

7-DEHYDROCHOLESTEROL¹

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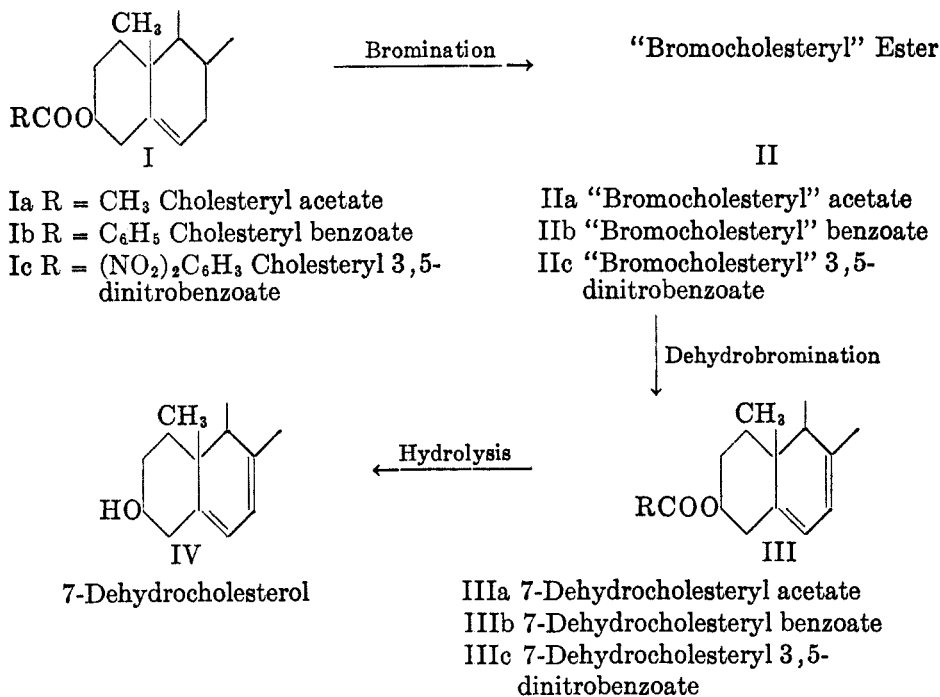
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In 1942, Ziegler and co-workers (1), in their classical paper on substitution in the "allylic" position with N-bromosuccinimide, mentioned that cholesteryl esters (I) had been brominated in a similar manner. Although no details were given, it was to be inferred that a dehydrobromination had also been carried out to give the desired 7-dehydrocholesteryl esters (III). This represented a novel method of preparing 7-dehydrocholesterol. The publication on this process mentioned by the German workers as being forthcoming, has not appeared. This undoubtedly may be ascribed to conditions created by the war.

Subsequently, several laboratories have undertaken a study of this novel preparation of 7-dehydrocholesterol (IV), and many publications have appeared on this subject (2-7).

We have been working on this synthesis independently and wish at this time to present a detailed account of our results.

The synthesis may be represented as follows:



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The discussion of the preparation may be conveniently divided into three parts; (A), bromination; (B), dehydrobromination; and, (C), isolation and estimation of yields.

A. BROMINATION

A systematic study of reaction conditions showed that the bromination with N-bromosuccinimide or N-bromophthalimide was affected by a number of factors: mole ratio of NBS⁴ to cholesteryl ester, type and boiling point of solvent and presence of catalysts, *e.g.* light. This work was done primarily on three esters of cholesterol, *i.e.* the acetate (Ia), benzoate (Ib) and 3,5-dinitrobenzoate (Ic), and of these three our attention has been concentrated on the benzoate. Remarks concerning the benzoate throughout the discussion will, for the most part, be true of the other two esters.

Our experience has shown that a 20% excess of NBS led to the best yields. Others (*loc. cit.*) have used varying mole ratios, varying from 0.8 to 1.5 equivalents of NBS. Results indicated that use of less than one equivalent of NBS led to difficulties in the isolation of the final product, due to the presence of unreacted cholesteryl ester, whereas use of more than 1.2 equivalents gave lower yields (see Tables II and III).

The time of bromination depends on the solvent and catalyst used. It was definitely demonstrated that the boiling point of the solvent and consequently the temperature at which the reaction was run, determines the bromination time. When the reaction was done in carbon tetrachloride (see Table II) the bromination time was of the order of ten minutes, whereas if a catalyst was present the reaction time could be reduced to, at the most, two minutes. [1.5 minutes with petroleum ether (see Table III).] It was found that the reaction could be catalyzed by irradiation of varying wavelengths.⁵ The most convenient light was generated by photospot lamps.

When the bromination was carried out in petroleum ether, b.p. 64–66°, with visible irradiation the yield was higher than when carried out in carbon tetrachloride under the same conditions. Others (*loc. cit.*) have used ether, petroleum ether of various boiling ranges, ethyl acetate, and carbon tetrachloride for this purpose.

The boiling point of the petroleum ether used played an important role in the time of bromination and the final yield of product. An examination of Table IV indicates that with the lower-boiling petroleum ethers, at least twenty minutes are required for sufficient bromination to obtain good yields. It should be pointed out that more extended times may increase the yields in these cases. Use of higher-boiling petroleum ethers tends to decrease the yield, *i.e.*, the use of petroleum ether b.p. 95–100° gave a crude yield of 16.7% as compared to a crude yield of 41.5% for petroleum ether, b.p. 55–60°.

Several experiments were carried out to determine at what stage in the bro-

⁴ NBS = N-Bromosuccinimide

⁵ Buisman, Stevens, and v.d. Vliet (5) have made a similar observation and have used ultraviolet light to catalyze the bromination reaction.

mination step the dehydrobrominating agent should be added to obtain the best results. Addition of an organic base such as dimethylaniline to the bromination mixture before reaction completely prevented bromination. It was found that addition of the amine to the refluxing bromination mixture immediately after complete reaction rather than waiting until after separation of the succinimide increased the yield by several per cent.

Mention should be made of the purity of the cholesteryl ester used in the bromination. It was found, especially in the case of the benzoate, that the ester must be extremely pure to give the maximum yield. This appeared to pertain less in the case of the acetate. The necessity for the absolutely pure benzoate is not well understood. Perhaps the answer lies in the possibility that traces of pyridine remaining from the benzoylation interfere. The adverse affect of amines on the bromination reaction has been discussed above.

