quantity k_2/k_3 is found to be greater than 200.[8] This substrate reacts only at the active site since the decarbamylation rate constant determined in this titration agrees with: (1) the value determined using *o*-nitroacetanilide (the first reported amide substrate of acetylcholinesterase) to assay the enzyme-catalyzed hydrolysis of *m*-nitrophenyl dimethylcarbamate[8]; and (2) the value determined using acetylcholine to assay the decarbamylation of the dimethylcarbamylenzyme, formed from the reaction of the enzyme with dimethylcarbamyl fluoride[4,9] (see Table I).

Table I. Titration of Acetylcholinesterase Solutions with *o*-Nitrophenyl Dimethylcarbamate[a]

$S_0 \times 10^4$, *M*	$N \times 10^6$,[d] *N*	$v \times 10^9$, *M* sec.$^{-1}$	$k_3 \times 10^4$,[b,c] sec.$^{-1}$
10.03	3.7 ± 0.15[e]	2.3 ± 0.3	6.0 ± 1.0
5.016	3.7 ± 0.15	2.3 ± 0.02	6.2 ± 0.3

[a] 4.29×10^{-2} *M* Na_2HPO_4 and 4.02×10^{-3} *M* KH_2PO_4, $I = 0.2$, pH 7.72 at 25.0 ± 0.1. [b] Calculated using the expression $v = k_3N$. [c] $k_3 = 6.7 \times 10^{-4}$ sec.$^{-1}$ at 25° and pH 7.85, $I = 0.2$, using *o*-nitroacetanilide to assay the enzyme-catalyzed hydrolysis of *m*-nitrophenyl dimethylcarbamate[8]; $k_3 = 5.3$[4] and 4.3×10^{-4}[9] sec.$^{-1}$ at pH 7.0, 25.0°. [d] V_m of phenyl acetate for this enzyme concentration was 6.14×10^{-2} *M* sec.$^{-1}$ (determined from a Lineweaver–Burk plot at pH 7.82, phosphate buffer, $I = 0.2$, 24.5°).[11] [e] The uncertainty is calculated from the uncertainty in the absorbance values of eq. 2.

The titrations reported in Table I were carried out using a Cary 14 PM recording spectrophotometer equipped with a 0.1 absorbance slide wire and a thermostated cell compartment. The enzyme was obtained from the Sigma Chemical Co., Type 3, lot 23B-7587, isolated from the electric eel and purified by the method of Lawler.[10] The enzyme solution was dialyzed against 0.2 *M* NaCl, 10^{-4} *M* phosphate at pH 7.0 at 4° for 24 hr. in order to remove ammonium sulfate. *o*-Nitrophenyl dimethylcarbamate was synthesized from the reaction of dimethylcarbamoyl chloride (Aldrich) with *o*-nitrophenol in pyridine; m.p. 56.7–57.0°. *Anal.* Calcd. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80; N, 13.33. Found, C, 51.63; H, 4.82; N, 13.17.

A quartz cuvette with a 10-mm. light path and a capacity of 1 ml. was filled with 500 μl. of phosphate buffer, pH 7.82, and 200 μl. of enzyme solution. After determining a base line, 25 μl. of the buffer was added to the solution resulting in a small decrease in absorbance; then 25 μl. of *o*-nitrophenyl dimethylcarbamate solution in 50% (v./v.) acetonitrile–water was added and the solution was stirred for *ca.* 30 sec. The liberation of *o*-nitrophenoxide ion at 415.0 mμ was recorded ($\epsilon_{415.0}$ 4.00×10^3, measured under the reaction conditions by adding 25 μl. of *o*-nitrophenol in water to the completed reaction using the 1.0 absorbance slide wire). An instantaneous increase in absorbance of about 0.015 unit occurred followed by a slow zero-order increase in absorbance, which was recorded for 1.3 hr. The pH of the solution, determined immediately after completion of the reaction, was 7.72, the change being caused by the buffering action of the enzyme.

Using the above procedure, the concentration (normality of the active sites in the solution) is[12]

$$N = \frac{-F(A_1 - A_2) + A_3 - A_4}{4.00 \times 10^3} \qquad (2)$$

where A_1 is the initial absorbance of the enzyme solution, A_2, the absorbance after the addition of 25 μl. of buffer (which measures the absorbance change due to the dilution of the enzyme solution), A_3, the extrapolated absorbance at zero time, and A_4, the absorbance of the carbamate (measured separately). The difference of A_1 and A_2 is corrected for dilution by the factor F.

