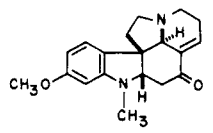
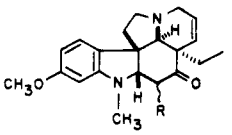
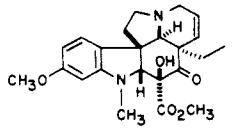


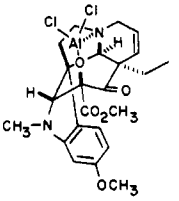
22



23 R=H
24 R=CO_2CH_3



25



c

205°, identical with natural material, mp 172–174°, according to chromatographic and spectral comparisons.

Acknowledgments. This work was supported generously by the National Institutes of Health (GM 09868) and the Hoffmann-La Roche Foundation. We are indebted to Drs. J. Belletire and G. Trammel for exploratory research on vindoline.

References and Notes

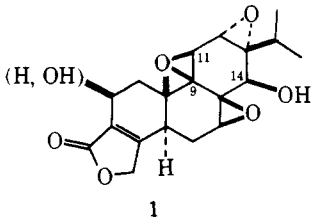
(1) M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).
(2) J. W. Moncrief and W. N. Lipscomb, *Acta Crystallogr.*, **21**, 322 (1966); N. Neuss, M. Gorman, W. Hargrove, N. J. Cone, K. Biemann, G. Büchi, and R. E. Manning, *J. Am. Chem. Soc.*, **86**, 1440 (1964).
(3) G. Büchi, K. E. Matsumoto, and H. Nishimura, *J. Am. Chem. Soc.*, **93**, 3299 (1971).
(4) Experiments by Dr. M. T. Cox.
(5) See R. Iyer, A. H. Jackson, and P. V. R. Shannon, *J. Chem. Soc., Chem. Commun.* 461 (1972).
(6) J. H. Looker, *J. Org. Chem.*, **24**, 1039 (1959).
(7) We are indebted to Drs. W. Leimgruber and P. Wehrli, Hoffmann-LaRoche Inc., Nutley, N.J., for this material.
(8) J. Szmuzkovicz, Belgium Patent, 621047 (1963).
(9) S. Hanessian, *Tetrahedron Lett.*, 1549 (1967).

Masayoshi Ando, George Büchi,* Takeshi Ohnuma
Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
Received August 11, 1975

Synthesis of (±)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,-9,10-tetrahydrobenzo[*a*]pyrene, a Potential Metabolite of the Carcinogen Benzo[*a*]pyrene with Stereochemistry Related to the Antileukemic Triptolides

Sir:

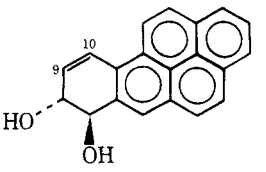
The antileukemic diterpenoid triepoxides, triptolide and tripdiolide (**1**), have been suggested[1] to effect their high biological activity through alkylation of biologically important macromolecular thiols at C-9 of the 9,11-epoxide. An-



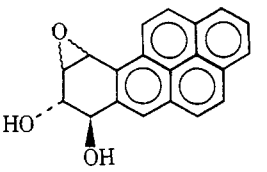
1

chimeric assistance by the proximate 14β-hydroxyl group markedly enhances the rate of adduct formation between **1** and simple thiols. A steroid in which a neighboring hydroxyl group enhances the rate of epoxide ring opening is also known.[2] The same stereochemical situation present in triptolide, an epoxide ring and a hydroxyl group two positions removed on the same face of a six-membered ring, may also be invoked to explain the metabolism induced binding[3] of carcinogenic polycyclic aromatic hydrocarbons to cellular macromolecules. We herein describe the synthesis and reactions of the title compound, a potential metabolite from the environmental carcinogen benzo[*a*]pyrene (BP).

Our interest in this synthesis was stimulated by the key observation of Borgen et al.[4] who demonstrated that *trans*-7,8-dihydroxy-7,8-dihydro-BP (**2a**) was much more extensively bound to DNA on further metabolism by liver microsomes than were either of two other metabolic dihydrodiols or BP itself. The above observation was confirmed by Sims et al.[5] who suggested diol epoxide **3** as the active binding agent and claimed its synthesis[6] by the action of *m*-chloroperoxybenzoic acid on diol **2a**. Although the question of rel-