B. DEHYDROBROMINATION

The yield in the dehydrobromination step was dependent on several factors: type and purity of dehydrobrominating agent, and presence or absence of a solvent. In our early work, dehydrobromination was done with no solvent other than the amine. Investigation showed that the use of an inert solvent as a diluent greatly facilitated this step and increased the yield. Examination of Tables II and III shows that *s*-collidine in xylene was the best combination of those tried. Several combinations used, such as pyridine in toluene, are not reported in the tables. Other workers (*loc. cit.*)⁶ have carried out this step without a diluent.

The use of a solvent greatly facilitated the work-up of the product. It was a simple matter to separate the amine hydrobromide and evaporate the diluent *in vacuo* to give the crude product.⁷

The purity of the amine had a bearing on the final yield. Highly purified *s*-collidine gave a slightly better yield than the material used as received.⁸

C. ISOLATION, ESTIMATION OF YIELDS

The crude 7-dehydrocholesteryl ester (III) was examined spectroscopically and, in this manner, a "crude" yield was obtained. The purity of the ester was determined by an estimation of the molecular extinction coefficient at the 282 $m\mu$ maximum. This method was found to be fairly reliable only when the 282 $m\mu$ maximum was equal to or higher than the 272 $m\mu$ maximum. Estimation of the purity of the product by this method is, we feel, open to a good deal of criti-

⁶ Buisman, Stevens, and v.d. Vliet (5) have reported one experiment with cholesteryl acetate where the dehydrobromination was carried out with *s*-collidine in xylene in the presence of calcium carbonate.

⁷ Removal of excess amine by steam distillation or work-up by extraction afforded no real advantage, in the case of the benzoate, over the process herein outlined. Although a purer "crude" product was obtained, the over-all yield was not appreciably affected. Extraction work-up, however, was preferred for the acetate.

⁸ A similar observation has been reported by N. V. Philips' Gloeilampenfabriken in British Patent Application 30908 (November 21, 1947).

cism; however, aside from isolation of the pure product, it is the only convenient way to determine the yield.

In many cases, pure 7-dehydrocholesteryl acetate (IIIa) or benzoate (IIIb) were isolated and, in this manner, the over-all yield could be accurately determined. It is felt that the high yields claimed by others are, in many cases, due to estimation of only the 282 $m\mu$ maximum without consideration of the effect of impurities on the absorption curve.

Since most of the experiments were carried out in a standard manner, isolation of the pure ester in several instances served as a check on the spectroscopic assay of the crude product. In eight out of the nine cases reported here (Tables II, III, and IV) where both "crude" and pure yields are given, the average difference between the two yields is 8.1%. It is reasonable to assume that purification losses are of this order of magnitude, and, therefore, the spectroscopic assays reported in the tables are reasonably reliable. In the ninth case (Run 13, Table III), where a discrepancy of 20% is reported, it may be said with certainty that the crude yield of 46.2% is in error.

By variation of reaction conditions, yields of over 40% (based on spectrophotometric assay of the crude product) have been obtained when the benzoate was used. Several recrystallizations from acetone gave a 30% yield of pure 7-dehydrocholesteryl benzoate (IIIb). Under similar conditions a 31% yield of crude 7-dehydrocholesteryl acetate (IIIa) was obtained; pure 7-dehydrocholesteryl acetate (IIIa) was isolated in 24% yield.

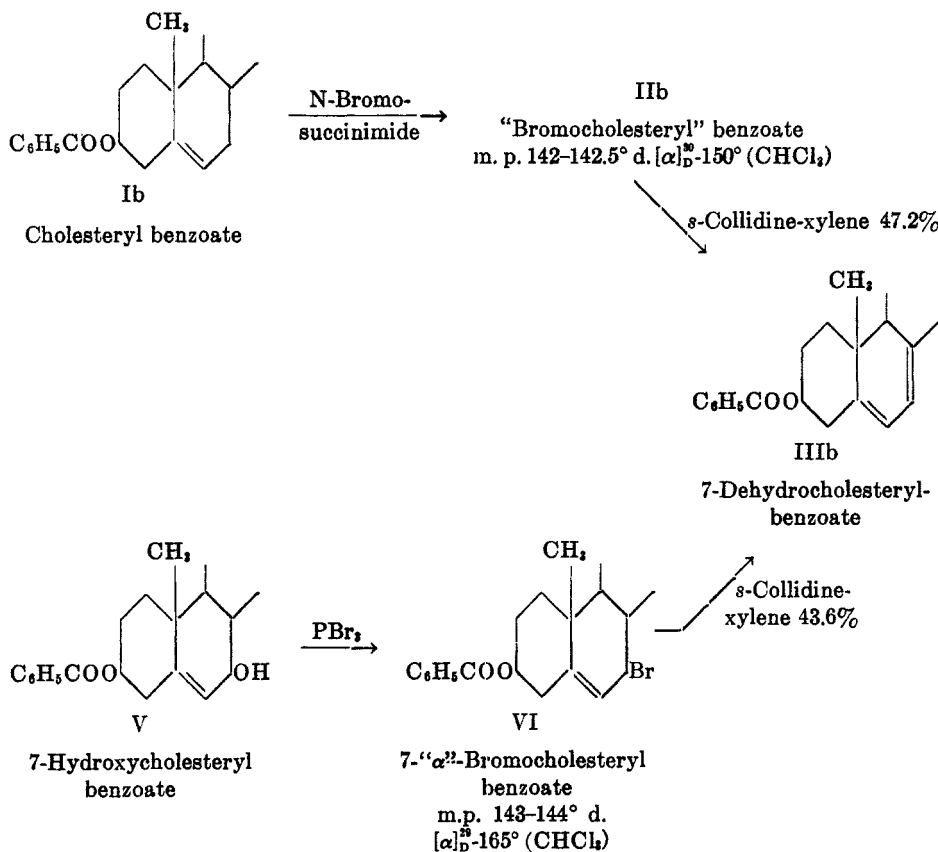
In earlier work we isolated pure 7-dehydrocholesteryl 3,5-dinitrobenzoate (IIIc) in 4.7% yield, but later process improvements should allow a considerable increase in that figure.

The Dutch workers (5) are the only investigators who previously have reported the isolation of a pure 7-dehydrocholesteryl ester directly from the dehydrobromination mixture by simple crystallization. These workers claimed a 29% yield of crude 7-dehydrocholesteryl acetate (IIIa), the melting point and spectrum of which were not stated, nor was the yield of the pure ester given. They also report in a footnote that yields of over 40% have been obtained, but no details have been reported.

