BRASSICASTEROL AND 22,23-DIHYDROBRASSICASTEROL FROM ERGOSTEROL VIA i-ERGOSTEROL

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Received January 26, 1965

Since the method most commonly employed for the preparation of brassicasterol gave brassicasterol acetate in an overall 4% to 5% yield, an alternate method for the preparation of gram quantities of brassicasterol and 22,23-dihydrobrassicasterol was investigated. The partial syntheses of brassicasterol and 22,23-dihydrobrassicasterol are described. Brassicasterol and 22,23-dihydrobrassicasterol were obtained in an overall yield of 25% and 20%, respectively, from ergosterol via i-ergosterol.

In connection with comparative studies on the utilization and metabolism of campesterol and 22,23-dihydrobrassicasterol (C-24 methyl isomeric sterols) by the house fly and other insects, gram quantities of 22,23-dihydrobrassicasterol were required. Since the method (1) most commonly employed for the preparation of brassicasterol (ergosta-5,22-diene-3β-ol) and 22,23-dihydrobrassicasterol (ergost-5-ene-3β-ol) gave the first intermediate in about 21% yield and the brassicasterol acetate in an overall 4% to 5% yield, an alternate method for the preparation of 22,23-dihydrobrassicasterol (VIIb) was investigated. This paper describes the preparation of gram quantities of VIIb from ergosterol.

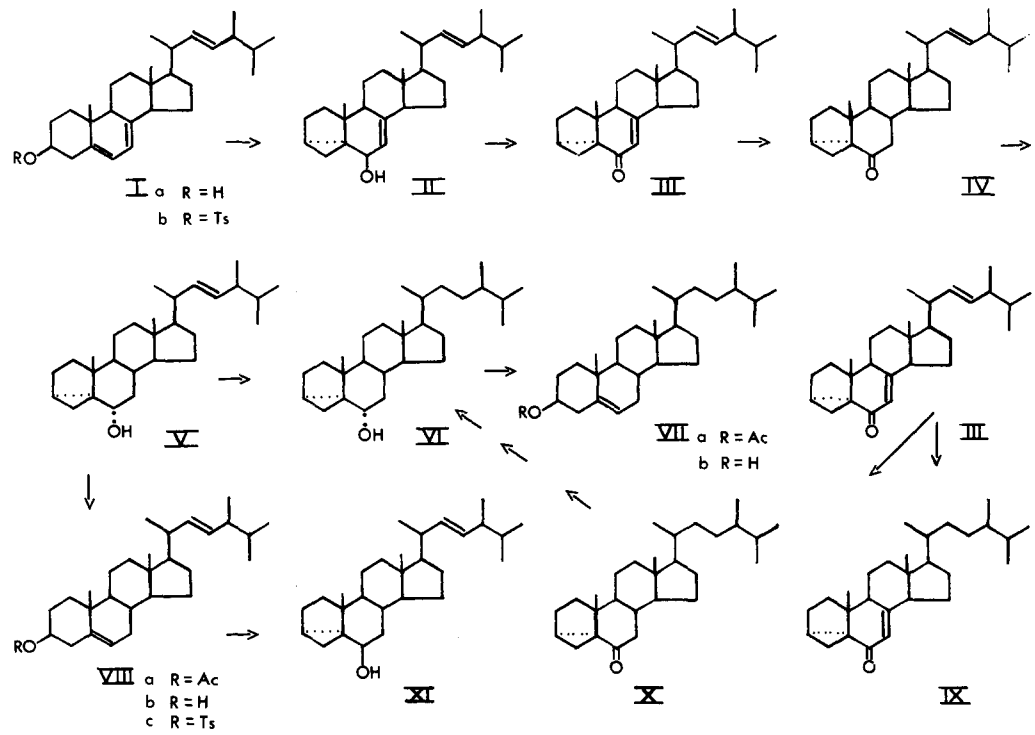
The solvolysis of ergosterol tosylate (Ib) according to the method of Nes and Steele (2) gave after chromatography on alumina i-ergosterol (II) in 55% yield. Oxidation of II with chromic acid in pyridine as previously reported (3) gave the ketone (III) in 70% yield. The strong

band at 1658 cm^{-1} and the ultraviolet absorption band at 250 mμ supports the assigned structure of III. Reduction of III with lithium and liquid ammonia yielded the 3,5-cycloergost-22-ene-6-one (IV). Compound IV showed a strong band at 1695 cm^{-1} indicative of the 6-ketone. The reduction of IV with lithium aluminum hydride gave the 3,5-cycloergost-22-ene-6α-ol (V). Analyses of V by thin layer chromatography (TLC) and gas-liquid chromatography (GLC) indicated only one compound. Catalytic hydrogenation of V afforded VI in 90% yield. The rearrangement of VI with zinc acetate in boiling acetic acid, followed by the saponification of the acetate VIIa, yielded 22,23-dihydrobrassicasterol (VIIb). The rearrangement of V with zinc acetate in boiling acetic acid gave VIIIa in nearly quantitative yield. The saponification of VIIIa with 2% methanolic potassium hydroxide gave brassicasterol (VIIIb).

