Table I. Carboxyl Carbon-13 Isotope Effects on the Enzymatic Decarboxylation of Glutamic Acid at pH 4.7, 37°

Enzyme spec act.[a]	Isotope ratios[b] 5% reacn	100% reacn	k^{12}/k^{13}
17	0.014062	0.014287	1.0170
17	0.014070[c]	0.014303[d]	1.0176
17	0.014071	0.014303[d]	1.0175
140	0.014079	0.014304	1.0169
140	0.014083[e]	0.014309	1.0170
140	0.014084	0.014305	1.0166
140	0.014067[c]	0.014307	1.0181
		Mean	1.0172 ± 0.0004

[a] Micromoles of glutamic acid decarboxylated per milligram of enzyme per minute in 3 ml of 0.025 *M* glutamic acid, pH 4.9, 37°. [b] *M/e* 45/44, corrected to tank standard 0.014150. [c] Carried to only 2.5% reaction. [d] Same sample. [e] 10^{-5} *M* pyridoxal 5′-phosphate added.

the flask was closed. The enzyme used in these studies (prepared as described previously[9]) was freed of low molecular weight contaminants before use by chromatography on a short column of Sephadex G-25 using freshly degassed buffer. Each flask containing the 0.01 *M* glutamic acid was equilibrated at 37.0° and an appropriate amount of enzyme was added through a serum cap. After a suitable length of time the reaction was stopped by the addition of concentrated H_2SO_4. The evolved carbon dioxide was freed of water, nitrogen, and other contaminants by standard procedures and its isotopic composition was measured on a Nuclide RMS 6-60 isotope-ratio mass spectrometer. All measurements were made relative to a standard CO_2 sample. The isotope effect was calculated by comparison of the isotopic composition of a sample of CO_2 obtained after 5% reaction with one obtained after complete decarboxylation. The isotope effect is then given by eq 1,[1]

$$k^{12}/k^{13} = (R_{100} - R_{17})/(R_5 - R_{17}) \qquad (1)$$

where k^{12} and k^{13} are the rate constants for the ^{12}C and ^{13}C species, respectively, R_5 and R_{100} are the measured isotope ratios (*m/e* 45/44) for the samples obtained at 5 and 100% conversion, respectively, and R_{17} is the isotope ratio for carbon dioxide containing ^{17}O, $^{12}C^{17}O^{16}O/^{12}C^{16}O_2$. The value 0.0008 has been used for R_{17}, and the calculated isotope effect is quite insensitive to the value of this ratio.

The correctness of the results was indicated by several tests: the isotope effect was constant from experiment to experiment; precisely the same isotope effect was obtained with different enzyme preparations; added 10^{-5} *M* pyridoxal 5′-phosphate had no effect on the isotope effect; the absolute isotope abundances were constant from experiment to experiment; exactly the same isotope effect was obtained when a sample was allowed to proceed to only 2.5% reaction instead of 5% reaction.

The results of seven determinations of the isotope effect are summarized in Table I. The isotope ratios given in this table are not actual isotopic abundances, but are instead uncorrected decade settings on the isotope-ratio mass spectrometer. Thus a standard CO_2 sample gives a ratio *m/e* 45/44 of 0.014150. The decade settings are directly proportional to isotopic abundances and can be used directly in eq 1. The reported ratios are estimated to be correct to ±0.000002. The mean of seven determinations is 1.0172 ± 0.0004. The pH of these measurements, 4.7, is within the range of maximum activity of the enzyme.[10]

The isotope effect can be related to the rate constants of the classical Michaelis–Menten formulation, eq 2, where E represents enzyme, G is glutamic acid, and P

$$E + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EG \xrightarrow{k_2} CO_2 + E + P \qquad (2)$$

is product. The first step in eq 2 is the formation of an enzyme-bound Schiff base between glutamic acid and pyridoxal 5′-phosphate. Since there is no change in bonding to the isotopic atom in this step, there will be no carbon isotope effect on k_1 or k_{-1}, and the observed isotope effect is given by eq 3.[5] Thus the observed

$$\frac{k^{12}}{k^{13}} = \frac{k_2^{12}(k_{-1} + k_2^{13})}{k_2^{13}(k_{-1} + k_2^{12})} \qquad (3)$$

isotope effect is equal to the isotope effect on k_2 reduced by a factor related to the extent to which k_2 is rate determining.[11] The presence of a sizable carbon isotope effect indicates that k_2 is not fast compared with k_{-1}. However, the mere occurrence of an isotope effect does not indicate that k_2 is rate limiting.