The pure 7-dehydrocholesteryl esters, acetate (IIIa), benzoate (IIIb), and 3,5-dinitrobenzoate (IIIc) obtained by the N-bromosuccinimide synthesis were hydrolyzed by alcoholic potash in the usual manner to give pure 7-dehydrocholesterol (IV) which was characterized by melting point, optical rotation and ultraviolet absorption spectrum. In turn 7-dehydrocholesterol (IV) was converted to its acetate (IIIa), benzoate (IIIb), dinitrobenzoate (IIIc) and *p*-nitrobenzoate. In Table I are listed the physical properties of 7-dehydrocholesterol (IV) and the above four esters.

It will be noticed in the process outlined above that the intermediate "bromocholesteryl" benzoate (IIb) was not isolated. Experience has shown that the less it is handled, the better the yield. However, we have concerned ourselves with the isolation and characterization of this intermediate. This product has been isolated, m.p. 142.0–142.5°d., $[\alpha]_D^{30} - 150^\circ$ (CHCl₃). 7-" α "-Bromocholes-

teryl benzoate (VI) (the assignment of configuration is arbitrary) prepared from 7-hydroxycholesteryl benzoate (V) (8) and phosphorus tribromide melted at 143–144° d., $[\alpha]_D^{20} - 165^\circ$ (CHCl_3).⁹ A mixed melting point determination showed only a slight depression. However, the difference of 15° in optical rotation is significant and suggests incomplete identity. We believe that “bromocholesteryl” benzoate (IIb) consists primarily of 7-“ α ”-bromocholesteryl benzoate (VI). The nature of the impurity has not been investigated. Both “bromocholesteryl” benzoate (IIb) and 7-“ α ”-bromocholesteryl benzoate (VI), on treatment with *s*-collidine in xylene gave about the same yield of 7-dehydrocholesteryl benzoate (IIIb) (47% and 44% respectively).



⁹ Buisman, Stevens, and v.d. Vliet (5) give as the melting point of 7-“ α ”-bromocholesteryl benzoate (VI), 139–140°, gas bubbles in the melt at 141°. For 7-“ β ”-bromocholesteryl benzoate they give the crude melting point of 132–133°, bubbles above the melting point. No melting point is given for the recrystallized material. The melting point of the crude bromo-compound which they isolated from the reaction mixture was 135–136°, bubbles at 138°.

Redel and Gauthier (7) report the intermediate “bromocholesteryl” benzoate (IIb) to have the melting point 135–137° d., $[\alpha]_D^{16} - 173^\circ$ (CHCl_3).

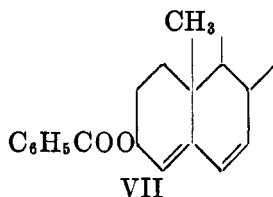
In one experiment with cholesteryl 3,5-dinitrobenzoate (Ic) the brominated product was isolated. However this intermediate has not been well characterized. Further work on this compound was abandoned in favor of more promising work on the benzoate.

When the isolated "bromocholesteryl" benzoate (IIb) was dehydrobrominated it was possible, after separation of the 7-dehydrocholesteryl benzoate, to isolate another product¹⁰ which melted at 125.5–126.5°, $[\alpha]_D^{28} = 90.7^\circ$ (CHCl₃), absorption maxima at 239 and 279–281 m μ , $\epsilon_{239} = 38,200$, $\epsilon_{279-81} = 1,100$. These properties agree best with those of $\Delta^{4,6}$ -cholestadienyl benzoate as described by Spring and Swain (9), m.p. 128–129°, $[\alpha]_D^{21} = 81^\circ$ (CHCl₃), single maximum at 239 m μ ,

TABLE I
PHYSICAL CONSTANTS OF 7-DEHYDROCHOLESTEROL AND ITS ESTERS

COMPOUND	M.P. °C	$[\alpha]_D$ (CHCl ₃)	PRINCIPAL U.V. ABSORPTION MAXIMA WITH MOLECULAR EXTINCTION COEFFICIENTS
7-Dehydrocholesterol	145–147.5 149–151.5	–120°	$\epsilon_{272} = 10,800$, $\epsilon_{282} = 11,570$, $\epsilon_{292} = 6,510$ $\epsilon_{272} = 11,250$, $\epsilon_{282} = 11,900$, $\epsilon_{292} = 6,650$
7-Dehydrocholesteryl acetate	128–130	–77.7°	$\epsilon_{272} = 10,350$, $\epsilon_{282} = 10,850$, $\epsilon_{294} = 6,140$
7-Dehydrocholesteryl benzoate	139–141, 189	–53.1°	$\epsilon_{272} = 13,270$, $\epsilon_{282} = 13,580$, $\epsilon_{294} = 7,400$ $\epsilon_{272} = 13,360$, $\epsilon_{282} = 13,510$, $\epsilon_{294} = 7,290$
7-Dehydrocholesteryl <i>p</i> -nitrobenzoate	151–153	–53.9°	$\epsilon_{271} = 22,940$, $\epsilon_{282} = 19,600$
7-Dehydrocholesteryl 3,5-dinitrobenzoate	210.5–212.5d	–42.1°	$\epsilon_{271} = 15,010$, $\epsilon_{282} = 13,730$, $\epsilon_{293} = 8,270$ $\epsilon_{271} = 14,840$, $\epsilon_{282} = 13,490$, $\epsilon_{293} = 8,020$ $\epsilon_{271} = 16,650$, $\epsilon_{282} = 14,940$, $\epsilon_{293} = 8,750$

$\epsilon = 33,000$. It is believed that the by-product isolated by us is most probably $\Delta^{4,6}$ -cholestadienyl benzoate (VII) but we have not pursued this feature of the problem further.

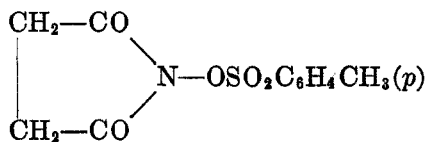


$\Delta^{4,6}$ -Cholestadienyl benzoate

¹⁰ Buisman, Stevens, and v.d. Vliet (5) have pointed to the presence of this compound in the reaction mixture and have indicated possible allylic rearrangements to account for its formation.

