Isolation of Brassicasterol from Steam Deodorizer Distillate of Rapeseed Oil: Some Properties of Its Acetate Tetrabromide and Its Reduction to 22,23-Dihydrobrassicasterol[1]

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ABSTRACT

Brassicasterol (5,22-ergostadien-3β-ol) was isolated from the steam deodorizer distillate of rapeseed oil and purified by acetylation, bromination, chromatography on 20% $AgNO_3/SiO_2$ columns and hydrolysis. Brassicasteryl and stigmasteryl (5,22-stigmastadien-3β-ol) acetates were brominated, and the yields of products and solubilities of the tetrabromides from the two steryl acetates were compared. Stigmasteryl acetate tetrabromide is less soluble than the corresponding brassicasteryl derivative; yet the latter precipitates selectively during bromination of a mixture of the two steryl acetates. This is explained on the basis of the stereochemistry of the bromine atoms in the side chains of the two steryl acetate tetrabromides. Hydrogenation of brassicasteryl acetate over Raney nickel gave the 22,23-dihydro derivative in excellent yield. The latter was separated from small amounts of ergostanyl acetate on a 20% $AgNO_3/SiO_2$ column.

INTRODUCTION

Brassicasterol was first obtained by Windaus and Welsch in 1909 (1) from the phytosterol fraction of rapeseed oil, and this source was used in two more recent isolations (2,3). The sterol has also been isolated from molluscs (4) and synthesized from ergosterol by two different procedures (5,6).

The sterols present in very small quantities in crude vegetable oils are concentrated during the steam deodorizing process and appear as substantial fractions of the deodorizer distillates (7-9). Our work with *Drosophila* (10) requires brassicasterol and its 22,23-dihydro derivative, so we chose the deodorizer distillate obtained during the commercial processing of rapeseed oil as a convenient source for these two compounds.

22,23-Dihydrobrassicasterol has been prepared by hydrogenation of the *i*-sterol methyl ether derived from brassicasterol (11,12), from ergosterol by two different multistep syntheses (5,6), from 3β-methoxy-5-cholenoyl chloride and an optically active cadmium reagent (13), and from pregenolone acetate and an optically active Grignard reagent (14).

The availability of pure brassicasterol together with the successful hydrogenation of stigmasterol to its 22,23-dihydro derivative in good yield over Raney nickel (15) prompted us to investigate the preparation of 22,23-dihydrobrassicasterol by direct hydrogenation of the parent sterol.

EXPERIMENTAL PROCEDURES

Methods and Materials

The gas liquid chromatography (GLC), thin layer chromatography (TLC) and titration of unsaturation with bromine of sterols and their esters has been described (16). Brassicasteryl acetate separates readily from a mixture of 22,23-dihydrobrassicasteryl acetate and ergostanyl acetate by GLC on a 5% OV-101 column; the latter two, however, have identical retention times. They were separated on $AgNO_3$/alumina 2:3 thin layer plates (17) with Skellysolve B (petroleum ether, bp 65-67 C) as solvent. Ethyl acetate was distilled before use; triethylamine (MCB) was used as received. Raney nickel (no. 28 active catalyst in water, W.R. Grace and Co., Raney Catalyst Div., Chattanooga, Tenn.) was solvent-exchanged through ethanol and ethyl acetate immediately before use. Melting points were taken in vacuo and are corrected.

Isolation of Sterols

Ten gallons of rapeseed oil steam deodorizer distillate (Fig. 1A) (Canada Packers, Ltd., Montreal) were filtered in two batches at room temperature through four large (3 liter), coarse porosity, sintered glass filters with an oil pump vacuum. Each filtration was hastened by scraping the surface of the funnel twice a day with a large flat-ended spatula. After 15 days (each batch), the precipitates on the four filters (ca. 5 liters total) were washed with an equal volume of ether to remove adhering oil, followed by a wash with absolute ethanol. The original fil-

