[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

MOLECULAR REARRANGEMENTS IN THE STEROLS. XI. THE ISOMERIC "*i*-CHOLESTANE-DIACIDS-6,7": A REINVESTIGATION

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Following the initial explanation of the *i*-steroid rearrangement by Wallis, Fernholz, and Gephardt (1), studies were initiated in this laboratory to support the proposed structure for *i*-cholestanone, and by inference, those of the *i*- or 3,5-cyclosteroids in general.

Ladenburg, Chakravorty, and Wallis (2) reported the preparation of three isomeric "cholestane-diacids", to which they assigned the structures II, IV, and VI. In addition, they suggested the possibility of a fourth isomer VII. The first acid "α_1-*i*-cholestane-diacid-6,7" (II) was prepared by the oxidation of *i*-cholestanone (3,5-cyclocholestan-6-one) (I) with potassium hypobromite in aqueous pyridine. The other two acids "β-*i*-cholestane-diacid-6,7" (IV), and "α_2-*i*-cholestane-diacid-6,7" (IV), were prepared by the dehydrohalogenation of 3-α-chlorocholestan-6//7-dicarboxylic acid (III) and 3-β-chlorocholestan-6//7-dicarboxylic acid (V) respectively.

It is immediately apparent that an error was made in this connection and that structural considerations limit the number of such acids having the 3,5-cyclo system to two, 3,5-cyclocholestan-6//7-dicarboxylic acid (hydrogen on $C_3\beta$, C_3–C_5 bond α) (II), and 3,5-cyclocoprostan-6//7-dicarboxylic acid (hydrogen on $C_3\alpha$, C_3–C_5 bond β) (IV). The other possibilities (VI, VII) would require the existence of a *trans* closure of the three-membered ring within the six-membered ring, which makes the probability of their real existence highly unlikely. The possibility of isomerism at C_8 is also to be considered, but the fact that the substituents at C_8 and C_9 are *trans* in the starting materials used in each case makes such a possibility extremely unlikely, since the conditions of the dehydrohalogenations were the same as those used for effecting *cis* to *trans* isomerizations of carboxylic acids.

Further consideration also suggests that if a cyclic system is formed in the dehydrohalogenation of the 3-β-chlorodiacid (V), the product should be identical to that obtained from the oxidation of 3,5-cyclocholestan-6-one (I).

Consequently it was decided to reinvestigate the work of Ladenburg, *et al.* (2) in order to determine the correct structure for their isomeric dicarboxylic acids.

The oxidation of I with potassium hypobromite to give 3,5-cyclocholestan-6//7-dicarboxylic acid ("α_1-diacid") (II), m.p. 232–233°, $[\alpha]_D^{25}$ +18° in absolute acetone, was repeated in this investigation with identical results. Examination of

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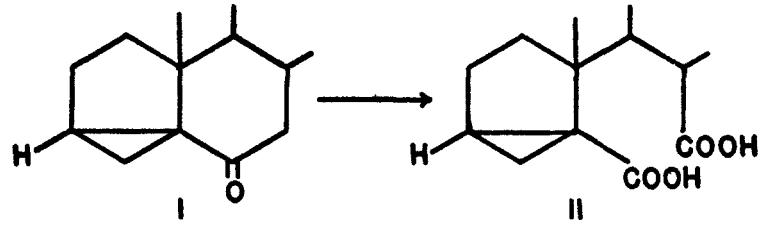
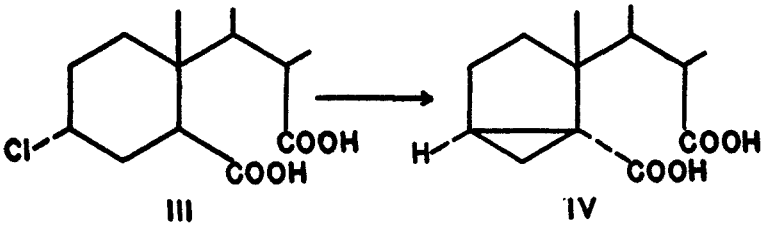
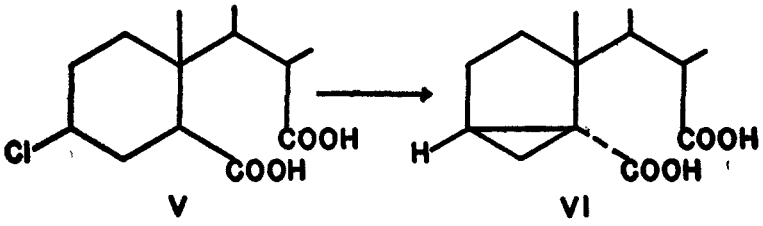
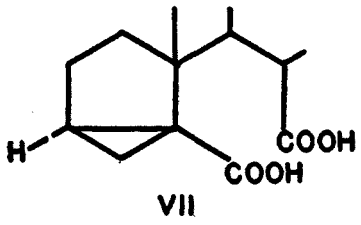
the infrared spectra of the acid (II) and its dimethyl ester showed fairly strong absorption at 9.88μ, characteristic of the cyclopropane ring (3) and the fused cyclopropane ring of 3,5-cyclosteroids (3a, 4), and no absorption in the 6μ