Redel and Gauthier (7) have isolated it by chromatography, m.p. 125–126°, $[\alpha]_D^{24} = 84.4^\circ$. On hydrolysis they obtained the free sterol which, in turn, was converted to the acetate. Both products were characterized by melting point and rotation.

During the early phases of our work we were interested in the activity of N-tosylsuccinimide (VIII) (10) under similar conditions to NBS and N-bromophthalimide. Mixtures of N-tosylsuccinimide (VIII) with cholesteryl benzoate were refluxed in carbon tetrachloride on the steam bath for 24 hours with and without benzoyl peroxide, and in both cases the starting material was recovered unchanged.



VIII

N-tosylsuccinimide

EXPERIMENTAL

Absorption spectra. All spectra were determined with a Beckmann quartz spectrophotometer (mfg'd. by the National Technical Laboratories, Pasadena, California), and were determined in 1% chloroform-absolute alcohol, *i.e.*, the weighed sample was dissolved in 1 ml. of reagent chloroform and this solution was rapidly diluted to 100 ml. with commercial absolute alcohol.

Melting points. All m.p.'s are uncorrected. When a compound has a cloudy melt, the clearing point is given after the m.p., *e.g.*, 139-141°, 189°. Melting points of the various bromo derivatives were taken by inserting the sample in the bath a few degrees below its m.p.

Yields. All "crude" yields are based on spectroscopic analysis. The value of the molecular extinction coefficient at the 282 m μ absorption maximum was taken as a measure of the purity of the material at hand, and was based on the following standards: 7-dehydroacetate, $\epsilon_{282} = 10850$; 7-dehydrobenzoate, $\epsilon_{282} = 13500$; and 7-dehydro-3,5-dinitrobenzoate $\epsilon_{282} = 13500$.

7-Dehydrocholesteryl acetate (IIIa). A mixture of 17.12 g. (0.04 M) of cholesteryl acetate (Ia), 8.56 g. (0.048 M) of NBS (1) and 200 ml. of petroleum ether, b.p. 64-66°, (purified with conc'd sulfuric acid and potassium permanganate¹¹) was heated to reflux with two photospot lamps (General Electric Co., RSP-2, 115 v) and was refluxed for 4 minutes with the lamps as the only source of heat. Eight ml. of *s*-collidine was then added to the refluxing mixture, which was cooled and filtered. The filtrate was evaporated *in vacuo* (nitrogen atmosphere); the distillation temperature was maintained at room-temperature and below. The residue was treated with about half of 100 ml. of xylene containing 4 ml. of *s*-collidine and the distillation was continued for a short time to ensure removal of traces of petroleum ether. The remainder of the *s*-collidine-xylene solution was added, the mixture was refluxed for 15 minutes (nitrogen atmosphere), cooled, and treated with water. The product was worked up in xylene and the extract was washed successively with cold, very dilute hydrochloric acid, water, sodium bicarbonate solution, and water. The extract was dried with magnesium sulfate, treated with Norit and filtered through Celite. Evaporation of the brown-yellow filtrate gave an oily solid which was dissolved in acetone. The solution was put under a nitrogen atmosphere and cooled. The crystals which separated were collected and washed with a small amount of cold acetone and a small amount of methanol; white solid intermixed with a very small amount of oily solid, wt. 5.78 g.; m.p. 114-123.5°; $\epsilon_{282} = 9860$ (90.8% pure); 30.8% yield. The crude 7-dehydroacetate was recrystallized twice from acetone-methanol, wt. 4.14 g.; m.p. 128-129.5°; 24.4% yield.

¹¹ All petroleum ethers used were similarly purified.

In another run with 4.28 g. (0.01 M) of cholesteryl acetate, 2.14 g. (0.012 M) of NBS and 50 ml. of petroleum ether, b.p. 64–66°, there was obtained 1.48 g.; m.p. 118–123°; $\epsilon_{252} = 10320$ (95.2% pure); 33% yield.

7-Dehydrocholesteryl benzoate (IIIb). A. A mixture of 19.6 g. (0.04 M) of cholesteryl benzoate, 8.55 g. (0.048 M) of NBS, and 200 ml. of petroleum ether, b.p. 64–66°, was refluxed for 4 minutes by the light and heat of two RSP-2 photospot lamps, placed two inches from the reaction vessel. Eight ml. of *s*-collidine was added to the boiling solution which was cooled, filtered with suction to remove succinimide, and distilled *in vacuo* at –10 to +10° to remove the petroleum ether. A dark red oil containing *s*-collidine and the crude "bromocholesteryl" benzoate remained in the flask.

TABLE II
CHOLESTERYL BENZOATE → 7-DEHYDROCHOLESTERYL BENZOATE
Bromination Solvent: CCl₄

	CB MOLES	NBS MOLES	BROMINA- TION TIME OF REFLUX MINUTES	IRRADIATION BROMINA- TION	STRIPPING AGENT—SOL- VENT	STRIPPING TIME OF REFLUX MINUTES	CRUDE YIELD, %	PURE YIELD, %
1	0.01	0.01	10	—	DMA-T	30	11.2	—
2	.01	.011	10	—	DMA-T	30	18.8	11.3
3	.01	.012	10	—	DMA-T	30	22.6	—
4	.01	.0125	10	—	DMA-T	30	17.3	13.1
5	.04	.048	2	V-1	DMA-T	30	27.0	18.3
6	.01	.012	3	PF	DMA-T	30	23.4	17.2
7	.01	.012	2	U.V.	DMA-X	10	25.6	—
8	.01	.012	2	I.R.	DMA-X	10	26.4	—
9	.04	.048	2	V-2	DMA-X	10	28.2	21.5
10	.01	.012	2	V-2	C-X	15	32.0	—

CB = Cholesteryl benzoate

NBS = N-Bromosuccinimide

V-1, V-2 = One and two, resp., photospot lamps; type RSP-2, 115 v, General Electric Co.

U.V. = Ultraviolet lamp; type 16200, 115 v, 125 w, Hanovia Chemical and Mfg. Co.

I.R. = Infra-red lamp; type R-40, 115 v, 250 w, Westinghouse Electric Co.

P.F. = Photoflood lamp; type 2A, General Electric Co.

