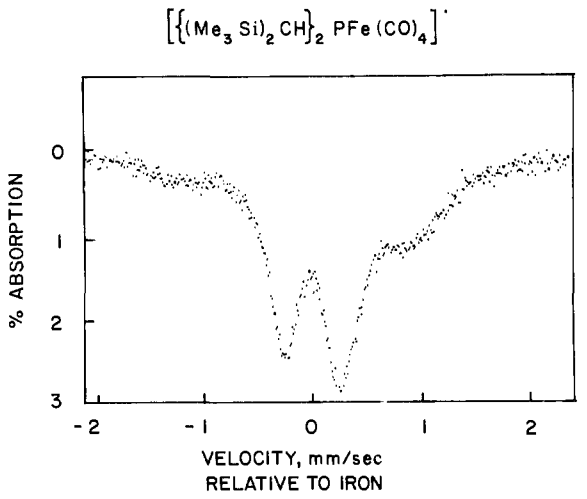
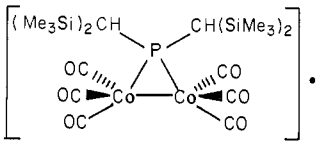


Figure 1. ^{57}Fe Mössbauer spectrum of $[\{(Me_3Si)_2CH\}_2PFe(CO)_4]\cdot$ (**2**) at 25 K.

of **2** (2040, 1930, 1920, and 1880 cm^{-1}) are ~100 cm^{-1} lower[14] than those of phosphenium complexes such as $[(Me_2N)_2PFe(CO)_4]^+$ (2123, 2063, 2014, and 1972 cm^{-1}).[15]

The reaction of **1** with $Co_2(CO)_8$ in toluene solution resulted in the rapid evolution of CO and the production of a dark purple solution, evaporation of which produced a purple solid radical of composition $[\{(Me_3Si)_2CH\}_2PCo_2(CO)_6]\cdot$ (**3**) for which μ_{eff} = 1.82 μ_B at 300 K. The 70-eV mass spectrum of **3** does not exhibit a parent peak; the highest m/e peaks appear at 495 and 464 and are attributable to $[\{(Me_3Si)_2CH\}_2PCo_2(CO)]^+$ and $[\{(Me_3Si)_2CH\}_2PCo(CO)_2]^+$, respectively. It is not possible to record satisfactory NMR spectra for **3**. The ESR spectrum of **3** in toluene solution consists of a 15-line pattern at g = 2.001; a_{Co} = 3.4 mT, thus indicating delocalization of the unpaired electron into the $Co_2(CO)_6$ moiety (^{59}Co, $I = {}^7/_2$, natural abundance = 100%).[16] The IR spectrum of **3** (cyclohexane solution) exhibits CO stretching frequencies at 2070, 2035, 2010, 1990, and 1975 cm^{-1}. There is no evidence for the presence of bridging CO ligands. The molecular weight determination was rendered inaccurate by slow decomposition in benzene solution; however, the observed value of 485 is indicative of the monomeric nature of **3** (calcd 636). The equivalence of the ^{59}Co nuclei in the ESR experiment and the absence of bridging CO's in the IR spectrum imply the following structure for **3**:[17]



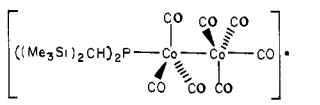
3

(14) ν_{CO} shifts of ~100 cm^{-1} are indicative of one-electron increase at iron. See: Nakamoto, K. "Infrared and Raman Spectra of Inorganic and Coordination Compounds"; Wiley: New York, 1978; pp 279-294.

(15) Montemayer, R. G.; Sauer, D. T.; Fleming, S., Sr.; Bennett, D. W.; Thomas, M. G.; Parry, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 2231-2233.

