Any liberated estradiol is recovered (90 ± 5%) in the filtrate. Aliquots of the filtrates are evaporated to dryness *in vacuo* at room temperature, and extracted with chloroform or acetone, which further removes chromogenic substances. After evaporation the residue is ready for the Folin–Ciocalteu reaction. The reaction mixture is taken to *p*H 7.0 before ethanol precipitation in order to prevent appreciable hydrolysis of unchanged estradiol benzoate during processing.

The following are typical results of estradiol liberated for 1 cc. of serum at 37.5°. Rabbit serum, *p*H 7.0 in albumin buffer for thirty minutes: 0.50 mg. estradiol. Control heated rabbit serum: 0.015 mg. estradiol. Human serum, *p*H 7.2 in albumin buffer for six hours: 0.18 mg. estradiol. Control heated human serum: 0.03 mg. estradiol. Bull serum, *p*H 8.3 for thirty minutes: 0.070 mg. estradiol. Control heated bull serum: 0.008 mg. estradiol.

The degree of hydrolysis of solutions of estradiol benzoate in aqueous albumin or sodium lauryl sulfate in the *p*H range and concentration used in the enzymatic reaction is of the low order found for serum heated one hour at 63° or ten minutes at 70°. The marked hydrolytic effect of unheated serum therefore proves the existence of a steroid hormone esterase.

The enzymatic action has also been demonstrated in rat, chicken and pigeon blood, and on the substrates estradiol diproprionate and estrone acetate.

CHEMICAL LABORATORY
SANTA BARBARA COTTAGE HOSPITAL
RESEARCH INSTITUTE
SANTA BARBARA, CALIFORNIA
RECEIVED AUGUST 1, 1949

A Hydrogen Transfer Reaction of the Butylenes and Butyl Alcohols in Sulfuric Acid

BY WILLIAM H. CALKINS AND T. D. STEWART

Isobutylene, 2-butene and the four butyl alcohols each react in 96% sulfuric acid at 0° to yield up to 20% isobutane. This reaction was observed by us during a study of the alkylation of isobutane by 2-butene.[1]

We had occasion to introduce 2-butene alone, as a gas, into a flask containing stirred sulfuric acid and nitrogen gas at atmospheric pressure; the reactor was fitted with a mercury manometer. With a rate of introduction of 10 ml./min. the pressure rose immediately to 7–8 mm., remained nearly constant for ten to fifteen minutes, and then started to rise again. When the 2-butene flow was shut off within five minutes the pressure immediately fell to practically its original value; but if it was shut off during the later rise, the pressure fell only 2–3 mm. and then continued to rise for an hour or more without further 2-butene input. The same behavior, and to the same degree, was observed with the rate of input 27 ml./min. The volume of the reactor system was 600 ml.; the sulfuric acid varied from 25–100 ml. The eventual pressure rise for a charge of about 150 ml. of 2-butene was 20–30 mm., which, when corrected to room temperature, corresponded to a 17% yield of gas.

A preliminary examination of the gas formed, by Orsat combustion, indicated butane. This was confirmed by an analysis performed for us by the Shell Development Company, using their Consolidated Mass Spectrometer, which indicated 4% by volume of isobutane, 1% isopentane and traces of possible hexanes, in the nitrogen. Routine analyses were later made by an infrared spectrophotometer (National Technical Laboratories), calibrated at the wave lengths 8.48, 9.78 and 10.2 μ against pure samples of normal butane, isobutane and isopentane, and following the procedure of Brattain, Rasmussen and Cravath.[2]

This isobutane evolution is related to the conversion of the four-carbon alkenes and alcohols to liquid saturated hydrocarbons under similar, but not identical, circumstances.[3] Its uniqueness lies in the high percentage of isobutane, which may be a precursor to the hydrocarbons of higher molecular weight. The reaction is characterized by the presence of an induction period of a few minutes, in the case of 2-butene, and by the fact that rate of gas evolution from any given solution is approximately first order. However, both the rate and amount of gas formation vary somewhat with different amounts of sulfuric acid used. Table I details typical results.