DMA = Dimethylaniline

C = *s*-Collidine

T = Toluene

X = Xylene

p.e. = petroleum ether

The oil was dissolved in a mixture of 100 ml. of xylene, b.p. 138–142° and 4 ml. *s*-collidine, refluxed in a nitrogen atmosphere for 15 minutes, and cooled; anhydrous magnesium sulfate was added, the slurry was filtered with suction, and the filtrate was distilled *in vacuo*. The partially crystalline red oil was treated with 100 ml. of acetone and cooled in an ice-bath, wt. 12.48 g.; m.p. 122–128°; $152^\circ \epsilon_{252} = 8700$ (64.5% pure); 41.3% "crude" yield. After four recrystallizations from acetone there remained 5.08 g., m.p. 138–140.5°, 187.5° and an additional 0.84 g., m.p. 138–140.5°, 185.5° was obtained from the mother liquors. The overall yield was 30.3%.

B. Numerous experiments (ca. 100) with cholesteryl benzoate were performed; a portion of these are reported in Tables II, III, and IV. All the experiments were carried out essentially as under (A).

7-Dehydrocholesteryl 3,5-dinitrobenzoate (IIIc). A. Five and eight-tenths grams

TABLE III
 CHOLESTERYL BENZOATE → 7-DEHYDROCHOLESTERYL BENZOATE
 Bromination Solvent: Petroleum Ether, bp. 64–66°

	CB MOLES	NBS MOLES	BROMINA- TION TIME OF REFLUX MINUTES	IRRADIATION BROMINA- TION	STRIPPING AGENT—SOL- VENT	STRIPPING TIME OF REFLUX MINUTES	CRUDE YIELD, %	PURE YIELD, %
1	0.01	0.0115	4	V-1	DMA-T	30	22.8	—
2	.01	.012	4	V-1	DMA-T	30	25.2	14.1
3	.01	.013	4	V-1	DMA-T	30	24.7	—
4	.01	.012	4	V-2	DMA-X	12	30.9	—
5	.01	.01175	4	V-2	C-X	15	37.0	—
6	.01	.012	1.5	V-2	C-X	15	36.6	—
7	.01	.012	2	V-2	C-X	15	39.0	—
8	.01	.012	2.5	V-2	C-X	15	36.8	—
9	.01	.012	3	V-2	C-X	15	38.1	—
10	.01	.012	3.5	V-2	C-X	15	36.4	—
11	.01	.012	4	V-2	C-X	15	37.2	—
12	.04	.048	4	V-2	C-X	15	41.3	30.3
13	.04	.048	4	V-2	C-X	15	46.2	26.2
14	.01	.012	4	V-2	C-X	15	42.7	—
15	.01	.01225	4	V-2	C-X	15	37.6	—
16	.01	.013	4	V-2	C-X	15	34.7	—

See legend to Table II.

TABLE IV
 CHOLESTERYL BENZOATE → 7-DEHYDROCHOLESTERYL BENZOATE
 Irradiation Bromination: V-2, 0.01 M C.B. 0.012 M N.B.S.

	BROMINATION SOLVENT	BROMINATION TIME OF REFLUX MINUTES	CRUDE YIELD, %	PURE YIELD, %
1	p.e. 45°	20	32.2	—
2	p.e. 50–55°	20	33.6	—
3	p.e. 55–60°	20	39.7	—
4	p.e. 55–60°	30	41.5	31.8
5	p.e. 60–65°	20	38.3	—
6	p.e. 65–70°	4	37.2	—
7	p.e. 70–75°	4	35.2	—
8	p.e. 75–80°	4	34.4	—
9	p.e. 85–90°	4	20.4	—
10	p.e. 90–95°	4	24.6	—
11	p.e. 95–100°	4	16.7	—

See legend to Table II.

(0.01 M) of cholesteryl 3,5-dinitrobenzoate was dissolved in 100 ml. of carbon tetrachloride by heating on the steam-bath. One and six-tenths grams (0.009 M) of NBS was added; the mixture was refluxed for 25 minutes, cooled, and filtered. The filtrate was concentrated *in vacuo*. This gave a yellow solid which was dissolved in 60 ml. of toluene, treated with 0.79 g. (0.01 M) of pyridine, and was refluxed for 2 hours in a nitrogen atmosphere. The resulting pyridine hydrobromide was removed and the filtrate was evaporated *in vacuo*.

This gave an orange, oily solid which was treated with acetone; the insoluble yellow solid was separated, wt. 0.71 g.; m.p. 176–178° d.; $\epsilon_{283} = 4900$. Three recrystallizations from benzene-acetone gave 258 mg. of pure 7-dehydro-dinitrobenzoate, m.p. 210–212° d. From the mother liquor 91 mg. more of product was obtained, m.p. 206.5–208° d.; 6.7% yield (based on NBS).

B. A mixture of 2.9 g. (0.005 M) of cholesteryl 3,5-dinitrobenzoate, 1.01 g. (0.0045 M) of N-bromophthalimide, and 50 ml. of carbon tetrachloride was reacted as above (A) and gave 90 mg. of 7-dehydro-dinitrobenzoate, m.p. 207–209.5° d.; 3.5% yield.

C. A mixture of 23.2 g. (0.04 M) of cholesteryl 3,5-dinitrobenzoate, 7.12 g. (0.04 M) of NBS, and 250 ml. of carbon tetrachloride was refluxed for 12 minutes, cooled and the succinimide was separated, wt. 3.95 g. (calc. wt. 3.96 g.); m.p. 115–122°.

The filtrate was evaporated *in vacuo* and the residue was treated with 10 ml. of dimethylaniline and 150 ml. of toluene. The mixture was refluxed for 0.5 hour. Anhydrous magnesium sulfate was added to the cooled mixture which was filtered through Celite. Evaporation of the toluene gave an oily residue which crystallized on treatment with acetone, wt. 6.3 g.; m.p. 180–182°; $\epsilon_{282} = 4940$ (36.8% pure). Seven recrystallizations from benzene-acetone gave 1.1 g. of pure dinitrobenzoate, m.p. 209–211° d.; 4.7% yield.

D. Twelve and seven-tenths grams (0.025 M) of cholesteryl 3,5-dinitrobenzoate was dissolved in 250 ml. of carbon tetrachloride and treated with 5.15 g. (0.029 M) of NBS. The mixture was refluxed for 15 minutes, cooled, and the succinimide was separated, wt. 2.82 g. (calc. wt. 2.87 g.); m.p. 119–123°.

