has been isolated in quantities of 0.3 mg. per kilo and its properties described.

2. Pro-γ-carotene is a naturally occurring stereoisomer of γ-carotene to which its relationship is analogous to that of prolycopene to lycopene. It is suggested that 6 or 7 double bonds of the pro-γ-carotene chromophore have *trans*- and 5 or 4 *cis*-configuration.

3. The following operations convert pro-γ-carotene into a mixture of stereoisomers which includes γ-carotene: melting of crystals, refluxing of solutions or treatment of a cold solution with iodine or concd. hydrochloric acid. If the latter is used as a catalyst, the chromatogram also includes minor pigments which do not belong to the stereochemical series discussed.

4. On addition of iodine to a solution of any stereoisomer of the γ-carotene series the spectrum shifts instantaneously to that of an equilibrium mixture, the components of which separate on the Tswett column.

PASADENA, CALIFORNIA RECEIVED JANUARY 20, 1942

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

7-Benzoxysterols and their Use in the Preparation of 7-Dehydrosterols

BY O. WINTERSTEINER AND WILLIAM L. RUIGH

In an attempt to secure free 7(α)-hydroxycholesterol by treatment of 7(α)-benzoxycholesteryl benzoate with a methanolic solution of sodium methylate at room temperature we found that the diester yielded almost quantitatively the 7-monoester, 7(α)-benzoxycholesterol. The resistance of the ester linkage in position 7 was also evident when the hydrolysis was conducted by boiling for three hours with 2% methanolic potassium hydroxide solution. The recovered material contained, according to the spectrographic assay, 35% of a monobenzoate which was undoubtedly the 7-monoester, since cholesteryl benzoate is completely saponified under these conditions, as well as by treatment with sodium methylate in the cold.

7(α)-Benzoxycholesterol, in contradistinction to the free diol, is not precipitable by digitonin in 90% ethanol. Though it is known that the presence of a benzoxy group in position 4, adjacent to a free 3-hydroxyl group, prevents the digitonin reaction,[1] the hindering effect of such a group in the more remote position 7 is noteworthy.

The position of the benzoxy group in the monoester follows from its conversion to (free) 7-dehydrocholesterol either by pyrolysis[2] or treatment with boiling dimethylaniline.[3,4] By contrast, the 3-monobenzoate of the diol[5,6] on pyrolysis forms a cholestatriene.[5] The dehydrosterol in the crude reaction mixture can be separated from unreacted material and by-products by means of digitonide, an advantage which is not obtained when the dibenzoate is used as the starting material.

The analogous conversion of 7(α)-benzoxystigmasteryl benzoate to 7-dehydrostigmasterol *via* the 7-monoester showed that the method can be employed generally for the preparation of 7-dehydrosterols.

Experimental

7(α)-Benzoxycholesterol.—To 10 g. of 7(α)-benzoxycholesteryl benzoate dissolved in benzene (200 cc.) a solution of sodium methylate (Mathieson) (6.66 g.) in absolute methanol (333 cc.) was added, and the mixture was allowed to stand at room temperature for three days. It then was poured on cracked ice and extracted repeatedly with cold water. Evaporation of the benzene yielded an oil which was redissolved in hexane and adsorbed on a column of aluminum oxide. Elution was effected with benzene containing increasing amounts (0.5 to 5%) of methanol. Most of the eluted material (6.74 g.) was essentially homogeneous, as shown by the specific rotations. The final fractions (673 mg. together) with rotations of +100 or lower were of gelatinous consistency and probably contained some 7(α)-hydroxycholesterol. Subsequent work on the corresponding stigmasterol derivative showed that with a shorter hydrolysis time, twelve to twenty-four hours, the amount of diol formed was negligible, so that the chromatographic purification could be dispensed with. It sufficed to recrystallize the crude material from benzene-pentane mixtures, or from hexane, from which it formed fine filamentous needles; m. p. 110–115°. The melting point varied somewhat with the rate of heating, $[\alpha]^{24}\text{D}$ +111°; 1.2% in chloroform.

Anal. Calcd. for $C_{34}H_{50}O_3$: C, 80.58; H, 9.95. Found: C, 80.41, 80.50; H, 9.85, 9.93.

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(1) Spring and Swain, *J. Chem. Soc.*, 83 (1941).