We cannot be sure without further consideration whether the isotope effect which we have observed is the one which would be expected if k_2 is much smaller than k_{-1}. It is instructive to compare this isotope effect with those obtained in nonenzymatic decarboxylations,[13] which are usually in the range 1.02 to 1.04. However, we do not necessarily expect the isotope effect on k_2 in eq 2 to be as large as those observed in nonenzymatic systems because of the greater transition-state stabilization provided by pyridoxal 5′-phosphate and the consequent smaller amount of carbon–carbon bond breaking expected at the transition state. Thus we can conservatively estimate that in this case k_2 is smaller than k_{-1} and decarboxylation is the rate-limiting step. Whether these two rate constants differ by a small amount or by an order of magnitude or more is the subject of continuing investigation.

Acknowledgment. This research was supported in part by grants from the Wisconsin Alumni Research Foundation and the National Institutes of Health (NB-07657).

(10) M. H. O'Leary and D. T. Richards, unpublished observations.

(11) It should be noted that Jencks' interpretation of the isotope effect on the decarboxylation of oxalacetate[5,12] is incomplete. The rate-limiting step in the overall reaction may not be the same as the rate-limiting step in the decarboxylation process, and a carbon isotope effect could be observed even if decomposition of the enzyme–product complex were rate determining.

(12) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969, p 244.

(13) P. E. Yankwich and W. E. Buddenbaum, *J. Phys. Chem.*, 71, 1185 (1967).

Marion H. O'Leary
Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706
Received July 3, 1969

A Novel Carbonium Ion Rearrangement in the *exo*-Tricyclo[4.2.1.$0^{2,5}$]non-3-ene Series

Sir:

Considerable interest has been focused recently on the extensive participation (*ca.* 10^{14} rate acceleration factor)

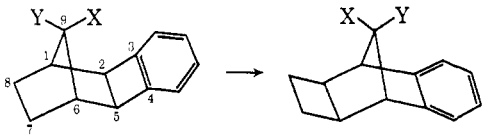
Table I. Acetolysis Rate Constants and Activation Parameters

ROBs	Temp, °C	$10^5 k_1$, sec^{-1}	Rel k_1 (165°)	$\Delta H^{\ddagger}$,[a] kcal/mol	$\Delta S^{\ddagger}$,[a] eu
1-OBs	165	2.98 ± 0.07	3.6	31.6 ± 1.0	−7.9 ± 2.2
	190	22.4 ± 0.7			
2-OBs	220	4.66 ± 0.14	0.06	35.2 ± 1.2	−7.7 ± 2.4
	240	19.9 ± 0.6			
7-Norbornyl	205	29.4[b]	1.0	35.7 ± 0.6	−3.5 ± 1.7

[a] Errors were computed according to the method of R. C. Peterson, J. H. Markgraf, and S. D. Ross, *J. Am. Chem. Soc.*, **83**, 3819 (1961). [b] Estimated on the basis of three times the rate for 7-norbornyl tosylate in ref 7.

by cyclopropane in the solvolytic reactions of *endo-anti*-tricyclo[3.2.1.0^{2,4}]oct-8-yl derivatives.[1] In contrast cyclopropane participation in the corresponding *exo-anti* case was found to be totally absent.[2]

The feasibility of similar "edge" as opposed to "face" participation by cyclobutane has prompted us to investigate the solvolytic behavior of a series of tricyclo-[4.2.1.0^{2,5}]non-9-yl derivatives, and in this communication we wish to report our results on the acetolysis of *exo-syn*- and *exo-anti*-3,4-benztricyclo[4.2.1.0^{2,5}]non-3-en-9-yl brosylates (**1**-OBs and **2**-OBs, respectively). In particular we describe an unusual and structurally striking skeletal rearrangement (eq 1) which offers prescience for future mechanistic and synthetic studies in bridged polycyclic systems.