Table I shows excellent agreement between two titrations of acetylcholinesterase carried out with different substrate concentrations. The accuracy of the titration is inversely proportional to the enzyme concentration. The lower limit of the titration is about 1.5×10^{-6} *N*,[11] at which concentration the uncertainty is about ±10% depending on the molar absorptivity of the *o*-nitrophenoxide ion.[14] The titration itself requires less than 2 min. since the steady-state reaction is reached in less than 30 sec. for the substrate concentrations used here. Thus the normality of acetylcholinesterase active sites may be determined in an accurate and convenient manner.[15]

(9) I. B. Wilson, M. A. Harrison, and S. Ginsburg, *J. Biol. Chem.*, **236**, 1498 (1961).

(10) H. C. Lawler, *ibid.*, **234**, 799 (1959).

(11) The relation between the titration presented here and a rate assay to determine the concentration of acetylcholinesterase, using phenyl acetate as a substrate, will be presented in the full report of this work.

(12) This equation follows that for a method B titration of α-chymotrypsin with N-*trans*-cinnamoylimidazole.[13] It should be noted that a dilution factor was mistakenly omitted in that previous equation.

(13) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961).

(14) Results consistent with those of Table I were obtained when the titration was determined at pH 8.20 at an enzyme concentration of 1.49×10^{-6} *N*.

(15) Previous titrations of acetylcholinesterase solutions have involved use of radioactive diisopropylphosphorofluoridate[16] and dimethylcarbamyl fluoride using an indirect procedure.[5]

(16) H. Michel and S. Krop, *J. Biol. Chem.*, **190**, 119 (1951).

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Rearrangement of Quadricyclanone. Genesis of the *cis*-Bicyclo[3.2.0]heptadiene System

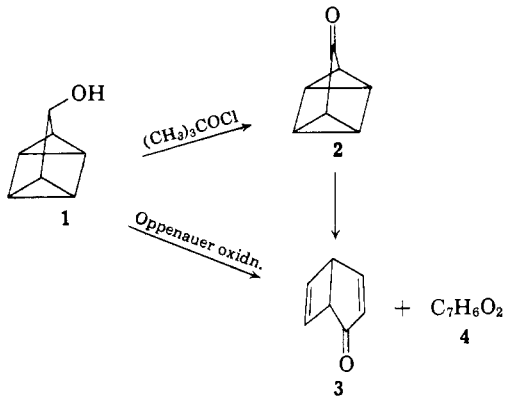
Sir:

As reported earlier,[1] oxidation of quadricyclanol (**1**) with *t*-butyl hypochlorite and pyridine yielded quadricyclanone (**2**). We now find that oxidation of **1** under Oppenauer conditions[2] generates, instead of quadricyclanone (**2**), *cis*-bicyclo[3.2.0]hepta-3,6-dien-2-one (**3**) in about 8% yield (isolated). The only other isolable product of this oxidation was an, as yet, unidentified ketone (**4**, 1.7%) whose empirical formula was determined as $C_7H_6O_2$.

We further observed that if the oxidation conditions[2] were modified so that the reaction was conducted in benzene at room temperature and for shorter periods,

(1) P. R. Story and S. R. Fahrenholtz, *J. Am. Chem. Soc.*, **86**, 1270 (1964).

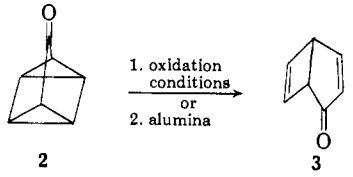
(2) For an example of the procedure followed see R. K. Bly and R. S. Bly, *J. Org. Chem.*, **28**, 3165 (1963).



(1)

e.g., 20 hr., we obtained only **2** and unreacted **1**, with no detectable amount of **3**. If, however, the room temperature oxidation were lengthened to 3 or 4 days, **3** and **4** began to appear. Accordingly, we placed pure **2** under the reaction conditions (a mixture of aluminum *t*-butoxide, quinone, hydroquinone, and refluxing benzene) and obtained **3** in good yield (55%, isolated). Aluminum *t*-butoxide alone in benzene had no effect on **2**.

