Summary

Two γ-globulin fractions, isoelectric at *p*H 5.85 and 7.35 in $\mu = 0.1$ buffers, were prepared by methods previously described, from sera of cows immunized to Brucella abortus and Newcastle virus. These fractions are inhomogeneous as indicated by electrophoretic spreading experiments and solubility studies, but are essentially monodisperse as regards sedimentation behavior. The antibodies studied are largely concentrated in the fraction of lower isoelectric point (γ_1-globulins). Concentration and separation of certain antibodies were obtained, indicating the feasibility of chemical separation of individual antibodies from polyvalent systems.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF MISSOURI]

Hydrodiethylstilbestrol Compounds. IV. The Unsaturated Ketones[1,2]

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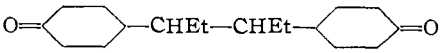
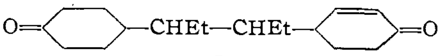
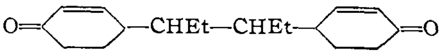
Unsaturated ketones derived from perhydrostilbestrol have been described by Schoeller and coworkers.[4] The di-unsaturated diketones are said to be colorless oils, the mono-unsaturated compounds have not been characterized nor is any criterion of purity disclosed in the pertinent examples. Since the compounds are claimed to possess androgenic activity up to one capon unit per mg., it appeared possible that purified preparations might possess considerable biological activity.

The substances have been prepared essentially as described by the previous investigators. The two known perhydrodiketones (I)[4,5] can be brominated rapidly in chloroform[4] or in glacial acetic acid[6] with the introduction of one or more atoms of bromine. In view of the possibility of replacing the tertiary hydrogens of the hexane chain in the diketones (I) the bromination has now been effected by means of N-bromosuccinimide which supposedly does not react with tertiary hydrogens in the absence of peroxides.[7]

Bromination of the diketones (I) with N-bromosuccinimide in carbon tetrachloride proceeds rapidly with the introduction of one atom of bromine. The second and third bromines are introduced more slowly, the second bromine entering preferentially the unbrominated nucleus. On the basis of recovered succinimide (98–100%) the reaction is nearly quantitative. The glassy bromodiketones are best dehydrobrominated by refluxing with collidine. Chromatographic adsorption of the crude glassy substances on alumina from benzene solution yields homogeneous products containing benzene of solvation which can be removed only by vacuum sublimation.


I


II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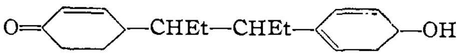
IV

Ultraviolet absorption spectra of the analytically pure sublimates (II) and (III)[8] show relatively low extinction values at 225 mμ and only a weak secondary maximum at 280–285 mμ. Both phenolic ketones (IV) give the expected phenolic band of 280 mμ. Traces of a phenolic by-product (V) have been isolated from the dehydrobromination of the *meso*-tribromodiketone regardless whether it is prepared by direct bromination or with N-bromosuccinimide. This substance has a strong maximum at 300 mμ which may be taken as evidence for the presence of a double bond conjugated with the phenolic ring.[9] A red ketonic by-product is obtained in the same reaction which must possess a multiple conjugated system in view of its color. The formation of these by-products and the small extinction values for the compounds (II) and (III) lend support to the assumption that N-bromosuccinimide may also attack tertiary hydrogens even in the absence of peroxides and that the substances (II), (III) and (IV) probably consist of mixtures of conjugated and non-conjugated unsaturated ketones.

The *meso* compounds (II) and (III) have been tested biologically. Both show little or no androgenic activity in the seminal vesicle test with three daily doses of 1.0 and 0.75 mg. of substance, respectively.[10]

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[1] Presented in part before the Division of Organic Chemistry of the American Chemical Society, Chicago, April, 1948.

[2] From the Ph.D. thesis of Paul W. Tucker, 1948.

[3] George Breon Fellow, 1946–1948. Present address: Research Dept., Phillips Petroleum Company, Bartlesville, Okla.

[4] Schoeller, Inhoffen, Steinruck and Höss, U. S. Patent 2,392,864.

[5] Ungnade and Ludutsky, *J. Org. Chem.*, **10**, 307 (1945).

[6] Ludutsky, Ph.D. thesis, University of Missouri, 1946.

[7] Schmid and Karrer, *Helv. Chim. Acta*, **29**, 573 (1946).

[8] Absorption spectra by Dr. E. E. Pickett, University of Missouri.

[9] Jones, *Chem. Rev.*, **32**, 36 (1943); Kaiser and Koenig, THIS JOURNAL, **68**, 740 (1946).

[10] Androgenic tests by Mr. K. D. Sprague, Control Department, Winthrop-Stearns, Inc., Rensselaer, N. Y.

