$W_2(CH_2SiMe_3)_6$, 36643-37-5; $W(CPr)(NMe_2)_3$, 82209-27-6; $W(CPr)(CH_2SiMe_3)_3$, 82209-28-7; $W(CCMe_3)(NMe_2)_3$, 82209-29-8; $W(CSiMe_3)(CH_2SiMe_3)_3$, 78638-62-7; $W_2Cl_6(THF)_4$, 77479-88-0; $W_2Cl_2(NMe_2)_4$, 63301-81-5; $Mo_2(OCHMe_2)_6(py)_2(C_2H_2)$, 78736-93-3; $Mo_2(\eta^5\text{-}C_5H_5)_2(CO)_4(\mu\text{-}C_2H_2)$, 64973-91-7; $W_2(OCHMe_2)_6(py)_2(C_2H_2)$, 82281-73-0; $Mo_2(OCMe_3)_6$, 60764-63-8; $Mo_2(OCH_2CMe_3)_6$, 62521-24-8; $Mo(CCMe_3)(OCMe_3)_3$, 82209-30-1; $Mo(N)(OCMe_3)_3$, 82209-31-2; 4-octyne, 1942-45-6; 3-hexyne, 928-49-4; 2-butyne, 503-17-3; diphenylacetylene, 501-65-5; 1-hexen-3-yne, 13721-54-5; acetonitrile, 75-05-8; benzonitrile, 100-47-0; ethyne, 74-86-2; propyne, 74-99-7; propionitrile, 107-12-0.

Structure of the Glycopeptide Antibiotic Vancomycin. Evidence for an Asparagine Residue in the Peptide

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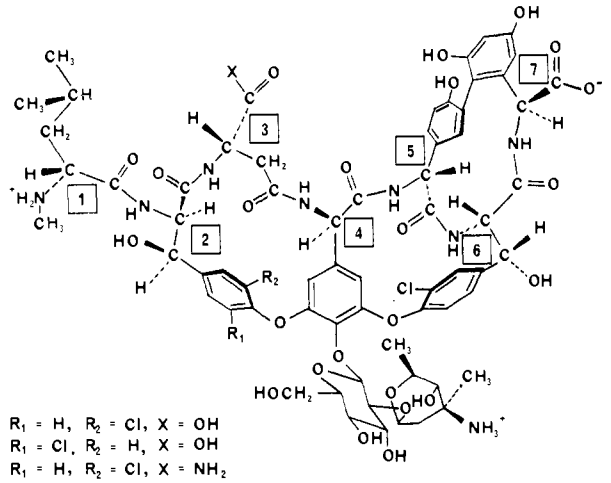
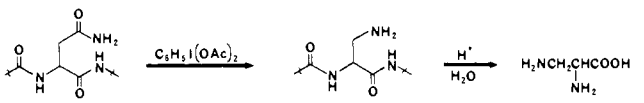
Received April 5, 1982

Vancomycin and related antibiotics have recently been the subject of intense investigation with respect to their structures and mechanism of action.[1] None of the antibiotics have yielded crystals of sufficient size and quality to permit structural solution by X-ray crystallography, but vancomycin undergoes a slow transformation on heating at pH 4–5 to a crystalline degradation product (CDP-I)[2] for which structure **1a** was obtained by Sheldrick et al.[3] Williams,[4,5b] Feeney,[5] and their co-workers have made extensive chemical and spectroscopic studies of vancomycin and have concluded that the only major structural difference between vancomycin and CDP-I is the presence of an isoasparaginyl residue in the antibiotic and an isoaspartyl residue in the degradation product. Although unusual structural features are common in peptide antibiotics, the vancomycin structure (**2**) attracted our attention because of the possibility that the unique isoasparagine residue might actually be the more common asparagine, with rearrangement to isoaspartate occurring during



1a, R_1 = H, R_2 = Cl, X = OH
b, R_1 = Cl, R_2 = H, X = OH
2a, R_1 = H, R_2 = Cl, X = NH_2
b, R_1 = Cl, R_2 = H, X = NH_2