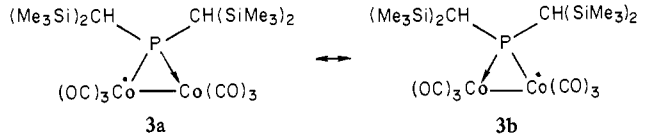
(16) Very similar ESR spectra have been observed for bridged $Co_2(CO)_6$ anion radicals. See: Peake, B. M.; Rieger, P. H.; Robinson, B. H.; Simpson, J. *J. Am. Chem. Soc.* **1980**, *102*, 156-163. *Inorg. Chem.* **1981**, *20*, 2540-2543.

(17) The analytical data for the cobalt product agree best with the formulation $\{(Me_3Si)_2CH\}_2PCo_2(CO)_6$. Anal. Calcd for $C_{20}H_{38}Co_2PO_6Si_4$: C, 37.79; H, 6.02. Found: C, 37.8; H, 6.3. However, these analytical data are also close to those for $\{(Me_3Si)_2CH\}_2PCo_2(CO)_7$. Anal. Calcd for $C_{21}H_{38}Co_2PO_7Si_4$: C, 38.00; H, 5.77. Since there are no bridging CO's (see text), the most likely structure for $\{(Me_3Si)_2CH\}_2PCo_2(CO)_7$ would be



However, two arguments render the $\{(Me_3Si)_2CH\}_2PCo_2(CO)_7$ structure unlikely: (i) the Co's are equivalent on the ESR time scale and (ii) no Co–Co stretch is detectable in resonance Raman experiments.

The above delocalized structure can be regarded as arising from canonical forms **3a** and **3b**.



3a **3b**

The reaction of stabilized phosphinyl radicals with other organometallic substrates is under active investigation.

Acknowledgment. We thank the National Science Foundation (Grant CHE79-10155) and the Robert A. Welch Foundation for generous financial support. We are also grateful to Professor R. L. Collins for the Mössbauer spectrum and Professor W. H. Woodruff for the resonance Raman spectrum.

Registry No. **1**, 63429-86-7; **2**, 80049-76-9; **3**, 80145-58-0; $Fe_2(CO)_9$, 15321-51-4; $Co_2(CO)_8$, 10210-68-1.

β-Methylene-DL-aspartic Acid: A Selective Inhibitor of Glutamate–Aspartate Transaminase

Arthur J. L. Cooper* and Susan M. Fitzpatrick

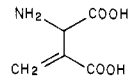
Departments of Neurology and Biochemistry
Cornell University Medical College
New York, New York 10021

Christopher Kaufman and Paul Dowd*

Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260

Received March 30, 1981

β-Methylene-DL-aspartic acid (I) has recently been synthesized[1] for use in the study of the β-methyl-L-aspartate ⇄ L-glutamate carbon skeleton rearrangement, a reaction catalyzed by the vitamin B_{12} dependent enzyme, methylaspartate mutase.[2] It occurred



I

to us that β-methylene-DL-aspartate (I) might have wider biological application.

The β,γ-unsaturated amino acids have been intensely studied as "suicide" inhibitors[3] of pyridoxal 5′-phosphate dependent and flavin dependent enzymes.[4,5] Active suicide inhibitors include propargylglycine[4] and vinylglycine.[5,6] DL-Vinylglycine irreversibly inactivates soluble pig heart glutamate-aspartate transaminase[6] and highly purified forms of mitochondrial and soluble rat brain glutamate-aspartate transaminase.[7] Glutamate-aspartate transaminase plays a central role in intermediary metabolism.[8] It

(1) Dowd, P.; Kaufman, C. *J. Org. Chem.* **1979**, *44*, 3956.

(2) Barker, H. A.; Weissbach, H.; Smyth, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 1093.

(3) Suicide (k_{cat} or mechanism based) inhibition was first described by: Helmkamp, G. M.; Rando, R. R.; Brock, D. J. H.; Bloch, K. *J. Biol. Chem.* **1968**, *243*, 3229. Bloch, K. *Acc. Chem. Res.* **1969**, *2*, 193. Bloch, K. *Enzymes, 3rd Ed.* **1971**, *5*, 441.