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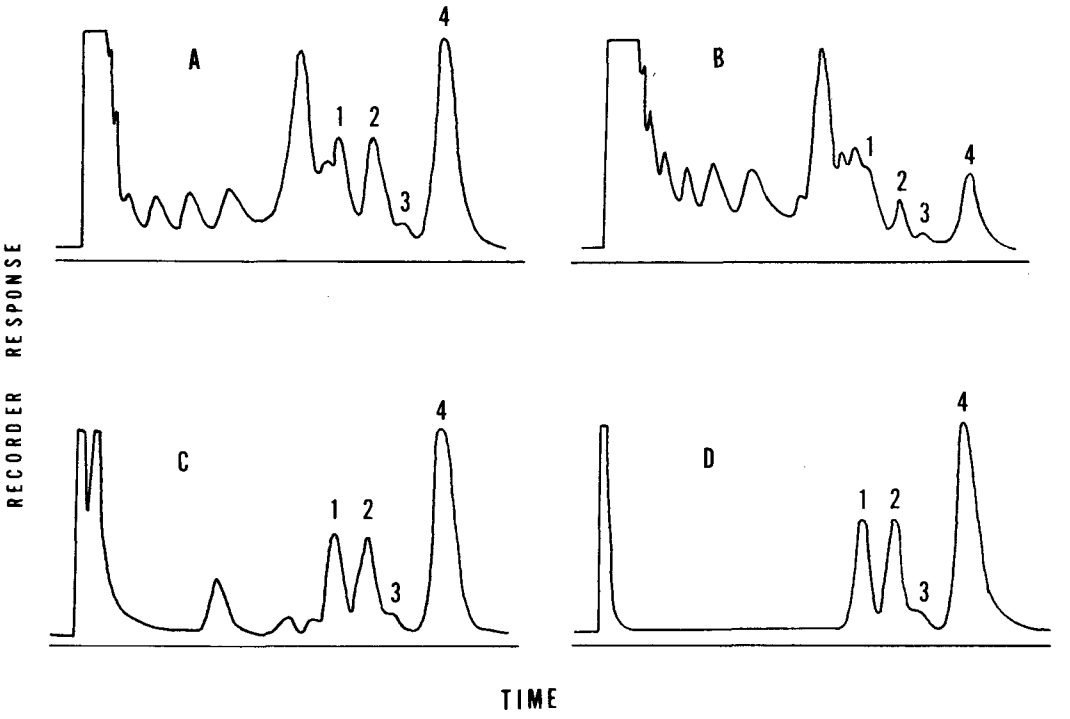

FIG. 1. Gas liquid chromatographic separation diagrams: (1) brassicasterol, (2) campesterol, (3) stigmasterol, (4) sitosterol. A. Crude rapeseed oil steam deodorizer distillate. B. Filtrate from A. C. Filter cake from A. D. Phytosterol mixture obtained from C by crystallization from toluene and isopropanol.

trates and washes (Fig. 1B) were discarded and the precipitates (Fig. 1C) combined in 16 liters hot $CHCl_3$ and filtered to remove a black, gritty material. The $CHCl_3$ was evaporated and the residue crystallized from 4 liters toluene and from isopropanol to yield 1690 g sterol mixture (Fig. 1D).

Acetylation

The sterol mixture was acetylated in six batches on steam baths in the hood with an equal weight of acetic anhydride and sufficient benzene to provide initial homogenity. When the mixtures had evaporated to dryness, the products were washed with methanol in a blender, filtered and dried in air; yield: 1540 g.

Bromination

The steryl acetates (100 g) in 550 ml ether were stirred on an ice bath while 500 ml 10% Br_2 in acetic acid was added dropwise during 30 min. The mixture was then stirred at room temperature for 3 hr and placed in a cold room (4 C) overnight. The precipitate was filtered in the cold, washed with acetic acid and methanol, and air-dried to yield 28 g crude brassicasteryl acetate tetrabromide.

The tetrabromides from 1540 g steryl acetates were combined and stirred with ether (1 liter/250 g), filtered and the procedure repeated. The resulting ether-insoluble precipitate (375 g) was boiled with 2.5 liters acetone, cooled and filtered to yield 344 g purified brassicasteryl acetate tetrabromide.