Scheme I



hydrolysis to form CDP-I. Examples of aspartyl → isoaspartyl rearrangements have been documented during acid and base treatment of peptides containing aspartate esters and asparagine.[6]

Two approaches were taken to search for the presence of a normal asparagine in vancomycin. In the first of these, vancomycin aglycone,[2b] treated with CH_2N_2 to protect the phenolic hydroxyl groups, was reduced with diborane in THF[7] and hydrolyzed.[8] Ion-exchange chromatography[8] of the hydrolysate gave a minor component having the same retention time, ninhydrin color, and TLC behavior as 2,4-diaminobutyric acid, which is the product that would result from reduction of the β-carboxamido group of asparagine. Acylation of the amino acid with benzoyl chloride followed by esterification (CH_2N_2) gave the *N,N*-dibenzoyl methyl ester, which was identical by TLC, MS, and ^1H NMR with authentic material. No 3,4-diaminobutyric acid (which would be the product from reduction of isoasparagine) was detected; neither was any 2,4-diaminobutyric acid detected in hydrolysates of unreduced material. The yield of 2,4-diaminobutyric acid was very low because diborane reduces secondary peptide linkages more rapidly than it reduces the primary amide of the asparaginyl group.

The second approach for establishing the presence of the asparaginyl residue involved a Hofmann-type oxidative degradation of the primary carboxamide (Scheme I).[9] Treatment of O-methylated aglycovancomycin with 5 equiv of (diacetoxyiodo)-benzene (18 h, 20 °C, 1:1 CH_3CN/H_2O) followed by peptide hydrolysis and ion-exchange chromatography[8] gave the asparagine degradation product 2,3-diaminopropionic acid, identical in all respects (TLC, ninhydrin color, ^1H NMR) with authentic material. Quantitative amino acid analysis was complicated by the fact that a significant portion of O-methylated aglycovancomycin is resistant to acid hydrolysis under the conditions that were employed; hydrolysis of unoxidized O-methylated aglycovancomycin gave only 51% of the theoretical yield of aspartic acid. Analysis of the products of oxidative degradation indicated an 18% yield of 2,3-diaminopropionic acid and 13% yield of unaltered aspartic acid. It was not feasible to force the oxidative degradation to completion by the use of a larger excess of oxidant or more vigorous conditions because the free amino group of the diaminopropionyl residue is also susceptible to oxidation. The oxidative degradation was repeated with vancomycin itself to avoid the risk that an isoasparagine → asparagine rearrangement occurs during formation of the protected aglycone.[10] The oxidation again gave 2,3-diaminopropionic acid although the yield was lower on account of depletion of the oxidant by reaction with the phenolic groups and/or condensation of the new amino group with quinoidal products resulting from phenolic oxidations. Isoasparagine, had