(4) Abeles, R. H.; Walsh, C. T. *J. Am. Chem. Soc.* **1973**, *95*, 6124.

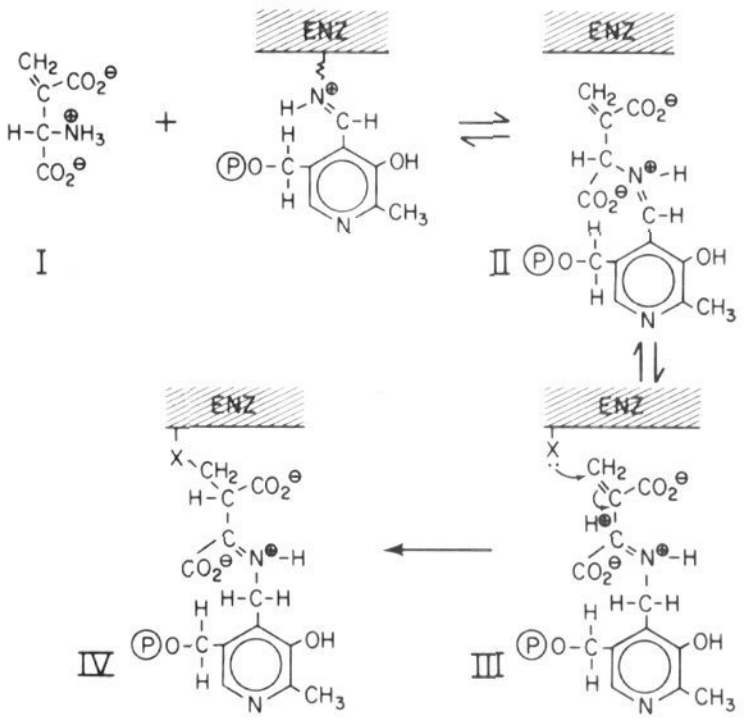
(5) Reviews: Rando, R. R. *Science (Washington, D.C.)* **1974**, *185*, 320. Rando, R. R. *Acc. Chem. Res.* **1975**, *8*, 281. Abeles, R. H.; Maycock, A. L. *Acc. Chem. Res.* **1976**, *9*, 313. Walsh, C. T. *Horiz. Biochem. Biophys.* **1977**, *3*, 36.

(6) Rando, R. R. *Biochemistry* **1974**, *13*, 3859.

(7) King, S.; Phillips, A. T. *J. Neurochem.* **1978**, *30*, 1399.

0002-7863/82/1504-0332$01.25/0

Scheme I



would be of value in a number of in vivo metabolic experiments if this enzyme could be inhibited selectively. Since β-methylene-DL-aspartate (I) more closely resembles aspartate than does vinylglycine, it seemed likely that β-methylene-DL-aspartate (I) might be a more potent and selective inhibitor of glutamate-aspartate transaminase than is vinylglycine.[9] The present work confirms this hypothesis.

Incubation[10] of soluble pig heart glutamate-aspartate transaminase[11] with β-methylene-DL-aspartate results in a time-dependent loss of enzyme activity.[12] From a plot of reciprocal pseudo-first-order rate constant vs. reciprocal inhibitor concentration,[13a,b] the inhibition constant and the rate-limiting constant for inactivation are calculated to be ~6.0 mM and ~0.4 min^{-1}, respectively, at 25 °C. Activity is not restored by prolonged dialysis in 10 mM potassium phosphate buffer (pH 7.2) containing 100 μM pyridoxal 5′-phosphate and 5 mM 2-mercaptoethanol. Inactivation is accompanied by loss of the absorption peak at 430 nm due to enzyme-bound pyridoxal 5′-phosphate and the appearance of a new peak at 335 nm due to the formation of the Schiff-based bound pyridoxamine form of the cofactor.[14] If the enzyme is first converted to the pyridoxamine form with cysteine sulfinate,[6] no inactivation by β-methyleneaspartate is observed. Incubation of the enzyme with β-methyleneaspartate (I) and α-ketoglutarate yielded no glutamate; 1 complete turnover for 50 inactivation events would have been detected.[15]