The yellow filtrate was evaporated *in vacuo*; the residue was treated with 25 ml. of benzene which was removed *in vacuo*. The benzene treatment was repeated. This gave 17 g. of brominated product which was practically all solid. Fractional recrystallization from ether, chloroform-ether and ether-absolute alcohol gave the following fractions: (a) most insoluble, wt. 3.44 g.; needles, m.p. 144–145°, transition, change in color, and final melt at 194–198°, dark melt;

Anal. Calc'd for $C_{24}H_{47}BrN_2O_8$: C, 61.90; H, 7.18; N, 4.25; Br, 12.12.

Found: C, 61.56; H, 7.18; N, 4.07; Br, 13.11.

(b) wt. 1.72 g., m.p. 137–144°, transition, change in color, gradual melt up to about 197°, dark melt; (c) mother liquor evaporated to dryness; (d) wt. 1.8 g., slight yellow tint, m.p. 130–132°, transition in color, final melt at 194°; (e) wt. 1.58 g., m.p. 125–135°, transition, final melt at about 192°; and (f) wt. 7.1 g., most soluble mother liquor evaporated to dryness, multi-colored solid with dark areas.

Three and eight-hundredths grams of Fraction (a) was treated with 0.74 g. of pyridine in 100 ml. of toluene. The mixture was refluxed for two hours in a nitrogen atmosphere, cooled and the pyridine hydrobromide was separated. The filtrate was evaporated to dryness *in vacuo* in a nitrogen atmosphere. The residue was treated with acetone and the insoluble orange solid was collected, wt. 0.38 g.; m.p. 194–201° d.; $\epsilon_{282} = 12020$ (89% pure). Recrystallization from benzene-acetone gave 200 mg. of pure 7-dehydro-dinitrobenzoate, m.p. 209–210° d.; $[\alpha]_D^{33} - 38.9^\circ$, $[\alpha]_D^{28.5} - 42.3^\circ$ (24.4 mg., 32.6 mg. in 2 ml. chloroform, 1 dem. semi-micro tube, gave $\alpha_D^{33} - 0.47^\circ$ and $\alpha_D^{28.5} - 0.69^\circ$ resp.); absorption maxima at 259, 272, 282 and 293.5–294 $m\mu$; $\epsilon_{259} = 14990$, $\epsilon_{272} = 15160$, $\epsilon_{282} = 13420$ and $\epsilon_{293.5-294} = 7690$.

The middle fractions (Fractions b, c, d, and e) were combined (wt. 6.7 g.), dissolved in 125 ml. of toluene, treated with 1.58 g. of pyridine, and the mixture was refluxed for two hours in a nitrogen atmosphere. The pyridine hydrobromide was removed by filtration and the filtrate was evaporated practically to dryness *in vacuo*. Acetone was added and the resulting solid was collected by filtration, wt. 0.6 g.; m.p. 160–162° d.; $\epsilon_{282} = 10040$ (74% pure). Recrystallization from acetone-benzene gave 0.27 g. of pure 7-dehydro-dinitrobenzoate, m.p. 209.5–211.5° d.; absorption maxima at 252, 259, 271, 282, and 293 $m\mu$; $\epsilon_{252} = 15430$, $\epsilon_{259} = 15020$, $\epsilon_{271} = 14840$, $\epsilon_{282} = 13490$ and $\epsilon_{293} = 8020$, $[\alpha]_D^{32} - 36.3^\circ$ (34.7 mg. in 2 ml. chloroform, 1 dem. semi-micro tube, gave $\alpha_D^{32} - 0.61^\circ$).

The most soluble fraction (f) on similar treatment with pyridine and toluene gave none of the desired 7-dehydro-dinitrobenzoate.

“Bromocholesteryl” benzoate (IIb). A. A mixture of 235.2 g. (0.48 M) of cholesteryl

benzoate, 85.4 g. (0.48 M) of NBS and 2 l. of carbon tetrachloride was refluxed for 15 minutes, cooled and the succinimide was removed by filtration, wt. 46.3 g. (calc'd wt. 47.6 g.); m.p., 120–125°. The filtrate was evaporated *in vacuo* with slight heating, and the red viscous oil so obtained was dissolved in 400 ml. of acetone. The solution was allowed to stand at room temperature overnight, and the crystals which separated were collected by filtration and were washed with acetone, wt. 45.9 g.; m.p. 130–136° d. From the mother liquor two more fractions of crystals were obtained, wt. 98.0 g. and 23.5 g.; m.p. 132–137° d. and 138–141° d. resp.

The first fraction consisted primarily of cholesteryl benzoate. The other two fractions were triangularly recrystallized from petroleum ether (60–80°) and gave 54.1 g. of "bromocholesteryl" benzoate, m.p. 142–143° d.

Anal. Calc'd for $C_{24}H_{49}BrO_2$: Br, 14.03. Found: Br, 14.04.

B. A mixture of 97.5 g. (0.20 M) cholesteryl benzoate, 37.4 g. (0.21 M) NBS and 1 l. of carbon tetrachloride was refluxed on the steam-bath for 3 minutes, cooled and the succinimide was separated, wt. 20.8 g. (calc'd wt. 20.8 g.); m.p. 110–123°.

The filtrate was evaporated *in vacuo* and the residual oil was taken up in ether. Thirty grams of solid separated, m.p. 115–123° d. The mother liquor on working with acetone gave 25.3 g.; m.p. 135–137.5° d. The mother liquor from this fraction gave 5.75 g.; m.p. 130–133° d.

The middle fraction was recrystallized three times from acetone to constant m.p., 142–142.5° d. with previous softening at 140–142°, $[\alpha]_D^{25} - 146^\circ$ (13.3 mg. in 2 ml. chloroform, 1 dm. semi-micro tube, gave $\alpha_D^{25} - 0.97^\circ$). The material was recrystallized twice from petroleum ether (b.p. 60–70°), m.p. 142–143° d.; $[\alpha]_D^{25} - 150^\circ$ (16.7 mg. in 2 ml. chloroform, 1 dm. semi-micro tube, gave $\alpha_D^{25} - 1.25^\circ$).

Dehydrobromination experiments with "bromocholesteryl" benzoate (IIb). *A.* A mixture of 2 g. of "bromocholesteryl" benzoate, 1 ml. of dimethylaniline, and 25 ml. of toluene was refluxed in a nitrogen atmosphere for 30 minutes, cooled, treated with anhydrous magnesium sulfate, and filtered through Celite. The filtrate was evaporated *in vacuo* in a nitrogen atmosphere. The residue was dissolved in acetone, the solution was concentrated and cooled. Crude 7-dehydro-benzoate separated and was collected by filtration, wt. 0.68 g.; m.p. 131–135°, 160.5°; $\epsilon_{282} = 10150$ (75.4% pure). From the mother liquor there was isolated an additional 0.3 g. of product, m.p. 110–114° (23.8% pure); yield 33.7%.