(1) For a recent review, see: Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojesen, G. *Top. Antibiotic Chem.* **1980**, *5*, 119.
(2) (a) Johnson, C. R. Thesis, University of Illinois, Urbana, 1962. (b) Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18.
(3) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature (London)* **1978**, *271*, 223.
(4) (a) Smith, K. A.; Williams, D. H.; Smith, G. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2369. (b) Smith, G. A.; Smith, K. A.; Williams, D. H. *Ibid.* **1975**, 2108. (c) Williams, D. H.; Kalman, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 2768. (d) Williams, D. H.; Butcher, D. W. *Ibid.* **1981**, *103*, 5697. (e) Williamson, M. P.; Williams, D. H. *Ibid.* **1981**, *103*, 6580.
(5) (a) Convert, O.; Bongini, A.; Feeney, J. *J. Chem. Soc., Perkin Trans. 2*, **1980**, 1262. (b) Bongini, A.; Feeney, J.; Williamson, M. P.; Williams, D. H. *Ibid.* **1981**, 201.
(6) (a) Battersby, A. R.; Robinson, J. C. *J. Chem. Soc.* **1955**, 259. (b) John, W. D.; Young, G. T. *Ibid.* **1954**, 2870. (c) Swallow, D. L.; Abraham, E. P. *Biochem. J.* **1958**, *70*, 364. (d) Sondheimer, E.; Holley, R. W. *J. Am. Chem. Soc.* **1954**, *76*, 2467. (e) Riniker, B.; Schwyzer, R. *Helv. Chim. Acta* **1964**, *47*, 2357. (f) Bernhard, S. A.; Berger, A.; Carter, J. H.; Katchalski, E.; Sela, M.; Shalitin, Y. *J. Am. Chem. Soc.* **1962**, *84*, 2421. (g) Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251. (h) Marshall, G. R.; Merrifield, R. B. *Biochemistry* **1965**, *4*, 2394. (i) Baba, T.; Sugiyama, H.; Seto, S. *Chem. Pharm. Bull.* **1972**, *21*, 207. (j) For a general discussion and additional references see: Barany, G.; Merrifield, R. B. In "The Peptides"; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 192–208.
(7) Brown, H. C.; Heim, P. *J. Org. Chem.* **1973**, *38*, 912.
(8) Peptide hydrolyses were carried out in sealed tubes at 105 °C for 22 h by using 1 mL of constant boiling HCl/10 mg of peptide. Ion-exchange chromatography was carried out on a 0.9 × 50 cm column of Aminex AG-50W-X2 at 35 °C with 0.1 M pyridine–acetate buffer, pH 4.50.
(9) Holt, L. A.; Milligan, B. *Aust. J. Biol. Sci.* **1981**, *34*, 395. For similar reactions employing [bis(trifluoroacetoxy)iodo]benzene see: (a) Radhakrishna, A. S.; Parham, M. E.; Riggs, R. M.; Loudon, G. M. *J. Org. Chem.* **1979**, *44*, 1746. (b) Soby, L. M.; Johnson, P. *Anal. Biochem.* **1981**, *113*, 149.
(10) Such a rearrangement appears unlikely since the aglycone retains much of the antibiotic activity of the parent antibiotic.[2b]

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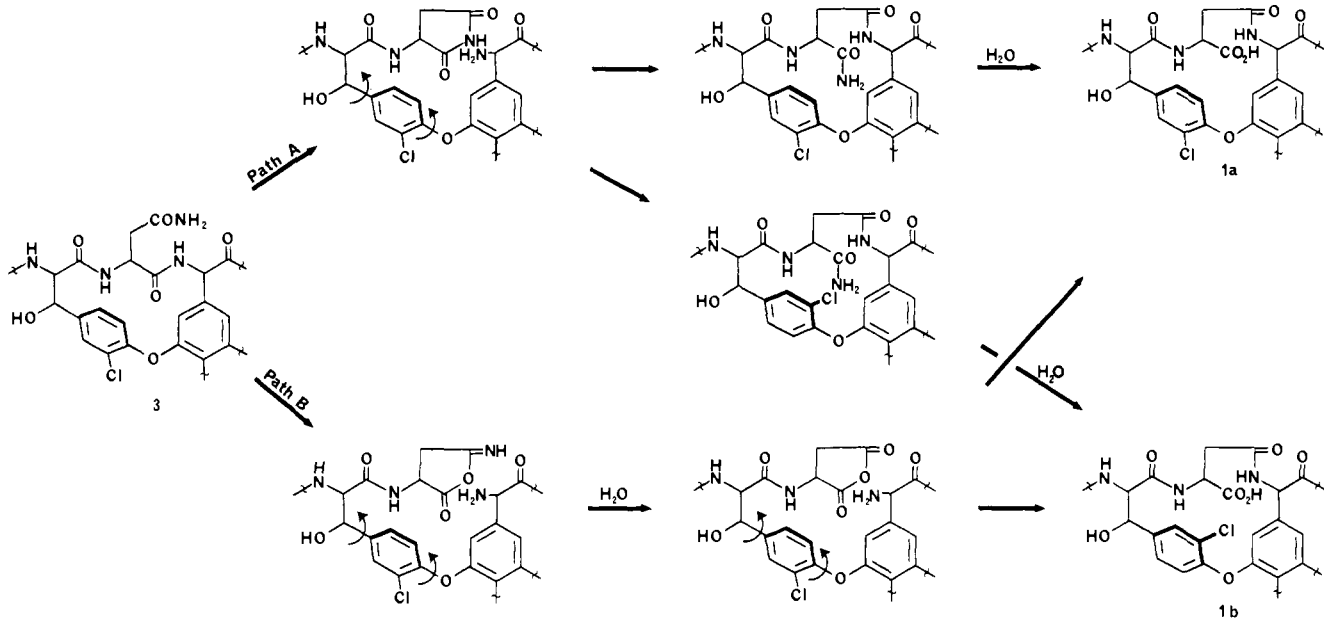
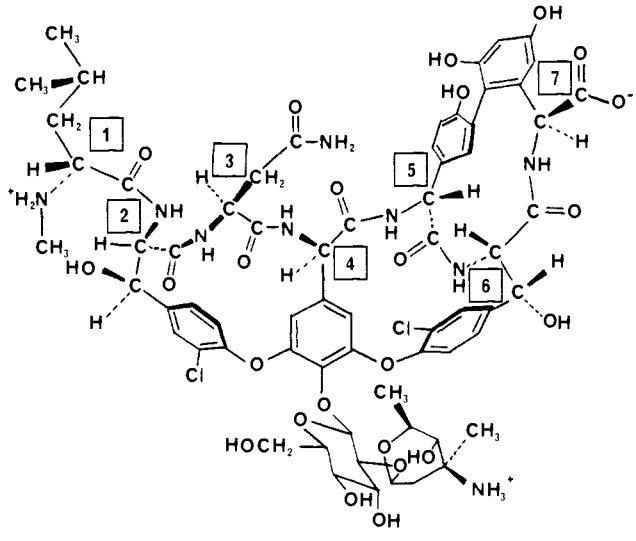
Scheme II