TABLE I

YIELDS OF GAS EVOLVED FROM BUTYLENES AND BUTYL ALCOHOLS IN CONCENTRATED SULFURIC ACID AT 0°

Alkenes	Volume of acid, ml.	Moles of reactant	Pressure increase, mm.	Molal conversion, %
2-Butene	25	0.0059	27	16
2-Butene	50	.0072	37	16
2-Butene	100	.0063	26	13
Isobutylene	25	.0061	32	18
Alcohols				
n-Butyl	25	.0066	15	7.4
t-Butyl	25	.0074	22	10
t-Butyl	100	.0063	30	15
s-Butyl	25	.0033	18	19
s-Butyl	100	.0066	32	14
Isobutyl	25	.0065	48	25
Isobutyl	100	.0065	49	22

In the case of the alcohols, solution was effected slowly at 0° in a small amount of the acid outside the stirred reactor; this solution was then added rapidly to the excess of acid. Gas evolution was followed manometrically and analysis showed it to be almost entirely isobutane.

The induction periods in the case of the alcohols were striking. For *n*-butyl it was seventy-five minutes, for isobutyl thirty minutes, for *s*-butyl five minutes, and for *t*-butyl no induction period was observable. This corresponds to induction periods for 2-butene and isobutylene of a few minutes and none, respectively.

The relative rates of isobutane formation, based upon that from 2-butene as unity and using 25 ml. of sulfuric acid, were approximately as follows: 2-butene, 1; isobutylene, *t*-butyl alcohol and isobutyl alcohol, 0.5; *s*-butyl alcohol, 1.7; *n*-butyl alcohol, 0.2. The half-life of the 2-butene reaction was twenty-five to thirty minutes. It may be noted also that whereas in general the gas evolution from a given solution seemed to be a first-order reaction, that from the tertiary alcohol or isobutylene was distinctly not, the end reaction being very slow.

It is tentatively suggested that this apparent dismutation and rearrangement is based, not upon simple reactions of the original materials, but involves some polymeric material, perhaps through dealkylation; until polymer is formed no isobutane is evolved. The problem then con-

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[1] Stewart and Calkins, THIS JOURNAL, **70**, 1006 (1948).

[2] Brattain, Rasmussen and Cravath, *J. Applied Phys.*, **14**, 418 (1943).

[3] Ormandy and Craven, *J. Soc. Chem. Ind.*, **47**, 317T (1928). The conversion of alkenes into alkanes during alkylation and polymerization, usually accompanied by rearrangement, has been observed, for instance by Ipatieff and Pines, *J. Org. Chem.*, **1**, 464 (1936), and McAllister, *et al.*, *ibid.*, **6**, 647 (1941). The polymerization studies have not been concerned with the low-boiling products; in alkylation an alkane–alkene transhydrogenation apparently occurs. In the present case a given alkene or alcohol is the sole initial reactant.

cerns the fundamental stability of alcohols and alkenes in sulfuric acid toward polymerization.

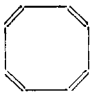
We wish to thank Drs. R. R. Brattain and D. Stevenson of the Shell Development Laboratories for their coöperation.

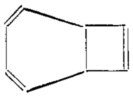
CHEMICAL LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA
RECEIVED JUNE 27, 1949

Some Observations on Certain Cycloöctatetraene Derivatives

BY S. L. FRIESS AND V. BOEKELHEIDE

In a recent publication, Reppe and his co-workers[1] have described the preparation of cycloöctatetraene (C.O.T.) and its conversion to many different derivatives. In attempting to explain the formation of the various reaction products of C.O.T., Reppe, *et al.*, have considered that C.O.T. may react as though it were present as any one of three different forms, I, II and III.


I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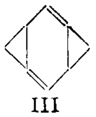
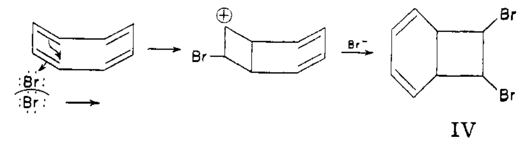

III

However, since I, II and III cannot very well be considered to be contributing forms to a resonance hybrid, it would appear desirable that the formation of unusual products from C.O.T. should be explicable on some other basis.