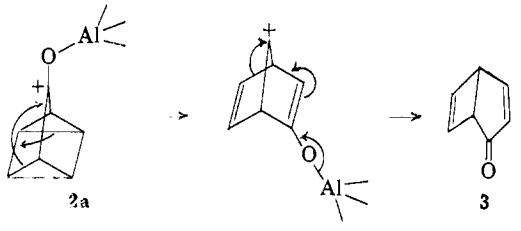
Quite inadvertently, we found that quadricyclanone (**2**) is rapidly converted to **3** (80%) by Woelm alumina (neutral or acidic, activity grade 1). A sequence utilizing this reaction is, in fact, the method of choice



(2)

for synthesis of bicycloheptadienone (**3**),[3] b.p. 60–62° (8 mm.); n.m.r. spectrum (carbon tetrachloride) of **3**: τ 2.40 (4), 3.46 (4), 3.70 (4), 4.05 (4), 6.11 (m), and 6.43 (m); infrared spectrum (neat, μ): 3.28 (m), 3.40 (w), 5.90 (s), 6.35 (w), 6.43 (m), 12.77 (s), 13.18 (s), 14.07 (s), and 14.90 (s); ultraviolet spectrum (cyclohexane, mμ): λ_{max} 208 (6800), and 349 (97, complex).

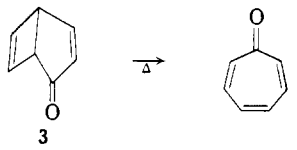
In view of the data outlined here, we propose, as the simplest path for the conversion of **2** to **3**, the mechanism pictured in eq. 3. It will be noted that we have chosen the first step to coincide exactly with the



(3)

mechanism proposed earlier for the 7-quadricyclic to 7-norbornadienyl carbonium ion rearrangement.[4]

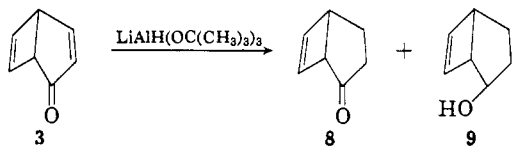
The structural assignment for **3** is derived from several pieces of information. They include the n.m.r. spectrum of **3** which is very similar, including coupling constants, to those of the methoxy-substituted bicyclo[3.2.0]heptadienones characterized by Chapman.[5] Quantitative hydrogenation (2 moles) of **3** yielded a saturated ketone, *cis*-2-bicyclo[3.2.0]heptanone (**5**), whose infrared spectrum was identical with that of authentic **5**.[6] Further evidence for the bicycloheptadienone structure **3** was provided by the observation that **3** was nearly quantitatively converted to tropone[7] at 350°.[8]



(4)

The unusual rearrangement of quadricyclanone (**2**) described in this report has provided a convenient synthesis of the hitherto unknown simple 2-substituted bicyclo[3.2.0]heptadienyl system. This structure is of special importance for it permits the study of yet another carbonium ion conjugate to the 7-norbornadienyl cation,[9] thereby allowing us to probe still deeper into the nature of bonding in these unusual ions. Furthermore, by means of a rather unique and fortuitous reaction of **3** with lithium aluminum tri-*t*-butoxy hydride,[10] a synthesis of the also previously unknown 2-substituted *cis*-bicyclo[3.2.0]-6-heptene system is provided (eq. 5). These compounds permit the generation of carbonium ions potentially strategically related to the *anti*-7-norbornenyl cation.[11]

Lithium aluminum hydride reduction of **3** proceeds normally, and in good yield, to give both epimeric alcohols, *endo-cis*-bicyclo[3.2.0]hept-3,6-dien-2-ol (**6**) and *exo-cis*-bicyclo[3.2.0]-hepta-3,6-dien-2-ol (**7**)[12] in the ratio 58:42, respectively. Reduction of **3** with lithium aluminum tri-*t*-butoxy hydride, however, yielded neither of these alcohols, but gave, as the major product (61%), *cis*-bicyclo[3.2.0]-6-hepten-2-one (**8**, infrared (neat, μ): 3.31 (w), 3.41 (m), 5.78(s), and 13.80 (s)) along with a small amount (9%) of the corresponding alcohol, *endo-cis*-bicyclo[3.2.0]-6-hepten-2-ol (**9**).[12] This reaction constitutes, as far as we can determine, the first example in which only the double bond of an α,β-unsaturated ketone is reduced by this



(5)

(3) Satisfactory elemental analyses were obtained for all new compounds.