it been present in the peptide, would have undergone oxidative degradation to give the unstable 3,3-diaminopropionic acid, which would not have been detected.

On the basis of these results we propose structure **3** for van-



3

comycin. The revised structure is more similar than the original one to the structures that have been deduced for ristocetin and other members of the class[1,11] in that it has the same size ring embodying residues 2–4.

The process by which the asparagine in vancomycin is converted into isoaspartic acid in CDP-I deserves comment. NMR studies by Williams and Kalman[4c] and by Convert et al,[5a] which involved monitoring changes in chemical shifts that occur during deprotonation of the N-terminus and during binding of aliphatic peptides to the antibiotic, indicated that the orientation of the chlorine atom on residue 2 was forward (i.e., as in **2a**) like that found in CDP-I (**1a**). A later study by Williamson and Williams,[4e] involving measurement of the rate of development and the magnitude of nuclear Overhauser effects, has indicated that the chlorine atom is on the back face of the molecule (i.e., as in **2b**); the conflict between this result and that obtained by the earlier methods has not yet been fully resolved. With presumption that the orientation of the chlorine established in the most recent study is the correct one, the formation of **1a** must involve cleavage and reclosure of the cyclophane ring embodying residue 2 since steric constraints would appear to prohibit ~180° rotation of the aromatic ring. A consequence of a seco intermediate is that CDP-I should arise as a mixture of the two orientations of the aromatic ring. Indeed, a careful examination of CDP-I by Williamson and Williams revealed that it is a mixture of two isomers, a major one (CDP-I-M), which without doubt is the one (**1a**) previously employed in the X-ray study, and a minor one (CDP-I-m), to which they assigned structure **1b**, having the aromatic ring of residue 2 oriented as in vancomycin.