Experimental[11]

Bromination of the Diketones.—The calculated amount of N-bromosuccinimide was added to a solution of the diketone dissolved in carbon tetrachloride. The mixtures were refluxed for four and one-half, five and one-half and nine hours, respectively, for introducing one, two or three atoms of bromine. Part of the solvent was removed by distillation and the reaction mixtures were allowed to cool. The succinimide was filtered off and washed with carbon tetrachloride. Evaporation of the solvents from the filtrates gave the crude bromo compounds which were used without further purification. The experimental values are listed in Table I.

TABLE I

BROMINATION OF THE DIKETONES

Diketone, g.		CCl_4, cc.	N-Bromosuccinimide, g.	Yield of bromodiketone, %
meso	4.0000	50	2.5632	98.2
meso	8.0000	100	10.2172	91.7
meso	12.0000	150	23.0688	98.9
dl	5.0000	50	3.2040	98.0
dl	10.0000	100	12.8160	98.0
dl	15.0000	150	19.2240	90.0

Dehydrobromination of the Bromodiketones.—The bromodiketones, dissolved in 4 to 6 parts by weight of collidine, were refluxed for one and one-half hours in an oil-bath. The collidine hydrobromide was filtered off and washed with benzene. The filtrates were extracted with benzene and the combined benzene solutions were washed with 10% aqueous hydrochloric acid and water, dried by distillation and evaporated. The dark colored glassy substances were dissolved in benzene and extracted with Claisen alkali. The remaining benzene solutions were washed, dried and adsorbed on alumina. The ketonic fractions were eluted with benzene and distilled to remove the solvent. The identity of the fractions was checked by means of their ultraviolet absorption spectra. Traces of starting material showed very low extinction values over the range of 215–335 mμ.

Phenolic substances were obtained only in minute quantities from mono- and dibromodiketones, but formed in fairly good yield from the tribromodiketones. They were regenerated by acidification of the Claisen alkali solutions with 1:1 aqueous hydrochloric acid, extracted with benzene, washed, dried and distilled. The residual material was adsorbed on alumina from benzene solution and eluted with the same solvent. The fraction with appropriate ultraviolet spectrum was sublimed under reduced pressure.

Vacuum sublimation of both phenolic and non-phenolic ketones was carried out in a molecular pot still with a path length of 1 cm. at 160–180° (1×10^{-4} mm.). Yields and constants of the unsaturated ketones are given in Table II.

Ultraviolet Absorption Spectra.—The unsaturated ketones were run in 95% alcohol in concentrations of 0.000185 mole per liter in a Beckman spectrophotometer.[8] The saturated *meso*-diketone (I) was run in a 0.005 molar solution. The extinction values are given in Table III.

TABLE II

DEHYDROBROMINATION OF THE BROMODIKETONES

Bromodiketone, g.	Crude unsaturated ketone, yield, %		Phenolic material, yield, %	Analyses C, %	H, %	M. p. °C.
5.00	*meso* (II)	71	Traces	78.09	10.21[a]	Glass
11.01	*meso* (III)	66.3	Traces	78.63	9.32[b]	Glass
22.00	*meso* (IV)	70.8	46.4	79.79	8.74[c]	Glass
7.00	*dl* (II)	74.4	Traces	78.00	10.02[a]	Glass
15.00	*dl* (III)	84	Traces	79.00	9.75[b]	Glass
25.57	*dl* (IV)	62.4	11.5	78.84	8.70[c]	52-53

[a] Calcd. for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. [b] Calcd. for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. [c] Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88.

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA

Compound	Maxima	ϵ_{max}[c]	Minima	$\epsilon_{min.}$
meso (I)	280	39	240	18
meso (II)	225	2890	220	2840
	280	1130	270	1080
dl (II)	230[a]	2290	225[b]	2460
	285	1180	270	1100
meso (III)	225	4580	220	4470
	285	2400	265	2040
dl (III)	235[a]	2790	225[b]	6030
	285	3240	270	2890
meso (IV)	225	12900	215	11500
	280	5250	255	3900
dl (IV)	225[a]	11300	220[b]	11500
	280	4900	255	3810
meso (V)	220	8300	265	3600
	300	6300		

[a] Shoulder. [b] Inflection point. [c] ϵ = molecular extinction coefficient.

Summary

Analytically pure unsaturated diketones and unsaturated phenolic ketones derived from perhydrostilbestrol have been prepared by bromination and dehydrobromination of the saturated diketones.

On the basis of the absorption spectra of the products and the by-products in the reaction it is proposed that N-bromosuccinimide may attack tertiary hydrogens even in the absence of peroxides and that the products may consist of mixtures of conjugated and non-conjugated unsaturated ketones.

The mono- and di-unsaturated *meso*-diketones fail to show any appreciable androgenic activity.

COLUMBIA, MO.

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[11] All temperatures uncorrected.