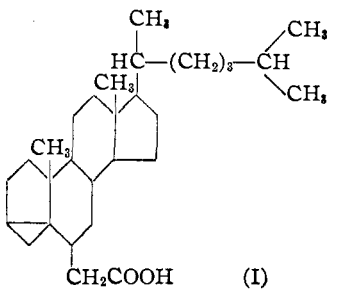
[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ARMOUR AND COMPANY]

The Preparation of Isomeric Cholesterylmalonic Acids. III. Rearrangement of *i*-Cholesterylacetic Acid and *i*-Cholesteryl malonamide

BY EMIL KAISER AND J. J. SVARZ

i-Sterol derivatives with a substituent connected by a carbon-to-oxygen bond to carbon atom six of the nucleus are known to rearrange to 3-substituted, 5–6 unsaturated sterols by treatment with acids.[1] In this paper the acid rearrangement of such *i*-cholesteryl derivatives is described in which a carbon-to-carbon bond links the substituent to position six of the ring. *i*-Sterols of this type are the *i*-cholesterylmalonic acid[2,3] and the *i*-cholesterylacetic acid (I).



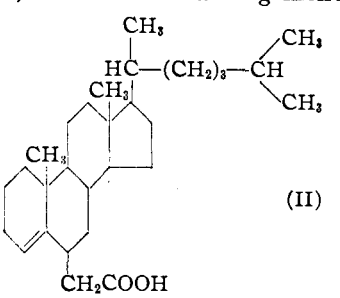
(I)

The *i*-cholesterylacetic acid was prepared by two different procedures. One was the decarboxylation of the *i*-cholesterylmalonic acid. The other method was the saponification of the *i*-cholesterylmalonamide in alkaline glycol solution. The *i*-cholesterylmalonamide not only saponified but was simultaneously decarboxylated to *i*-cholesterylacetic acid.

The *i*-cholesterylacetic acid was dextrorotatory $[\alpha]^{25}D$ + 32°, m.p. 111–112°. When treated with small amounts of sulfuric acid in glacial acetic acid solution a rearrangement occurred. Two compounds were isolated from the reaction mixture. One product was an isomeric cholesterylacetic acid, m.p. 173–174°, $[\alpha]^{25}D$ + 46°. Perbenzoic acid titration showed the presence of one double bond in the compound. The other rearrangement product was also an isomer of the *i*-cholesterylacetic acid, but was found to be neutral to alkali. Perbenzoic acid titration indicated a saturated ring structure. The isomeric neutral product had a m.p. 108–109°, $[\alpha]^{25}D$ + 80.5°.

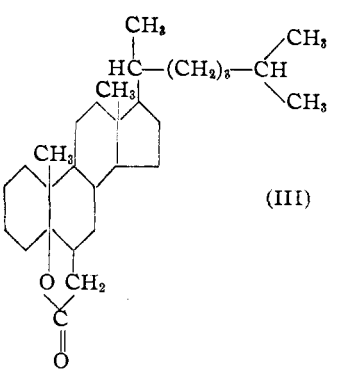
The acid and neutral products of the *i*-cholesterylacetic acid rearrangement were found to be interchangeable. Either of these compounds yielded a mixture of the two derivatives when heated in glacial acetic acid solution in the presence of sulfuric acid. Such an interchange is known to be typical for γ-unsaturated acids and their corresponding lactones.[4,5]

The mechanism of the *i*-cholesterylacetic acid rearrangement is different from the rearrangement mechanism of *i*-derivatives having substituents connected by carbon-to-oxygen[1] or carbon-to-nitrogen[6] bonds to the six position of the sterol nucleus. Cholesteryl-3-acetic acid[2] was not formed in the rearrangement of the *i*-cholesterylacetic acid and both products isolated from the rearrangement mixture were strongly dextro-rotatory. Therefore, a shift of the substituent from the six to the three position did not take place. Only the cyclopropane ring opened up and a γ-unsaturated acid, the Δ^4-cholestene-6-acetic acid (II) was formed. This acid partly lactonized and yielded the cholestane-6-acetic acid lactone, the neutral rearrangement product.



(II)

The cholestane-6-acetic acid lactone was heated to temperatures above 200°[5] to prove a γ or δ configuration. Since cholestene-6-acetic acid was not formed by heating, the lactone was assigned the structure of cholestane-6-acetic acid-γ-lactone (III).



(III)

The acid rearrangement procedure was also applied to the *i*-cholesterylmalonamide. The reaction product was separated into two fractions

(1) (a) Wallis, Fernholz and Gephart, THIS JOURNAL, **53**, 137 (1937). (b) Wagner-Jauregg and Werner, *J. physiol. Chem.*, **213**, 119 (1932). (c) Beynon, Heilbron and Spring, *J. Chem. Soc.*, 907 (1936).