Debromination

The acetate tetrabromide (100 g) was refluxed with 100 g Zn dust in 1.5 liters 1:1 ethanol-acetic acid for 4 hr. The mixture was decanted from unreacted Zn, the latter washed with a small amount of ether and the combined solutions placed in a cold room overnight. The resulting precipitate was filtered and washed with methanol to give 47.5 g crude brassicasteryl acetate. An additional 4.5 g of a mixture of steryl acetates and free sterols was obtained by addition of water to the filtrate. In all, 170 g brassicasteryl acetate containing ca. 12% stigmasteryl acetate, 8% sitosteryl acetate and 2% campesteryl acetate (Fig. 2A) was isolated from 10 gal rapeseed oil steam deodorizer distillate.

Purification of Brassicasteryl Acetate

A solution of 300 g $AgNO_3$ in 4.5 liters water was mixed with 1500 g silica gel (Mallinckrodt Silicic Acid, A.R., 100 mesh) and 600 g

Celite (Johns-Manville). The mixture was dried on a steam bath for 2 days and in an oven at 110 C overnight. One kilogram was screened (80 mesh), slurried in Skellysolve F-dry diethyl ether 100:1, and poured into a large chromatographic tube (adsorbent dimensions 6 x 85 cm). Ten grams of the crude brassicasteryl acetate mixture (Fig. 2A) was placed on the column in a small volume of benzene and eluted with Skelly F-ether 100:1. After 14 liters had eluted from the column, the steryl acetates began to emerge. Fractions (500 ml) were collected, evaporated and analyzed by GLC. After elution of 2.32 g of sitosteryl, campesteryl and stigmasteryl acetates, fractions rich in brassicasteryl acetate were collected (1.92 g). Stigmasteryl acetate was identified by its melting point (143.5-144.5 C, lit. [18] 144 C) and IR spectrum (superimposable on that of an authentic specimen). When stigmasteryl acetate was no longer evident in the eluate (GLC), the column was eluted with ether to yield 5.61 g brassicasteryl acetate. The 1.92 g of brassicasteryl acetate-rich fraction was rechromatographed on a smaller (250 g) column in the same way to yield an additional 1.53 g of product. The combined materials (7.14 g) were recrystallized from isopropanol to yield 6.25 g brassicasteryl acetate, mp 157.5-158.5 C (Fig. 2B) (highest melting point reported: 158-159 C [6]); double bonds by bromine titration (16): 2.14/mol.

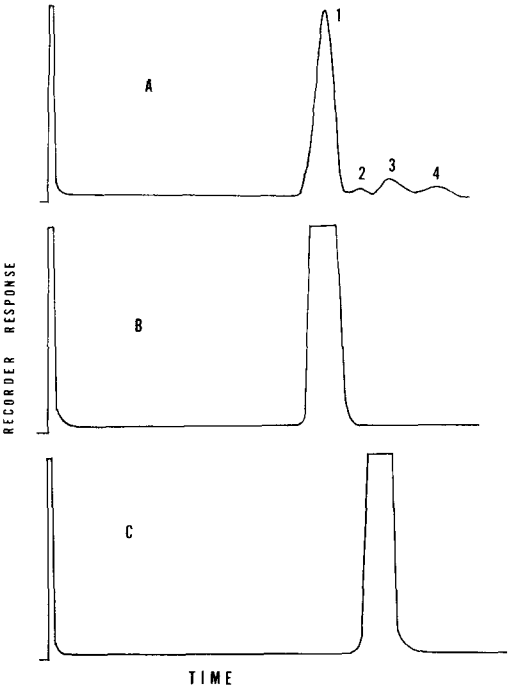

FIG. 2. Gas liquid chromatographic separation diagrams. A. Crude brassicasteryl acetate obtained from ID by bromination and debromination of the insoluble steryl acetate tetrabromides. B. Purified brassicasteryl acetate. C. Purified 22,23-dihydrobrassicasteryl acetate.

