[CONTRIBUTION FROM THE ARMOUR LABORATORIES]

The Preparation of Isomeric Cholesteryl Malonic Acids

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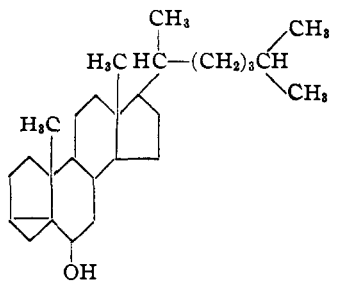
Although the literature discloses that many new esters or acids have been prepared from sterols, the malonic ester condensation has not been applied to them. The sole method for the introduction of the malonic ester group into the sterol nucleus was carried out through the Reformatsky reaction, as described by Schwenk and Whitman.[1]

Our attempts to prepare sterol substituted malonic esters by treating cholesteryl halides with sodium malonic ester in xylene solution were without success, but the condensation between sodium malonic ester and cholesteryl *p*-toluenesulfonate led to the formation of two isomeric forms of cholesteryl malonic acid ester.

Cholesteryl *p*-toluenesulfonate was first prepared by Freudenberg and Hess.[2] Its use under certain experimental conditions led to the preparation of a new series of cholesterol derivatives which were designated with the prefix *i*. Formation of an *i*-cholesteryl derivative was first observed by Stoll,[3] who prepared two isomeric cholesteryl methyl ethers which showed opposite optical rotations.

The influence of anhydrous potassium acetate on the formation of *i*-cholesteryl derivatives from cholesteryl *p*-toluenesulfonate was further investigated by Wallis, Fernholz and Gephart.[4] These authors found that by heating cholesteryl *p*-toluenesulfonate in acetic anhydride solution in the presence of anhydrous potassium acetate, two isomeric compounds, cholesteryl acetate and *i*-cholesteryl acetate, were formed. The *i*-cholesteryl acetate, like the *i*-cholesteryl methyl ether, was dextrorotatory $[\alpha]^{20}D$ +47.8°. When *i*-cholesteryl acetate was titrated with perbenzoic acid, oxygen was not consumed by this compound while the cholesteryl acetate took up one atom of oxygen per mole.

To account for the difference in the behavior of cholesterol and *i*-cholesterol, Wallis, Fernholz and Gephart proposed a structural formula for the *i* form



Proof of this structural configuration was furnished by Ford and Wallis,[5] Ford, Chakravorty and Wallis,[6] and by Butenandt and Suranyi.[7]

To investigate the reaction between cholesteryl *p*-toluenesulfonate and sodium malonic ester, a condensation between these two compounds was carried out in xylene solution. In the course of the reaction cholesterylmalonic ester and sodium *p*-toluenesulfonate were formed according to the equation

$$C_{27}H_{45}O\cdot SO_2C_6H_4CH_3 + CHNa(COOC_2H_5)_2 \longrightarrow C_{27}H_{45}CH(COOC_2H_5)_2 + NaSO_3\cdot C_6H_4CH_3$$

The cholesterylmalonic ester was saponified and cholesterylmalonic acid set free from the saponification product. The cholesterylmalonic acid could be separated into a petroleum ether soluble and petroleum ether insoluble fraction. Both fractions proved to be mono-cholesterylmalonic acids by their neutralization equivalents. The petroleum ether insoluble cholesteryl malonic acid, which is designated as Acid A, was crystallized. The petroleum ether soluble cholesterylmalonic acid, which will be called Acid B, could not be crystallized nor completely freed from impurities. Neutralization equivalents even of the purest preparations of this acid were only about 85% of the calculated values.

From the physical properties of several derivatives of these acids, conclusions have been drawn about the difference in structure of Acid A and Acid B.

The first derivatives prepared were di-adrenalin salts. If only one of the carboxyl groups was neutralized by adrenalin, then alcohol soluble mono-adrenalin salts were obtained from both acids. Addition of ether to the alcohol solutions of the mono-adrenalin salts gave precipitates, which when isolated and analyzed checked well with those calculated for di-adrenalin cholesterylmalonic acids, indicating a conversion of the mono salt to the di salt and giving further proof that Acid A and Acid B are isomeric dicarboxylic acids.