(2) Windaus, Lettré and Schenk, *Ann.*, **520**, 98 (1935).

(3) Haslewood, *J. Chem. Soc.*, 224 (1938); *Biochem. J.*, **33**, 454 (1939).

(4) Rosenberg, U. S. Patent 2,209,934 (1940).

(5) Eckhardt, *Ber.*, **71**, 461 (1938).

(6) Rosenberg, U. S. Patent 2,215,725 (1940).

The absorption spectrum showed maxima at 230 mμ (ε, 12,750) and 272 mμ (ε, 740), corresponding to the presence of one benzoxy group. The extinction coefficients given by the dibenzoate were 24,300 and 1590, respectively. Benzoylation in pyridine yielded 7(α)-benzoxycholesteryl benzoate; m. p. 171–172°. A solution of 20 mg. of the monobenzoate in 10 cc. of 90% alcohol, containing 100 mg. of digitonin, failed to deposit a precipitate on standing overnight.

7(α)-Benzoxycholesteryl 3,5-Dinitrobenzoate.—7(α)-Benzoxycholesterol (60 mg.) was treated with 3,5-dinitrobenzoyl chloride (250 mg.) in 7 cc. of pyridine and worked up in the usual manner; needles from benzene–methanol, m. p. 162–163°; $[\alpha]^{24}D$ +80.5° (1.3% in chloroform).

Anal. Calcd. for $C_{41}H_{52}O_8N_2$: N, 4.00. Found: N, 4.21, 4.00.

7(α)-Benzoxycholesteryl *p*-Toluenesulfonate.—A mixture of 7(α)-benzoxycholesterol (1 g.) and tosyl chloride (1 g.) in dry pyridine (10 cc.) was allowed to stand overnight. The reaction product was isolated by ether extraction in the usual manner. Crystallization from hexane yielded needles melting with decomposition from 90 to 100°, depending on the rate of heating. The compound decomposed gradually on standing; $[\alpha]^{29}D$ +83.8° (1.1% in chloroform).

Anal. Calcd. for $C_{41}H_{56}O_5S$: C, 74.50; H, 8.54; S, 4.85. Found: C, 74.58; H, 8.64; S, 4.50.

An attempt to convert the tosyl derivative into the *i*-methyl ether by treatment with potassium acetate in boiling methanol[7] resulted in the formation in low yield of a crystalline product (m. p. 153.5–155.5°; $[\alpha]^{26}D$ +104°) which analyzed correctly for carbon and hydrogen, but did not possess the required methoxyl content (Calcd. for $C_{35}H_{52}O_3$: OCH_3, 5.96. Found: OCH_3, 4.49, 4.64).

7-Dehydrocholesterol.—In order to determine the optimal conditions for the reaction in dimethylaniline a solution of 7-benzoxycholesterol (250 mg.) in 10 cc. of redistilled dimethylaniline was boiled in an atmosphere of carbon dioxide. One-cc. samples rapidly withdrawn at intervals were diluted with ether and freed from dimethylaniline by thorough extraction with hydrochloric acid. The ether residues were treated with digitonin in 90% alcohol, and the precipitates formed were collected and weighed. The results showed that the reaction had become stationary after three hours with 57% of the theoretical amount of 7-dehydrocholesterol formed.

In another experiment the reaction mixture was refluxed for three hours and worked up in a single batch. Calculated from the weight of the digitonide (empirical conversion factor 0.20) the yield was 68%, while the extinction at 282 mμ indicated 65%.

The monoester was also cleaved thermally according to Windaus in a vacuum of 2 mm. The yield according to the spectrographic assay was 53%.

A sample of the digitonide was decomposed by the pyridine method and worked up in the usual way. The recovered material was recrystallized repeatedly from acetone and yielded needles melting at 142.5–143.5°; $[\alpha]^{26}D$ −121° (0.8% in chloroform); $\epsilon_{282\ m\mu}$ 11,100. Windaus, *et al.*,[2]: m. p. 142–143.5°, $[\alpha]_D$ −113.6°; Boer, *et al.*[8]: m. p. 149–150°, [α] −122.5° (in benzene).