The inactivation process differs from the vinylglycine-promoted inactivation of bacterial D-amino acid transaminase, where turnover occurs hundreds of times for every inactivation event.[13a] Low molecular weight thiols reduce the rate of inactivation but do not reverse the inactivation process.[16]

A possible mechanism[6,13a] is outlined in Scheme I. β-Methylene-L-aspartate (I) reacts with pyridoxal 5′-phosphate at the active site yielding an aldimine (II) which is converted to a ketimine (III). Nucleophilic attack on the methylene carbon of the conjugated ketimine (anticipated to be an outstanding Michael acceptor) by a juxtaposed nucleophile leads to an inactive structure IV.[17]

The requirements for an effective in vivo enzyme inhibitor are[5] (a) the binding constant must be such that protection from natural substrates is not overwhelming, (b) the inhibitor must not be rapidly metabolized, and (c) the inhibitor must be specific for the enzyme of choice. β-Methylene-DL-aspartate exhibits a K_i of ~3

(8) Since NADH does not readily cross the mitochondrial membrane, net transfer of reducing equivalents must occur via a carrier system. In rat liver, heart and possibly brain, reduced equivalents generated by glycolysis in the cypoplasm are thought to be transported into the mitochondrion via the malate-aspartate shuttle. This shuttle was first described by: Borst, P. In "Funkionelle and Morpholigische Organisation der Zelle"; Karlson, P., Ed.; Spinger-Verlag: Berlin, 1963; pp 137–162. Both soluble and mitochondrial forms of glutamate-aspartate transaminase are important components of this shuttle system. See for example, Williamson, J. R.; Jakob, A.; Refino, C. *J. Biol. Chem.* **1971**, *246*, 7632.

(9) Various bulky α- and β-substituted aspartates, such as α-methyl-*erythro*-β-hydroxy- and α-hydroxymethylaspartate exhibit binding constants similar to or *less* than the Michaelis constant of L-aspartate: Hammes, G. G.; Haslam, J. L. *Biochemistry* **1969**, *8*, 1591. Fonda, M. L.; Johnson, R. J. *J. Biol. Chem.* **1970**, *245*, 2709. Cheng, S.; Michuda-Kozak, C.; Martinez-Carrion, M. *J. Biol. Chem.* **1971**, *246*, 3623. Walsh, J. J.; Metzler, D. E.; Powell, D.; Jacobson, R. A. *J. Am. Chem. Soc.* **1980**, *102*, 7136. The absolute Michaelis constant of L-aspartate has been estimated to be 4.4 mM. Hensen, C. P.; Cleland, W. W. *Biochemistry* **1963**, *3*, 338.

(10) Enzyme (5 μg) was incubated in 0.1 mL of 100 mM potassium phosphate buffer (pH 7.2) containing β-methylene-DL-aspartate at 25 °C. At intervals, aliquots (1–4 μL) were withdrawn and assayed by the method of: Bergmeyer, H. U. "Methods of Enzymatic Analysis", 2nd ed.; Academic Press: New York, 1974.

(11) Pig heart glutamate-aspartate transaminase was obtained from Boehringer-Mannheim. The stated specific activity was ~200 U/mg which we verified. This value may be contrasted to the specific activity of ~350 U/mg obtained for the pure α subform (the most active subform): Birchmeier, W.; Wilson, K. J.; Christen, P. *J. Biol. Chem.* **1973**, *248*, 1751.

