with the observations of Campbell, *et al.*,[4] who also prepared purified antibody by exposure to acid *p*H (3.5). Controls involving the treatment with R-stromata of normal rabbit serum, a mixture of bovine γ-globulin and purified antibody, and bovine γ-globulin alone, showed no appreciable non-specific precipitation. Protein concentrations were determined by ultraviolet absorption[5]; the validity of this procedure was assured by low R-stromata blanks, and by the characteristic shape of the absorption curves.

The binding of homologous haptenic dye *p*-(*p*-hydroxyphenylazo)-phenylarsonic acid by purified antibody was determined by equilibrium dialysis. One ml. aliquots of an antibody solution of known protein concentration (0.15%) containing buffer and salt (*p*H 7.4, 0.01 *M* PO_4, 0.16 *M* NaCl) were placed inside 0.25-in. dialysis bags. These were equilibrated against equal volumes of hapten in buffered saline whose initial dye concentrations were accurately known. The free dye concentrations in equilibrium with the bound dye were determined by measuring the spectral absorption, at 440 mμ, of the outside solutions, after suitable dilution and adjustment to strongly alkaline *p*H. The concentrations of bound dye were calculated from the values for the free equilibrium and initial dye concentrations, the former ranging from 5×10^{-6} *M* to 8×10^{-5} *M*. This calculation required a correction, due to adsorption of dye on the dialysis bag, amounting to 11% of the free dye concentration. The following (which corresponds to the third point in Fig. 1) represents a typical calculation: initial dye concentration $= 3.96 \times 10^{-5}$ *M*, free equilibrium dye concentration $= 1.18 \times 10^{-5}$ *M*, concentration of bound dye $= 1.35 \times 10^{-5}$ *M*, moles hapten bound per mole protein[6] (r) = 1.45.

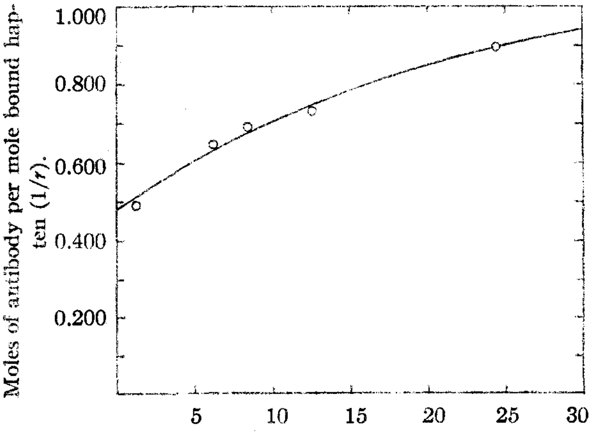

Fig. 1.

By plotting the reciprocal of moles hapten bound per mole antibody ($1/r$) against the reciprocal of free hapten concentration ($1/c$) the binding capacity is obtained from the extrapolated value of $1/r$.[7] As could be anticipated from the known heterogeneity of antibodies,[3,8] the curve obtained was not linear. In such a situation the average intrinsic association constant can be shown to be equal to the value of $1/c$ at which one-half the binding sites are occupied, if it is assumed that the variation in the free energy of binding among the various sites can be described by a Gaussian distribution function.[9] Such an assumption has been made previously by Pauling, *et al.*,[8] to describe hapten inhibition data.

The binding data obtained at room temperature (29°) with the hapten indicated above are summarized in Fig. 1. Extrapolation yields a value of 2 for the binding capacity ("valence") of the antibody, within an accuracy of 10%. The value of the intrinsic association constant is 3.5×10^5, corresponding to $\Delta F^0 = -7.7$ kcal. per mole hapten.

Antisera prepared as in these experiments by Campbell, *et al.*,[4] contained about 1.0% antibody for R-group, whereas our yields of purified antibody correspond to an initial antibody concentration in antiserum of about 0.04%. Either our antisera were far less potent than Campbell's, or the antibody removed by our purification procedure comprised only a small fraction of the total antibody present in the antiserum, in which case the above association constant would measure the average binding energy of only the most reactive antibody molecules.

