which agrees with that reported by Ruzicka and Wettstein.[9]

Δ^5-Androstene-3,17-dibenzoate.—The dibenzoate was prepared by heating on the water-bath a mixture of Δ^5-androstene-3,17-diol, benzoyl chloride and pyridine. The crude dibenzoate was recrystallized from methanol; m. p. 203–205°.

Anal. Calcd. for $C_{33}H_{38}O_4$: C, 79.5; H, 7.6. Found: C, 79.3; H, 7.8.

Dehydrogenation of Δ^5-Androstene-3,17-diacetate with Benzoquinone.—A mixture of 0.3 g. of the diacetate and 0.094 g. of benzoquinone was heated in a sealed evacuated tube at 130° for three hours. The product from this reaction was dissolved in ether and the solution thoroughly shaken with a 20% solution of sodium hydrosulfite, then extracted several times with a 10% sodium hydroxide solution, dried, and the ether removed. The slightly discolored residue was purified from methanol and a product was obtained the spectrum of which showed a single band with a maximum at 288 mμ having a molecular extinction coefficient of 1116. Δ^5-Androstene-3,17-diacetate exhibited no selective absorption in this region. A comparison of the extinction coefficient with that of ergosterol (10,600 at 282 mμ) indicated that the material contained the desired dehydrogenated product to the extent of about 10%.

Irradiation of $\Delta^{5,7}$-Androstadiene-3,17-diol.—A sample of the partially dehydrogenated diacetate was hydrolyzed with methyl alcoholic potash and the non-saponifiable diol irradiated in ether for four hours using a quartz-mercury lamp. The product was then assayed biologically by Professor R. S. Harris who reported no activity at a level of 10,500 U. S. P. XI vitamin D units per gram. Almost a year after these experiments were performed, Dimroth and Paland[8] reported similar biological results with this substance made by a different procedure.

The authors wish to express their appreciation for a generous sample of dehydroandrosterone supplied to one of us (N. A. M.) by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

Summary

1. Δ^5-Androstenol-3(*cis* 3:10) and its acetate and benzoate were prepared in the pure state.

2. The partial dehydrogenation of Δ^5-androstenol-3-benzoate and Δ^5-androstene-3,17-diacetate with benzoquinone has been studied. The irradiated dehydrogenation products were devoid of antirachitic action when tested at levels of 4000 and 10,500 U. S. P. XI vitamin D units per gram, respectively.

CAMBRIDGE, MASSACHUSETTS

RECEIVED NOVEMBER 30, 1945

[CONTRIBUTION FROM THE ARMOUR LABORATORIES]

The Ultraviolet Absorption Spectra of Allyl and Propenyl Substituted Derivatives of Diethylstilbestrol and Hexestrol

BY EMIL KAISER AND VIRGIL L. KOENIG

In a previous communication[1] the preparation of 3,3′-allyl and 3,3′-propenyl diethylstilbestrol and hexestrol are described. The 3,3′-allyl substituted derivatives were rearranged by heating in alkaline solution to compounds which were assumed to be 3,3′-propenyl derivatives in analogy to reactions reported in the literature.[2]

Obviously, the reaction described by Balbiano[3] for the differentiation between allyl and propenyl groups cannot be applied here inasmuch as the large number of double bonds would introduce complications. For this reason it was decided to examine the ultraviolet absorption spectra of these compounds. An important difference in the structure of the 3,3′-allyl and 3,3′-propenyl derivatives under discussion here resides in the position of the double bonds of the side chains. In the propenyl compounds the double bonds are in a conjugated position to the double bonds of the aromatic nuclei; in the allyl derivatives the double bonds are not conjugated. Since the presence of the double bonds is shown by the shape of the ultraviolet absorption curves of organic compounds, it was thought possible to demonstrate the shifting of the double bonds by comparing the absorption spectra of 3,3′-allyl derivatives of diethylstilbestrol and hexestrol with the absorption spectra of 3,3′-propenyl derivatives of diethylstilbestrol and hexestrol.

Acknowledgment.—The authors are indebted to Professor F. C. Koch for his helpful criticism of the manuscript and for his encouragement during the work.