The 3,5-dinitrobenzoate was prepared according to Windaus and recrystallized from chloroform–acetone; m. p. 209.5–210.5°; $[\alpha]^{26}D$ −38.3° (0.76% in chloroform). Windaus, *et al.*,[2] m. p. 207°, $[\alpha]^{20}D$ −45.7°; Boer, *et al.*,[8] m. p. 207°. The discrepancies in the melting points and specific rotations of the free sterol are probably referable in part to differences in solvent content, *cf.* Schenk, Buchholz and Wiese.[9]

7(α)-Benzoxystigmasterol.—Three grams of 7(α)-benzoxystigmasteryl benzoate, m. p. 183.5–185° (Linsert,[10] 156–158°; Haslewood,[3] 184–186°) was dissolved in benzene (60 cc.); a solution of sodium methylate (2 g.) in absolute methanol (100 cc.) was added and the mixture allowed to stand for twenty-four hours at 23–25°. Crystallization of the reaction product from benzene–hexane yielded 2.3 g. of needles, which melted at 154.5–156.5°, but resolidified on further heating and finally melted again at 193°: $[\alpha]^{24}D$ +100.8° (0.98% in chloroform).

Anal. Calcd. for $C_{36}H_{52}O_3$: C, 81.15; H, 9.84. Found: C, 81.30, 81.26; H, 9.94, 10.01.

The monoester was not precipitated by digitonin.

7(α)-Benzoxystigmasteryl 3,5-dinitrobenzoate, prepared as described for the corresponding cholesterol derivative, formed fine fluffy needles from benzene–ethanol; m. p. 150.5–152.5°.

Anal. Calcd. for $C_{43}H_{54}O_8N_2$: N, 3.86. Found: N, 4.24.

7-Dehydrostigmasterol.—One gram of the monobenzoate, boiled for three hours in 10 cc. of dimethylaniline and worked up as previously described, yielded 1.871 g. of digitonide. The free sterol obtained after four crystallizations from acetone-methanol weighed 189 mg.; m. p. 147.5–152.5°; $[\alpha]^{24}D$ −96.5°; $\epsilon_{282\ m\mu}$ 9700 (in ether); ϵ_{280} for ergosterol 11,200. The material from the mother liquors and rotation measurements was benzoylated in pyridine. Repeated recrystallization from benzene–alcohol yielded needles melting at 178.5–180°; $[\alpha]^{26}D$ −48.5° (1.2% in chloroform).

Anal. Calcd. for $C_{36}H_{50}O_2$: C, 83.99; H, 9.79. Found: C, 83.92, 83.73; H, 9.88, 9.96.

Hydrolysis of the benzoate yielded dehydrostigmasterol; m. p. 150–152.5°; $[\alpha]^{27}D$ −104.0° (0.87% in chloroform); −109.8° (0.94% in benzene); $\epsilon_{282\ m\mu}$ 10,800 (in ether). Linsert,[10] $[\alpha]^{20}D$ −113.15° in benzene.

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 85.16, 85.06; H, 11.17, 11.05.

We are greatly indebted to Dr. N. H. Coy of the Vitamin Laboratory of E. R. Squibb and Sons for carrying out all spectrographic measurements and assays.

The microanalyses were carried out by J. F. Alicino, Fordham University.

Summary

The dibenzoates of 7(α)-hydroxysterols are hydrolyzed by cold sodium methylate solution selec-

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[7] Stoll, *Z. physiol. Chem.*, **207**, 147 (1932); Wallis, Fernholz and Gephart, THIS JOURNAL, **59**, 137 (1937).

[8] Boer, Reerink, Van Wijk and Van Nierkerk, *Konikl. Akad. Wetenschappen Amsterdam.*, **39**, 622 (1936).

[9] Schenk, Buchholz and Wiese, *Ber.*, **69**, 2696 (1936).

[10] Linsert, *Z. physiol. Chem.*, **241**, 116 (1936).

tively at the 3-benzoxy group to yield almost quantitatively the 7-monobenzoates. 7(α)-Benzoxycholesterol and 7(α)-benzoxystigmasterol thus prepared have been converted to the corresponding free 7-dehydrosterols.