(12) The enzyme was not completely inactivated; approximately 3–6% activity always remained; in the presence of 20 mM glutathione, residual activity is ~0.5%. The commercial enzyme is prepared by a method involving a step that contains a maleate buffer. Martinez-Carrion et al. (Martinez-Carrion, M.; Turano, C.; Chiancone, E.; Bossa, F.; Giartosio, A.; Riva, F.; Fasella, P. *J. Biol. Chem.* **1967**, *242*, 2397) have shown that this treatment may result in some alkylation. While it is possible that the residual activity is a result of some modification by maleate treatment, it is worth pointing out that the highly purified nonmaleate treated mitochondrial and soluble forms of rat brain glutamate-aspartate transaminase are inactivated by vinylglycine in a nonmonoexponential fashion.[7] The soluble and mitochondrial preparations contained ~20% and ~8%, respectively, of a component resistant to inactivation. Similarly, a 1% residual activity was reported for DL-vinylglycine-inactivated bacterial D-amino acid transaminase;[13a] the residual activity may possibly be due to alkylated enzyme.[13a] The explanation for the residual activity in the various preparations of glutamate-aspartate transaminase must await further study. We thank a referee for the reference above.

(13) (a) Soper, T. S.; Manning, J. M.; Marcotte, P. A.; Walsh, C. T. *J. Biol. Chem.* **1977**, *252*, 1571. (b) Inhibitor concentrations employed in the present study were 2, 5, 10, 20, and 70 mM.

(14) A solution of 2.4 mg of enzyme[11] in 1 mL of 100 mM potassium phosphate buffer (pH 6.2) showed an absorbance at 435 nm of 0.175 and absorbance at 335 nm of 0.070. Following incubation with 2.0 mM β-methyleneaspartate for 5 h at 25 °C, the absorbance at 435 nm was reduced to 0.051 and a new peak appeared at 335 nm with absorbance 0.240.

(15) Enzyme (5 mg) was incubated with 2.0 mM β-methylene-DL-aspartate and 0.2 mM α-ketoglutarate in 0.7 mL of 100 mM potassium phosphate buffer (pH 7.2) for 24 h at 25 °C. The solution was deproteinized with 0.2 mL of 3 M perchloric acid followed by neutralization of the supernatant with 2 M sodium bicarbonate. No glutamate was detected in the neutralized extract when assayed according to the spectrophotometric method of: Lowry, O. H.; Passonneau, J. V. "A Flexible System of Enzymatic Analysis"; Academic Press: New York, 1972; pp 184–186. It is of interest that Soper et al.[13] also showed that inactivation of commercial pig heart glutamate-aspartate transaminase by DL-vinylglycine is accompanied by "at most 0.1 to 0.2 nmol of vinylglycine oxidized/nmol of enzyme".

(16) In the presence of 40 mM β-methylene-DL-aspartate, the enzyme loses 50% activity in about 3 min. If 60 mM 2-mercaptoethanol, dithiothreitol, or glutathione is included, the time to reach 50% inactivation is 90, 45, and 4 min, respectively. The presence of 4 mM α-ketoglutarate and 60 mM 2-mercaptoethanol slows the process even further ($t_{1/2} > 5$ h). These data suggest that Michael addition of 2-mercaptoethanol to structure III competes effectively with addition of enzyme –X:. Presumably, dithiothreitol is less efficient than 2-mercaptoethanol because it is more bulky: glutathionine is too large to interact at the active site. Rando found that 2-mercaptoethanol has no effect on the rate of inactivation of glutamate-aspartate transaminase by vinylglycine.[6] That the ketimine structure III is expected to be a better Michael acceptor than the analogous one from vinylglycine may account for the differing behavior toward mercaptoethanol. No reaction occurred (24 h, pH 7) between mercaptoethanol and β-methyleneaspartate (I). We thank a referee for suggesting the mercaptan experiment.