(2) Kaiser and Svarz, THIS JOURNAL, **67**, 1309 (1945).

(3) Svarz and Kaiser, *ibid.*, **69**, 847 (1947).

(4) Linstead, *J. Chem. Soc.*, 115 (1932).

(5) Linstead and Rydon, *ibid.*, 580 (1933).

(6) Julian, Magnani, Meyer and Cole, THIS JOURNAL, **70**, 1834 (1948).

of different solubilities in methanol. Both fractions were saponified separately with alkali in propylene glycol. From the methanol less soluble fraction the Δ^4-cholestene-6-acetic acid was isolated. The methanol soluble fraction yielded the cholestane-6-acetic acid lactone as the main product.

Experimental[7,8]

i-Cholesterylacetic Acid from i-Cholesterylmalonic Acid.—In a 500-cc. flask immersed in an oil-bath, 10 g. of *i*-cholesterylmalonic acid was heated in a vacuum maintained by a mercury vapor pump. At 168° bath-temperature evolution of carbon dioxide started. The bath-temperature was slowly raised to 175° and kept there for ten minutes. Then the temperature was slowly raised again to 200° and heating continued for ten more minutes. After that the flask was cooled to room temperature and the reaction product crystallized from acetone. After recrystallization from acetone 6.8 g. of *i*-cholesterylacetic acid, m.p. 111.5–112.5°, was obtained.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.27; H, 11.28, neut. equiv., 428.0. Found: C, 81.29; H, 11.34; neut. equiv., 428; $[\alpha]^{25}D$ + 32° (in abs. alcohol). Perbenzoic acid titration: one-tenth of a gram dissolved in chloroform did not consume oxygen after seventy-two hours at −4°.

i-Cholesterylmalonamide.—The previous procedure was slightly modified. In 10 cc. of thionyl chloride 5 g. of *i*-cholesterylmalonic acid was suspended and the mixture kept at room temperature for two hours. Material still undissolved was brought into solution by heating on the steam-bath for ten minutes. The excess of thionyl chloride was distilled off under reduced pressure and the crude acid chloride transformed into the amide as described before.[3] Yield of crystalline *i*-cholesterylmalonamide was 2.5 g., m. p. 224–226°.

Saponification of i-Cholesterylmalonamide.—Twenty grams of potassium hydroxide was dissolved by warming in 100 cc. of propylene glycol. Seven grams of *i*-cholesteryl malonamide was added and the mixture refluxed for ten hours. Saponification of the amide was indicated by evolution of ammonia during the heating. The clear propylene glycol solution was then diluted with water, filtered and acidified with hydrochloric acid. A precipitate was formed which was extracted with ether. The ether extract was washed with water and dried over sodium sulfate. The ether was evaporated, the residue dissolved in methanol and the solution treated with charcoal. After filtration and chilling needles were obtained which were twice recrystallized from methanol; yield 4.2 g., m.p. 110.5–111.5°. The melting point remained unchanged when mixed with *i*-cholesterylacetic acid prepared by pyrolysis of *i*-cholesterylmalonic acid.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.27; H, 11.28; neut. equiv., 428.0. Found: C, 81.55; H, 11.40; neut. equiv., 429.8; $[\alpha]^{25}D$ + 32°.

Attempts were made to saponify the *i*-cholesterylmalonamide in isopropyl alcohol. Two grams of potassium hydroxide and 0.5 g. of *i*-cholesterylmalonamide was dissolved in 10 cc. of isopropyl alcohol. The solution was refluxed for ten hours, cooled, diluted with water and filtered. The filtrate was acidified; no precipitation occurred. The solid residue was crystallized from ethyl acetate and identified as *i*-cholesterylmalonamide.

The same result was obtained when heating of the isopropyl alcohol solution was done under pressure at 130°.

Attempt to Decarboxylate i-Cholesterylmalonic Acid.—One gram of *i*-cholesterylmalonic acid and 3 g. of potassium hydroxide were refluxed with 10 cc. of propylene glycol for ten hours. *i*-Cholesterylmalonic acid was the sole product isolated from the propylene glycol solution.