Williamson and Williams proposed that the transformation involves retroaldol cleavage of residue 2, free rotation of the resulting aryl aldehyde, and reclosure of the severed bond with complete retention of configuration at the α and β positions of the amino acid. In view of our results, it appears more likely that residue 3, the asparagine, is the site of cleavage rather than residue 2. Cleavage of the cyclophane ring system would occur by attack of the primary amide group of asparagine on the secondary one (Scheme II). If the attacking atom is amide nitrogen (path A), a succinimide would result that could yield **1a** and **b** by reacylation of the freed amino group of residue 4 and hydrolysis of the new primary amide group. Attack by the amide oxygen (path B) would give an isoimide[12] that would form **1a** and **b** by hydrolytic loss of ammonia followed by acylation of the free amino group of residue 4 by the resulting succinic anhydride.[13] Insufficient evidence exists to make a choice between the two pathways at this time. An alternative pathway, in which the asparagine rearranges by attack of the secondary amide on the primary one to give a succinimide moiety in the peptide chain followed by reopening by nucleophilic attack on the α-carbonyl group, is more attractive on mechanistic grounds and has been demonstrated many times in simpler peptides[14] although not under the exact conditions employed here.[15] However, such a process would not accom-

(11) For a recent revision in the structure of ristocetin see: Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1982**, *104*, 363.

(12) Isoimides have been postulated as intermediates in the dehydration of *N*-acyl asparagines (having the α-carboxyl group free) by reagents such as dicyclohexylcarbodiimide (see ref 6j); nitriles are formed under these conditions and also upon treatment of unsubstituted isoimides with base.

(13) Aspartate-derived succinimides and succinic anhydrides normally are attacked by nucleophiles predominantly at the α-carbonyl group. In both path A and path B, attack by the reentering peptide amino group must occur at the β-carbonyl group to give the isoaspartyl residue of CDP-I; steric control may be involved.

(14) See, e.g.: Bornstein, P.; Balian, G. In "Methods in Enzymology"; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: New York, 1977; Vol. 47, pp 132–145, as well as ref 6.

modate flipping the aromatic ring of residue 2. We are presently pursuing further experiments to confirm the orientation of the aromatic ring in vancomycin and to delineate the mechanism of the rearrangement process.

CDP-I (M?) has been reported by Marshall to lack antibiotic activity,[2b] and we have confirmed that a mixture of the two forms of CDP-I does not have activity against *Staphylococcus aureus*. This result was difficult to reconcile with the previously held structure **2b** for vancomycin since the structure and that of CDP-I-m (**1b**) were identical except for the carboxamide group, which was distant from the putative site of antibiotic activity. Major differences between the revised structure **3** for vancomycin and the structures of CDP-I-M and -m can account for the absence of activity in CDP-I.

Acknowledgment. We thank Dr. A. M. Thomas (Abbott Laboratories) for helpful discussions, Dr. F. T. Wolf (Vanderbilt University) for the antibiotic assays, and Dr. P. Fletcher (East Carolina University) for the amino acid analyses. This research was supported by the U.S. Public Health Service (GM-25793).

Registry No. Vancomycin, 1404-90-6; vancomycin aglycone, 82198-76-3; O-methylated aglycovancomycin, 82198-77-4.

(15) Under the conditions for CDP-I formation (pH 4.11, 75 °C) the model peptide Ac-L-Asn-D-Ala showed no detectable reaction after 70 h, but the rate of rearrangement is known to be quite sequence dependent (see, e.g., ref 6i).

Synthesis and Thermal Behavior of 6-Methoxytricyclo[5.3.0.0^{2,5}]deca-3,6,8,10-tetraene (Methoxy-Dewar Azulene). Similarity between Valence Isomers of Azulene and Benzene

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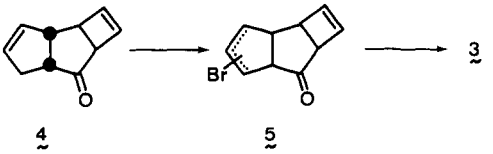
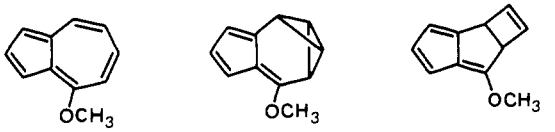
Received April 5, 1982