Experimental

The ultraviolet absorption curves of the following compounds were determined: diethylstilbestrol, diallyl ether of diethylstilbestrol, 3,3′-allyl diethylstilbestrol, 3,3′-propenyl diethylstilbestrol and hexestrol, hexestrol diallyl ether, 3,3′-allylhexestrol and 3,3′-propenylhexestrol. The diethylstilbestrol and hexestrol were recrystallized from commercial preparations. The other compounds were prepared according to a previous communication.[1] Acetone-free absolute methanol, distilled from alkali, was used as solvent. The concentration of the solutions was 0.005%.

The measurements were made on a Beckman spectrophotometer using the hydrogen discharge tube as the source of light. Transmittance values were determined at 10 millimicron increments from wave length 220 millimicrons to 400 millimicrons. Extinction values were obtained from the transmission values and, from these, molecular extinctions were calculated and used for plotting the graphs.

Discussion

In Fig. 1 the absorption spectra of diethylstilbestrol, diallyl ether of diethylstilbestrol, 3,3′-

(1) Kaiser and Svarz, THIS JOURNAL, **68**, 636 (1946).

(2) "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 19.

(3) Balbiano, *Ber.*, **48**, 394 (1915).

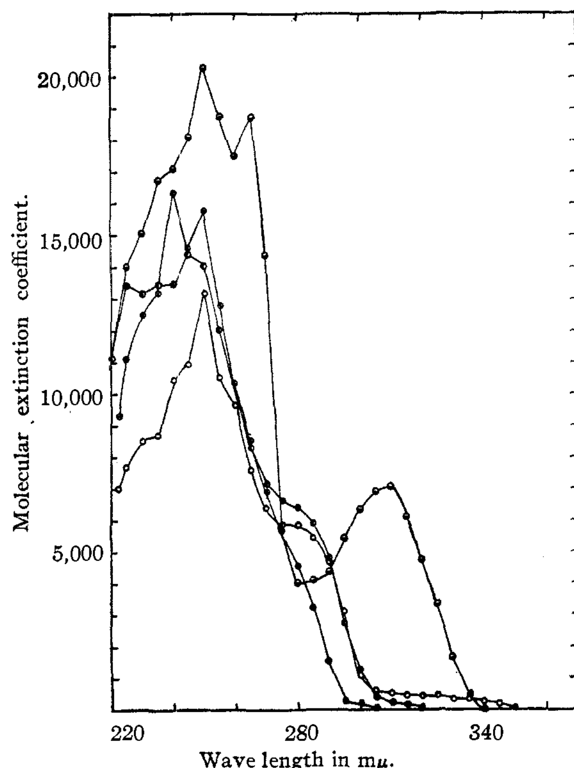

Fig. 1.—O, diethylstilbestrol; ⊗, diethylallyl ether; ◐, 3,3′-allyldiethylstilbestrol; ◒, 3,3′-propenyldiethylstilbestrol.

allyldiethylstilbestrol and 3,3′-propenyldiethylstilbestrol are shown. The diethylstilbestrol absorption curve was very similar to the absorption curves reported for this compound by Elvidge,[4] Kharasch and Kleiman[5] and by Solmsson.[6] The maximum of the absorption curve was close to 250 millimicrons, a value somewhat different from Elvidge, who found the maximum close to 240 millimicrons. The highly purified diethylstilbestrol of Kharasch and Kleiman[5] has a maximum close to 250 millimicrons in accordance with our measurements. The inflection at 280 millimicrons, pointed out by Solmsson,[6] is shown in Fig. 1. The absorption curve of the diallyl ether of diethylstilbestrol shows a maximum in the region of 240–250 millimicrons and has no inflection at 280 millimicrons. Another ether of diethylstilbestrol, the dimethyl ether, has an inflection at 280 millimicrons in the absorption curve.[5] The maximum of the curve of the 3,3′-allyldiethylstilbestrol was very close to the maximum of the diethylstilbestrol curve and the inclination at 280 millimicrons was very marked. An entirely different picture is presented by the absorption curve of 3,3′-propenyldiethylstilbestrol. The absorption of light is greater than in the case of diethylstilbestrol and its allyl derivatives.

(4) Elvidge, *Quart. J. Pharm. Pharmacol.*, **12**, 347 (1937).

(5) Kharasch and Kleiman, THIS JOURNAL, **65**, 11 (1943).

(6) Solmsson, *ibid.*, **65**, 2370 (1943).

A maximum of the absorption is observed close to 250 millimicrons, then a drop in the absorption occurs until a wave length of about 280 millimicrons. There instead of an inflection of the curve the light absorption increases again until a maximum is reached at 310 millimicrons. The presence of conjugated double bonds in the molecule shows up very definitely by this particular peak of the absorption curve.