2a

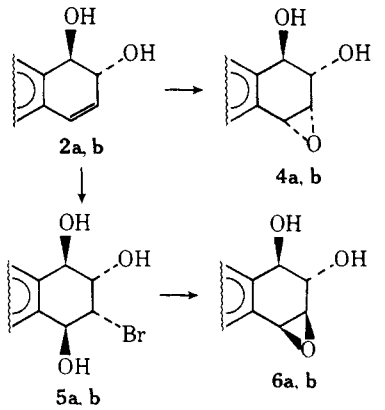


3

ative stereochemistry between the hydroxyl groups and the 9,10-oxirane was not considered in this study,[5] there is ample precedent to expect that epoxidation should occur on the face of the molecule which bears the 8-OH[7] to produce the isomer of diol epoxide **3** in which anchimeric assistance of nucleophilic attack on the oxirane by the 7-OH is impossible as the oxirane and 7-OH are trans. The corresponding epimer of triptolide has low biological activity and is 20-fold slower on reaction with propanethiol.[1] The isomeric sterol epoxides display an 18-fold difference in rates of reaction with azide.[2]

trans-1,2-Dihydroxy-1,2-dihydronaphthalene[8] (**2b**) was chosen as a simple model compound to test possible synthetic routes to the isomers of the BP diol epoxide **3**. In solution, the dihydrodiol prefers the conformation in which the hydroxyl groups occupy pseudo-equatorial positions,[9] the conformation in which both hydroxyl groups should act in concert[7] to direct epoxidation such that the 1-OH and the oxirane are trans (Scheme I). Reaction of **2b** with *m*-chloroperoxybenzoic acid (CH_2Cl_2, 0°, 2 hr) cleanly produced 1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydronaphthalene[10] (**4b**) in 60% yield (mp 153–155°). As anticipated, the reaction was highly stereoselective, and only the stereoisomer **4b** was isolated.

Scheme I



a = BP series
b = naphthalene series

Synthesis of the stereoisomeric diol epoxide (**6b,** Scheme I,) from **2b** presented a synthetic challenge. Fortunately, approach of *N*-bromoacetamide (NBA) to the diol substrate occurs at the same face of the molecule as does peroxyacid. Reaction of **2b** with NBA (20% aqueous-THF, 0°, 3 hr) provided the halohydrin **5b** (154–156° dec) in 79% yield.[11] None of the undesired isomer in which the 2-OH and 3-Br are trans was detected. Cyclization of **5b** to the diol epoxide **6b**[12] was accomplished with Amberlite IRA-400 (OH form) in dry THF thus generating (95%) the triptolide like stereochemistry. The trimethylsilyl ethers[13] of **4** and **6** were found particularly useful in obtaining spectral data.

Peroxyacid epoxidation of the BP dihydrodiol **2a** was conducted[5] exactly as described ($CHCl_3$, 0° for 48 hr) except on much larger scale with synthetic diol.[14] Direct silylation of the crude reaction mixture in the cold followed by mass spectrometry indicated the presence of diol epoxide **3** (presumably **4a**). Work-up as described[5] resulted in substantial production of a *m*-chlorobenzoic acid adduct.[15] Preparative TLC as described[5] did not allow identification of **3** by mass spectrometry after silylation. Although epoxidation in $CHCl_3$ does appear to proceed cleanly, conditions for isolation of pure **3** have yet to be found.[24] Rigorous assignment of the stereochemistry in **3** and the adduct will require further study.

Synthesis of the triptolide like isomer (**6a**) proceeded as described in the model studies; the halohydrin **5a**[16] (94% from **2a,** 128–130° dec) was cyclized to the diol epoxide **6a**[17] (85% yield, 226–228° dec) either by treatment with the resin or by reaction with 1 equiv of NaH in THF at 0°. This compound is extremely reactive but can be stabilized as the disilyl ether.[18]

Relative reactivity of the diol epoxides **4b** (0.22 M^{-1} sec^{-1}), **6b** (0.10 M^{-1} sec^{-1}), as well as phenanthrene 9,10-oxide[19] (2.1 M^{-1} sec^{-1}) was established by measurement of the second-order rate constants for reaction with *p*-nitrothiophenolate in water–alcohol.[20] Failure to observe enhanced reactivity of **6b** relative to **4b** in the naphthalene series may be a consequence of conformational effects in water–alcohol. Notably, both **1** and the sterol epoxide[1,2] are locked in the conformation for which hydrogen bonding to the epoxide is possible. Accurate comparison of the reactivity of the diol epoxides **4b** and **6b** (naphthalene-series) with **6a** in water–alcohol is not possible due to a high solvolysis rate for **6a.** However, **6a** is estimated to be more than two orders of magnitude more reactive than **4b** and **6b.** Further studies are in progress to establish the origin of this enhanced reactivity. In *tert*-butyl alcohol solvent, a high degree of anchimeric assistance has been detected in both the naphthalene and BP series.[24,25]