Next dimethyl esters of Acid A and Acid B were prepared. The dimethyl ester of Acid A could be crystallized, the dimethyl ester of Acid B was a viscous oil.

The preparation of cholesterylacetic acids by splitting off one of the carboxyl groups in the form of carbon dioxide was also attempted. Although crystalline Acid A lost carbon dioxide between 202–206°, crystalline material could not be isolated from this transformation product. The

(1) U. S. Patent 2,247,822 (1941).
(2) Freudenberg and Hess, *Ann.*, **448**, 121 (1926).
(3) Stoll, *Z. physiol. Chem.*, **207**, 147 (1932).
(4) Wallis, Fernholz and Gephart, THIS JOURNAL, **59**, 137 (1937).
(5) Ford and Wallis, *ibid.*, **59**, 1415 (1937).
(6) Ford, Chakravorty and Wallis, *ibid.*, **60**, 413 (1938).
(7) Butenandt and Suranyi, *Ber.*, **75**, 591 and 597 (1942).

observation was made that when the decomposition temperature of Acid A was lowered to 170–180° by the addition of Acid B, a crystalline cholesteryl acetic acid melting at 212–213° was obtained, which yielded upon esterification with methanol a crystalline cholesteryl acetic acid methyl ester.

Acid B, when heated, decomposed at a lower temperature than Acid A. From the decomposition product a crystalline compound could be isolated. This compound had the same melting point of 212–213° and the same neutralization equivalent as cholesteryl acetic acid prepared from Acid A. It probably came from Acid A as a contaminant in Acid B due to incomplete separation of the acids. Other crystalline substances could not be obtained from the decomposition product of Acid B. This finding together with the neutralization equivalent, which was lower than that calculated for cholesterylmalonic acid, indicates that the Acid B preparation is a mixture of three components (a) cholesterylmalonic acid, which is an isomer of the crystalline Acid A; (b) cholesterylmalonic acid A, and (c) neutral impurities. Purification and isolation of the petroleum ether soluble isomeric cholesterylmalonic acid will be described in a later paper.

The opical rotation of Acid A was $[\alpha]^{25}D$ −22.5°, that of Acid B was $[\alpha]^{25}D$ +39.5°. The fact that Acid A was levorotatory and Acid B dextrorotatory is a very significant indication of their respective structures, because cholesterol derivatives, as was shown in the lieterature survey, are levorotatory and *i*-cholesterol derivatives dextrorotatory compounds. To get further proof of the structure of both acids their dimethyl esters were titrated with perbenzoic acid. One mole of the Acid A dimethyl ester consumed 1.19 gram atoms of oxygen. This value shows the presence of one double bond in the ester and hence Acid A is considered as cholesteryl-3-malonic acid. One mole of Acid B dimethyl ester consumed only 0.31 gram atoms of oxygen in the perbenzoic acid titration, one fourth of the amount of oxygen consumed by Acid A dimethyl ester. As Acid B contains about 20% of impurities there is a strong possibility that this oxygen consumption is due to the impurities alone. Regardless of this possibility the low value of the perbenzoic acid titration of Acid B dimethyl ester indicates that *i*-cholesterylmalonic acid is the main component of Acid B since compounds of the *i*-cholesteryl series do not consume oxygen.

Acknowledgment.—The authors wish to thank Dr. F. C. Koch for his helpful advice during the course of this work.

Experimental[8,9]

Preparation of Cholesteryl *p*-Toluenesulfonate.—The method of Freudenberg and Hess with the modification of Wallis, Fernholz and Gephart was used for the preparation of this compound.[4]