Rearrangement of i-Cholesterylacetic Acid.—Four grams of *i*-cholesterylacetic acid was dissolved in 120 cc. of glacial acetic acid. Ten drops of concentrated sulfuric acid was added and the solution heated on the steam-bath for two hours. The reaction mixture was cooled and poured into one liter of 10% aqueous sodium chloride solution. The precipitate was filtered off, dried and dissolved in 20 cc. of glacial acetic acid. After filtration the solution was kept at room temperature for several days. Crystals deposited and were removed by filtration. The mother liquor was concentrated to half its volume under reduced pressure. A second crop of crystals was obtained; yield 1.9 g. of Δ^4-cholestene-6-acetic acid, m.p. 173°. After recrystallization from glacial acetic acid the melting point was 173–174°.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.27; H, 11.28; neut. equiv., 428.0. Found: C, 81.28; H, 11.36; neut. equiv., 427.3; $[\alpha]^{25}D$ + 46° (in abs. alcohol). Perbenzoic acid titration: one-tenth of a gram dissolved in chloroform consumed 3.32 mg. of oxygen after ninety-six hours at −4°. *Anal.* Calcd. for $C_{29}H_{48}O_2$: 1 g. atom of oxygen (16 g.). Found: 0.89 g. atom of oxygen (14.2 g.). Control: one-tenth of a gram of cholesteryl acetate dissolved in chloroform consumed 3.29 mg. of oxygen after ninety-six hours at −4°. Calcd. for $C_{29}H_{48}O_2$: 1 g. atom of oxygen (16 g.). Found: 0.88 g. atom of oxygen (14.1 g.).

After the Δ^4-cholestene-6-acetic acid crystallized out from the concentrated glacial acetic acid solution, the mother liquor was evaporated to dryness under reduced pressure. The oily residue was dissolved in ether and repeatedly extracted with a 5% potassium hydroxide solution. The combined alkaline extracts, after acidification and crystallization of the precipitates from glacial acetic acid, yielded an additional amount 0.5 g. of Δ^4-cholestene-6-acetic acid, m.p. 173°.

The ether layer was washed with water, dried over sodium sulfate and the ether evaporated. The residue was crystallized from methanol. The cholestane-6-acetic acid-γ-lactone crystallized in beautiful needles; yield 0.3 g., m.p. 108–109°, mixed melting point with *i*-cholesterylacetic acid 86–88°. Two-tenths of a gram dissolved in ethanol did not consume 0.1 *N* sodium hydroxide solution at room temperature.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.27; H, 11.28. Found: C, 81.21; H, 11.28; $[\alpha]^{25}D$ + 80.5°. Perbenzoic acid titration: one-tenth of a gram in chloroform did not consume oxygen after seventy-two hours at −4°.

Rearrangement of i-Cholesterylmalonamide.—In 200 cc. of glacial acetic acid 2.25 g. of *i*-cholesterylmalonamide was dissolved and 1 cc. of concentrated sulfuric acid added. The mixture was kept on the steam-bath for one hour. A slight purple color developed. Then 500 cc. of water was added. The precipitate was filtered off, washed with water and dried. The dry material was dissolved in 15 cc. of methanol and the solution kept at −4° for twelve hours. Swollen lumps deposited and were separated by filtration. After drying, 0.55 g. of a slightly colored powder was obtained. This substance was soluble in ether and benzene and was designated as Fraction I.

The mother liquor filtered off from Fraction I was diluted with water. A white precipitate was formed. This precipitate, Fraction II, amounted to 1.55 g.[9]

Hydrolysis of Fractions I and II.—One gram of potassium hydroxide was dissolved in 10 cc. of propylene glycol and 0.50 g. of Fraction I was added. The solution was refluxed for two hours, cooled and diluted with 100 cc. of water. The precipitate was filtered off, washed with water and dried. The dry material, weighing 0.3 g., was crystallized from glacial acetic acid. After recrystallization from methanol 0.12 g. of a substance melting 173–174° was obtained. The melting point was not depressed when mixed with Δ^4-cholestene-6-acetic acid, which was obtained from *i*-cholesterylacetic acid.

(7) All melting points are uncorrected.

(8) Microanalyses were made by Mr. C. W. Beazley, Skokie, Illinois.

(9) The rearrangement of *i*-cholesterylmalonamide resulted in loss of nitrogen. Found in *i*-cholesterylmalonamide: N, 6.01; in Fraction I: N, 4.66; in Fraction II: N, 3.02

Anal. Calcd. for $C_{29}H_{48}O_2$: neut. equiv., 428.0. Found: neut. equiv., 428.5.

One and a half gram of Fraction II was refluxed in 25 cc. of propylene glycol containing 2.5 g. of potassium hydroxide. After two hours of refluxing the solution was cooled, diluted with 400 cc. of water (clear solution) and acidified. The precipitate was filtered off, washed with water and dried. This substance, weighing 0.95 g. was dissolved in 20 cc. of methanol. After dilution with 100 cc. of water the mixture was extracted with petroleum ether. The petroleum ether was evaporated. The residue was twice crystallized from methanol. Three-tenths of a gram of large needles was obtained, m.p. 108–109°. No melting point depression was observed when mixed with the cholestane-6-acetic acid-γ-lactone, isolated from the *i*-cholesterylacetic acid rearrangement.