(17) Structure IV, Scheme I, suggests that the inactivated enzyme contains pyridoxamine 5′-phosphate in a Schiff's base linkage. Evidence for this linkage was obtained as follows: (a) β-Methylene-DL-aspartate-inactivated glutamate-aspartate transaminase was assayed for pyridoxal 5′-phosphate content according to the method of: Wada, H.; Snell, E. E. *J. Biol. Chem.* **1961**, *236*, 2089. Only 8% pyridoxal 5′-phosphate relative to an equal amount of enzyme lacking inhibitor was found. In another control 5% pyridoxal 5′-phosphate was found when the enzyme was converted to the pyridoxamine 5′-phosphate form with cysteine sulfinate: Jenkins, W. T.; D'Ari, L. *J. Biol. Chem.* **1966**, *241*, 2845. (b) Following deproteinization of such samples, pyridoxamine 5′-phosphate was found by paper chromatography in β-methylene-DL-aspartate-inactivated enzyme and cysteine sulfinate-treated enzyme samples but not in control samples. It may be noted that pyridoxamine 5′-phosphate was found following paper electrophoresis of glutamate-aspartate transaminase inactivated by L-2-amino-4-methoxy-*trans*-3-butenoic acid. Rando, R. R.; Relyea, N.; Cheng, L. *J. Biol. Chem.* **1976**, *251*, 3306.

mM (L form) which is less than the absolute Michaelis constant of L-aspartate (4.4 mM). L-Aspartate at a concentration of 2.0 mM provides only modest protection[18] against inactivation by 1.8 mM β-methylene-DL-aspartate.

Vinylglycine is a good substrate of L-amino acid oxidase[19,20] and D-amino acid oxidase[20] and a poorer one of beef heart glutamate-alanine transaminase.[20] β-Methylene-DL-aspartate is not a substrate of these enzymes. γ-Cystathionase and L-threonine deaminase catalyze rapid double-bond migration, resulting in conversion of vinylglycine to α-ketobutyrate and ammonia.[20] Rat organ homogenates do not catalyze α-keto acid formation from β-methylene-DL-aspartate under conditions which result in rapid conversion of L-homoserine (the commonly used substrate of γ-cystathionase) to α-ketobutyrate and ammonia.[21]

Vinylglycine irreversibly inactivates snake venom L-amino acid oxidase.[19,20] No inactivation of D-amino acid oxidase, L-amino acid oxidase, pig heart glutamate-alanine transaminase, soluble rat kidney glutamine transaminase, *E. coli* glutamate decarboxylase, *P. fluorescens* GABA transaminase, and rat brain GABA transaminase (homogenates) was observed with β-methylene-DL-aspartate.[22,23]

In vivo experiments were carried out as follows: Six hours following an intraperitoneal injection of 100 mM β-methylene-DL-aspartate in 0.9% saline into six mice (5 mmol/kg),[24] kidney glutamate-aspartate transaminase activity was decreased by 40% ($P < 0.0005$) and the liver enzyme activity was decreased by 23% ($P < 0.01$) compared to six saline injected controls.[25-27]

Acknowledgment. This work was supported by United States Public Health Service Grant AM 16739 (A.J.L.C.) and Grant GM 19906 from the Institute for General Medical Sciences of the National Institutes of Health (P.D.). A.J.L.C. is a recipient of a United States Public Health Service Career Development Award NS 00343.

Registry No. I, 71195-09-0.

(18) Incubation at 25 °C in 100 mM potassium phosphate buffer (pH 7.2) with 1.8 mM β-methylene-DL-aspartate led to 50% inactivation after 7 min. In the presence of 2 mM L-aspartate and 1.8 mM β-methylene-DL-aspartate, the time required to reach 50% inactivation was 19 min.

(19) Cooper, A. J. L.; Stephani, R. A.; Meister, A. *J. Biol. Chem.* **1976**, *251*, 6674.

(20) Marcotte, P.; Walsh, C. *Biochemistry* **1976**, *15*, 3070.