Examination of the mutagenicity of metabolites of BP (phenols and arene oxides) has established that BP 4,5-oxide is highly mutagenic toward histidine dependent *Salmonella typhimurium* and 8-azaguanine sensitive Chinese hamster V-79 cells in culture.[21] Preliminary studies of diol epoxide **6a** indicate it is markedly more active (>40 times) than BP 4,5-oxide in these tests. In contrast, BP 7,8-oxide is the only metabolite of the phenols and arene oxides which have been tested[22] that displays marked carcinogenicity in vivo. Since BP 7,8-oxide may be first hydrated to diol **2a** and then converted to diol epoxide **6a** prior to initiation of the oncogenic event, an attractive hypothesis for the mechanism of carcinogenesis by BP emerges. Diol **2a** functions as a stable precarcinogen in the body while the highly reactive ultimate carcinogen (**6a**) is generated in situ, possibly by drug metabolizing enzymes in the nuclear envelope surrounding DNA. We are presently attempting to establish whether **2a** and **6a** are carcinogens in vivo.[23–25]

References and Notes

(1) S. M. Kupchan, W. A. Court, R. G. Dailey, C. J. Gilmore, and R. F. Bryan, *J. Am. Chem. Soc.*, **94,** 7194 (1972); S. M. Kupchan and R. M. Schubert, *Science,* **185,** 791 (1974).

(2) D. H. R. Barton and Y. Houminer, *J. Chem. Soc., Chem. Commun.,* 839 (1973).

(3) For a discussion of the relationship between covalent binding to biopolymers and chemically induced oncogenesis see E. C. Miller and J. A. Miller in "Molecular Biology of Cancer", H. Bush, Ed., Academic Press, New York, N.Y., pp 377–402. See also, D. M. Jerina and J. W. Daly, *Science,* **185,** 573 (1974), for a discussion of the possible involvement of arene oxides in such binding reactions.

(4) A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, *J. Med. Chem.,* **16,** 502 (1973).

(5) P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature (London),* **252,** 326 (1974).

(6) Since the reaction was conducted on 0.2 mg of diol **2a,** adequate structural characterization of the product was not possible.

(7) H. B. Henbest, *Proc. Chem. Soc., London,* 159 (1963), and P. Chamberlain, M. L. Roberts, and G. H. Whitham, *J. Chem. Soc. B,* 1374 (1970), have established that cyclohex-2-en-1-ols are epoxidized to produce the cis isomer particularly when the hydroxyl group occupies a pseudo-equatorial position. Furthermore, cyclohex-3-en-ols show a preference for the trans isomer on epoxidation.

(8) J. Booth, E. Boyland, and E. E. Turner, *J. Chem. Soc.,* 1188 (1950).

(9) A. M. Jeffrey, H. J. C. Yeh, D. M. Jerina, T. R. Patel, J. F. Davey, and D. T. Gibson, *Biochemistry,* **14,** 575 (1975).

(10) The NMR spectrum (HR 220, DMSO-d_6) of diol epoxide **4b** indicates that the conformer in which the hydroxyl groups are pseudo-equatorial is preferred: H_1 δ 4.31, H_2 3.66, H_3 3.60, H_4 3.99, C_1–OH 5.55, C_2–OH 5.47 with $J_{1,OH}$ = 6.5, $J_{1,2}$ = 9.0, $J_{2,OH}$ = 5.0, $J_{2,3}$ = 1.0, $J_{3,4}$ = 4.5 Hz. The aromatic protons $H_{5,8}$ appear as downfield doublets at δ 7.50 and 7.56 due to the proximate hydroxyl group and oxirane ring. This compound as well as all other new compounds gave acceptable mass spectra either directly or as the trimethylsilyl ethers.