(1)

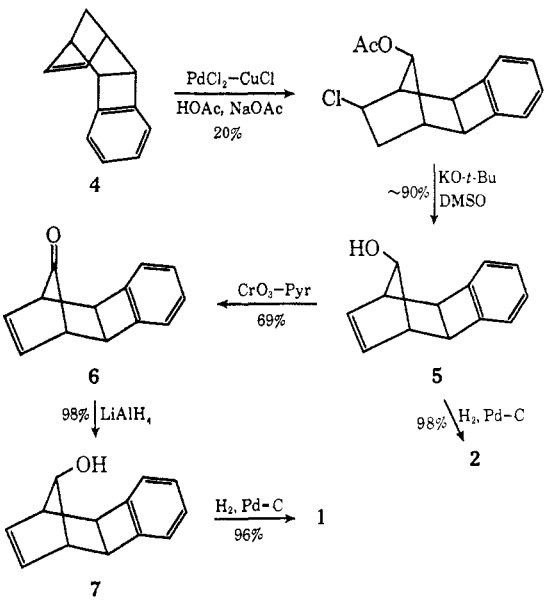
1, X = OH; Y = H
2, X = H; Y = OH

3, X = OH; Y = H
8, X; Y = O

Synthesis of the requisite alcohols **1**,[3] mp 101–102°, and **2**, mp 93–94°, was accomplished according to the scheme outlined in Chart I.[4] Proof that compounds **5**–**7** as well as **1** and **2** have the *exo*-tricyclononyl skeleton is provided by their nmr spectra which show a relatively sharp two-proton singlet in the region τ 6.45–6.82 assignable only to the *endo* benzcyclobutyl protons.[5] Ketone **6**, mp 133–134°, showed the characteristic bridged carbonyl stretching frequency at 1765 cm^{-1} and in solution at 150° smoothly decarbonylated to benzcyclooctatetraene. The *exo-syn* configuration of alcohol **1** was established by its infrared spectrum which, in contrast to **2**, exhibited a sharp intramolecularly bonded O–H absorption band at 3590 cm^{-1}.[6]

The first-order rate constants (k_1) and activation parameters for acetolysis of **1**-OBs and **2**-OBs in acetic acid (containing 0.102 *M* sodium acetate) are compared with the corresponding values for 7-norbornyl brosylate[7] in Table I. Ionization of the *exo-syn* brosylate is

Chart I



clearly accelerated over that for **2**-OBs as well as 7-norbornyl. The origin of the acceleration is not immediately obvious; however, some insight is provided by examination of the solvolyses products.

Product studies were conducted under conditions identical with those in the kinetic runs with analyses performed on the alcohol products obtained after lithium aluminum hydride reduction of the crude acetate mixture. Glpc analysis of the products from acetolysis of **1**-OBs at 190° for ten half-lives revealed three major components for a combined yield of 93%. The major component (79%) was isolated by fractional recrystallization to give **3**: mp 117.5–119°; nmr ($CDCl_3$) τ 2.90 (s, 4 H), 6.06 (bs, 1 H), 6.72 (d, 2 H, J = 1.5 Hz), 7.5–8.10 (m, 7 H); mass spectrum (70 eV) *m/e* (rel intensity) 186 (16), 158 (53), 157 (78), 129 (100), 128 (52), 115 (36). Although isomeric with **1** and obviously tricyclic in structure, alcohol **3** cannot have the carbon skeleton of **1** or **2** as the nmr spectrum, particularly the sharp doublet at τ 6.72, clearly shows. Oxidation of **3** with CrO_3–pyridine afforded ketone **8** [mp 69–70°; $\nu_{C=O}^{KBr}$ 1775 cm^{-1}; nmr ($CDCl_3$) τ 2.83 (s, 4 H), 6.83 (s, 2 H), 7.5–8.5 (m, 6 H); mass spectrum (70 eV) *m/e* (rel intensity) 184 (1), 156 (23), 129 (23), 128 (100), 115 (14)] which yields alcohol **3** exclusively on reduction with lithium aluminum hydride. The above chemical and spectral data are consistent only with structure **3** for the major product alcohol. The remaining products from **1**-OBs were identified as **1** (12%) and **2** (2%).

(1) (a) H. Tanida, T. Tsuji, and T. Irie, *J. Am. Chem. Soc.*, **89**, 1953 (1967); (b) M. A. Battiste, C. L. Deyrup, R. E. Pincock, and J. Haywood-Farmer, *ibid.*, **89**, 1954 (1967); (c) M. J. S. Dewar and J. M. Harris, *ibid.*, 90, 4468 (1968); (d) Y. E. Rhodes and T. Takino, *ibid.*, **90**, 4469 (1968); (e) R. M. Coates and J. L. Kirkpatrick, *ibid.*, 90, 4162 (1968).

(2) J. Haywood-Farmer, R. E. Pincock, and J. I. Wells, *Tetrahedron*, 22, 2007 (1966).