B. This reaction when carried out under the same conditions with *s*-collidine and xylene gave 1.0 g. of crude 7-dehydro-benzoate, m.p. 133–139°, 171°, $\epsilon_{282} = 10950$ (81.2% pure); 47.2% yield.

7-" α "-Bromocholesteryl benzoate (VI). *A.* A mixture of 1.9 g. of 7-hydroxycholesteryl benzoate, 0.25 ml. of phosphorus tribromide, and 50 ml. of benzene was heated to about 50° three times in four hours. Water was added and the benzene extract was washed with water until a negative Congo Red test was obtained, washed twice with saturated saline solution, dried with magnesium sulfate, filtered and distilled *in vacuo*.

The residue was recrystallized four times from petroleum ether, m.p. 143–144° d.; $[\alpha]_D^{25} - 165^\circ$ ($CHCl_3$) (12.7 mg. in 2 ml. chloroform, 1 dm. semi-micro tube, gave $\alpha_D^{25} - 1.05^\circ$). A mixed melting point with another sample prepared from the reaction of NBS and cholesteryl benzoate gave only slight depression, m.p. 139.5–141.5° d.

Anal. Calc'd for $C_{24}H_{49}BrO_2$: C, 71.86; H, 8.67; Br, 14.03.

Found: C, 71.49, 71.51; H, 9.07, 9.10; Br, 14.48, 13.78.

Recrystallization of the bromo compound was unsatisfactory if the material was allowed to stand in warm acetone for any length of time as decomposition occurred.¹²

B. Seven-tenths of a gram of 7-hydroxycholesteryl benzoate in 20 ml. of benzene was treated with 0.15 ml. of phosphorus tribromide; the mixture was allowed to stand at room temperature for four hours. The product was worked up as in (A) and gave 0.45 g. of the desired product, needles, m.p. 143–145° d., bubbles.

¹² Buisman, Stevens, and v.d. Vliet (5) have made a similar observation.

Dehydrobromination of 7- α -bromocholesteryl benzoate (VI). A. Three-tenths of a gram of the 7- α -bromo-benzoate was refluxed with 0.2 ml. of dimethylaniline in 15 ml. of toluene for 0.5 hour (nitrogen atmosphere), cooled, filtered through Celite and distilled *in vacuo*. Recrystallization from acetone gave pure 7-dehydro-benzoate, m.p. 139.5–141°, 188.5°; $\epsilon_{272} = 12750$, $\epsilon_{282} = 13000$, $\epsilon_{294} = 7200$.

B. 7- α -Bromo-benzoate (0.45 g.) was refluxed for 20 minutes with 0.5 ml. of *s*-collidine in 20 ml. of xylene (nitrogen atmosphere), and the product was worked up in the usual manner. This gave 0.25 g. of 7-dehydro-benzoate, m.p. 131–135°, 162°; $\epsilon_{282} = 9150$ (67.7% pure); 43.6% yield.

7-Dehydrocholesterol (IV).¹³ A mixture of 7.5 g. of pure 7-dehydrocholesteryl benzoate (prepared by the N-bromosuccinimide method) and 150 ml. of filtered 5% alcoholic potash was refluxed on the steam-bath for one hour in a nitrogen atmosphere. The mixture was cooled in an ice-bath; the resulting crystals were filtered and washed with cold alcohol, water, and alcohol. During this operation crystals separated in the filtrate. They were combined with the main batch of crystals. Recrystallization from acetone gave 4.5 g., m.p. 142–145° (dried over Drierite). More material was isolated from the mother liquors. An aliquot was further recrystallized from acetone, m.p. 145–147.5°; $[\alpha]_D^{20} = -120^\circ$ (14 mg. in 2 ml. of chloroform, 1 cm. semi-micro tube, gave $\alpha_D^{20} = -0.84^\circ$).

In another hydrolysis the initially isolated dehydrocholesterol melted at 145–148° (dried 2 hours *in vacuo*, oil pump); absorption maxima at 272, 282 and 294 μ , $\epsilon_{272} = 9500$, $\epsilon_{282} = 10000$ and $\epsilon_{294} = 5700$. Recrystallization from acetone gave m.p. 148–151°, $\epsilon_{272} = 10800$, $\epsilon_{282} = 11570$ and $\epsilon_{293} = 6510$. The material was further recrystallized from acetone, m.p. 149–151.5°, $\epsilon_{272} = 11250$, $\epsilon_{282} = 11900$ and $\epsilon_{293} = 6650$. However, one more recrystallization lowered the melting point and extinction coefficients, m.p. 147–148°, $\epsilon_{272} = 10500$, $\epsilon_{282} = 10660$ and $\epsilon_{293} = 6030$.

7-Dehydrocholesterol was converted into the following derivatives in the usual manner. These compounds were recrystallized to constant melting point, optical rotation and absorption spectrum.

(a) *Acetate*,¹⁴ m.p. 128–130°; $[\alpha]_D^{20} = -77.7^\circ$, (CHCl₃), absorption maxima at 272, 282 and 294 μ , $\epsilon_{272} = 10350$, $\epsilon_{282} = 10850$ and $\epsilon_{294} = 6140$.

(b) *Benzoate*,¹⁵ m.p. 139–141°, 189°; $[\alpha]_D^{20} = -53.1^\circ$ (CHCl₃); absorption maxima at 272, 282 and 294 μ , $\epsilon_{272} = 13270$, $\epsilon_{282} = 13580$ and $\epsilon_{294} = 7400$.

(c) *p*-Nitrobenzoate,¹⁶ m.p. 151–153° (cloudy melt); $[\alpha]_D^{20} = -53.9^\circ$ (CHCl₃); absorption maxima at 271 and 281 μ , $\epsilon_{271} = 22940$ and $\epsilon_{281} = 19600$.

¹³ (a) Windaus, Lettré, and Schenck, *Ann.*, **520**, 98 (1935); m.p. 142–143.5°; $[\alpha]_D^{20} = -113.6$ (CHCl₃).

(b) Boer, *et al.*, *Koninkl. Akad. Wetenschap. Amsterdam*, **39**, 672 (1936); m.p. 149–150°; $[\alpha]_D = -122.5^\circ$ (C₆H₆).