One possible explanation for the formation of certain of these products would be to consider that under attack by a reagent C.O.T. may undergo molecular rearrangement. The reaction of C.O.T. with bromine and with perbenzoic acid has been considered from this viewpoint and the results obtained in the present study are in support of such a postulation.

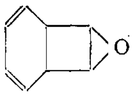
If the assumption is made that the strongest case yet presented for the structure of C.O.T. is the X-ray study[2] pointing to the "tub" or all-*cis* form, then it seems possible to explain certain of the reactions of the compound, and in particular those involving transitions from an initial eight-membered ring to a six–four bicyclic system, by a direct participation of a suitably situated neighboring double bond[3] in these reactions. The bromination process, for example, could be represented by the following scheme, involving the participation of a neighboring double bond in the reaction form presented by the molecule to the attack of a reagent;



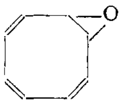
IV

This formulation of the bromination of C.O.T. is in accord with the chemical evidence which Reppe, *et al.*,[1] have presented in support of structure IV.

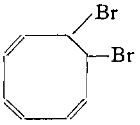
A similar formulation could be postulated for epoxidation, using the electrophilic entity $:\ddot{O}:H^+$ from the perbenzoic acid. The result of participation of the neighboring double bond during epoxidation would be that C.O.T. oxide should be represented by formula V.



V



VI



VII

In this case, however, Reppe, *et al.*,[1] have assigned structure VI to C.O.T. oxide. A reinvestigation of the oxide has been made and evidence supporting structure V has been found.

The ultraviolet absorption spectrum of C.O.T. oxide in ethanol is shown in Fig. 1. It is seen that the position of the absorption peak for the oxide (λ_{max} = 241 mμ, log ε = 3.60) is compatible with a cyclic diene structure, V, as inferred by comparison with the peak of cyclopentadiene[4] in isoöctane (λ_{max} = 241, log ε = 3.50). The maximum of the dibromide (λ_{max} = 260, log ε = 3.70) is somewhat displaced to longer wave lengths, but is not quite as high as would be expected for the conjugated triene form VII.

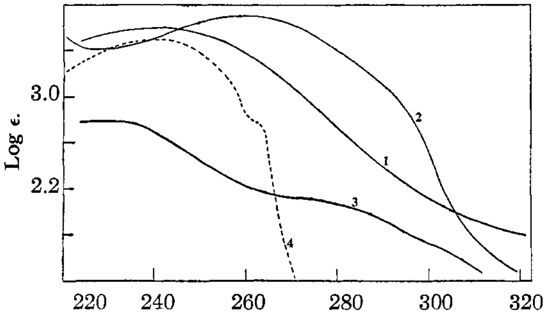


Fig. 1.—Ultraviolet absorption spectra in 95% ethanol of: 1, C. O. T. oxide; 2, C. O. T. dibromide; and 3, C. O. T. oxide hydrogenation product. Curve 4 is for cyclopentadiene in isoöctane.[5]

Further evidence for the diene character of the oxide was obtained from its maleic anhydride adduct. It would be expected that V would yield VIII, whereas structure VI should give IX. When the Diels–Alder reaction was carried out in benzene under anhydrous conditions, an exothermic

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[1] Reppe, Schlichting, Klager and Toepel, *Ann.*, **560**, 1 (1948).

[2] Kaufman, Fankuchen and Mark, *Nature*, **161**, 165 (1948).

[3] For participation of a neighboring double bond in replacement reactions, see Winstein and Adams, THIS JOURNAL, **70**, 838 (1948).

[4] The ultraviolet absorption spectrum for cyclopentadiene in isoöctane solution is given for illustration since it is recorded in the literature,[5] whereas the complete solution spectrum of 1,3-cyclohexadiene was not available to us. A study of the variation of λ_{max} and log ε with increasing ring size in the conjugated cyclic dienes is contemplated.

[5] Data for cyclopentadiene were obtained from Ultraviolet Spectrograms, National Bureau of Standards, A.P.I. Research Project 44, serial no. 53.