Acknowledgment.—We are indebted to Professor R. Keith Cannan for the laboratory facilities so generously made available to us during the conduct of this investigation.

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[4] D. H. Campbell, R. H. Blaker and A. B. Pardee, THIS JOURNAL, **70**, 2496 (1948).

[5] H. N. Eisen, *J. Immunol.*, **60**, 77 (1948).

[6] Assuming a molecular weight for rabbit antibody of 160.000

[7] I. M. Klotz, F. M. Walker and R. B. Pivan, THIS JOURNAL, **68**, 1486 (1946)

[8] L. Pauling, D. Pressman and A. L. Grossberg, *ibid.*, **66**, 784 (1944).

[9] F. Karush and M. Sonenberg, *ibid.*, in press.

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Preparation and Properties of Several Cyclohexyl-alkyl-substituted Ketene Dimers

BY CARL M. HILL AND GILBERT W. SENTER

It has been demonstrated that tertiary aliphatic amines dehydrohalogenate acid halides with the formation of ketene monomers and dimers.[1] Sauer[2] has reported the dehydrohalogenation of several fatty acid halides by tertiary aliphatic amines to yield ketene dimers.

This paper describes the dehydrohalogenation of five omega-cyclohexyl-substituted acid chlorides (of type $C_6H_{11}\cdot(CH_2)_n\cdot COCl$) by triethylamine.

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[1] Hanford and Sauer, "Organic Reactions," Vol. III, John Wiley and Sons, New York, N. Y., 1946, pp. 138–140.

[2] Sauer, THIS JOURNAL, **69**, 2444 (1947)

TABLE I

KETENE DIMERS

-Cyclohexyl, ketene dimers	B. p. °C.	B. p. Mm.	Yield,[a] %	n^{20}_D	Formula	Analyses, % Mol. wt.[b] Found	Mol. wt. Calcd.	Carbon Found	Carbon Calcd.	Hydrogen Found	Hydrogen Calcd.
,ketene	108–111	2	28	1.5001	$C_{16}H_{24}O_2$	250.7	248.4	76.68	77.36	10.08	9.74
,methyl	190–191	6	40	1.4925	$C_{18}H_{28}O_2$	281.9	276.4	78.09	78.22	10.38	10.21
β-, ethyl	115–120	2	18	1.4850	$C_{20}H_{32}O_2$	278.2	304.5	79.15	78.91	10.64	10.52
γ-, propyl	150–152 (m. p., 16–17)	1	25	1.4860	$C_{22}H_{36}O_2$	331.8	332.5	79.53	79.39	11.04	10.90
δ-, butyl	128–130 (m. p., 33–35)	1	36	....	$C_{24}H_{40}O_2$	351.6	360.5	80.46	79.88	11.56	11.18

[a] Based upon the amount of unrecovered acid chloride. [b] Determined cryoscopically.

The ketene dimers thus formed have not been previously reported.

The dimers gave a negative test with silver nitrate; positive tests with potassium permanganate, and bromine. *p*-Nitrophenylhydrazine reacted with the dimers to yield the hydrazones.

Experimental

The procedure followed in dehydrohalogenation of the the acid chlorides was similar to that reported by Sauer.[2] Equal molar quantities of acid chloride and triethylamine were allowed to react in dilute ether solution. Each dimer was isolated in its pure form by distillation under diminished pressure. A quantity of unreacted acid chloride was recovered from each experiment.

Analytical data and physical constants of the five ketene dimers are shown in Table I.

***p*-Nitrophenylhydrazones.**—The hydrazones were prepared according to the procedure described by McElvain.[3] The color of the hydrazones varied from lemon to deep orange.

Analytical data and physical properties of the hydrazones are given in Table II.

