a crystalline residue was obtained
which melted at 224.5–226°. Yield, 236 gm. Recrystallization from
alcohol gave a product which melted at 227° and agreed in other proper-
ties with the same succinoxy derivative described by Schwenk et al. (4).

3(α)-SUCCinoxy-12-ketochoLanic Acid—A portion (58.4 gm.) of 3(α)-
succinoxy-12-hydroxycholanic acid prepared by the method described
above was dissolved in 610 cc. of glacial acetic acid and treated at 15°
during the course of 45 minutes with 14 gm. of chromic acid dissolved in
175 cc. of 90 per cent acetic acid. Stirring was continued for another 45
minutes after the chromic acid had been added. The resulting 57.2 gm.
of crude crystalline material melted at 234–238°. Recrystallization from
alcohol gave a pure product of melting point 236–238°, identical in proper-
ties with the same compound prepared by Schwenk et al. by a similar
method.

3(α)-Hydroxy-12-ketochoLanic Acid—10 gm. of 3(α)-succinoxy-12-keto-
cholanic acid were saponified for 1 hour on the steam bath with 6.6 gm.
of potassium hydroxide in 50 cc. of water. The mixture was poured into
an ice-acid mixture; the white precipitate when washed and dried weighed
7.9 gm. and melted unsharply at 157–159°. Recrystallization from ethyl
acetate gave needles melting at 159–161.5°. Esterification with a 1 per
cent solution of hydrogen chloride in methanol gave the methyl ester in
97 per cent yield. Acetylation of the methyl ester with acetic anhydride
in pyridine gave methyl 3(α)-acetoxy-12-ketocholanate, m.p. 148.5–
150.5°. [α]D = +107.5° (57.2 mg. in 5 cc. of absolute alcohol solution).
The over-all yield from desoxycholic acid was 86 per cent.
3(α)-Hydroxy-Δ⁹⁻¹¹-ketocholenic Acid. From 3(α)-Succinoxy-11-bromo-12-ketocholenic Acid—10 gm. of 3(α)-succinoxy-12-ketocholenic acid were dissolved in 25.6 cc. of glacial acetic acid and treated with 25.6 cc. of a 1.05 M bromine-acetic acid solution. The reaction was allowed to continue for 1 hour, after which time most of the color had disappeared. The resulting crystalline monobromide melted at 234°. 4 gm. of this material dissolved in 20 cc. of hot absolute alcohol were added carefully to a boiling solution of sodium ethylate prepared from 4 gm. of sodium and 47.5 cc. of absolute alcohol. As the reaction generally was not satisfactory and very little sodium bromide was liberated, the mixture was refluxed for 10 minutes. When the product was worked up in the usual manner, the residual oil on crystallization from ethyl acetate gave low yields of 3(α)-hydroxy-Δ⁹⁻¹¹-ketocholenic acid of melting point 158–161°. Recrystallization gave crystals melting at 160–163°, compared with 171° for the pure compound first prepared by Chakravorty and Wallis (1).

From Methyl 3(α)-Benzoxy-11-bromo-12-ketocholanate—100 gm. of methyl 3(α)-hydroxy-12-ketocholanate in 500 cc. of benzene were treated with 24.4 cc. of benzoyl chloride dissolved in 50 cc. of dry pyridine. After completion of the reaction 92 gm. of methyl 3(α)-benzoxy-12-ketocholanate of melting point 94–95° were obtained. 20 gm. of this product in 54 cc. of glacial acetic acid were brominated with 54 cc. of 1.05 M bromine-acetic acid solution at 85° for 45 minutes. The resulting 20 gm. of oily product were dissolved in 100 cc. of hot absolute alcohol and added slowly to a boiling solution of sodium ethylate (20 gm. of sodium in 240 cc. of absolute alcohol). The reaction proceeded smoothly, giving sodium bromide immediately. Water was added after 15 minutes of refluxing and the product worked up. Two recrystallizations from ethyl acetate gave 4.9 gm. of 3(α)-hydroxy-Δ⁹⁻¹¹-ketocholenic acid of melting point 172–173°. [α]₂⁰ = +113° (15.4 mg. in 2 cc. of absolute alcohol). Further crops of crystals increased the yield.

From 3(α)-Acetoxy-11-bromo-12-ketocholanic Acid—A solution of 90 gm. of 3(α)-hydroxy-12-ketocholanic acid in 200 cc. of dry pyridine and 200 cc. of acetic anhydride was heated for 18 hours on the water bath. The amorphous product on crystallization from aqueous acetic acid gave 63 gm. of material melting at 192°. From the mother liquor additional amounts of the acetoxy derivative were obtained. To 60 gm. of this compound dissolved in 600 cc. of glacial acetic acid at 50–60° was added over a period of 3 hours a solution of 12 cc. of bromine in 450 cc. of acetic acid. After standing 4 more hours at 55° it was worked up in the usual manner to yield a non-crystalline bromo derivative. 20 gm. of this product dissolved in 130 cc. of absolute alcohol were added slowly to a refluxing solution of sodium ethylate (24 gm. of sodium in 275 cc. of absolute alcohol).
The reaction proceeded smoothly and after 10 minutes the product was worked up. Crystallization from ether and from ethyl acetate gave 4 gm. of 3(α)-hydroxy-Δ9,11-12-ketocholenic acid which melted at 168-170°. From the mother liquor additional amounts of this compound were obtained to increase the yield to 65 per cent. In one case this was accomplished by dissolving in ether the material in the mother liquors and treating the solution with gaseous hydrogen bromide. The addition product so obtained was crystallized and filtered. On solution in warm acetic acid the compound lost hydrogen bromide and on cooling additional amounts of 3(α)-hydroxy-Δ9,11-12-ketocholenic acid crystallized.

