

were dried at room temperature; yield 43 g. This substance was called Fraction BII.

The propylene dichloride filtrate from Fraction BII was evaporated to dryness under reduced pressure on the steam-bath. A light brown sirup remained, which weighed 22 g. Attempts to crystallize this sirup failed.

Fifteen grams of the above sirup was dissolved in a mixture of 80 cc. of absolute ether and 80 cc. of anhydrous benzene. Three drops of pyridine was added followed by 15 cc. of freshly distilled thionyl chloride. This mixture was allowed to stand at room temperature for five hours. The solvents were then distilled off under reduced pressure at about 40 to 45°. The residue was treated twice with 25-cc. portions of dry benzene, each portion being distilled off as before. Finally 150 cc. of anhydrous ether was added and anhydrous ammonia gas passed into the ether solution for forty-five minutes. Some of the ether evaporated and a precipitate separated out, which was removed by filtration. The filtrate was chilled and more material obtained. From the concentrated mother liquor still more of this substance crystallized out, when petroleum ether was added. The fractions were combined, washed with water and dried; yield 8 g.

About 70% of the starting material was recovered in the fractionation. The *i*-cholesterylmalonic acid amounted to about one-half of the weight of the whole Acid B mixture.

Crystallization of Fraction BI.—Seven grams of Fraction BI obtained from the Fraction B mixture was crystallized from methanol. After three recrystallizations from methanol a compound melting at 67–68° was obtained. When heated to 220° and recrystallized from methanol the melting point remained the same; equivalent weight in two different preparations 298 and 310.

The combined methanol mother liquors were kept overnight at –4°. The crystalline material had a melting point of 52 to 57°; equivalent weight 250.

Perbenzoic Acid Titration—Fraction Melting 67–68°.—One-tenth of a gram dissolved in chloroform did not consume oxygen after seventy-two hours at –4°. Fraction melting 52–57°: one-tenth of a gram dissolved in chloroform did not consume oxygen after seventy-two hours at –4°.

The Fraction BI obtained in the separation procedure had an optical rotation of $[\alpha]^{25}_D -0.17^\circ$ in 95% ethanol.

Crystallization of *i*-Cholesterylmalonic Acid.—Fraction BII was twice recrystallized from propylene dichloride and dried *in vacuo* at 100°. The crystalline *i*-cholesterylmalonic acid melts with decomposition at 173–174°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.23; neut. equiv., 236.3. Found: C, 76.20; H, 10.70; neut. equiv., 236.15; $[\alpha]^{25}_D +65^\circ$ (in 95% ethanol).

Perbenzoic acid titration: one-tenth of a gram dissolved in chloroform did not consume oxygen after seventy-two hours at –4°.

Preparation of Crystalline Dimethyl *i*-Cholesterylmalonate.—Two grams of *i*-cholesterylmalonic acid was dissolved in 20 cc. of methanol. One-half cc. of concentrated sulfuric acid was added and the solution refluxed

for six hours. The ester was isolated in the usual manner and crystallized out slowly from methanol after standing for several days at –4°. The white needles were recrystallized from methanol and dried, m. p. 69–71°.

Anal. Calcd. for $C_{32}H_{52}O_4$: C, 76.80; H, 10.40. Found: C, 76.90; H, 10.98. $[\alpha]^{25}_D +58.5^\circ$ (in 95% ethanol).

Perbenzoic acid titration: one-tenth gram dissolved in chloroform did not consume oxygen after seventy-two hours at –4°.

Preparation of *i*-Cholesterylmalonamide.—One gram of *i*-cholesterylmalonic acid was dissolved in 20 cc. of absolute benzene and 10 cc. of absolute ether. One drop of pyridine was added, followed by one cc. of freshly distilled thionyl chloride. The mixture was allowed to stand for four hours at room temperature with occasional swirling. The solvents were distilled off under reduced pressure at 40°, then 10 cc. of anhydrous benzene was added and also removed *in vacuo*. The residue, which remained after removal of the benzene, was taken up in 25 cc. of absolute ether. The ether solution was filtered and dry ammonia gas passed into the filtrate for seventy minutes. The ether was removed and the residue washed with water. After drying *in vacuo* the *i*-cholesterylmalonamide was crystallized from ethyl acetate, m. p. 224–225°.

Anal. Calcd. for $C_{30}H_{50}N_2O_2$: C, 76.58; H, 10.72; N, 5.95. Found: C, 76.23; H, 10.84; N, 6.01.

Preparation of *i*-Cholesterylmalonamide from the Fraction B Mixture.—Five grams of the original Acid B mixture was transformed to an acid chloride and treated with ammonia as described in the preparation of the amide from pure *i*-cholesterylmalonic acid. The ether-benzene mixture was distilled off under reduced pressure and the residue suspended in ether. The ether was filtered off and the solids thoroughly washed with ether. The product was dried at 60°, then washed with hot water and dried at 100°. The dry substance was crystallized from ethyl acetate; yield 2.2 g., melting point 224–225°. When mixed with *i*-cholesterylmalonamide prepared from pure *i*-cholesterylmalonic acid no melting point depression was observed.

Summary

The petroleum ether soluble cholesterylmalonic acid fraction was freed from 3-cholesterylmalonic acid and thereafter separated into two fractions. The first fraction proved to be a sterol acid, which does not possess a double bond, nor does it belong into the *i*-series. The second fraction, about 50% of the starting material, was the *i*-cholesterylmalonic acid, which was transformed into its amide and dimethyl ester. *i*-Cholesterylmalonamide could also be prepared directly from the petroleum ether soluble cholesterylmalonic acid.

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