Brassicasterol

The acetate (16 g) was hydrolyzed for 2 hr on the steam bath with 900 ml 5% KOH in ethanol. After cooling and one recrystallization from ethanol, 13.4 g brassicasterol was obtained, mp 150-151 C (highest melting point reported: 151 C [6]); double bonds by bromine titration (16): 2.18/mol.

Preparation of Stigmasteryl and Brassicasteryl Acetate Tetrabromides and Determination of their Solubilities

Stigmasteryl acetate (13.5 g, 29.7 mmol) in 180 ml ether was treated with Br_2 (11 g, 69 mmol) in 90 ml acetic acid on an ice bath, and the mixture was kept in the cold room overnight. The precipitate was washed with ether, refluxed with 300 ml ether, filtered and dried to yield 9.15 g (40%) stigmasteryl acetate tetrabromide. The latter was debrominated with 10 g Zn dust in 80 ml acetic acid and the product crystallized from ethanol to yield 4.1 g (78% from tetrabromide, 31% overall) stigmasteryl acetate.

Brassicasteryl acetate (3.1 g, 7 mmol) in 40 ml ether was treated in a like manner with Br_2 (2.4 g, 15 mmol) in 20 ml acetic acid to yield 2.9 g (54%) brassicasteryl acetate tetrabromide.

Samples (ca. 1 g) of the acetate tetrabromides of stigmasterol and brassicasterol were stirred with the bromination reaction solvent (ether-acetic acid 2:1) and with the wash solvent (ether) at room temperature and in the cold room for 18 hr. The stirring was stopped, and a 25 ml aliquot of each run was removed and evaporated in vacuo in a tared flask. The solubilities thus obtained are given in Table I.

Bromination of Mixtures of Brassicasteryl and Stigmasteryl Acetates

Two by weight mixtures of brassicasteryl and stigmasteryl acetates were prepared, 1:1 and 6.7:1, respectively. A sample of each mixture (1.32 g, 3 mmol) was treated with either 1.5 or 2.2 equivalents of Br_2/mol in the usual way, and the sterol acetate tetrabromide mixtures isolated. The yields of precipitates from the various runs and the composition of the products as determined by quantitative GLC analysis after debromination are given in Table II.

Hydrogenation of Brassicasterol

Brassicasterol (0.399 g, 1 mmol) was added

TABLE I

Solubilities of Brassicasteryl and Stigmasteryl Acetate Tetrabromides

Sample[a]	Solvent	Solubilities, g/100 ml solvent 25 C	4 C
Brassicasterol	2:1 Ether-HOAc	0.280	0.238
	Ether	0.387	0.324
Stigmasterol	2:1 Ether-HOAc	0.128	0.084
	Ether	0.218	0.157

[a]Acetate tetrabromides.

to 0.8 ml Raney nickel slurry in 50 ml ethyl acetate and 1 ml triethylamine, and hydrogenated until 1 mmol H_2 had been absorbed (mercury filled gas buret). The product showed no brassicasterol by GLC, a trace of ergostanol by thin layer chromatography (TLC) and contained 0.98 double bonds per mole of sterol by titration with bromine.

Hydrogenation of Brassicasteryl Acetate

Raney nickel (7 ml slurry) was equilibrated with hydrogen in 500 ml ethyl acetate for 1 hr, after which 4.40 g (10 mmol) brassicasteryl acetate was added. Hydrogen uptake ceased after 10.2 mmol had been absorbed (Fig. 3); the reaction mixture was then filtered and evaporated. The residue contained no brassicasteryl acetate (GLC), a small amount of ergostanyl acetate (TLC), and gave 0.929, 0.933 double bonds per mole steryl acetate by bromine titration.

Purification of 22,23-Dihydrobrassicasteryl Acetate

A 20% $AgNO_3$/silica gel column was prepared with Skellysolve F-diethyl ether 100:1 as described above. Crude 22,23-dihydrobrassicasteryl acetate (10 g) was placed on the column in a minimum quantity of benzene and eluted with the 100:1 solvent. After 10 liters had passed through the column, ergostanyl acetate began to emerge. When it was no longer evident in the eluate (TLC), the column was eluted with ether to yield 8.15 g 22,23-dihydrobrassicasteryl acetate, mp 145-146.5 C after recrystallization from ethanol (Fig. 2C).