The difference between the light absorption of the allyl and propenyl compounds is even more pronounced in the hexestrol series, Fig. 2. The maxima of absorption of hexestrol, hexestrol diallyl ether and 3,3′-allylhexestrol are all close to 230 millimicrons and 280 millimicrons, in good agreement with the values for hexestrol reported by Elvidge[4] and by Goetze and Serf.[7] The absorption curve of 3,3′-propenylhexestrol, however, had maxima at 250 and 310 millimicrons. The maxima are at the same wave lengths as the absorption maxima of 3,3′-propenyldiethylstilbestrol and seem to be characteristic for the conjugated double bonds of 3,3′-propenyldiethylstilbestrol and hexestrol.

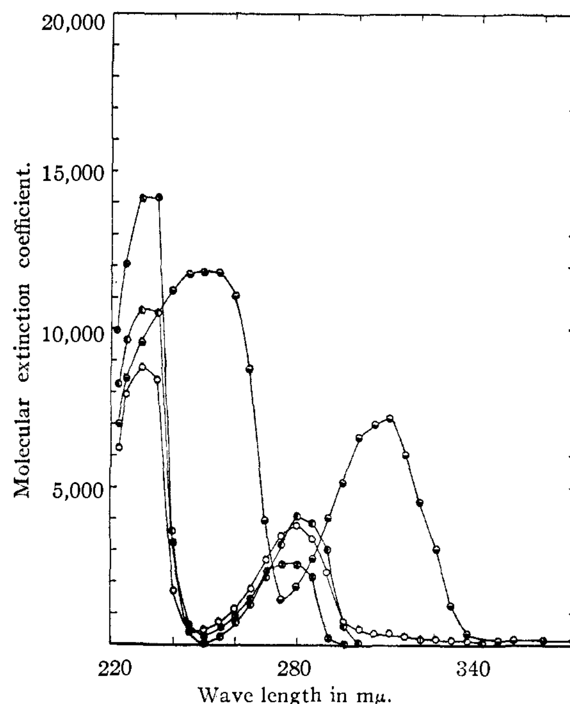

Fig. 2.—O, hexestrol; ⊗, hexestrol allyl ether; ◐, 3,3′-allylhexestrol; ◒, 3,3′-propenylhexestrol.

Summary

Ultraviolet absorption curves are reported for diethylstilbestrol, diethylstilbestrol diallyl ether, 3,3′-allyldiethylstilbestrol, 3,3′-propenyldiethylstilbestrol, hexestrol, hexestrol diallyl ether, 3,3′-allylhexestrol and 3,3′-propenylhexestrol. The shifting of the double bonds from the allyl to the

(7) Goetze and Serf, *J. Am. Pharm. Assoc.*, XXXIV, 209 (1945).

propenyl grouping is accompanied by a shift of the absorption maxima in the ultraviolet. The absorption maxima of 3,3′-propenyldiethylstilbestrol and of 3,3′-propenylhexestrol are at identical wave lengths.

CHICAGO, ILLINOIS RECEIVED JANUARY 5, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Osmotic Pressure of β-Lactoglobulin Solutions

BY HENRY B. BULL AND BYRON T. CURRIE[1]

A number of values for the molecular weight of β-lactoglobulin from cow's milk have been reported. There is, however, a disconcerting lack of agreement between these values as is shown in Table I.

TABLE I

MOLECULAR WEIGHT OF β-LACTOGLOBULIN AS REPORTED BY VARIOUS WORKERS

Method	Molecular weight	Workers
Ultracentrifuge (equilibrium) and diffusion	38,000	Pedersen[a]
Ultracentrifuge (rate sedimentation)	41,500	Pedersen[a]
X-Ray (wet crystals)	33,000	Crowfoot[b]
		McMeekin and Warner[c]
X-Ray (air dried crystals)	35,000	Crowfoot[b]
		McMeekin and Warner[c]
Chemical analysis } mini-	42,000	Brand and Kassel[d]
Chemical analysis } mum	42,000	Chibnall[e]
Chemical analysis } (m. w.)	42,020	Brand *et al.*[f]
Osmotic pressure	38,000	Gutfreund[g]
Film pressure	2 × 17,100	Bull[h]
Light scattering	35,000	Heller and Klevens[i]
Osmotic pressure	35,020	Bull and Currie