Condensation of Cholesteryl *p*-Toluenesulfonate with Diethyl Malonate.—To a 2-liter, three-necked flask, equipped with a mercury-sealed stirrer, dropping funnel and calcium chloride tube, were transferred 11.5 g. (0.5 mole) of metallic sodium and 200 cc. of dried xylene. The flask was immersed in an oil-bath kept at about 105°. Through the dropping funnel a mixture consisting of 96 g. (0.6 mole) of diethyl malonate and 90 cc. of dried xylene was added slowly with constant stirring. When the addition was complete and all the sodium had dissolved, a solution of 135 g. of cholesteryl *p*-toluenesulfonate (0.25 mole) in 270 cc. of xylene was poured into the flask. The temperature of the oil-bath was maintained at 105° while heating and stirring for ten hours. During this time a voluminous precipitation of sodium *p*-toluenesulfonate took place. The color of the reaction mixture turned brown. After cooling to room temperature the precipitate was filtered off and washed several times with xylene. The filtrates were combined and the xylene was distilled off *in vacuo* on the steam-bath. The cooled residue was a very viscous oil, which consisted of cholesteryl diethylmalonate, unreacted diethyl malonate and unsaponifiable materials.

Separation of Crude Isomeric Cholesterylmalonic Acids.—The xylene free residue of the vacuum distillation, containing the cholesteryl diethylmalonate, was dissolved in 200 cc. of isopropyl alcohol by heating. Sixty grams of potassium hydroxide dissolved in 200 cc. of methanol was added and the mixture refluxed for six hours. After cooling, 1500 cc. of water and 1000 cc. of ether were poured into the reaction mixture. The mixture was transferred to a separatory funnel and mixed well by shaking. The ether formed an emulsion with the alkaline layer and separated only after addition of more isopropyl alcohol with gentle shaking. The alkaline layer was drawn off and extracted with two more 800-cc. portions of ether in a similar manner. The alkaline solution was freed from the remaining ether by cautious heating and then cooled to room temperature. Addition of 10% hydrochloric acid to the cooled alkaline solution yielded a precipitate which after standing overnight could be filtered off through a folded filter paper or removed by centrifugation. The residue was washed with water and dried in a desiccator. This is the crude cholesterylmalonic acid.

The dry cholesterylmalonic acid was dissolved in sufficient hot ether to obtain a clear solution and then 1000 cc. of low boiling petroleum ether (boiling range 30–60°) was added. The mixture was kept for forty-eight hours at −4°. Petroleum ether insoluble material separated which was filtered off and washed on the filter with petroleum ether. The filtrate was concentrated to about one-third of its volume and two more volumes of petroleum ether were added. More insoluble material separated at −4°. The precipitate was removed, the filtrate concentrated again, mixed with petroleum ether and kept several days at −4°. After this last precipitation the petroleum ether insoluble fractions were combined and dried in air; yield 20–30 g. in different runs. Neutralization equivalents in several runs showed some variation, indicating different amounts of neutral impurities in the product.

Anal. Calcd. for $C_{50}H_{48}O_4$: neut. equiv., 236.3. Found: neut. equiv., 283, 291, 258.7.

The petroleum ether solution containing the petroleum ether-soluble fraction of the cholesterylmalonic acid was concentrated to a thick sirup on the steam-bath and the sirup dried in a current of air. The product was pulverized and then dried in a vacuum desiccator. Yields were about the same as that of the petroleum ether insoluble part, varying from 20 to 30 g. Variations in the neutralization equivalents of several runs were greater than in the case of the petroleum ether insoluble acid.

Anal. Calcd. for $C_{50}H_{48}O_4$: neut. equiv., 236.3. Found: neut. equiv., 280, 283, 297, 341.

Purification of the Petroleum Ether Insoluble Cholesterylmalonic Acid (Acid A).—The crude Acid A, obtained by

[8] Microanalyses were made at The California Institute of Technology, Pasadena, California.

[9] All melting points are uncorrected.

petroleum ether separation from the mixture with Acid B, was dissolved in ether. According to the purity of the product different amounts of ether have to be used to dissolve the substance.

In one run 12 g. of Acid A (neutralization equivalent 291) was dissolved by warming in 50 cc. of ether, the solution filtered and the residue brought into solution by washing the filter with ether. To the ether solution 200 cc. of low-boiling petroleum ether was added and the mixture kept for forty-eight hours in the cold. During this time Acid A crystallized out in small needles which were filtered off and washed with petroleum ether. To the filtrate, concentrated to about 100 cc., was added 100 cc. of petroleum ether. After filtration the mother liquor was concentrated again and petroleum ether added. A third crop of crystals was obtained. The combined fractions weighed 8.9 g. After recrystallization from ether–petroleum ether mixture the crystals of Acid A were dried in an Abderhalden dryer over phosphorus pentoxide at the boiling point of methanol. The dried substance decomposes with darkening at 202 to 206° and melts completely at 206°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.23, neut. equiv., 236.3. Found: C, 76.27; H, 10.05, neut. equiv., 237.1; $[\alpha]^{25}D$ −22.5° (in 95° alcohol).