From 3(α)-Formoxy-11-bromo-12-ketocholanic Acid—When 7.9 gm. of 3(α)-hydroxy-12-ketocholanic acid were dissolved in 25 cc. of 98 per cent formic acid and the solution allowed to stand for 12 hours, a crystalline 3(α)-formoxy derivative in 97 per cent yield was obtained, m.p. 203-204°. Recrystallization from alcohol gave 7 gm. of product, m.p. 203-204.5°, [α]D = +116.5° (17.3 mg. in 2 cc. of absolute alcohol). A solution of 25 gm. of this product in 75 cc. of glacial acetic acid was treated for 16 hours at room temperature with 75 cc. of a 1.05 M bromine-acetic acid solution. The product was then worked up in the usual manner. From a concentrated ether solution (250 cc.) a small amount of crystalline material separated. Weight, 2 gm.; m.p. 135-142° (with decomposition). Recrystallization from alcohol gave crystals which melted at 145.5-147°, [α]D = +24.2° (49.4 mg. in 5 cc. of acetone solution).

Analysis—C35H45O6Br3. Calculated, C 45.80, H 5.39; found, C 46.20, H 5.58

The ether filtrate was evaporated to dryness to give a brominated non-crystalline residue. A solution of 3.6 gm. of this material in 20 cc. of absolute alcohol was poured slowly into a refluxing sodium ethylate solution (4 gm. of sodium dissolved in 46 cc. of absolute alcohol). Refluxing was continued for 15 minutes, after which the product was worked up. Great difficulty was encountered in getting a crystalline material of high purity. The first recrystallization from ether gave a product which melted unsharply at 158-163°. Five recrystallizations from ethyl acetate gave 0.5 gm. of 3(α)-hydroxy-Δ9,11-12-ketocholenic acid, m.p. 168-169°. The 3(α)-formoxy derivative was prepared by dissolving a portion of this material in 98 per cent formic acid. Recrystallization from dilute acetone gave white plates which melted at 169-170°.

3(α)-Hydroxy-Δ9,11-cholenic Acid—To 15.7 gm. of pure 3(α)-hydroxy-Δ9,11-12-ketocholenic acid dissolved in 120 cc. of alcohol were added 9.5 gm. of sodium acetate and 7.8 gm. of semicarbazide hydrochloride in 15 cc. of water. The solution was refluxed for 8 hours and the alcohol was removed by distillation. The crystalline residue was leached with hot
water, dried, and repeatedly washed with ether. The product melted at 218–221° and weighed 15 gm. To 2 gm. of this semicarbazone was added a solution of sodium ethylate (6 gm. of sodium in 70 cc. of absolute alcohol) and the contents were sealed in a tube and placed in an oven at 180° for 12 hours. The material was worked up and 0.9 gm. of crude product, m.p. 173–178°, was obtained. This yield was increased to 70 per cent when the time of heating at 180° was cut to 8 hours. Four recrystallizations from acetone gave plates which melted at 181–184°. \([\alpha]_D^{20} = +54.2°\) (15.5 mg. in 2 cc. of absolute alcohol solution).

The crystalline acetoxy methyl ester was prepared in excellent yield by the action of diazomethane followed by acetylation. It melted at 133–135° and had \([\alpha]_D^{23} = +62.9°\) (92.4 mg. in 10 cc. of absolute alcohol).

**Methyl 3(α)-Hydroxy-Δ^9,11,-12-ketocholenate—** Methylation of 1.0 gm. of 3(α)-hydroxy-Δ^9,11,-12-ketocholenic acid with 0.34 gm. of diazomethane in ether gave an oil which, on crystallization from ether, melted at 116–117°. \([\alpha]_D^{22} = +109.5°\) (21.0 mg. in 2 cc. of absolute alcohol solution).

**Analysis—** C_{32}H_{49}O_4. Calculated, C 74.54, H 9.51; found, C 74.83, H 9.53

**3(α)-Acetoxy-Δ^9,11,-12-ketocholenate—** 1 gm. of the methyl ester prepared by the method described above was treated on the water bath with 5 cc. of acetic anhydride in 10 cc. of dry pyridine. When the product was worked up, an oil was obtained which, on crystallization from methanol, gave crystals of melting point 138–139°; \([\alpha]_D^{18} = +110.8°\) (22.4 mg. in 2 cc. of absolute alcohol solution).

**Analysis—** C_{27}H_{48}O_3. Calculated, C 72.94, H 9.07; found, C 72.90, H 9.17

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**SUMMARY**

Improved methods have been developed by which the preparation of 3(α)-hydroxy-Δ^9,11-cholenic acid from deoxycholic acid may be effected in yields of 50 per cent.

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IMPROVED METHODS FOR THE PREPARATION OF 3(\(\alpha\))-HYDROXY-\(\Delta^9,11\)-CHOLENIC ACID
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