region. Josien, Fuson, and Cary (4) report characteristic but weak absorption due to the cyclopropane ring of 3,5-cyclosteroids at *ca.* 11.2μ and *ca.* 11.6μ. In the present case, only the band at 11.2μ was observed. At higher concentrations a band at 3.23μ and a shoulder at 3.31μ were found, which have been reported as characteristic of the three-membered ring (5a). The band at *ca.* 5.8μ due to ester carbonyl was an incompletely resolved complex structure with two maxima,

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA

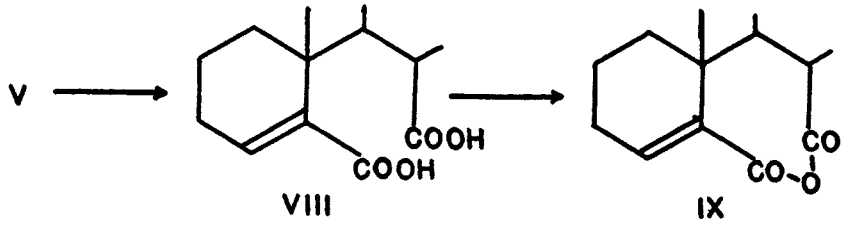
Acid			Ester		
c(moles/liter)	$\lambda_{max.}$, mμ	log ε	c(moles/liter)	$\lambda_{max.}$, mμ	log ε
2.33×10^{-3}	217	3.89	4.35×10^{-4}	218	4.01
2.33×10^{-4}	215	3.91	8.70×10^{-5}	217	4.04
2.33×10^{-5}	207	4.37	4.35×10^{-5}	207	4.18

one at 5.76μ, characteristic of a saturated ester, and a second at 5.80μ, compatible with the presence of an ester group on the three-membered ring. This is concluded by analogy with the lowering of the frequency shown for a ketone carbonyl at C_6 on *i*-steroids as compared to normal steroids (5b, 4).

A second acid, m.p. 265°, $[\alpha]_D^{25}$ +46° in dioxane, designated as "α_2-*i*-cholestane-diacid-6,7" (VI), believed to be isomeric with 3,5-cyclocholestan-6//7-dicarboxylic acid (II), was isolated by Ladenburg, *et al.* (2) after heating 3-β-chlorocholestan-6//7-dicarboxylic acid (V) with sodium ethoxide in absolute ethanol at 150° in a sealed tube for eight hours. Repeated efforts in this investigation failed to give this acid, but instead resulted in the isolation of a new acid, m.p. 226–227°, $[\alpha]_D^{25}$ + 74° in acetone. Refluxing the β-chloro-diacid (V) with sodium isoamoxide in dry isoamyl alcohol also yielded this new acid.

The ultraviolet spectra of the acid, (Table I), m.p. 226–227°, and its non-crystalline dimethyl ester showed marked deviations from Beer's Law, both as to the value of the extinction coefficient and the wave length of maximum absorption.

A failure to obey Beer's Law, and the presence of maxima whose wave-lengths are concentration dependent, lying at or below 225mμ, and decreasing with decreasing concentration, are characteristic of an α,β-unsaturated acid or ester (6). In view of the mode of preparation, the analytical data, and the ultraviolet spectrum, the acid, m.p. 226–227°, was assigned the structure of Δ⁴-cholesten-6//7-dicarboxylic acid (VIII).