Preparation of the Di-adrenalin Salt of Acid A.—Ninety-four hundredths of a gram of crystalline Acid A was dissolved in 15 cc. of benzyl alcohol by gentle heating. Next 0.27 g. of adrenalin was added and the mixture heated on the steam-bath for a few minutes. The adrenalin dissolved almost completely. When the filtered solution was poured into 200 cc. of ether, a white precipitate settled out. The precipitate was separated, washed with ether and dried in the vacuum desiccator at room temperature.

Anal. Calcd. for $C_{48}H_{72}O_{10}N_2$: C, 68.78; H, 8.83; N, 3.35. Found: C, 68.04; H, 8.70; N, 3.61.

Preparation of Crystalline Cholesteryl Dimethyl Malonate from Acid A.—Forty grams of Acid A was dissolved in 400 cc. of methanol and 10 cc. of concentrated sulfuric acid was added. The solution was refluxed for eight hours then cooled to room temperature and diluted with 600 cc. of water. The precipitated ester was separated from the unesterified acid and crystallized from absolute alcohol. Clustered needles were formed; yield, 21.5 g. of cholesteryl dimethyl malonate; m. p. 88–89° but remains turbid and clears up at 106°.

Anal. Calcd. for $C_{32}H_{52}O_4$: C, 76.80; H, 10.40. Found: C, 76.70; H, 10.44.

Perbenzoic acid titration.—Sample 1—One-tenth of a gram dissolved in chloroform consumed 3.84 mg. of oxygen after seventy-two hours at 5°. Sample 2.—Five hundredths of a gram consumed 1.87 mg. oxygen. Calcd. oxygen consumption for $C_{32}H_{52}O_4$: 1 g. atom of oxygen (16 g.). Found: 1.19 g. atoms of oxygen (18.95 g.).

Preparation of Cholesterylacetic Acid from Acid A.—The preparation of cholesterylacetic acid was successfully carried out starting with crude Acid A. Degradation of crystalline Acid A to cholesterylacetic acid was also attempted.

First the degradation of crude Acid A, which started to decompose at 170°, will be described. This crude material was kept for one hour in an oil-bath of 170–180°. During this time evolution of carbon dioxide took place and the substance was transformed into a clear brown melt. This melt was cooled and dissolved in 200 cc. of isopropyl alcohol. The solution was boiled with charcoal, filtered and kept at −4° for forty-eight hours. The precipitate formed during this time was filtered off, the mother liquor concentrated and mixed with the same volume of methanol. Again precipitation took place in the cold and the solid was removed by filtration. A total of 11.2 g. of crude cholesterylacetic acid was isolated from 20 g. of Acid A. This material was dissolved in absolute alcohol, a small amount of decolorizing charcoal added, and the solution refluxed for half an hour. The solution was filtered and treated twice more with charcoal in the same manner. The filtrate was colorless after these charcoal treatments and was kept overnight at −4°. Crystals formed and were filtered off. The mother liquor was concentrated, cooled, and a second crop of crystals obtained. The combined crystalline fractions were recrystallized from absolute alcohol, yield, 2.4 g. of cholesterylacetic acid; m. p. 212–213°.

Anal. Ash, 0.013. Calcd. for $C_{29}H_{48}O_2$: C, 81.27; H, 11.28, neut. equiv., 428. Found (without ash): C, 81.30; H, 11.30, neut. equiv., 428.8.

Crystalline Acid A decomposing at 202–206° was worked up under identical conditions. Cholesterylacetic acid crystals could not be isolated. However, when a mixture of 0.1 g. of Acid B and 0.5 g. of Acid A was decomposed at 175–180° bath temperature, cholesterylacetic acid crystals melting at 212–213° were obtained from the reaction product.