(11) The NMR spectrum (HA 100, CD_3OD) of halohydrin **5b** suggests the isomer in which the C_2–OH and C_3–Br are cis due to the small value of $J_{2,3}$: H_1 δ 4.99, H_2 4.58, H_3 4.22, H_4 4.71, and four aromatic hydrogens at δ 7.20–7.60 with $J_{1,2}$ = 6.3, $J_{2,3}$ = 2.49, and $J_{3,4}$ = 6.3 Hz. The indicated stereochemistry of **5b** is required by the subsequent conversion to **6b.**

(12) The NMR spectrum (HR 220, DMSO-d_6) of the diol epoxide **6b** indicates that the hydroxyl groups are pseudo-axial in this stereoisomer, the conformation in which internal hydrogen bonding between the C_1–OH and the oxirane ring is possible: H_1 δ 4.38, H_2 4.15, H_3 3.69, H_4 3.98, C_1–OH 4.14, and C_2–OH 5.47 with $J_{1,OH}$ = 8.3, $J_{1,2}$ = 3.0, $J_{1,3}$ = 1.5, $J_{2,OH}$ = 5.0, $J_{2,3}$ = 2.5, and $J_{3,4}$ = 4.0 Hz. The upfield shift for C_1–OH and the larger value of $J_{1,OH}$ in **6b** relative to **4b** indicates that intramolecular hydrogen bonding is present in **6b** but may not be as strong as in **1** where J_{H-C-OH} = 11 Hz. Additional proof for the conformation of **6b** is found in the fact that only H_5 at δ 7.55 moves downfield from the aromatic multiplet.

(13) Silylation was conducted with *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce) in DMF for 3 hr at room temperature under argon.

(14) D. T. Gibson, V. Mahadevan, D. M. Jerina, and H. Yagi, *Science,* **189,** 295 (1975).

(15) The *m*-chlorobenzoic acid adduct of **4a** was identified by mass spectrometry after silylation (M^+ 674, chemical ionization with NO gas). The NMR spectrum of **4a** indicated that H_{10} was shifted into the aromatic region (H_{10} δ 7.35) by decoupling experiments. Such a downfield shift is indicative of a benzylic ester in a bay region of the hydrocarbon; i.e., attack occurs at C_{10} of the epoxide. Direction of the opening of the oxirane ring in **3a** parallels the sterol epoxide but not triptolide. H. E. Audier, J. F. Dupin, and Jullien, *Bull. Soc. Chim. Fr.,* 3844 J. J (1968), have established that solvolysis of tetralin 1,2-epoxide occurs at the benzylic position under acidic and basic conditions.

(16) The NMR spectrum (HR 220, $CDCl_3$–CD_3OD) of **5a** showed H_7 δ 5.10, H_8 4.25, H_9 4.28, H_{10} 5.94 and eight aromatic hydrogens at δ 7.80–8.60 with $J_{7,8}$ = 10.0, $J_{8,9}$ = 3.0, and $J_{9,10}$ = 3.0 Hz. The C_{10} –OH is pseudo-axial, thus avoiding severe steric interaction in the "bay region".

(17) The NMR spectrum (HR 220, DMSO-d_6) of **6a** indicates that intramolecular hydrogen bonding may not be as strong as in **6b:** C_7–OH δ 5.25, C_8–OH 5.85, H_7 4.96, H_8 3.99, H_9 3.88, H_{10} 4.98, H_{11} 8.62, and seven aromatic hydrogens at δ 8.0–8.4 with $J_{7,OH}$ = 7.0, $J_{7,8}$ = 6.0, $J_{8,OH}$ = 4.0, $J_{8,9}$ = 1.5, and $J_{9,10}$ = 4.0 Hz. The upfield shift of δ 0.6 for the C_7–OH argues for the intramolecular hydrogen bond when compared to **4a,b** (notes 10 and 24).

(18) The NMR spectrum (HR 220, $CDCl_3$) of the disilyl ether of **6a** indicates that the oxygen substituents at C_7 and C_8 move toward pseudo-equatorial positions once the possibility for intramolecular hydrogen bonding is removed: H_7 δ 5.16, H_8 3.85, H_9 3.80, H_{10} 4.66, H_{11} 8.44, and seven aromatic hydrogens at δ 7.8–8.3 with $J_{7,8}$ = 9.0, $J_{8,9}$ = 2.5, and $J_{9,10}$ = 4.25 Hz.

(19) For a kinetic comparison of the reactions of K-region and non-K-region arene oxides with nucleophiles, see P. Y. Bruice, T. C. Bruice, H. Yagi, and D. M. Jerina, submitted.

(20) Kinetics were conducted under N_2 at 30° in a mixture of 25% ethanol and 75% of buffer which was μ = 0.1 in phosphate and 10^{-4} *M* in EDTA at pH 7.4 and were monitored by loss of absorption at 415 nm due to thiolate.