The products from three such column runs were combined (24 g) and recrystallized from ethanol to yield 21.5 g 22,23-dihydrobrassicasteryl acetate, mp 147.5-148.5 C (Table III), 0.984, 0.991 double bonds per mole by bromine titration.

22,23-Dihydrobrassicasterol

The acetate (13.5 g) was hydrolyzed with 5% KOH in ethanol (1 liter) and the product recrystallized once from ethanol to yield 10.2 g 22,23-dihydrobrassicasterol, mp 159.5-160.5 C (Table III); 1.002, 0.994 double bonds per mole by bromine titration. A benzoate (Table III) was prepared in the usual way.

RESULTS AND DISCUSSION

Brassicasterol occurs together with stigmasterol, campesterol and sitosterol in the steam deodorizer distillate from the commercial processing of rapeseed oil (Fig. 1A). The isolation of the phytosterol fraction (Fig. 1C) was simply accomplished by filtration of the semisolid distillate as obtained. Although a portion of the sterols was lost in the filtrate and washings of the precipitate (Fig. 1B), the ready availability of the inexpensive starting material made extensive work-up procedures unprofitable at this point.

Brassicasterol was readily separated from the bulk of the campesterol and sitosterol by virtue of the insolubility of its acetate tetrabromide

TABLE II

Bromination of Mixtures of Brassicasteryl and Stigmasteryl Acetate

	Brassicasteryl-stigmasteryl ratio (start) 1:1		6.7:1	
Mol Br_2/mol acetate	1.5	2.2	1.5	2.2
Tetrabromide precipitate, % yield	20	57	23	59
Brassicasteryl-Stigmasteryl ratio in precipitate	3.3:1	1.2:1	28:1	10:1
Brassicasteryl-stigmasteryl ratio in product recovered from filtrate	0.6:1	0.5:1	5:1	3:1

(1). Since stigmasterol also forms an insoluble acetate tetrabromide, separation of brassicasterol from this sterol is more difficult. Crystallization of the acetate mixture derived from the large scale brominations-debrominations (Fig. 2A), the free sterols derived from this mixture or the acetate tetrabromides of the sterols from numerous solvents failed to give pure brassicasterol. Some purification was achieved by a repetition of the bromination-debromination procedure (80 → 90% pure brassicasterol), but this was very wasteful; ca. 50% of the starting material was lost in the process (19,20).

Chromatography of 10 g batches of the crude brassicasteryl acetate on 1 kg 20% $AgNO_3$/silica gel (3) with 1% ether in low boiling petroleum ether (Skellysolve F) was finally used as the best way to obtain gram quantities of brassicasterol free from stigmasterol and traces of campesterol and sitosterol. The average yield of 6.25 g pure brassicasteryl acetate from 10 g crude mixture represents an 80% recovery of the sterol from the mixture. Both brassicasterol and its acetate consumed close to 2.15 mol Br_2 per mole of sterol, as did stigmasterol and its esters in a standardized titration procedure (16).

A number of experiments were performed to explain the results obtained from bromination of crude brassicasteryl-stigmasteryl acetate mixtures. We had found in numerous trials (unpublished work) that the tetrabromide acetate precipitates were always enriched in the brassicasterol derivative when compared to the starting material. Determination of the solubilities of the two tetrabromides (Table I) showed that this property does not provide the answer; the stigmasterol derivative had the lesser solubility under all conditions.