Preparation of the Methyl Ester of the Crystalline Cholesterylacetic Acid.—One gram of the crystalline cholesterylacetic acid, m. p. 212–213°, was dissolved in 20 cc. of methyl alcohol, 0.5 cc. of concentrated sulfuric acid added and the solution refluxed for six hours. The ester was isolated in the usual manner and recrystallized three times from absolute alcohol. The cholesterylacetic acid methyl ester crystallized in needles, m. p. 107–108°

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38. Found: C, 81.34; H, 11.32.

Purification of Petroleum Ether Soluble Cholesterylmalonic Acid (Acid B).—To 10 g. of crude Acid B dissolved in 150 cc. of methanol was added 15 g. of lead acetate dissolved in 150 cc. of methanol. The lead salt of Acid B precipitated immediately, and was filtered off and washed several times with methanol. To the lead salt dissolved in chloroform, methanol was added until no more precipitate was formed. The white solid was filtered off, mixed with methanol to a thick paste, and suspended in water and hydrochloric acid. Hydrogen sulfide was bubbled through the suspension until the lead salt was completely decomposed. The hydrogen sulfide was removed in a stream of air from the suspension and the precipitate filtered. The black filtration residue which consisted of lead sulfide and Acid B was extracted with ether. The ether was dried over sodium sulfate and concentrated to a sirup. This sirup was dried in a current of air at room temperature. Acid B was obtained in the form of a cream-colored powder, which still contained varying amounts of impurities in the different runs as could be shown by the varying neutralization equivalents.

Anal. Calcd. for $C_{30}H_{48}O_4$: neut. equiv., 236.3. Found: neut. equiv., 265, 278, 283; $[\alpha]^{25}D$ +39.5° (95% alcohol, sample with neutralization equivalent 278 was used).

Preparation of the Di-adrenalin Salt of Acid B.—Ninety-four hundredths of a gram of Acid B and 0.37 g. of adrenalin were dissolved in 15 cc. of benzyl alcohol by gentle heating. The solution was filtered and the di-adrenalin salt precipitated by adding first 150 cc. of ether and then 100 cc. of petroleum ether to the benzyl alcohol solution. The precipitate was filtered off, washed with ether, and dried in a vacuum desiccator at room temperature.

Anal. Calcd. for $C_{48}H_{72}O_{10}N_2$: C, 68.78; H, 8.83; N, 3.35. Found: C, 68.21; H, 8.93; N, 3.23.

Preparation of Cholesteryl Dimethylmalonate from Acid B.—Twenty grams of Acid B was esterified with a mixture of 200 cc. of methanol and 5 cc. of concentrated sulfuric acid by heating on the steam-bath for six hours. The cholesteryl dimethylmalonate of Acid B was obtained as a viscous oil which could not be crystallized.

Perbenzoic acid titration: One tenth of a gram dissolved in chloroform consumed 1.04 mg. of oxygen after seventy-two hours at 5°.

Anal. Calculated oxygen consumption for $C_{32}H_{52}O_4$: 1 gram atom of oxygen (16 g. of oxygen). Found: 0.31 gram atom (5.2 g. of oxygen).

Attempt to Prepare Cholesterylacetic Acid from Acid B.—Thirty grams of cholesterylmalonic acid B was kept

for one hour in an oil-bath at 140°. The substance melted with strong foaming. The clear melt was cooled, dissolved in ether and extracted several times with a 2% potassium hydroxide in water solution. Emulsions were broken up by the addition of isopropyl alcohol and gentle shaking of the separatory funnel. The combined alkaline layers were washed with ether, then freed from the remaining ether by heating. The cooled alkaline solution was acidified with a 10% hydrochloric acid. The precipitate formed upon acidification was filtered off, washed with water and dried in the vacuum desiccator. The dry substance weighed 24.3 g. and was dissolved by heating in 300 cc. of methanol. The methanol solution was refluxed with charcoal, then filtered hot and left for twenty-four hours at 5°. Crystals were formed which were filtered off and recrystallized from methanol. One and three-tenths grams of crystalline material was obtained, m. p. 212–213°. Melting point depression was not observed with cholesterylacetic acid prepared previously from low melting Acid A. Additional crystalline material could not be isolated from the mother liquor.