The physical properties of VIIb, VIIIb, and their derivatives are in agreement with published data. Their relative retention times on three GLC systems also agreed with those of authentic naturally-occurring material.

By isolating only the intermediates II and III, brassicasterol can be obtained in an overall purified yield of 25%. 22,23-Dihydrobrassicasterol can be obtained in an overall yield of 20% by isolating the intermediates II, III, and V. With impure V the hydrogenation of the 22-double-bond proceeds slowly or not at all.

The reduction of the double bond at C-7 of compound III with lithium and liquid ammonia afforded as expected the naturally-occurring β-configuration at C-8. Barton and Robinson (1) have shown that the reduction of 3β-acetoxyergosta-7,22-diene-6-one by lithium and liquid ammonia afforded the product with the β-configuration at C-8. These



authors demonstrated further that carbanion reduction processes in alkaline media which involve the creation of asymmetric centers usually appear to afford the sterochemically more stable products.

The catalytic hydrogenation of III with palladium on charcoal gave a mixture of compounds, IX and X. The conversion of X to VI and subsequently to 22,23-dihydrobrassicasterol (VIIb) indicates that the more stable β-configuration also was formed at C-8, under the hydrogenation conditions employed.

The hydroxyl group of compounds V and VI have been assigned the 6α-configuration. Wagner and Wallis (4) found that reduction of i-cholestanone with lithium aluminum hydride afforded exclusively epi-i-cholesterol and that epi-i-cholesterol afforded cholesterol on rearrangement with zinc acetate and acetic acid, followed by saponifi-

cation. Thus, the 3β-hydroxy products VIIb and VIIIb would be obtained from the rearrangement of V and VI regardless of the orientation of the hydroxyl group at C-6. The more positive specific rotation of +35° for V further supports the assignment of the 6α-hydroxyl, as compared to a +15° for XI which has the 6β-hydroxyl configuration.

The conversion of ergosterol to brassicasterol and 22,23-dihydrobrassicasterol via the i-ergosterol is a convenient procedure and affords an improved yield of brassicasterol and 22,23-dihydrobrassicasterol of very high purity. This method of preparation also eliminates the high temperature sealed tube reaction employed in the previous synthesis (1).

EXPERIMENTAL

All melting points were determined on the Kofler (5) block. Rotations were determined in approximately 1% solutions in chloroform at 23°. Infrared spectra were obtained in CS_2 with a Perkin-Elmer (5) model 221 prism grating double-beam spectrophotometer. Gas-liquid chromatography analyses were made on Barber-Colman (5) models 10 and 15. A radium sulfate ionization source was used in the detector cell and argon was the carrier gas. The inert support was prepared and coated according to the method of VandenHeuvel et al. (6), and the gas-liquid chromatography systems used were SE-30, QF-1, and NGS. Activity grade II neutral alumina (Woelm) was used for chromatography.

i-Ergosterol (3,5-Cycloergosta-7,22-diene-6β-ol) (II) - A mixture of 10.0 g of dried ergosterol (Ia), 60 ml of pyridine, and 12.0 g of p-toluene-sulfonyl chloride was allowed to stand at room temperature in the dark for 18 hr and was poured into a liter of cracked ice and water. The crystalline ergosterol tosylate (Ib) was collected and washed with 2% ice cold potassium carbonate solution and ice cold water. The wet tosylate (theoretical yield of 13.88 g) was dissolved immediately in 1 liter of acetone at 20° C and was added to a boiling solution of 7.0 g of potassium carbonate in 875 ml of water and 2.1 liters of acetone over a 5 min. period. The mixture was refluxed for an additional 15 min. and distilled at atmospheric pressure. Approximately 2.0 liters of acetone was collected. The mixture was diluted with 1.5 liters of water and kept overnight at 0°. The semicrystalline solid was collected, dissolved in ether, and the ethereal solution was dried over sodium sulfate and evaporated to dryness in vacuo. The residue (10.0 g) was dissolved in 100 ml of hexane, adsorbed on a column of 220 g of hexane-washed alumina, and eluted as follows: 1-2, 250 ml fraction of hexane; 3-4, 250-ml fractions of hexane-benzene (3:1); 4-11, 250 ml fractions of hexane-benzene (1:1) and 250 ml fraction of benzene.

Fraction 1 yielded 1.6 g of crystalline i-hydrocarbon, and fractions 3-4 gave 1.0 g of a yellow oily residue. Crystallization of the crystalline fraction (6-10) from dilute acetone yielded 5.5 g of needles, m.p. 131-132.5° αD-11° (lit (2) m.p. 132-133°, αD-11°).