In recent years, keen interest has developed in the chemistry of the valence isomers of benzenoid aromatic hydrocarbons,[1] many of which possess not only novel strained structures[2] but also valued ground- and excited-states properties.[3] However, little is known for the valence isomers of nonalternant hydrocarbons.[4] We have recently reported the synthesis of 6-methoxytetracyclo[5.3.0.0^{2,4}.0^{3,5}]deca-6,8,10-triene (methoxyazulvalene) (**2**)[5] as a first example of the valene-type valence isomer of a representative nonalternant hydrocarbon azulene derivative, **1**. In connection with the study on the thermal and photochemical behavior of **2**, 6-methoxytricyclo[5.3.0.0^{2,5}]deca-3,6,8,10-tetraene (methoxy-Dewar azulene) (**3**) is required. To date known compounds having the Dewar azulene skeleton are confined only to the heavily substituted derivatives prepared through cycloaddition reactions of pentalenes with acetylenes.[6] We report here the synthesis and some properties of **3**.

Scheme I



4 **5** **3**



1 **2** **3**

As outlined in Scheme I, **3** was prepared conveniently from *cis-anti-cis*-tricyclo[5.3.0.0^{2,5}]deca-3,9-dien-6-one (**4**) previously used as a synthetic intermediate for **2**.

Bromination of **4** with *N*-bromosuccinimide in dry carbon tetrachloride in the presence of azodiisobutyronitrile under reflux for 1.5 h gave a mixture of allylic bromides **5** ($\nu_{C=O}$ 1720 cm^{-1}) which, without separation, was treated with 2 equiv of potassium *tert*-butoxide in HMPA at 0 °C for 5 min and then quenched with freshly distilled methyl fluorosulfonate for 5 min to give **3** as a yellow oil in 60% yield.[7] Dewar azulene **3** was too unstable to allow its combustion analysis (highly sensitive to air and acids; however, it could be stored at ambient temperature under argon atmosphere); however, available spectroscopic data are consistent with the proposed structure; MS, *m/e* 158 (M$^+$, 64%), 128 (azulene cation, 66%), 115 (indenium ion, 100%); ^{1}H NMR (100 MHz, $CDCl_3$) δ 4.11 (s, 3 H, OCH_3), 4.11 (m, 1 H, H-2), 4.24 (dd, 1 H, J = 2.7, 1.0 Hz, H-5), 6.02 (m, 1 H, H-10), 6.25 (dd, 1 H, J = 4.8, 1.1 Hz, H-8), 6.49 (dd, 1 H, J = 2.6, 1.0 Hz, H-3 or -4), 6.55 (dd, 1 H, J = 2.6, 1.0 Hz, H-4 or -3), 6.73 (dd, 1 H, J = 4.8, 2.2 Hz, H-9); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 44.8, 59.9, 60.1, 110.2, 113.2, 124.9, 135.2, 138.6, 144.1, 146.3, 172.4; UV (cyclohexane) λ_{max} 289 nm (ϵ 12 000), 357 (950). The ultraviolet spectrum of **3** needs comment. In spite of the increased strain involved in the fulvene chromophore, the long-wavelength maximum of **3** exhibits a blue shift by 10 nm compared to that of **2**. This finding implies that there is a conjugation effect between the fulvene and bicyclobutane moieties in **2** to some extent.[8]

In sharp contrast to **2**, which undergoes clean isomerization to **1** on irradiation, photolysis of **3** resulted in slow decomposition instead of isomerization to **1**.[9] On the other hand, the thermal

(1) For Dewar benzenes, see: (a) van Tamelen, E. E.; Pappas, S. P. *J. Am. Chem. Soc.* **1963**, *85*, 3297. (b) Breslow, R.; Napierski, J.; Schmidt, A. H. *Ibid.* **1972**, *94*, 5906. van Tamelen, E. E.; Carty, D. *Ibid.* **1967**, *89*, 3922, **1971**, *93*, 6102. (c) McDonald, R. N.; Frickey, D. G. *Ibid.* **1968**, *90*, 5315. (d) Yang, N. C.; Carr, R. V.; Li, E.; McVey, J. J.; Rice, S. A. *Ibid.* **1974**, *96*, 2297. For benzvalenes, see: (e) Katz, T. J.; Wang, E. J.; Acton, N. *Ibid.* **1971**, *93*, 3782. (f) Gandillon, G.; Bianco, B.; Burger, U. *Tetrahedron Lett.* **1981**, 51. (g) Burger, U. *Chimia* **1979**, *33*, 147. For prismane, see: (h) Katz, T. J.; Acton, N. *J. Am. Chem. Soc.* **1973**, *95*, 2738.