[a] Pedersen, *Biochem. J.*, **30**, 961 (1936). [b] Crowfoot, *Chem. Rev.*, **28**, 215 (1941). [c] McMeekin and Warner, THIS JOURNAL, **64**, 2393 (1942). [d] Brand and Kassel, *J. Biol. Chem.*, **145**, 365 (1942). [e] Chibnall, *Proc. Roy. Soc. London*, **B131**, 136 (1942). [f] Brand, Saidel, Goldwater, Kassell and Ryan, THIS JOURNAL, **67**, 1524 (1945). [g] Gutfreund, *Nature*, **155**, 237 (1945). [h] Bull, THIS JOURNAL, **68**, 745 (1946); [i] Heller and Klevens, private communication.

The present paper reports the result of 20 osmotic pressure measurements on solutions of β-lactoglobulin along with the calculation of the molecular weight of this protein.

Experimental

The osmotic pressure apparatus was a modification of that described by Bull.[2] This modification is diagrammed in Fig. 1. The capillary tube had an inner diameter of 0.2 mm. and was furnished by Corning Glass Works. About 5 cc. of the outside solution (solution whose composition was identical with that of the protein solution except for the absence of protein) was placed in the bottom of the apparatus. Toluene was added from the top of the clean, dry capillary and forced down the capillary with gentle air pressure. Suction was then applied to the capillary to remove the trapped air. The apparatus was then filled with outside solution. The sack which contained the protein solution was made from Visking sausage casing whose flat width was 1.5 cm. and was supplied through the courtesy of Dr. C. J. B. Thor. The moist casing was securely knotted at one end and the other end was slipped over a rubber stopper which had been trimmed to size, and attached to the end of the tube which was to contain the protein. The stopper had been previously coated with stopcock grease. The end of the sack which had been slipped over the rubber stopper was wrapped tightly with a rubber band to complete the attachment to the rubber stopper. The sack was then filled with protein solution through the larger glass tube to which was attached a second, larger rubber stopper which was coated with stopcock grease. The apparatus was then assembled as shown in Fig. 1. The larger rubber stopper was held in place by a metal clamp which is not shown in Fig. 1. Small one-hole rubber stoppers were placed in the tubes containing the outside solution and the protein solution. Their purpose was to decrease the evaporation of these solutions during an experiment.

The whole apparatus was clamped in a fixed position in a constant temperature bath at 25°. The stopcock was allowed to remain open until the apparatus had come to temperature. The position of the toluene level in the capillary was marked by appropriate means and the stop cock was closed. The toluene meniscus immediately began to rise or fall depending on whether the hydrostatic pressure was greater or less than the osmotic pressure. Protein solution was added to or removed from the protein solution column to bring the toluene meniscus to its original level. Adjustment of the protein solution level was made from time to time as needed. The toluene column thus acted essentially as an indicator and in the ideal case there is no net motion of liquid across the sausage casing membrane. Usually, no further adjustment of the protein solution column was necessary after about two hours. In all cases, however, the experiment was allowed to continue overnight. At the end of this time, the position of the toluene column had usually shifted one or two mm. The drift of the toluene level was multiplied by the density of toluene and applied as a correction to the osmotic pressure. The difference in level between the outside solution and the protein solution was measured with a cathetometer. This difference in level when multiplied by the density of the protein solution gave, after applying the correction due to the small excursion of the toluene column, the osmotic pressure of the protein solution in centimeters of water.

The β-lactoglobulin was prepared by a modification of a method suggested in a private communication by Dr. A. H. Palmer. Raw whole milk was brought to 50% saturation with ammonium sulfate. The solution was filtered and the filtrate brought to 80% saturation with ammonium sulfate. A small amount of water along with some toluene was added to the precipitate. The precipitate was transferred to sausage casing and dialyzed against frequent changes of distilled water at 5° for four or five days. The protein solution was then removed from the sausage casing and any extraneous solid material filtered off. The filtrate was adjusted to *p*H 5.1 by the cautious addition of dilute hydrochloric acid. The turbid solution was then dialyzed against distilled water and the dialysis continued until no further separation of the "oily" phase took place. The protein was then crystallized as described by Palmer.[3]

(1) On leave of absence from the Corn Products Refining Company.

(2) Bull, *J. Biol. Chem.*, **137**, 143 (1941).

(3) Palmer, *ibid.*, **104**, 359 (1934).