(3) All new compounds reported gave elemental analyses and spectral data (ir, nmr, mass) in accord with their assigned structures.

(4) The procedure employed in the palladium(II)-catalyzed addition reaction of olefin **4** was essentially that of W. C. Baird, Jr., *J. Org. Chem.*, **31**, 2411 (1966).

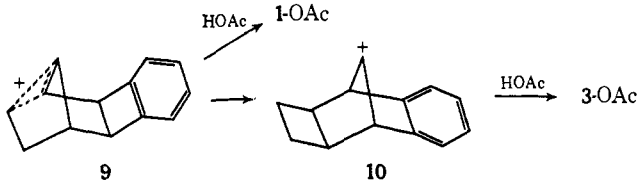
(5) *Cf.* H. E. Simmons, *J. Am. Chem. Soc.*, **83**, 1657 (1961).

(6) M. Tichy in "Advances in Organic Chemistry: Methods and Results," Vol. 5, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., John Wiley & Sons, Inc., New York, N. Y., 1965, p 115.

(7) S. Winstein, M. Shatavsky, C. Norton, and R. B. Woodward, *J. Am. Chem. Soc.*, 77, 4183 (1955).

The product situation with regard to **2**-OBs (240°; seven half-lives) was considerably more complex with at least 17 components detected by glpc analysis. Of the various products, five were isolated in pure form by preparative glpc and identified by their spectral properties as **1** (19%), **2** (6%), **3** (28%), 5,6-benzindan[8] (18%), and 5,6-benzindene (2%). Although the acetates **1**-OAc, **2**-OAc, and **3**-OAc are stable to the acetolysis conditions (HOAc containing NaOAc; 240°), considerable charring with resulting variation in the relative yields of the above products occurred on prolonged heating of the actual acetolysis solution.

The formation of **3** from **1**-OBs requires a double Wagner–Meerwein rearrangement with ion **9** visualized as the first intermediate. This ion may either react with solvent to give retained acetate or rearrange further *via* a phenonium ion shift to give the more stable benznorbornenyl ion **10**. Although it is unlikely that



10 is formed concertedly from **1**-OBs, it is conceivable that formation of ion **9** is electronically assisted by overlap of the incipient p orbital at C-1 with the π orbitals of the aromatic ring.[9]

Acknowledgment. Financial support of this work by the Air Force Office of Scientific Research and the National Science Foundation is gratefully acknowledged.

(8) S. C. Sen-Gupta, *Current Sci.*, **5**, 133 (1936); *Chem. Abstr.*, **31**, 5789 (1937).

(9) Mechanistic speculation on the origin of products obtained from **2**-OBs is not warranted at this time, although it is reasonable to assume that **1**-OAc and **3**-OAc are formed after leakage to **9** from an ion originally generated by backside participation of the C-1–C-2 bond in the ionization step.

(10) Alfred P. Sloan Foundation Fellow, 1967–1969.

Merle A. **Battiste**,[10] **John W. Nebzydoski**
Department of Chemistry, University of Florida
Gainesville, Florida 32601
Received July 22, 1969

Synthesis of Chlorobiumquinone

Sir:

The isolation and characterization of chlorobiumquinone as a 2-methyl-3-(1′-alkenyl)-1,4-naphthoquinone (**1**), $C_{45}H_{62}O_2$, has been reported.[1] Subsequently mass spectrometry established the molecular weight as 662, requiring 28 mass units more than in the proposed structure. Based on similar mass spectral observations and similarity in the uv of chlorobiumquinone and 2-acetyl-1,4-naphthoquinone, a 1′-oxomenaquinone-7 (**2**), $C_{46}H_{62}O_3$, structure was proposed for chlorobiumquinone.[2] Although uv analogies in this series can be misleading (*e.g.*, the close similarity of vinylquinones[3] to chlorobiumquinone), nevertheless structure **2** is reasonable for chlorobiumquinone. We have turned to synthesis for proof and now report the synthesis of both **1** and **2**, utilizing as a common side-chain intermediate *all-trans*-farnesylfarnesylacetone (**20**).[4]