(4) P. R. Story and S. R. Fahrenholtz, *J. Am. Chem. Soc.*, **86**, 527 (1964).

(5) (a) O. L. Chapman, *ibid.*, **85**, 2014 (1963); (b) W. G. Dauben, K. Koch, S. L. Smith, and O. L. Chapman, *ibid.*, **85**, 2616 (1963).

(6) S. Winstein, F. Gadient, E. T. Stafford, and P. E. Klinedinst, *ibid.*, **80**, 5895 (1958).

(7) H. J. Dauben, Jr., and H. J. Ringold, *ibid.*, **73**, 876 (1951); W. von E. Doering and F. L. Detert, *ibid.*, **73**, 877 (1951).

(8) A similar thermal reorganization has been observed with methoxy-substituted bicycloheptadienones. See ref. 5b.

(9) P. R. Story and M. Saunders, *J. Am. Chem. Soc.*, **84**, 4876 (1962); P. R. Story, L. C. Snyder, D. C. Douglass, E. W. Anderson, and R. L. Kornegay, *ibid.*, **85**, 3630 (1963).

(10) H. C. Brown and R. F. McFarlin, *ibid.*, **80**, 5372 (1958).

(11) S. Winstein, M. Shatavsky, C. Norton, and R. B. Woodward, *ibid.*, **77**, 4183 (1955).

(12) The configurational assignments of the alcohols were made on the basis of relative retention times (g.p.c.) (*cf.* C. H. DePuy and P. R. Story, *Tetrahedron Letters*, **No. 6**, 20 (1959)) and by hydrogenation to the known saturated alcohols (see ref. 6).

reagent, although lithium aluminum hydride has been used to achieve similar results in some special cases.[13]

We have concluded, after examination of Dreiding molecular models, that the carbonyl of **3** is not reduced by lithium aluminum tri-*t*-butoxy hydride because it is sterically hindered by the four-membered ring; *i.e.*, the reagent is too bulky for *endo* approach to the carbonyl and the salt resulting from *exo* approach of the hydride donor cannot be accommodated in the *endo* position. Consequently, *exo* hydride donation in the β-position is favored, yielding the enolate anion which is subsequently hydrolyzed to the ketone (**8**).

The results of our studies with both the bicyclo[3.2.0]heptadienyl and bicyclo[3.2.0]heptenyl carbonium ions will be reported later.

Acknowledgment. We thank Professor S. Winstein for furnishing us with an infrared spectrum of authentic bicycloheptanone (**6**).

(13) O. L. Chapman, D. J. Pasto, and A. A. Griswold, *J. Am. Chem. Soc.*, **84**, 1213 (1962); N. G. Gaylord in "Reduction with Complex Metal Hydrides," Interscience Publishers, Inc., New York, N. Y., 1956, Chapter 15.

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Formation of Monochlorocarbene by the Gas-Phase Decomposition of Dihalomethane Molecules Excited through Recoil Tritium Substitution Reactions[1]

Sir:

We have formed monochlorocarbene in the gas phase through dehydrohalogenation of highly excited dihalomethane molecules and have detected the carbene through the formation of cyclopropyl chloride in the presence of ethylene. The observation of the cyclopropane-forming reaction with olefins has been an important feature of the earlier liquid-phase experiments on monochlorocarbene from various sources.[2,3]

The dihalomethane molecules were excited in our system through the hot atom substitution of tritium for hydrogen, forming $CHTClX^*$ from CH_2ClX.[4,5] The monochlorocarbene thus created, after the elimination of HX,[6] is actually CTCl, and is detected through the radioactivity of cyclopropyl-*t* chloride. Reactions have thus far been carried out with $CHTCl_2^*$ and $CHTClF^*$, representing the elimination of HCl and HF, respectively, in the dehydrohalogenation step.