TABLE II

p-NITROPHENYLHYDRAZONES OF DIMERS

-Cyclohexyl, ketene dimer	Hydrazone	N, % Found	N, % Calcd.	M. p.,[a] °C.	Yield, %
,ketene	$C_{28}H_{34}O_4N_6$	16.29	16.21	102–103 d.	19
,methyl	$C_{30}H_{38}O_4N_6$	14.89	15.38	104–105 d.	41
β-, ethyl	$C_{32}H_{42}O_4N_6$	14.05	14.63	110–111 d.	53
γ-, propyl	$C_{34}H_{46}O_4N_6$	14.10	13.95	113–114 d.	44
δ-, butyl	$C_{36}H_{56}O_4N_6$	[b]	...	84–85	57

[a] Melting points are uncorrected. [b] Compound decomposed upon prolonged drying.

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(3) McElvain, "Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1945, p. 199.

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The Preparation and Vapor Pressure of Bromopentachloroethane; the Vapor Pressures of Heptachloropropane and Dibromotetrachloroethane

BY TAKERU HIGUCHI,[1] NOBORU ENDOW[2] AND JOHN E. WILLARD

When confronted with a need for a sample of bromopentachloroethane we were not able to find any record of its preparation in the literature. We have prepared a sample by the photobromination of pentachloroethane at elevated temperature, using a technique similar to that previously employed in this laboratory for the preparation of bromotrichloromethane from chloroform and bromine.[3] The over-all reaction is

$$C_2Cl_5H + Br_2 \longrightarrow C_2Cl_5Br + HBr$$

Eastman Kodak Co. technical pentachloroethane was washed several times with concentrated sulfuric acid, with water, with 20% potassium hydroxide solution and again with water. After drying with phosphorus pentoxide, approximately 1 mole (125 cc.) was then mixed with one mole (55 cc.) of bromine and 75 cc. of carbon tetrachloride. This mixture was illuminated by two 500-watt Mazda projection bulbs placed next to the flask which was sealed to a water-cooled reflux condenser. The heat from the bulbs was sufficient to maintain the solution at or near the boiling temperature. Since the photobromination of chloroform has been found to be inhibited by both oxygen and water, the following precautions were taken to exclude gross amounts of these substances: (a) a slow stream of nitrogen was bubbled through the solution and allowed to pass out through the condenser throughout the course of the reaction; (b) 5 g. of phosphorus pentoxide was added to the reaction flask.

Initial attempts to prepare bromopentachloroethane by this method, from mixtures of bromine and pentachloroethane without carbon tetrachloride, led to a mixture of products. Because of the possibility that this might be the result of reactions favored by the relatively high temperature of the boiling point of the pentachloroethane (155°), carbon tetrachloride was added to reduce the boiling point.

After a week of continuous illumination the reaction solution solidified on cooling. The weight of this crude product was 200 g. Following three recrystallizations from small amounts of carbon tetrachloride large white crystals were obtained which melted with decomposition in the range of 180 to 200° when heated in sealed evacuated tubes. An estimate of the molecular weight and vapor pressure was obtained from this sample by measuring the gas pressure produced in a known volume by a weighed sample as a function of temperature. This was accomplished by placing the sample in an evacuated sealed bulblet in a sidearm of an evacuated thermostated flask which was connected to an open-end mercury manometer,[4] the middle section of which was made of flexible rubber suction tubing. The sample tube was broken with a glass-enclosed magnetic hammer. By means of a leveling bulb the mercury was maintained at an index mark on the glass side arm of the flask which connected with the manometer, thus maintaining a constant gas volume as the pressure

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(3) (a) Bohlmann and Willard, THIS JOURNAL, 64, 1342 (1942); (b) Higuchi, Ph.D. Thesis, University of Wisconsin, 1943.

(4) The apparatus used in these determinations was constructed by Norman W. Rosenberg with the aid of funds from the National Youth Administration. Details of modifications and applications are given in the Masters' degree thesis of Noboru Endow filed with the Library of the University of Wisconsin in January, 1948.