Geraniol (**4**)[5] was converted to geranylacetone (**5**),[6] homogeneous by glpc,[7b,8] and treatment with ethylene glycol and *p*-toluenesulfonic acid in benzene afforded ethylene ketal **6**. Selective epoxidation of the terminal double bond of **6** was achieved using N-bromosuccinimide[9] to give the bromohydrin **7** followed by KOH–methanol to give epoxide **8** in 79% yield. The nmr spectrum showed six methyl hydrogens at δ 1.2 and 1.23 (s), three methyl hydrogens on a *trans* double bond at 1.64, and one α-epoxy hydrogen at 2.62 (t, J = 7 Hz).[10]

Treatment of **8** with acetic acid–sodium acetate–acetic anhydride (8:1:1) gave the monoacetate **9** in 77% yield; nmr δ 2.08 (s, 3), 4.86 (b, 1), consistent with the acetate ester being at C-9. Hydrolysis in KOH–methanol gave glycol **10** (87% yield) which was cleaved to aldehyde **11** (97% yield) with sodium periodate in aqueous dioxane; δ 10.45 (t). Reduction of aldehyde **11** with sodium borohydride gave alcohol **12** in 43% overall yield from ethylene ketal **6**. Alternatively alcohol ethylene ketal **12** was obtained from diene ketal **6** in methanol at −78° with 1 equiv of O_3 followed by reduction with sodium borohydride–sodium hydroxide.[11] The desired alcohol **12** was obtained in 25% yield after separation of the complex product mixture and was homogeneous by glpc;[7a] nmr: two methylene hydrogens at δ 3.5 (t, J = 6 Hz), three methyl hydrogens on a *trans* double bond at δ 1.62.

Alcohol **12** was converted to *p*-toluenesulfonate **13**, iodoketal **14**, and triphenylphosphonium salt **15** by standard procedures. The ylide **16** was generated from salt **15** in dimethyl sulfoxide by addition of butyllithium and allowed to react with ketone **5** at 25°. Geranylgeranylacetone ethylene ketal (**17**) was isolated in 73%

(4) G. I. Samokhalov and E. A. Obol'nikova, *Usp. Khim.*, **36**, 413 (1967), and references therein.

(5) A generous gift of Givaudan Corp.

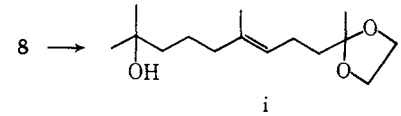
(6) O. Isler, R. Ruegg, L. Chopard-dit-Jean, H. Wagner, and K. Bernhard, *Helv. Chim. Acta*, **39**, 897 (1956).

(7) Glpc analyses were performed on (a) 30% QF-1 on acid-washed, DMCS-treated, 60–80 Chromosorb P, 10 ft × 0.25 in.; (b) 20% Carbowax 20 M on 60–80 Firebrick, 10 ft × 0.25 in.; (c) Apiezon J on 60–80 Chromosorb P, 5 ft × 0.25 in.; (d) Apiezon L, capillary column, 100 ft × 0.1 mm.

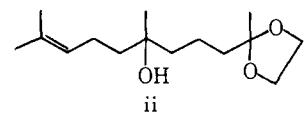
(8) Satisfactory elemental analyses and confirmatory mass spectral data were obtained for all compounds. Structures assigned are consistent with ir and nmr spectra (δ values in $CDCl_3$) and homogeneity was established by glpc[7] and tlc (ethyl acetate–benzene on Kiesel Gel G). All final purifications were by column chromatography on silica gel, eluting with ethyl acetate–benzene.

(9) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962); E. E. van Tamelen and K. B. Sharpless, *ibid.*, 2655 (1967).

(10) The isomeric purity of **8** was established by conversion to i with lithium aluminum hydride in tetrahydrofuran [H. C. Brown, P. M Weissman, and N. M. Yoon, *J. Am. Chem. Soc.*, **88**, 1458 (1966)] and glpc[7a] comparison of the trimethylsilyl ether with the ethers of a mixture of i and ii.



i



ii

(11) C. G. Overberger and H. Kaye, *ibid.*, **89**, 5640 (1967).

(1) B. Frydman and H. Rapoport, *J. Am. Chem. Soc.*, **85**, 823 (1963).

(2) R. Powls, E. R. Redfearn, and S. Trippett, *Biochem. Biophys. Res. Commun.*, **33**, 408 (1968); R. Powls and E. R. Redfearn, *Biochim. Biophys. Acta*, 172, 429 (1969).

(3) W. E. Bondinell, S. J. Di Mari, B. Frydman, K. Matsumoto, and H. Rapoport, *J. Org. Chem.*, **33**, 4351 (1968).