Bioactive Marine Metabolites. Part 38. Aurantosides A and B: Cytotoxic Tetramic Acid Glycosides from the Marine Sponge Theonella sp

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Aurantosides A and B: Cytotoxic Tetramic Acid Glycosides from the Marine Sponge *Theonella* sp.[1]

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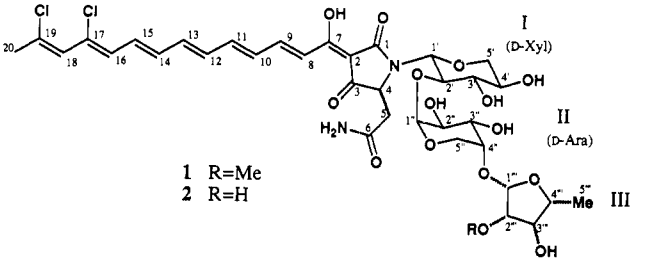
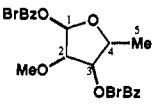
Certain sponges contain microbial symbionts including blue-green algae and bacteria[2] and possess secondary metabolites, some of which may be of microbial origin.[3] A *Theonella* sponge collected off Hachijo Island, from which we isolated bioactive cyclic peptides,[4,5] also contained orange pigments possessing cytotoxic activity. Here we describe the isolation and structure elucidation of the pigments named aurantosides A and B, which are superficially reminiscent of the streptolydigins.[6]

The MeOH extract of the sponge (15 kg) was partitioned between water and ether, and the aqueous phase was extracted with *n*-BuOH. The *n*-BuOH phase was gel-filtered over Sephadex LH-20 with MeOH. The cytotoxic orange band was purified by reverse-phase chromatography[7] to furnish aurantoside A (**1**, 1.3 $\times 10^{-3}$% yield based on the wet weight) and aurantoside B (**2**, 1.5×10^{-3}% yield),[8] both as orange amorphous powders. They are cytotoxic against P388 and L1210 leukemia cells (**1**, IC_{50} 1.8 and 3.4 μg/mL, respectively; **2**, IC_{50} 3.2 and 3.3 μg/mL).

The UV-visible spectrum of aurantoside A was pH-sensitive.[8] **1** showed intense $(M + Na)^+$ cluster ions with small $(M + H)^+$ ion species in the FAB mass spectrum. A molecular formula of $C_{36}H_{46}Cl_2N_2O_{15}$ was established by the FAB mass and NMR spectral data, as well as from combustion analysis. A conjugated hexaene (C8-C20) was inferred from the COSY, HMQC, and HMBC spectra. Assignments for the olefinic protons and Me-20 were straightforward: signals were well separated, and long-range couplings were observed between H16 and H18 and between H18 and Me-20 in the normal COSY spectrum. Two chlorine atoms could be placed on C17 and C19 on the basis of their ^{13}C chemical shifts (δ 129.8 and 137.4). Judging from the ^1H-^1H coupling constants and NOESY data, the double bonds have all-trans geometry.

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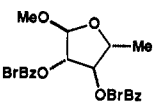
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Two apparent anomeric protons were shown in the ^1H and COSY spectra. Starting from the higher field signal (δ 5.04, d, J = 2.8 Hz), we could deduce an arabinopyranose structure (sugar II): H1″ and H4″ were equatorial, whereas H2″ and H3″ were axial. Another anomeric proton at δ 5.06 (H1‴) was that of 5-deoxypentofuranose (sugar III), which had a methoxy group on C2‴ as revealed by the HMBC spectrum. Interpretation of the NMR data for this unit was unexceptional. Though H1′ and H2′ signals of the sugar unit I were both broad and overlapping, ^1H-^1H coupling constants and NOESY data allowed us to assign the xylopyranose with an axial anomeric proton.[9] This was supported by the COSY and NOESY spectra in CD_3OD at -30 °C, which gave well-separated and sharper signals for H1′ and H2′.