(21) Tissue extracts were prepared and γ-cystathionase was assayed with L-homoserine as substrate according to the methods of: Greenberg, D. M. *Methods Enzymol.* **1962**, *5*, 936. In a separate experiment L-homoserine was replaced by β-methylene-DL-aspartate. No α-keto acid formation from β-methylene-DL-aspartate was detected in rat brain, liver, and kidney homogenates. Conditions were such that a rate of α-keto acid formation from β-methylene-DL-aspartate as low as 0.0001% the rate of α-ketobutyrate formation from DL-homoserine would have been detectable in rat liver homogenates. One qualification is required. The enamine derived from β-methyleneaspartate may be more stable than that derived from homoserine; hydrolysis to the α-keto acid or attack at the α carbon by 2,4-dinitrophenylhydrazine may be slow reactions. If so, the negative 2,4-DNP result would not rule out the possibility of interaction of β-methyleneaspartate with γ-cystathionase.

(22) β-Methylene-DL-aspartate is neither a substrate nor an inhibitor of bacterial D-amino acid transaminase: Soper, T. S.; Manning, J. M., personal communication.

(23) Glutamate-alanine transaminase in rat liver homogenates and glutamate decarboxylase in rat brain homogenates are, however, slowly inactivated when incubated with 5 mM β-methylene-DL-aspartate in 100 mM potassium phosphate buffer, pH 7.2, 25 °C.

(24) No obvious behavioral difference between mice injected with β-methylene-DL-aspartate and controls was discernible.

(25) No inactivation of brain enzyme was noted; L-aspartate is known to cross the blood-brain barrier only poorly: Oldendorf, W. H. *Am. J. Physiol.* **1971**, *221*, 1629. The skeletal muscle enzyme and the heart muscle enzyme were also not affected.

(26) Further evidence that β-methylene-DL-aspartate is active in vivo is provided by the findings that 1 h after intraperitoneal administration of β-methylene-DL-aspartate into mice (2.5 mmol/kg), the initial rate of exhaled $^{14}CO_2$ (derived from L-[1-^{14}C]aspartate) is diminished significantly. Owen W. Griffith, personal communication.

(27) In experiments in which the blood-brain barrier is circumvented, i.e., in tissue slices, cerebral glutamate-aspartate transaminase is strongly inhibited by β-methylene-DL-aspartate. The inhibition of enzyme activity is accompanied by a marked reduction in oxygen consumption. Fitzpatrick, S. M.; Cooper, A. J. L.; Duffy, T. E. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 1843.

Bimolecular Thermal Reactions of 5-Methylene-1,3-cyclohexadiene (*o*-Isotoluene) and 3-Methylene-1,4-cyclohexadiene (*p*-Isotoluene)

Joseph J. Gajewski* and Andrea M. Gortva

Department of Chemistry, Indiana University
Bloomington, Indiana 47405
Received August 17, 1981

Despite intensive study of the pyrolysis of C_7H_8 compounds, the methylenecyclohexadienes, *o*- and *p*-isotoluenes, **1** and **2**, respectively, have received little attention.[1] Both **1** and **2** are ca. 23 kcal/mol less stable than toluene,[2] and so their pathways for isomerization are of concern. Further, these species may be involved in retro-ene reactions occurring in coal liquefaction.[3]

While both **1** and **2** have been prepared,[4,5] their sensitivity to acid and base have precluded or obscured efforts to observe their thermal behavior. We report here the benzene solution second-order pyrolytic reactions of these materials which are preparatory to our efforts to examine their dilute gas-phase isomerization.

o-Isotoluene has been prepared in different ways by Bailey,[4a] Kopecky,[4b] and Pryor[4c] who also reported that it disappears in a second-order process; all previous workers have reported that toluene is formed.[4d] *p*-Isotoluene was prepared by Plieninger and Maier-Borst who pyrolyzed (1,4-dihydrobenzyl)trimethylammonium hydroxide,[5] but the oxide of (1,4-dihydrobenzyl)dimethylamine has been found to eliminate smoothly to **2** (and toluene) at 60 °C under vacuum. *o*-Isotoluene could not be purified by GC without ca. 20% conversion to toluene under conditions which allowed purification of the para isomer, but **1** is remarkably pure upon pyrolytic generation from 5-methylenecicyclo[2.2.1]hept-2-en-7-one,[4c] and so it was used directly after vacuum line transfers.