VIII IX

The infrared spectra of the acid VIII, its dimethyl ester, and its anhydride (IX) lent further support to the proposed structure. Absorption at 6.16, 6.15, and 6.09μ respectively, characteristic of a conjugated double bond, was observed. In addition, absorption at 12.2μ characteristic of a triply substituted double bond was also observed. The carbonyl absorption in the spectrum of the dimethyl ester showed two bands, one at 5.76μ, characteristic of the saturated

ester group at C_3, and a second at 5.84μ, characteristic of the α,β-unsaturated ester group at C_5.

A comparison of the acid VIII with a sample of the 265° acid prepared by Ladenburg (2) revealed some striking differences in physical properties. Δ^4-cholesten-6//7-dicarboxylic acid (VIII) is moderately soluble in non-polar solvents, as is 3,5-cyclocholestan-6//7-dicarboxylic acid (II). However, the 265° acid is almost completely insoluble in such solvents.

The ultraviolet spectrum of the dimethyl ester of the 265° acid obeyed Beer's Law, and showed three maxima, the first at 229mμ, log ϵ 3.84, the second at 273mμ, log ϵ 2.67, and the third at 280mμ, log ϵ 2.58. In addition, there was a shoulder at 266–270mμ, log ϵ *ca.* 2.64. The spectrum was not compatible with that to be expected from the product of a simple dehydrohalogenation of the 3-β-chlorodiacid (V), and as a matter of fact bore a striking resemblance to that of an ester of an aromatic acid.

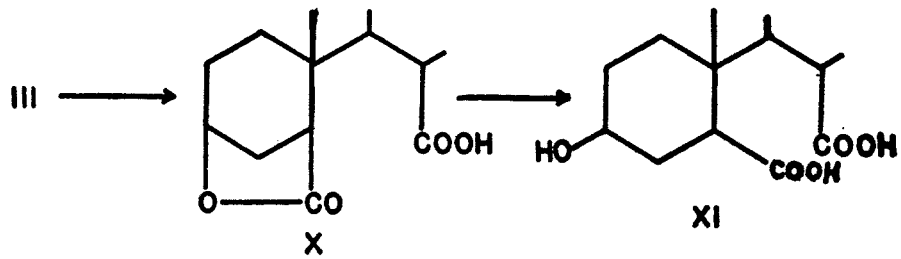
The possibility that the 265° acid might indeed be an aromatic acid was borne out by the infrared spectrum of its ester. The carbonyl absorption was at 5.83μ, indicative of a conjugated unsaturated ester. In the 6–7μ region there were three bands, at 6.23, 6.31, and 6.71μ, characteristic of a conjugated phenyl group (7). Unfortunately there was not a sufficient amount of Ladenburg's material to allow the spectrum of a highly concentrated solution to be taken, in which case the absorption in the 5–6μ region, if not obscured by carbonyl absorption, might have shown the orientation of substituents on the aromatic ring (8). The absorption in the 10–15μ region, in which absorption characteristic of ring orientation also occurs (9), was too complex to allow any definite conclusions to be drawn.

The third acid obtained in the earlier work (2) "β-*i*-cholestanediacid-6,7", m.p. 230–231°, was prepared by Ladenburg (2) by heating 3-α-chlorocholestan-6//7-dicarboxylic acid (III) with sodium ethoxide in absolute alcohol in a sealed tube for one hour at 120°. Owing to the insolubility of this free acid in suitable solvents, it was necessary to prepare the dimethyl ester in order to obtain its infrared spectrum. A moderately strong band at 2.9μ strongly indicated that the acid was a hydroxy acid rather than a hydrate as was suggested by the earlier workers. The most likely possibility seemed to be that the compound was actually 3-β-hydroxycholestan-6//7-dicarboxylic acid (XI), despite the disparity in its melting point, 230–231°, with the value reported in the literature (10). However, Shoppee (11) notes that the free acid (XI) is extremely difficult to dry, and that without exercising precautions, material melting in the vicinity of 230° is obtained.

An authentic sample of XI was prepared (10), and the infrared spectrum of its dimethyl ester was found to be identical with that of the so called "β-*i*-cholestanediacid-6,7 dimethyl ester" of Ladenburg.

In the light of these observations the mode of formation of the hydroxy acid (XI) deserves consideration. Lane and his co-workers (12) have studied the kinetics of hydrolysis of the epimeric acids (III) and (V). Their work indicates that a different mechanism is followed by the two isomers. In the hydrolysis of

the α-isomer, accomplished with sodium bicarbonate, the kinetics were compatible with an S_N1 mechanism, involving the displacement of chloride at C_3 by the C_5 carboxylate anion, with the formation of the lactone X, followed by hydrolysis of the lactone to the acid XI by the aqueous base. In the case of the β-isomer, which required the use of sodium hydroxide, the kinetics of an S_N1 reaction were also followed, the results indicating that the retention of configuration was due either to a shielding effect caused by the C_5 carboxylate anion and/or the departing chloride ion forcing the incoming hydroxyl ion to assume the α-configuration.