The NMR spectra contained signals for a $CHCH_2$ unit (C4, C5) with a broadened methine proton. Two primary amide protons were observed in DMSO-d_6 which showed NOESY correlations with the C5 methylene protons, suggesting that a primary amide was attached to C5. The remaining portion (C1-C3 and C7) consisted of four nonprotonated carbons, generating broad signals at δ 195.0, 176.1, 174.8, and 102.0, one nitrogen and three oxygens. This constellation was reminiscent of a tetramic acid moiety, in which C4 is incorporated into the five-membered ring. The ^{13}C chemical shifts agreed well with those reported for the relevant portion of streptolydigin.[10] The above-mentioned structural units were connected, on the basis of HMBC spectral data (Table I). Structure **1** was fully consistent with the FAB-MS/MS data.[11]

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(6) (a) Brill, G. M.; McAlpine, J. B.; Whittern, D. *J. Antibiot.* **1988**, *41*, 36-44. (b) Meyers, E.; Cooper, R.; Dean, L.; Johnson, J. H.; Slusarchyk, D. S.; Trajo, W. H.; Singh, P. D. *J. Antibiot.* **1985**, *38*, 1642-1648 and references cited therein.

(7) The orange solids were first applied to an open column of ODS (70-230 mesh) and eluted with 30, 50, 70, 90, and 100% MeOH in water. The 90 and 100% MeOH fractions were combined and subjected to ODS HPLC with MeCN-H_2O (1:1) with 0.1% TFA to afford **1** and **2**.

(8) The name was coined from the Latin word auranticus, which means orange. **1**: amorphous solid, $[\alpha]^{23}_D$ -568° (c = 0.1, MeOH); UV-vis (H_2O) 414 (ϵ 46 700), 242 nm (12 100); UV-vis (0.01 N HCl) 456 (ϵ 32 500), 324 nm (8500); UV-vis (0.01 N NaOH) 412 (ϵ 52 000), 241 nm (11 500); FAB-MS (positive) m/z 843, 841, 839 $(M + Na)^+$, 821, 819, 817 $(M + H)^+$; FAB-MS (negative) m/z 819, 817, 815 $(M - H)^-$; IR (KBr) 3330, 2920, 1650, 1600, 1540, 1390, 1240, 1130, 1060, and 1000 cm^{-1}. **2**: amorphous solid, $[\alpha]^{23}_D$ -492° (c = 0.1, MeOH); UV-vis (H_2O) 414 (ϵ 49 100), 242 nm (10 900); UV-vis (0.01 N HCl) 456 (ϵ 36 100), 301 nm (9800); UV-vis (0.01 N NaOH) 412 (ϵ 56 200), 242 nm (10 900); FAB-MS (positive) m/z 829, 827, 825 $(M + Na)^+$, 807, 805, 803 $(M + H)^+$; FAB-MS (negative) m/z 805, 803, 801 $(M - H)^-$; IR (KBr) 3320, 2900, 1650, 1610, 1540, 1390, 1230, 1130, 1070, 1040, and 995 cm^{-1}.

(9) Judging from the coupling constants, H2′, H3′, and H4′ were all axial, so that this unit must be xylopyranose. In the NOESY spectrum, a strong correlation was observed between H5′a and a broad signal at δ 4.52 (H1′ and H2′). H5′a and H2′ project axially in opposite directions from the tetrahydropyran ring; therefore, this cross peak was assignable to the NOE between H5′a and H1′.

(10) Lee V. J.; Rinehart, K. L., Jr. J. *Antibiot.* **1980**, *33*, 408-415.

(11) The pseudomolecular ion peak at m/z 817 gave rise to ions at m/z 685, 555, and 423, which were generated by the cleavage of the three glycosidic bonds. Though the NMR data with broad ^1H and ^{13}C signals indicate that aurantoside A exists as a mixture of four possible tautomers (2,3 enol or 2,7 enol with cis or trans 2,7 bond), X-ray study of a tetramic acid (Nolte, M. J.; Steyn, P. S.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1057-1065) suggests that the tautomer depicted in formula **1** is the predominant one.