Interchange of Δ⁴-Cholestene-6-acetic Acid and Cholestane-6-acetic Acid-γ-lactone.—(a) Two-tenths of a gram of cholestane-6-acetic acid-γ-lactone was dissolved in 20 cc. of glacial acetic acid containing one-tenth of a cc. of concentrated sulfuric acid. The mixture was heated for one hour on the steam-bath, then diluted with a saturated sodium chloride solution. The precipitate was filtered off, washed with water and dissolved in about 20 cc. of boiling methanol. Crystals deposited in the cold and were separated from the liquid. After recrystallization from glacial acetic acid, 0.05 g. of a substance was obtained with a m.p. of 173–174°; mixed melting point with Δ⁴-cholestene-6-acetic acid 173–174°. From the methanol mother liquor 0.06 g. of unchanged cholestane-6-acetic acid-γ-lactone was isolated.

(b) Half a gram of Δ⁴-cholestene-6-acetic acid was treated with sulfuric acid in glacial acetic acid solution in an identical manner as the lactone. The acid treated product was first crystallized from glacial acetic acid. Unchanged Δ⁴-cholestene-6-acetic acid was recovered, yield, 0.25 g. From the mother liquor a solid was precipitated with saturated sodium chloride solution. This was twice crystallized from methanol. Cholestane-6-acetic acid-γ-lactone was obtained, m.p. 108–109°, yield, 0.1 g.

(c) Two-tenths of a gram of cholestane-6-acetic acid-γ-lactone was heated for eight hours to 205–210°.[5] The brown-colored product was three times recrystallized from methanol, m.p. 108–109°, mixed melting point with cholestane-6-acetic acid-γ-lactone 108–109°. The mother liquors yielded an additional amount of cholestane-6-acetic acid-γ-lactone.

Summary

i-Cholesterylacetic acid was prepared by pyrolysis of *i*-cholesterylmalonic acid and by alkaline hydrolysis of *i*-cholesteryl malonamide. The *i*-cholesterylacetic acid was rearranged in acid solutions. Two products were obtained; the Δ⁴-cholestene-6-acetic acid and the cholestane-6-acetic acid-γ-lactone. The mechanism of the *i*-cholesterylacetic acid rearrangement was shown to be different from the rearrangement mechanism of *i*-compounds connected by carbon-to-oxygen or carbon-to-nitrogen bonds to substituents in the six position.

i-Cholesterylmalonamide was also rearranged and the rearrangement products saponified. The same compounds were isolated as obtained from the *i*-cholesterylacetic acid rearrangement mixture.

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Amine Bisulfite Addition Products of Aldehydes and Ketones. I

BY ROGER ADAMS AND R. D. LIPSCOMB[1]

Aldehydes and ketones are frequently purified through their sodium bisulfite addition products. The adducts are seldom used for identification purposes since they usually do not have sharp melting or decomposition points and suffer the shortcoming of being essentially insoluble in organic solvents. Amine bisulfite addition products of aldehydes and ketones, which have been inadequately investigated, should be formed with equal ease. They offer several possibilities for utilization (1) as compounds for characterization of ketones and aldehydes; (2) for the resolution of aldehydes and ketones and (3) for the replacement of aldehyde and ketone adducts of sodium bisulfite as intermediates in various organic syntheses. The preparation of several representative tertiary amine bisulfites with typical aldehydes and ketones and the characterization of the adducts are described in this first communication.

The procedure usually followed for preparation of the amine bisulfites was to pass sulfur dioxide into the tertiary amines mixed with sufficient water to form the bisulfites. Invariably heat was evolved when sulfur dioxide was first introduced and in many of the conversions, a white solid was observed to separate. This white solid, probably the amine sulfite, gradually dissolved in the reaction mixture as the addition of sulfur dioxide was continued. Whether or not this solid was observed, the reaction mixture always became viscous during the intermediate stage of the conversion. In the final stages, the color of the solution changed to yellow or orange and the viscosity decreased. The reaction was no longer exothermic after the intermediate viscous stage had been reached. The volume of product was often nearly double the volume of starting materials. This volume increase was most noticeable during the latter stages of the reaction.

The resulting mixtures were obviously not pure amine bisulfites but complexes of amine bisulfites with sulfur dioxide. They are relatively stable to air oxidation over long periods of time, probably due to the presence of the excess sulfur dioxide. These mixtures were kept for several years in ordinary reagent bottles in a refrigerator; they may be kept in tightly closed containers at room tem-

(1) An abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry. General Aniline and Film Corporation Fellow, 1942–43.