(21) A. W. Wood, R. L. Goode, W. Chang, W. Levin, A. H. Conney, H. Yagi, P. M. Dansette, and D. M. Jerina, *Proc. Nat. Acad. Sci. USA.,* **72,** 3176 (1975), and studies in progress.

(22) A. H. Conney, A. Wood, W. Levin, A. Y. H. Lu, R. Chang, P. Wislocki, G. M. Molder, P. M. Dansette, H. Yagi, and D. M. Jerina, Abstracts of the

IUPHAR Satellite Symposium on "Active Intermediates: Formation, Toxicity and Inactivation," Turku, Finland, July 1975, p 15. See also W. Levin, A. W. Wood, H. Yagi, P. M. Dansette, D. M. Jerina, and A. H. Conney, *Proc. Nat. Acad. Sci.*, in press.

(23) The biological testing is being done in collaboration with Drs. A. H. Conney, A. Wood, and W. Levin, Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche.

(24) Since submission of the present manuscript, P. B. Hulbert, *Nature (London)*, **256**, 146 (1975), has come to similar conclusions regarding the importance of diol epoxide **6a** for which anchimeric assistance to attack by nucleophiles is possible. In addition, D. J. McCaustland and J. F. Engel, *Tetrahedron Lett.*, 2549 (1975), described a modification of the original synthesis[5] of **3**. Although this procedure also results in impure material based on NMR and mass spectra after silylation, Dr. McCaustland kindly suggested the use of THF as solvent for the oxidation. Pure **4a** results and has the following NMR spectrum (HA 100, DMSO-d_6): C_7–OH δ 5.98, C_8–OH 5.75, H_7 4.83, H_8 4.00, H_9 3.91, H_{10} 5.24, H_6 8.56, H_{11} 8.70, and six aromatic hydrogens at δ 7.96–8.35 with $J_{7,OH}$ = 7.5, $J_{7,8}$ = 9.0, $J_{8,OH}$ = 5.0, $J_{8,9}$ = 1.0, and $J_{9,10}$ = 4.5 Hz. The hydroxyl groups in **4a** occupy mainly pseudo-equatorial positions, and the stereochemistry of **6a** is thus unequivocally established

(25) Once both **4a** and **6a** were available, *tert*-butyl alcohol was found to be sufficiently nonnucleophilic to allow measurement of reaction rates with sodium *p*-nitrothiophenolate (loss of absorbance at 450 nm). Rates were measured at 30° in 3.0 ml of dry *tert*-butyl alcohol plus 0.05 ml of DMSO containing the diol epoxides: **4a** (0.43 M^{-1} sec^{-1}), **6a** (70 M^{-1} sec^{-1}), **4b** (0.01 M^{-1} sec^{-1}), and **6b** (3.3 M^{-1} sec^{-1}). In this solvent, the isomers **6a,b** with stereochemistry similar to triptolide are more than 100-fold more reactive than **4a,b**, presumably due to anchimeric assistance by the benzylic hydroxyl group. Evidence to support this was found by examination of 7,8,9,10-tetrahydrobenzo[*a*]pyrene 9,10-oxide which has a comparatively low rate constant (0.15 M^{-1} sec^{-1}). The hydroxyl groups in **6a** cause a >400-fold increase in rate on reaction with the thiolate.

H. Yagi, O. Hernandez, D. M. Jerina*

National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health
Bethesda, Maryland 20014

Received July 22, 1975

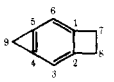
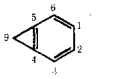
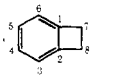
Cyclopropa[4,5]benzocyclobutene[1]

Sir:

Benzene has been bent,[2,3] twisted,[3] and strained.[2-4] As a system it has shown that its properties are remarkably resilient to such treatment. One of the more acute sources of strain has been provided by annelating benzene with small rings. Initial interest was in the fusion of four-membered rings as exemplified by the synthesis of benzo[1,2:4,5]dicyclobutene,[5] but more recently the dramatic success achieved in annelating benzene with a three-membered ring[6,7] has led to a considerable effort in the synthesis of benzocyclopropenes.[4] We would now like to report a further intensification of the strain on benzene by the synthesis of cyclopropa[4,5]benzocyclobutene (**8**),[1] the first compound known in which benzene is annelated by both a three- and a four-membered ring.