0002-7863/91/1513-9690$02.50/0

Table I. NMR Data for Aurantosides A and B (MeOH-d_4)

	aurantoside A (1)				aurantoside B (2)		
	^{13}C mult	^{1}H mult	J, Hz	HMBC (C no.)	^{13}C mult	^{1}H mult	J, Hz
1	174.8 s				174.9 s		
2	102.0 s				102.0 s		
3	195.0 s				195.1 s		
4	65.5 d	4.33 br			65.3 d	4.32 br	
5a	38.5 t	2.67 dd	7.0, 16.0	4, 6	38.1 t	2.67 dd	7.2, 16.0
5b		2.79 dd	4.5, 16.0	3, 4, 6		2.79 dd	4.1, 16.0
6	174.3 s				174.3 s		
7	176.1 s				176.2 s		
8	122.1 d	7.21 br d	15.1		122.0 d	7.22 br d	15.4
9	146.4 d	7.59 dd	10.9, 15.1	10, 11	146.5 d	7.61 dd	11.4, 15.4
10	133.4 d	6.59 dd	10.9, 14.6	8, 9, 12	133.4 d	6.61 dd	11.4, 14.5
11	145.2 d	6.87 dd	11.2, 14.6	12, 13	145.2 d	6.88 dd	11.3, 14.5
12	135.8 d	6.56 dd	11.2, 14.5	10, 11, 13	135.7 d	6.57 dd	11.3, 14.6
13	140.1 d	6.68 dd	11.1, 14.5	12, 15	140.1 d	6.69 dd	11.2, 14.6
14	137.6 d	6.59 dd	11.1, 14.6	12, 16	137.6 d	6.61 dd	11.2, 14.5
15	132.0 d	6.76 dd	10.7, 14.6	14, 16, 17	131.9 d	6.77 dd	10.7, 14.5
16	131.5 d	6.44 d	10.7	14, 15, 18	131.4 d	6.45 d	10.7
17	129.8 s				129.7 s		
18	127.8 d	6.32 s		16, 17, 19	127.8 d	6.33 s	
19	137.4 s				137.4 s		
20	23.6 q	2.38 d	1.1	18, 19	23.5 q	2.39 br s	
1′	86.5 d	4.52 br			86.0 d	4.52 br	
2′	81.5 d	4.52 br			81.1 d	4.52 br	
3′	79.2 d	3.48 dd	9.0, 9.0	2′, 4′	79.2 d	3.48 t	9.0
4′	70.4 d	3.63 ddd	5.4, 9.0, 10.5	3′	70.5 d	3.62 m	
5′a	69.4 t	3.22 dd	10.5, 11.3	3′, 4′	69.2 t	3.21 t	11.0
5′b		3.88 dd	5.4, 11.3	1′, 3′, 4′		3.88 m	
1″	103.8 d	5.04 d	2.8	2′, 3″, 5″	103.9 d	5.04 d	2.5
2″	71.5 d	3.79 dd	2.8, 9.4	3″	71.7 d	3.79 dd	2.5, 9.4
3″	70.7 d	3.77 dd	2.9, 9.4	2″	70.7 d	3.77 m	
4″	75.9 d	3.90 ddd	1.4, 2.9, 3.9	2″, 3″, 1‴	76.5 d	3.92 m	
5″a	61.4 t	3.57 dd	3.9, 12.6	1″, 3″, 4″	61.9 t	3.61 m	
5″b		3.71 dd	1.4, 12.6	1″, 4″		3.75 m	
1‴	98.7 d	5.06 d	4.4	4″, 3‴, 4‴	100.7 d	4.92 d	4.7
2‴	87.2 d	3.65 dd	4.4, 7.9	4‴, OMe	78.9 d	3.88 m	
3‴	79.7 d	3.88 dd	6.6, 7.9	2‴, 4‴	79.5 d	3.76 m	
4‴	79.5 d	3.74 dq	6.6, 6.4	1‴, 3‴	81.1 d	3.78 m	
5‴	20.9 q	1.31 d	6.4	3‴, 4‴	20.7 q	1.31 d	6.0
OMe	58.3 q	3.35 s		2‴			

The absolute stereochemistry of arabinose and xylose were determined to be D by GC analysis of the hydrolysis product on a Chirasil Val III column.[12] Acid hydrolysis of **1**, followed by *p*-bromobenzoylation, yielded the 1,3-bis-*O*-(*p*-bromobenzyl) derivative of the 5-deoxypentose (**3**).[13] ^{1}H NMR coupling constants and NOE data indicated that this sugar unit was 5-deoxy-2-*O*-methylarabinofuranose.[14] $NaIO_4/KMnO_4$ oxidation of **1**, followed by acid hydrolysis, provided L-Asp as identified by HPLC after derivatization with Marfey's reagent,[15] thereby revealing that aurantoside A had 4*S* stereochemistry.