Each system contained the dihalomethane parent compound, He^3, and ethylene, while some contained O_2 in addition. The recoil tritium atoms are formed by thermal neutron reactions with He^3, and are able to react chemically with both C_2H_4 and CH_2ClX in energetic atomic reactions.[7,8] These irradiated systems are analyzed for their radioactive components by the usual techniques of radio-gas chromatography, utilizing a sufficient variety of separation columns to ensure isolation and identification of all of the radioactive components.[9] Each of the compounds found from recoil tritium reactions with C_2H_4 and CH_2ClX separately is also observed in the irradiated mixtures. In addition cyclopropyl-*t* chloride is found both in the presence or absence of oxygen as a scavenger molecule.[10] The yield of cyclopropyl-*t* chloride is given in Table I, relative to the yield of CHTClX as 1.0.[11] No activity was observed in the form of chloropropylene-*t*, and a particular search was made for the product expected from the insertion of CTCl into the C–H bond of ethylene. The maximum yield of $CHTClCH{=}CH_2$ was less than 0.02 times the yield of cyclo-C_3H_4TCl.

Table I. Relative Yields of Cyclopropyl-*t* Chloride from Recoil Tritium Reactions with Ethylene-Scavenged Dihalomethanes[a]

	Gas pressures, cm.					Rel. radioactivity *c*-C_3H_4TCl
CH_2Cl_2	25.0	C_2H_4	9.5			0.30 ± 0.01
	26.2		33.3			0.28 ± 0.01
	5.0		28.1			0.32 ± 0.03
	23.6		9.1	O_2	4.2	0.20 ± 0.01
	27.8		29.8		2.8	0.27 ± 0.02
	12.5		22.1		3.2	0.22 ± 0.02
CH_2ClF	56.1	C_2H_4	17.5	O_2	3.4	0.10 ± 0.01
	47.3		17.7		3.4	0.11 ± 0.02

[a] Radioactivity of CHTClX = 1.0

The amount of cyclopropyl-*t* chloride observed among the products is relatively insensitive to the presence or absence of O_2, and to the ratio of CH_2Cl_2/C_2H_4. These observations indicate semiquantitatively that the CTCl formed in this decomposition reacts approximately as well with the double bond of ethylene as with O_2, and much better than with the C–H bond of CH_2Cl_2. By analogy with current CH_2 hypotheses, we conclude that the CTCl is largely in the singlet state.[12] Quantitative evaluation of the significance of the variations in the ratios of cyclo-$C_3H_4TCl/CHTClX$ will require further experiments on the effects of pressure, mole fraction, oxygen, etc.

The absence of $CHTClCH{=}CH_2$ as an observed product in CH_2Cl_2–C_2H_4 mixtures indicates not only that no insertion occurs for CTCl into the C–H bond of ethylene but also that no isomerization of the cyclopropyl-*t* chloride occurs. The failure to insert into

(1) This research was supported by A.E.C. Contract No. AT-(11-1)-407 with the University of Kansas.

(2) See, for example, W. Kirmse, "Carbene Chemistry," Academic Press Inc., New York, N. Y., 1964.

(3) G. L. Closs and G. M. Schwartz, *J. Am. Chem. Soc.*, **82**, 5729 (1960); G. L. Closs and L. E. Closs, *ibid.*, **82**, 5723 (1960); G. L. Closs and J. J. Coyle, *ibid.*, **84**, 4350 (1962).

(4) E. K. C. Lee and F. S. Rowland, *ibid.*, **85**, 897 (1963).

(5) Y. N. Tang, E. K. C. Lee, and F. S. Rowland, *ibid.*, **86**, 1280 (1964).

(6) The dehydrohalogenation step also goes by the alternate path of elimination of TX, with the formation of CHCl. The carbene formed in this manner is not labeled and, since it is formed in the system in amounts of the order of 10^{-10} mole fraction, is not detectable with present analytical procedures.

(7) For example, see "Chemical Effects of Nuclear Transformations," Vol. 2, International Atomic Energy Agency, Vienna, 1961.

(8) Y. N. Tang, Ph.D. Thesis, University of Kansas, 1964.

(9) J. K. Lee, E. K. C. Lee, B. Musgrave, Y. N. Tang, J. W. Root, and F. S. Rowland, *Anal. Chem.*, **34**, 741 (1962).

(10) Other radioactive compounds, including *n*-propyl-*t* chloride, are found in the absence of O_2.

(11) The yield of CHTClX is not an absolute standard, since it is somewhat dependent upon the pressure and composition of the system, especially the former through collisional de-excitation competition with the dehydrohalogenation reaction.[8]

(12) See, however, P. O. Gaspar and G. Hammond, Chapter 12 in ref. 2, for a discussion of the validity of these presumptions for CH_2.