(c) Wintersteiner and Ruigh, *J. Am. Chem. Soc.*, **64**, 1177 (1942); m.p. 142.5–143.5°; $[\alpha]_D^{20} = -121^\circ$ (CHCl₃); $\epsilon_{282} = 11100$.

¹⁴ (a) Schenck, *et al.*, *Ber.*, **69**, 2696 (1936); m.p. 130°.

(b) Windaus and Bock, *Z. physiol. Chem.*, **245**, 168 (1937); m.p. 130°; $[\alpha]_D = -84.9^\circ$ (C₆H₆).

(c) Bernstein, Hicks, Clark, and Wallis, *J. Org. Chem.*, **11**, 646 (1946), predicted a rotation of -77.6° (CHCl₃) for 7-dehydro-acetate.

(d) Buisman, Stevens, and v.d. Vliet, *Rec. trav. chim.*, **66**, 87 (1947); m.p. 130.0–130.5°, $[\alpha]_D^{20} = -87^\circ$ (C₆H₆).

¹⁵ (a) Windaus, Lettré, and Schenck, *Ann.*, **520**, 98 (1935); m.p. 139–140°, 183°; $[\alpha]_D^{20} = -53.2^\circ$ (CHCl₃).

(b) Buisman, Stevens, and v.d. Vliet (5), m.p. 140–141°, 189°; $[\alpha]_D^{19} = -55^\circ$ (CHCl₃).

¹⁶ Huber, *et al.*, *J. Am. Chem. Soc.*, **67**, 609 (1945); m.p. 153–154°; $[\alpha]_D = -49.8^\circ$ (CHCl₃); absorption maxima at ca. 271 and 281 μ , $\epsilon_{271} = \text{ca. } 23000$, and $\epsilon_{281} = \text{ca. } 19500$ (estimated from curve, values not given).

(d) *Dinitrobenzoate*,¹⁷ m.p. 210.5–212.5° d.; $[\alpha]_D^{20}$ -42.1° (CHCl₃); absorption maxima at 252, 260, 271, 282 and 293 m μ , plateau at 230–231 m μ , $\epsilon_{230-1} = 23650$, $\epsilon_{252} = 16320$, $\epsilon_{260} = 15950$, $\epsilon_{271} = 16650$, $\epsilon_{282} = 14940$, and $\epsilon_{293} = 8750$.

$\Delta^4, 6$ -*Cholestadienyl benzoate* (VII) (?).¹⁸ Fifteen and seventeen-hundredths grams, m.p. 139–141.5° d. (Found Br, 14.00), of "bromocholesteryl" benzoate was treated in the usual manner with 10 ml. of pyridine in 250 ml. of toluene. The crude 7-*dehydro-benzoate*, m.p. 114–127.5°, 147° (32.7% pure); wt. 6.67 g.; was recrystallized three times from acetone, wt. 1.21 g., m.p. 138.5–140°, 183°; $\epsilon_{272} = 13740$, $\epsilon_{282} = 13800$ and $\epsilon_{294} = 7350$.

From the mother liquors by a triangular recrystallization from acetone the following fractions were obtained: (a) most insoluble m.p. 122–124°, wt. 1.75 g.; (b) m.p. 117–120°, wt. 1.75 g.; $\epsilon_{240} = 42500$, $\epsilon_{281} = 2800$; (c) m.p. 122–124°, 2.52 g.; $\epsilon_{240} = 36000$ and $\epsilon_{281} = 1680$ and (d) mother liquor which was discarded.

Fractions (b) and (c) were combined and recrystallized from acetone to constant melting point, m.p. 124.5–126.5°, $\epsilon_{239} = 38200$ and $\epsilon_{273-81} = 1100$, $[\alpha]_D^{25} -90.7^\circ$ (20.5 mg. in 2 ml. chloroform, 1 cm. semi-micro tube, gave $\alpha_D^{25} -0.93^\circ$).

Anal. Calc'd for C₂₄H₃₆O₂: C, 83.55; H, 9.90.

Found: C, 83.54; H, 9.88.

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Addendum. While this manuscript was in process of publication there appeared a series of papers on 7-dehydrocholesterol and related subjects by a group of English workers, BIDE, *et al.*, *J. Chem. Soc.*, 1783, 1788 (1948); and, HENBEST AND JONES, *J. Chem. Soc.*, 1792, 1798 (1948). A 30% over-all yield of 7-dehydrocholesterol from cholesterol by the Ziegler method was claimed. They have also presented evidence which shows that the product obtained from the bromination of cholesteryl benzoate with N-bromosuccinimide is identical with 7-" β "-bromocholesteryl benzoate. We have subsequently re-investigated this matter, and confirmed the identity of these two compounds. A detailed report will be included in a forthcoming publication on 7-amino-steroids and related compounds.

SUMMARY

1. The reaction between cholesteryl esters, in particular the benzoate, and N-bromosuccinimide, with subsequent elimination of hydrogen bromide to give 7-dehydrocholesteryl esters has been studied in great detail.

2. A procedure has been developed which gave consistently high yields of the 7-dehydrocholesteryl esters. 7-Dehydrocholesteryl benzoate has been prepared in 41–43% yield (spectroscopic determination on crude material); pure

¹⁷ (a) Windaus, Lettré, and Schenck, *Ann.*, **250**, 98 (1935); m.p. 207°, $[\alpha]_D^{20} -45.7^\circ$ (CHCl₃).

(b) Wintersteiner and Ruigh, *J. Am. Chem. Soc.*, **64**, 477 (1942); m.p. 209.5–210.5°, $[\alpha]_D -38.3^\circ$ (CHCl₃).

(c) Huber, Ewing, and Kriger, *J. Am. Chem. Soc.*, **67**, 609 (1945); m.p. 210–212°, $[\alpha]_D -45.7^\circ$ (CHCl₃).

¹⁸ (a) Spring and Swain, *J. Chem. Soc.*, 320 (1941); m.p. 128–129°, $[\alpha]_D^{25} -81^\circ$ (CHCl₃), absorption maximum at 239 m μ , $\epsilon_{239} = 33000$.

(b) Redel and Gauthier (7), m.p. 125–126°; $[\alpha]_D^{25} -84.4^\circ$ (CHCl₃).

benzoate was isolated in 30% yield. Under similar conditions, 7-dehydrocholesteryl acetate was obtained in crude yields of 31–33%, and in a pure yield of 24%.

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