The stoichiometric amount of water required for complete hydrolysis of any lactone formed in the dehydrohalogenation reaction of the 3-β-chlorodiacid (III) would have been about forty milligrams, or a concentration of 0.16% in the amount of solvent used under the conditions of this investigation. Therefore it is deemed quite likely that the lactone is the primary product of the reaction, and that the hydroxy acid (XI) found arises from secondary hydrolysis of the lactone by traces of water present.

With this possibility in mind, the disodium salt of the chloroacid (III) was prepared using sodium carbonate in methanol at room temperature. The dry sodium salt then was refluxed in dry isoamyl alcohol. The product, m.p. 208–210°, was isolated and its infrared spectrum was taken. A strong band at 5.62μ, characteristic of a five membered lactone was observed, and a second of equal intensity was observed at 5.84μ, characteristic of the free carboxyl group.

EXPERIMENTAL

All melting points were taken on a Kofler block, and are uncorrected. Analyses were performed by Clark Microanalytical Laboratory, Urbana, Illinois.

3,5-Cyclocholestan-6//7-dicarboxylic acid (II) was prepared in quantitative yield from 3,5-cyclocholestanone-6 (I) by the method of Ladenburg, Chakravorty, and Wallis (2). Melting point 233° from aqueous ethanol, $[\alpha]_D^{28}$ +10° in acetone (*c*, 1).

Dimethyl 3,5-cyclocholestan-6//7-dicarboxylate. The dimethyl ester was prepared by adding an excess of ethereal diazomethane to a solution of 1 g. of the diacid in ether. The solution was allowed to stand overnight, and the ether was removed under an air jet. The residue was taken up in a small quantity of petroleum ether and chromatographed on alumina. Elution was accomplished with a 4:1 mixture of petroleum ether and benzene. All fractions showed identical infrared spectra. The chromatographed material could not be crystallized.

3-β-Chlorocholestan-6//7-dicarboxylic acid (V). The 3-β-chlorodiacid was prepared from 3-β-chlorocholestanone-6 by oxidation with fuming nitric acid according to the directions

of Windaus and von Staden (13). The yield was about 20%, m.p. 260–262° from aqueous ethanol or aqueous acetic acid.

Δ^4-Cholesten-6//7-dicarboxylic acid (VIII). (*a*) *With sodium ethoxide in absolute ethanol.* Sodium (2 g.) was dissolved in 25 ml. of absolute ethanol in a bomb tube. One gram of 3-β-chlorocholestan-6//7-dicarboxylic acid (V) was dissolved in the clear solution, and the tube was sealed off and heated in a furnace at 150° for eight hours. After cooling, the seal was broken and the contents of the tube were diluted with water. The alkaline solution was acidified with dilute sulfuric acid. A white precipitate formed immediately, which was filtered off and recrystallized from aqueous methanol to give a quantitative yield of Δ^4-cholesten-6//7-dicarboxylic acid (VIII), m.p. 226–227° with gas evolution. $[\alpha]_D^{28}$ +74° in acetone (*c*, 1).

Anal. Calc'd for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25.

Found: C, 74.64, 74.60; H, 10.20, 10.49.

(*b*) *With sodium isoamoxide in isoamyl alcohol.* Sodium (2 g.) was dissolved in 25 ml. of dry isoamyl alcohol. One gram of 3-β-chlorocholestan-6//7-dicarboxylic acid (V) then was added, and the clear solution was refluxed for 8–12 hours. The solution then was allowed to cool, and 250 ml. of ether was added. The resulting mixture then was extracted with water until acidification of the aqueous extract no longer caused turbidity. The combined aqueous extracts were washed once with ether and then were acidified with dilute hydrochloric acid. The precipitate which formed immediately was allowed to coagulate, and then was filtered off and recrystallized from aqueous methanol. The yield was quantitative, m.p. 226–227° with gas evolution.

The infrared spectra of the compounds prepared by methods *a* and *b* were identical.