3,5-Cycloergosta-7,22-diene-6-one (III) - To a mixture of 5.0 g of chromium trioxide in 35 ml of pyridine was added 5.0 g of i-ergosterol (II) in 35 ml of pyridine and the mixture was left overnight at room temperature. The mixture was diluted with ether and filtered. The ethereal solution was washed three times with water, dried over sodium sulfate, and concentrated to dryness in vacuo. The crystalline product was crystallized from dilute acetone to give 3 g of 3,5-cycloergosta-7,22-diene-6-one (III) as plates m.p. 168-169°, αD +43°. λmax 250 mμ E 13,800, νmax 1658 cm^{-1} (Lit (3) m.p. 168-169°, αD +43°, λmax 249 mμ (log E 4.13). A second crop of material weighing 0.5 g, m.p. 165-169° was obtained.

3,5-Cycloergost-22-ene-6-one (IV) - To a solution of 200 mg of lithium in 70 ml of liquid ammonia was added rapidly with vigorous stirring 3.44g of 3,5-cycloergosta-7,22-diene-6-one (III) in 120 ml of dry ether. The mixture was stirred for 2 min. after the addition was completed. If the dark blue color was still present in the reaction mixture, the excess lithium was destroyed immediately with solid ammonium chloride. Usually this step was not necessary. If the blue color disappeared before the addition of III was completed, the addition was stopped and a few milligrams more of lithium was added and the addition was continued. The reaction mixture was allowed to come to room temperature, diluted with water and extracted with ether three times. The ethereal extract was washed twice with water, and dried over sodium sulfate. The solution was evaporated to dryness in vacuo to give 3.3 g of semicrystalline residue. For characterization and analysis a 0.5-g portion of the residue was chromatographed over 15 g of hexane-washed alumina. Crystallization from dilute acetone of the fraction eluted with a total volume of 300 ml of hexane gave 400 mg of IV, m.p. 108-110°, αD +5°. νmax 1695 cm^{-1} (6 ketone).

Anal. Calcd. for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.66; H, 10.93.

3,5-Cycloergost-22-ene-6α-ol (V) - To a solution of 3.4 g of crude 3,5-cycloergost-22-ene-6-one (IV) in 140 ml of dry ether was added 0.6 g of solid lithium aluminum hydride and the mixture was refluxed for 2 hr. The mixture was cooled and the excess lithium aluminum hydride was destroyed immediately with ethyl acetate and then treated with water (IV allowed to stand overnight in the presence of lithium aluminum hydride resulted in the cleavage of the 3,5-cyclopropane ring). The aqueous layer was separated and extracted with ether and the extracts combined. The ethereal extract was washed with water, dried over sodium sulfate, and evaporated to dryness in vacuo. Crystallization of the oily residue from acetone-acetonitrile yielded 3.0 g of V as spears, m.p. 92-93°, αD +35°, νmax sharp bands at 3000, 3070, shoulder at 3030 cm^{-1} (cyclopropane ring).

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.70; H, 11.38.

3,5-Cycloergostan-6α-ol (VI) - A mixture of 2.9 g of 3,5-cycloergost-22-ene-6α-ol (V), 1.0 g of 10% palladium on charcoal, and 75 ml of ethyl acetate was shaken with hydrogen at room temperature and atmospheric pressure for 5 hr, at which time one mole equivalent of hydrogen had been absorbed. The catalyst was removed by filtration and the solution was concentrated to dryness in vacuo. The crystalline residue was crystallized from acetone-acetonitrile and yielded 2.6 g of VI, m.p. 96-98°, αD +65°, νmax sharp bands at 3010, 3070 cm^{-1}, shoulder 3030 cm^{-1} (cyclopropane ring).

Anal. Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.07. Found: C, 84.20; H, 11.90.

22,23-Dihydrobrassicasterol (VIIb) - A mixture of 2.2 of 3,5-cycloergostan-6α-ol (VI), 4.4 g of freshly fused zinc acetate in 40 ml of acetic acid was refluxed with magnetic stirring for 3 hr. The solution was cooled, diluted with water, and filtered to give the crude acetate (VIIa). Saponification of VIIa with 2% methanolic potassium hydroxide gave 2.0 g of VIIb. The material was adsorbed on 60 g of hexane-washed alumina and eluted as follows: fraction 1, 400 ml of hexane; fraction 2, 400 ml of benzene-hexane (1:1); fraction 3, 400 ml of benzene; and fraction 4, 100 ml of ether. Fraction 4, crystallized from dilute acetone, yielded 1.7 g of 22,23-dihydrobrassicasterol, m.p. 158-159°, αD -44° (Lit (7), m.p. 158°, αD -46°).