In degassed benzene-d_6 solution in NMR tubes sealed under vacuum both **1** and **2** disappear with second-order kinetics: $\log k_1$ (L/mol·s) = (4.6 ± 1.0 − 11 800 ± 2000)/(2.3RT) and $\log k_2$ (L/mol·s) = (8.1 ± 0.2 − 21 800 ± 300)/(2.3RT).[6] Thus at 56 °C **1** reacts ca. 1500 times faster than **2** at equivalent concentrations. The activation parameters, especially the A factor for reaction of **1**, suggest a concerted reaction for **1** but not for **2**. The cyclopentadiene dimerization has a log A factor between 3.5 and 6.8.[7] The A factor for loss of **2** suggests little orientational demand by the transition state.

The product distribution from each material reinforces the kinetic observations. *o*-Isotoluene gives 75% of ene products **3** and **4** in a 2:1 ratio along with 12% of two preparative GC inseparable unknowns with the residual material apparently being trimeric;[8] however, little, if any, toluene is formed in contrast to

(1) For a review, see: Gajewski, J. J. "Hydrocarbon Thermal Isomerizations"; Academic Press: New York, September 1981.

(2) Bartmess, J. E. *J. Am. Chem. Soc.*, following communication in this issue.

(3) This is particularly true with **1**: Virk, P. S. *Fuel* **1979**, *58*, 149.

(4) (a) Bailey, W. J.; Baylouny, R. A. *J. Org. Chem.* **1962**, *27*, 3476. (b) Kopecky, K. R.; Lau, M. P. *Ibid.* **1978**, *43*, 524. (c) Graham, W. D.; Green, J. G.; Pryor, W. A. *Ibid.* **1979**, *44*, 907 and references contained therein. (d) *o*-Isotoluene is apparently extremely sensitive to acids giving toluene.

(5) Pleininger, H.; Maier-Borst, W. *Chem. Ber.* **1965**, *98*, 2504. *o*-Isotoluene is the thermal isomerization product of 5-methylenebicyclo[2.2.0]-hex-2-ene. Hasselmann, D.; Loosen, K. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 606.

(6) The average deviation reported is that for two separate runs of a sample of **1** or **2** which was divided into separate tubes and examined over a 40–42° range of temperatures. E_a is reported in kcal/mol.

(7) Benson, S. W. "Foundations of Chemical Kinetics"; McGraw-Hill: New York, 1960; p 302. Benson, S. W.; O'Neal, H. E. "Kinetic Data on Gas-Phase Unimolecular Reactions; National Bureau of Standards, Washington, DC, 1970; NBS-21, p 342.

(8) The major products were identified after GC separation on SE-30 column. 220-MHz NMR of **3**: δ 7.2 (m, 5 H), 6.07 (d, J = 10 Hz, 1 H), 5.42 (d, J = 10 Hz, 1 H), 4.72 (s, 1 H), 4.69 (s, 1 H), 2.7–2.0 (m, 5 H), 1.77 (sym m, 1 H, 1.36 (sym m, 1 H). 220-MHz NMR of **4**: δ 7.2 (m, 5 H), 5.8 (m, 3 H), 5.46 (d, J = 10 Hz, 1 H), 2.57 (ABq, J = 13 Hz, 2 H), 2.07 (ABq, J = 17 Hz, 2 H) (the downfield lines are doubled with J = 5 Hz and the upfield lines are doublets of doublets with J = 4, 2 Hz), 0.93 (s, 3 H). One of the unknowns appears to be 1-methyl-5-benzyl-1,3-cyclohexadiene, a 10-electron ene product.

0002-7863/82/1504-0334$01.25/0