Dimethyl Δ^4-cholesten-6//7-dicarboxylate. The dimethyl ester was prepared in the usual manner using ethereal diazomethane. After the removal of the ether, the residual oil was taken up in petroleum ether and chromatographed. Elution was accomplished with a 4:1 mixture of petroleum ether and benzene. The infrared spectra of all fractions were identical. The combined eluates were evaporated to give a colorless oil which could not be crystallized. A portion of the oil was sublimed at 0.05 mm. pressure and a bath temperature of about 220°.

Anal. Calc'd for $C_{29}H_{48}O_4$: C, 75.59; H, 10.50.

Found: C, 75.62, 75.89; H, 10.52, 10.55.

Δ^4-Cholesten-6//7-dicarboxylic acid anhydride (IX). The unsaturated acid (VIII) (200 mg.) was refluxed with 5 ml. of acetic anhydride for two hours. The solution then was concentrated under reduced pressure to give an oil, which partially crystallized on standing. Reerystallization from methanol gave a quantitative yield of the anhydride (IX), m.p. 126–127°.

Anal. Calc'd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21.

Found: C, 78.44; H, 10.12.

3-α-Chlorocholestan-6//7-dicarboxylic acid (III). The 3-α-chlorodiacid was prepared from 3-α-chlorocholestanone-6 by oxidation with fuming nitric acid according to the directions of Windaus and Stein (10) in 60–70% yield. Melting point 243° from glacial acetic acid.

3-β-Hydroxycholestan-6//7-dicarboxylic acid (XI). The hydroxy acid was prepared in quantitative yield from 3-α-chlorocholestan-6//7-dicarboxylic acid (III) by the method of Windaus and Stein (10). Melting point 231–232° from aqueous ethanol, $[\alpha]_D^{28}$ +35° in acetone (*c*, 1).

Dimethyl 3-β-hydroxycholestan-6//7-dicarboxylate. The dimethyl ester was prepared in the usual manner using ethereal diazomethane. The ester was a colorless oil which could not be crystallized.

3-β-Hydroxycholestan-6//7-dicarboxylic acid 6-3 lactone (X). 3-α-Chlorocholestan-6//7-dicarboxylic acid (III) (300 mg.) and 70 mg. of anhydrous sodium carbonate were shaken overnight in 50 ml. of anhydrous methanol. The solvent then was removed at room temperature under reduced pressure. Dry isoamyl alcohol (25 ml.) was added to the residue, and

the mixture was refluxed for two hours. At the end of that time, the alcohol was removed under reduced pressure, and the salts which remained were dissolved in water. Upon acidification with dilute hydrochloric acid a precipitate formed, which was filtered off and repeatedly recrystallized from methanol to give a white crystalline solid, m.p. 208–210°, which gave an unreproducibly positive Beilstien test. In an attempt to remove the remaining traces of chloroacid the material was esterified with diazomethane, and chromatographed on alumina. About 20 mg. of a white crystalline solid, identified as the dimethyl ester of the chloroacid, was the only material which could be eluted.

SUMMARY

The preparations and structure of the isomeric "cholestane-diacids" of Ladenburg, Chakravorty, and Wallis (2) have been reinvestigated. The preparation of 3,5-cyclocholestan-6//7-dicarboxylic acid by the oxidation of *i*-cholestanone has been confirmed, and infrared spectral data confirm the assigned structure. All attempts to prepare "α_2-*i*-cholestane-diacid-6,7" m.p. 265° descrided by Ladenburg were unsuccessful. Instead a new acid Δ^4-cholesten-6//7-dicarboxylic acid was isolated. Ultraviolet and infrared spectral data support the proposeb structure of the acid. Spectral data obtained from the original sample of Ladenburg's acid m.p. 265° indicate that this acid is not a product of simple dehydrohalogenation of 3-β-chlorocholestan-6//7-dicarboxylic acid, but rather an aromatic acid of unknown structure. The "β-*i*-cholestane-diacid-6,7" of the earlier workers has been shown to be 3-β-hydroxycholestan-6//7-dicarboxylic acid. Evidence is given that the hydroxy acid is a secondary reaction product arising from the hydrolysis of the lactone, 3-β-hydroxycholestan-6//7-dicarboxylic acid 6-3 lactone, which is the primary product formed by an intra-molecular displacement of the 3-α-chlorine atom by the C_5 carboxylate anion in the dehydrohalogenation of 3-α-chlorocholestan-6//7-dicarboxylic acid.

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