(2) For a review, see: Greenberg, A.; Liebman, J. F. "Strained Organic Molecules"; Academic Press: New York, 1978.

(3) (a) For degenerate photoinduced valence isomerizations and quantum chain processes, see: Renner, C. A.; Katz, T. J.; Pouliquen, J.; Turro, N. J.; Waddel, W. H. *J. Am. Chem. Soc.* **1975**, *97*, 2596. Turro, N. J.; Ramamurthy, V.; Katz, T. J. *Nouv. J. Chim.* **1977**, *1*, 363. (b) For adiabatic photoreaction, see: Turro, N. J.; McVey, J.; Ramamurthy, V.; Lechtken, P. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 572. (c) For chemoexcitations, see: Lechtken, P.; Breslow, R.; Schmidt, A. H.; Turro, N. J. *J. Am. Chem. Soc.* **1973**, *95*, 3025.

(4) Notable early examples of such isomers were naphtho[1,8]tricyclo[4.1.0.0^{2,7}]heptene ((a) Murata, I.; Nakasuji, K. *Tetrahedron Lett.* **1973**, 47. (b) Pagni, R. M.; Watson, C. R., Jr. *Ibid.* **1973**, 59. (c) Pagni, R. M.; Burnett, K.; Hasell, A. C. *Ibid.* **1977**, 163; *J. Org. Chem.* **1978**, *43*, 2750) and naphtho[1,8]bicyclo[3.2.0]heptene ((d) Meinwald, J.; Samuelson, G. E.; Ikeda, M. *J. Am. Chem. Soc.* **1970**, *92*, 7604. (e) Gleiter, R.; Haider, R.; Murata, I.; Pagni, R. M. *J.Chem. Res., Synop.* **1979**, 72. (f) Watson, R., Jr.; Pagni, R. M.; Dodd, J. R.; Bloor, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 2551. (g) Turro, N. J.; Ramamurthy, V.; Pagni, R. M.; Butcher, J. A., Jr. *J. Org. Chem.* **1977**, *42*, 92).

(5) Sugihara, Y.; Sugimura, T.; Murata, I. *J. Am. Chem. Soc.* **1981**, *103*, 6738.

(6) Suda, M.; Hafner, K. *Tetrahedron Lett.* **1977**, 2453. For examples of intermediate formation of Dewar azulene skeleton, see: Hafner, K.; Diehl, H.; Suss, H. U. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 104. LeGoff, E. *J. Am. Chem. Soc.* **1962**, *84*, 3975.

(7) Preparation of **3** has to be done under very strictly controlled conditions. All solvents used were deoxygenated by bubbling with dry argon before use. Potassium *tert*-butoxide and methyl fluorosulfonate were purified by sublimations and distillations, respectively, just before use.

(8) Conjugation effects between bicyclobutane and some π systems have been suggested in benzvalene (Griffith, D. W. T.; Kent, J. E.; O'Dwyer, M. F. *Aust. J. Chem.* **1975**, *28*, 1397. Griffith, D. W. T.; Kent, J. E.; O'Dwyer, M. F. *J. Mol. Spectrosc.* **1975**, *58*, 427. Harmon, P. J.; Kent, J. E.; O'Dwyer, M. F.; Smith, M. H. *Ibid.* **1979**, *32*, 2579) and in tropovalene (Sugihara, Y.; Morokoshi, N.; Murata, I. *Tetrahedron Lett.* **1977**, 3887).

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