Summary

Condensation of cholesteryl *p*-toluenesulfonate and sodium malonic ester in xylene solution yielded cholesterylmalonic ester. From the cholesterylmalonic ester cholesterylmalonic acid was prepared, which was separated into a petroleum ether soluble fraction and a petroleum ether insoluble fraction. The two fractions were identified as isomeric cholesterylmalonic acids.

The petroleum ether insoluble cholesterylmalonic acid was crystallized and from it were prepared: di-adrenalin salt, dimethyl ester, cholesterylacetic acid and cholesterylacetic acid methyl ester.

The petroleum ether soluble cholesterylmalonic acid was obtained in impure form only. The di-adrenalin salt and dimethyl ester thereof were prepared.

The petroleum ether insoluble cholesterylmalonic acid was found to be levorotatory and the petroleum ether soluble acid dextrorotatory. From the difference in the direction of the optical rotation and differences in the consumption of oxygen of the dimethyl esters in the perbenzoic acid titration it was concluded that the petroleum ether insoluble cholesterylmalonic acid is cholesteryl-3-malonic acid and the petroleum ether soluble cholesterylmalonic acid has the structure of *i*-cholesterylmalonic acid.

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Vapor Phase Methylation of Aromatic Hydrocarbons over Solid Catalysts

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Friedel and Crafts[1] in 1877 demonstrated the methylation of aromatic hydrocarbons in the liquid phase by methyl halides under the catalytic influence of anhydrous aluminum halides. Although many methylation studies have been subsequently published, this reaction has never been accomplished in the vapor phase over solid catalysts, although it has been attempted.[2]

Thinking that vapor phase methylation of aromatic hydrocarbons over solid catalysts might offer certain advantages, we studied this mode of operation and obtained satisfactory yields of methylated aromatics from benzene, toluene, and naphthalene, using methyl chloride and methyl bromide as methylating agents, and alumina, alumina–silica, activated clay, and aluminum chloride adsorbed on activated carbon as catalysts. Methyl iodide was ineffective as methylating agent under the conditions tried.

Contrary to expectation, certain dehydrohalogenation catalysts (barium chloride and ferric chloride) were ineffective. It was thought that the methylation reaction might go via the methylene group and that a dehydrohalogenation catalyst might catalyze the methylation reaction by removing hydrogen halide from methyl halide to form methylene. "Solid" phosphoric acid catalyst also was ineffective for the catalysis of the methylation reaction.

(1) Friedel and Crafts, *Compt. rend.*, **84**, 1392 (1877).

(2) Schwartz and Pflugmacher, *J. prakt. Chem.*, **156**, 205 (1916).

Experimental

Materials.—The methyl halides were commercial products which were used without further purification. The benzene and toluene were of nitration grade and the naphthalene was of chemical grade.

Catalysts.—Alumina catalyst was made from dried alumina gel which had been precipitated from dilute aqueous aluminum chloride by freshly distilled aqueous ammonia and dried at 110°. The dried gel was crushed and screened to 20–30 mesh and then molded in the form of $1/8'' \times 1/8''$ pellets with 4% of aluminum stearate as die-lubricant. These pellets were heated in a stream of air at 600° to remove carbon before being used as catalyst.

Silica gel was precipitated from diluted water glass by hydrochloric acid and washed six times with distilled water (filtering by suction after each wash and slurrying the filter cake in each subsequent wash) until the sodium content of the silica gel was lowered to 0.1%. The washed silica gel cake (about 12% silicon dioxide) was dried at 110° and used in granular form (6–10 mesh).

Alumina–silica catalysts were made by dispersing freshly-prepared, washed silica gel in dilute aqueous aluminum chloride and adding aqueous ammonia to the vigorously stirred mixture to precipitate aluminum hydroxide intimately with the silica gel. The precipitate was washed about five times with distilled water (until the filtrate showed only a faint test for chloride ion) and dried at 110°; the dried material was crushed, screened, pelleted, and burned at 600°, as described above.

Barium chloride catalyst was prepared by soaking granular pumice (6–10 mesh) in a concentrated aqueous solution of the salt and drying the impregnated pumice