Anal. Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.07. Found: C, 83.68; H, 12.33.

GLC analyses on three chromatographic systems showed only one peak.

22,23-Dihydrobrassicasterol acetate (VIIa) - A mixture of 1.06 g of 3,5-cycloergostan-6α-ol VI, 2.0g of fused zinc acetate, 25 ml. of acetic acid was refluxed with magnetic stirring for 2 hr. The solution was cooled, diluted with water, and filtered. Recrystallization of the crystalline material from acetone-methanol gave 1.0 g of VIIa as plates, m.p. 146-148°, αD -49° (Lit (7) m.p. 145°, αD -46°).

Anal. Calcd. for $C_{30}H_{50}O$: C, 81.39; H, 11.38. Found: C, 81.60; H, 11.20.

Brassicasterol (VIIIb) - A mixture of 1.0 g of 3,5-cycloergost-22-ene-6α-ol (V), 2.0 g of fused zinc acetate, 25 ml of acetic acid was refluxed for 2 hr and worked up in a similar manner as in the preparation of VIIb. After the saponification of the acetate and recrystallization from dilute acetone, 856 mg of VIIIb was obtained, m.p. 149-151°, αD -66° (Lit (8,1), m.p. 148° αD -64°, m.p. 150-151°, αD -60°).

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.50; H, 11.59.

The GLC analyses of brassicasterol on a SE-30, QF-1, and HGS column gave identical relative retention time as authentic brassicasterol. Its infrared spectrum was identical with that of authentic brassicasterol.

3,5-Cycloergostan-6-one (X) and 3,5-cycloergost-7-ene-6-one (IX) - A mixture of 2.4 g of 3,5-cycloergosta-7,22-diene-6-one (III), 1.0 g of 10% palladium on charcoal, and 250 ml of ethanol was shaken with hydrogen at room temperature and atmospheric pressure for 8 hr. The catalyst was removed by filtration and the solution was concentrated to dryness in vacuo. An infrared analysis of the crystalline material showed that the double bond at the 22-position had been completely reduced. Carbonyl absorption bands at 1695 cm^{-1} and 1658 cm^{-1} indicated an incomplete hydrogenation at the Δ^7 position. The material was chromatographed over 60 g of hexane-washed alumina and the material was eluted from the column with 100 ml fractions of hexane. Fractions 1-3 exhibiting only the carbonyl absorption bands at 1695 cm^{-1} were combined and recrystallized from dilute acetone to give 914 mg of 3,5-cycloergostan-6-one (X), m.p. 108-110°, αD +33°.

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.32; H, 11.55.

Fractions 11-14 exhibiting carbonyl absorption band at 1658 cm^{-1} were combined and recrystallized from dilute acetone to give 570 mg of IX, m.p. 142-144°, αD +77°, λmax 249 mμ E 11,987.

Anal. Calcd. for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.50; H, 11.31.

Fractions 4-10 still consisted of a mixture of IX and X.

The reduction of X with lithium aluminum hydride gave VI and subsequent treatment with zinc acetate in acetic acid and saponification of the acetate yielded 22,23-dihydrobrassicasterol (VIIb). The physical properties of VIIb were identical to those of VIIb obtained by the lithium-liquid-ammonia reductive synthetic pathway to 22,23-dihydrobrassicasterol. Their infrared spectrum and GLC relative retention times on three GLC systems were indistinguishable.

3,5-Cycloergost-22-ene-6β-ol (XI) - To 140 mg of brassicasterol (VIIIb) in 3 ml of dry pyridine was added 210 mg of p-toluenesulfonyl-chloride. The mixture was kept in the dark for 18 hr at room temperature and was poured into 25 ml of cracked ice and water. The crystalline brassicasterol tosylate VIIIc was collected and washed with ice cold 2% potassium carbonate solution and water. An infrared analysis showed that the tosylate VIIIc had been formed. The crude tosylate in 56 ml of acetone, 5.6 ml of water, and 131 mg of potassium bicarbonate was refluxed for 4 hr. The solution was reduced in volume to about 10 ml and stored at 5° overnight. The precipitate was collected, dried, and chromatographed over hexane-washed alumina. The column was washed with 100 ml of hexane and further eluted with 25-ml fractions of hexane-benzene (1:1). Fractions 4-7 crystallized from acetonitrile-acetone gave 79.0 mg of XI, m.p. 113-115°, αD +15°, ν max sharp band 3015, 3060 cm^{-1} (cyclopropane ring).

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.40; H, 11.33.

ACKNOWLEDGMENT

Thanks are extended to Dr. William Alford and the staff of the Microanalytical Department of the National Institutes of Health, Bethesda, Md., for the carbon and hydrogen analyses.

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5. Mention of proprietary products herein does not necessarily imply their endorsement by the U. S. Department of Agriculture.

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