Bromination of stigmasteryl and brassicasteryl acetate individually and in two synthetic mixtures suggested reasons for the above observations. The yield of solvent-insoluble acetate tetrabromide was 40% from stigmasterol and 54% from brassicasterol. In addition, when

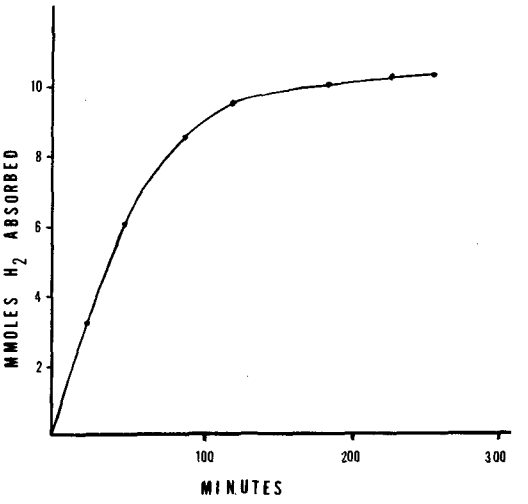

FIG. 3. Hydrogenation of 10 mmol brassicasteryl acetate in ethyl acetate over Raney nickel.

mixtures of the two acetates were brominated with only 1.5 mol Br_2 per mole steryl acetate, brassicasteryl acetate tetrabromide was largely enriched in the resulting precipitates (Table II); whereas when an excess of Br_2 was added (2.2 mol Br_2 per mole acetate), the enrichment was much smaller (Table II).

Theoretically, four isomers of each sterol acetate tetrabromide are possible by *trans* addition (21) of bromine to the Δ^5 and Δ^{22} double bonds: 5α, 6β, 22R, 23S; 5α, 6β, 22S, 23R; 5β, 6α, 22R, 23S; and 5β, 6α, 22S, 23R. However the preferential attack of Br_2 on the Δ^5 bond in a $\Delta^{5,22}$ diene (22) from the α-side of the sterol molecule (23), together with the low temperatures maintained in this work during bromination and work-up to inhibit 5α,6β → 5β,6α isomerization (24), suggests that the 3β-acetoxy-5α,6β-dibromo configuration is the same in both brassicasteryl and stigmasteryl acetate tetrabromides and that each sterol yields only two isomers. The results are then best explained on the basis of the stereochem-

TABLE III

Melting Points (C) of 22,23-Dihydrobrassicasterol, Its Acetate and Benzoate

Source	Sterol	Acetate	Benzoate
Fernholz and Ruigh (11)	158	145	162
Barton and Robinson (6)	146-148	152-154	
Clayton and Bloch (12)	155-156		
Thompson et al. (5)	158-159	146-148	
Martinez et al. (13)	148-149	153-155	
Ikan et al. (14)		140	
Present work[a]	159.5-160.5	147.5-148.5	164-165

[a]In vacuo, corrected.

istry of the side chain in the two sterols.

The rates of formation and the solubilities of the four tetrabromo derivatives are probably quite different. From the data in Table II, it is reasonable to conclude that the rate of bromination of the Δ^{22} bond in brassicasteryl acetate is greater than that of the stigmasteryl derivative or the formation of an insoluble 5α, 6β, 22ξ, 23ξ tetrabromide in the former case is sterically favored. The enrichment of brassicasterol in precipitates obtained by bromination of mixtures of the two steryl acetates is then a function of the rates of reaction of the Δ^{22} bond with bromine in the two compounds, together with the relative insolubility of a sterically favored brassicasteryl acetate tetrabromide.

Hydrogenation of brassicasterol or its acetate over Raney nickel is a convenient way to prepare 22,23-dihydrobrassicasterol. The sterol and its acetate contained 1.00 double bond per mole by a standardized bromine titration; in this way it resembles cholesterol and sitosterol (16). The melting points observed in the present work are in accord with some of those recorded in the literature, but at variance with others (Table III). It is possible that the compounds prepared in three earlier studies (6,13,14) either were not pure or were isomers of the desired dihydrobrassicasterol.

The hydrogenations of brassicasteryl acetate (Fig. 3) were much more facile than those observed for stigmasterol or its esters (15). In the latter cases, 20-30% of stigmastanol formed when the reductions were run until almost all stigmasterol had been consumed. By contrast, only 3-7% of ergostanyl acetate was formed in reaction mixtures that contained not a trace of brassicasteryl acetate. This increased activity of the Δ^{22} bond in brassicasteryl acetate over that in the stigmasteryl derivative was also observed during bromination of the two steryl acetates.

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