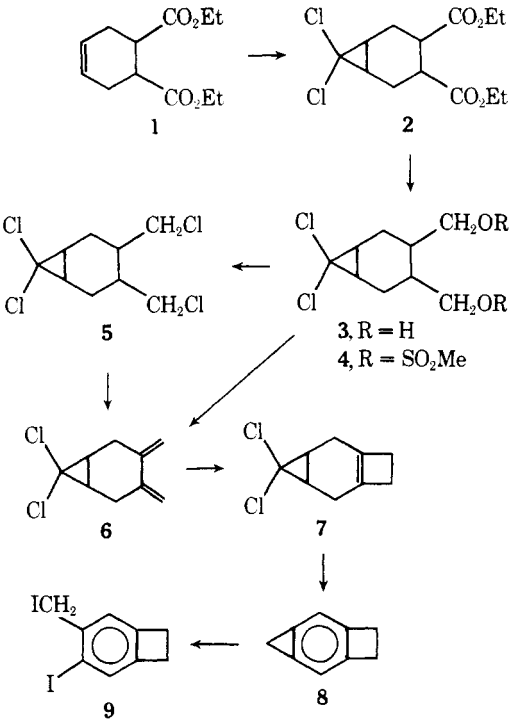
Dichlorocarbene addition to the diester **1** was effected by the phase transfer method[8] using triethylbenzylammonium chloride and gave **2** in 85% yield.[9-11] Reduction of **2** with $LiAlH_4$ in Et_2O for 8 hr gave the diol **3**, mp 75–79°, 66%.[9,10] Treatment of **3** with methanesulfonyl chloride, NEt_3 at 0°[12] for 30 min, gave the dimesylate **4**, mp 99–100°, 80–85%.[9,10] Reaction of the diol **3** with thionyl chloride in boiling pyridine for 20 min gave the tetrachloride **5**, mp 74–75°, 20%.[9,10] When either the dimesylate **4** or the tetrachloride **5** was treated with 3 equiv of KO*t*-Bu in THF at room temperature the diene **6**, bp 50–60°, 0.02 mm, was obtained in 65% yield.[9] The ^{1}H NMR spectrum showed two bands at τ 4.80 and 5.20 due to the exocyclic methylene protons, and the electronic spectrum showed an absorption at 243 nm (ϵ 7000).[13] Photoirradiation of **6** in pentane with an Hanovia 250-W medium pressure lamp through quartz under argon for 8 hr gave the cyclobutene **7**, bp 40–46°, 0.01 mm, in 50% yield.[9,14] The ^{1}H NMR spectrum showed a singlet (τ 7.62) superimposed on a multiplet τ 7.4–8.0, and a multiplet at τ 8.25 in the ratio 4:1, and the ^{13}C NMR spectrum showed bands at 19.4, 25.1, 30.3, 66.3, and 137.4 ppm.[15] Treatment of **7** (100 mg, 0.5 mmol) with KO*t*-Bu (225 mg, 2.0 mmol) in DMSO (1 ml)[16] gave cyclopropa[4,5]benzocyclobutene (**8**) in 30–40% yield.[17] The mass spectrum (20 eV) had *m/e* 116 (M^+, 100%), 115 (M − 1, 95%); high resolution (70 eV) 116.0609 (C_9H_8 requires 116.0625). The ^{1}H NMR spectrum showed only two singlets at τ 3.15 (2 H) and 6.92 (6 H),[18] and the ^{13}C spectrum had five absorptions (see Table I).

Table I. ^{13}C NMR Shifts in **8**, Benzocyclobutene, and Benzocyclopropene[a]

	C-1,2	C-3,6	C-4,5	C-7,8	C-9	Ref
	145.5	110.0	122.8	29.0	19.2	
	128.8	114.7	125.4		18.4	21
	145.2	122.1	125.8	29.5		21

[a] The numbering of benzocyclopropene has been chosen for ease of comparison with 8.

The electronic spectrum (cyclohexane) showed a broad band with maxima at 284 nm (ϵ ca. log 3.0) 287.5 (ϵ ca. log 3.0) and 294 (ϵ ca. log 2.8).[19,20]



The above data are clearly in accord with the assigned structure. A comparison of the ^{13}C spectrum with those of benzocyclopropene[21] and benzocyclobutene[21] is made in Table I. The chemical shifts observed for **8** are very close to those observed in these compounds, except that carbons-3,6 in **8** are at higher field than the corresponding carbon atoms in benzocyclopropene and benzocyclobutene.[22] This upfield shift is presumably due to the increase of strain in **8**.

Treatment of **8** with iodine at room temperature caused cleavage of the cyclopropene ring to give **9**, mp 138–139°.[7,9,10]