NEW BRUNSWICK, N. J. RECEIVED FEBRUARY 7, 1942

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

The Dipole Moment of a Free Radical

BY JOHN TURKEVICH, PETER F. OESPER AND CHARLES P. SMYTH

Before the development of our present ideas of resonance, the dipole moments of α,α-diphenyl-β-picrylhydrazine and the free radical, α,α-diphenyl-β-picrylhydrazyl, were measured,[1] as were those of triphenylchloromethane and triphenylcarbinol.[2] The moment value found for triphenylchloromethane was close to those of the butyl chlorides, while that for triphenylcarbinol was about 0.4 higher than the moments of most of the alcohols. Moment values for 1,1,1-triphenylethane,[3] triphenylmethane,[4,5] and tri-*p*-nitrophenylmethane[5] showed no abnormality. Absence of marked abnormality in the moments of these molecules, which are closely related to free radical-forming structures, is to be expected in view of the fact that the resonating structures particularly responsible for the stabilization of the free radicals cannot contribute to these structures. Karagunis and Jannakopoulos[6] have recently found zero moment for the solutes in solutions of hexaphenylethane and tribiphenylmethyl and have attributed the absence of moment to the existence of the free radicals in symmetrical, planar structures.

In the present investigation, the early measurements upon α,α-diphenyl-β-picrylhydrazine and α,α-diphenyl-β-picrylhydrazyl have been repeated. The two substances were prepared by methods given in the literature.[7,8]

Experimental Results

The dipole moments were obtained by means of the apparatus and methods previously described,[9] the dielectric constants of benzene solutions of mole fractions, c_2, being measured at a frequency of 520 kilocycles and the densities, *d*, of the solutions being determined with an Ostwald–Sprengel pycnometer. These data are listed in Table I, together with the polarizations, P_2, calculated for the solute, the values of P_∞ obtained by extrapolation of the P_2 values, the molar refractions for the *D* sodium line, MR_D, calculated as the sums of atomic and group refractions, and the dipole moments, μ.

TABLE I

DIELECTRIC CONSTANTS AND DENSITIES OF BENZENE SOLUTIONS AND POLARIZATIONS AT 25° AND DIPOLE MOMENTS

c_2	ϵ	d	P_2
	α,α-Diphenyl-β-picrylhydrazine		
0.00000	2.2756	0.87321	...
.00265	2.3249	.87768	355
.00556	2.3824	.88264	361
.00659	2.4017	.88417	361
P_∞, 370; MR_D (calcd.), 106; μ, 3.59 × 10^{-18}			
	α,α-Diphenyl-β-picrylhydrazyl		
.000000	2.2726	.87313	...
.000792	2.3002	.87455	587
.001558	2.3272	.87600	588
.003292	2.3854	.87903	574
.004927	2.4367	.88168	558
.005351	2.4537	.88242	564
.009027	2.5768	.88912	550
P_∞, 600; MR_D (calcd.), 105; μ, 4.92 × 10^{-18}			

Discussion of Results

The moment value 3.59 in Table I for α,α-diphenyl-β-picryl-hydrazine is distinctly larger than the value 3.08 found by Wolf, but the value 4.92 for the free radical is in excellent agreement with Wolf's value, 4.88. The molecules of hydrazine, phenylhydrazine, α,α-, and α,β-diphenylhydrazine have moments between 1.53 and 1.87.[10] Since the three nitro groups of the picryl group are symmetrically located around the ring, their moments, in the absence of complicating effects, should cancel as do the similar moments in 1,3,5-trinitrobenzene, which has zero moment. In the

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[1] Wolf, Diplomarbeit, Karlsruhe, 1930.

[2] Smyth and Dornte, THIS JOURNAL, **53**, 545 (1931).

[3] Smyth and Dornte, *ibid.*, **53**, 1296 (1931).

[4] Lautsch, *Z. physik. Chem.*, **B1**, 115 (1928).

[5] Bergmann, Engel and Wolff, *ibid.*, **B17**, 81 (1932).

[6] Karagunis and Jannakopoulos, *ibid.*, **B47**, 343 (1940).

[7] Goldschmidt, *Ber.*, **55**, 628 (1922).

[8] Turkevich and Selwood, THIS JOURNAL, **63**, 1077 (1941).

[9] Lewis and Smyth, *J. Chem. Phys.*, **7**, 1085 (1939); THIS JOURNAL, **61**, 3063 (1939).

[10] Audrieth, Nespital and Ulich, *ibid.*, **55**, 673 (1933).