NMR spectra of aurantoside B, which is more polar than **1**, were almost superimposable on those of aurantoside A, except for the absence of a methoxy signal. Interpretation of the NMR and FAB mass data indicated that **2** was the 2‴-des-*O*-methyl derivative of aurantoside A.[16] Acidic methanolysis, followed by *p*-bromobenzoylation and HPLC separation, afforded 1-*O*-methyl-2,3-bis-*O*-(*p*-bromobenzoyl)-5-deoxyarabinose (**4**).[17] The CD spectrum of **4** exhibited a negative exciton split, indicating that this sugar unit was in the D form.[18] Thus, it is most likely that 5-deoxy-2-*O*-methylarabinose in **1** is in the D form.

Aurantosides are the first tetramic acid glycosides isolated from a marine organism. While the tetramic acid moiety is reminiscent of some terrestrial microbial metabolites,[19] the acetamide and 14-carbon side chains as well as the *N*-trisaccharide-derivatized tetramic acid are unique structural features of the aurantosides.[21]

Acknowledgment. We thank Professor P. J. Scheuer, University of Hawaii, for editorial comments and Ms. C. Nohara and Dr. Y. Numazaki, Central Research Laboratories of Yamanouchi Pharmaceutical Co., Ltd., for cytotoxicity tests. We are indebted to Dr. N. Morisaki and Professor S. Iwasaki, Institute of Applied Microbiology of the University of Tokyo, for the FAB-MS/MS analysis and to Dr. T. Kusumi, Tsukuba University, and Dr. K. Watanabe, Tokyo College of Pharmacy, for preliminary NMR

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(13) ^{1}H NMR data for 3 ($CDCl_3$): δ 6.48 (s, H1), 5.08 (d, J = 3.3 Hz, H3), 4.51 (dq, J = 3.3, 6.6 Hz, H4), 4.10 (s, H2), 3.55 (3 H, s, 2-OMe), 1.49 (3 H, d, J = 6.6 Hz, H_35). Aromatic protons are omitted.

(14) Stevens, J. D.; Fetcher, H. G., Jr. *J. Org. Chem.* **1968**, *33*, 1799–1805. Small coupling constants between H1 and H2 and between H2 and H3 in **3** indicated that both H1/H2 and H2/H3 are trans. An NOE observed for H1 on irradiation of Me-5 showed that they were on the same face of the molecule. ^{1}H-^{1}H coupling constants of the 5-deoxy-2-*O*-methylarabinose residue in aurantoside A revealed that H1‴ and H2‴ are cis.

(15) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.

(16) 2D NMR data (COSY, ROESY, HMQC, and HMBC) supported the structure **2** for aurantoside B. C4 stereochemistry and the configuration of Ara and Xyl residues were established as in the case of **1**.

(17) ^{1}H NMR data for 4 ($CDCl_3$): δ 5.40 (s, H2), 5.14 (d, J = 5.0 Hz, H3), 5.05 (s, H1), 4.34 (dq, J = 5.0, 6.5 Hz, H4), 3.44 (3 H, s, 1-OMe), 1.51 (3 H, d, J = 6.5 Hz, H_35). Aromatic protons are omitted. CD spectrum (MeCN): 251 ($\Delta\epsilon$ = −27.2), 240 (0), 234 (+6.9) nm.

(18) Nakanishi, K.; Kuroyanagi, M.; Nambu, H.; Oltz, E. M.; Takeda, R.; Verdine, G. L.; Zask, A. *Pure Appl. Chem.* **1984**, *56*, 1031–1048.

(19) Aurantosides have a conjugated polyene structure similar to the structures of erythroskyrine, lipomycins, and oleficin,[20] none of which are chlorinated.

(20) (a) Shoji, J.; Shibata, S.; Sankawa, U.; Taguchi, H.; Shibanuma, Y. *Chem. Pharm. Bull.* **1965**, *13*, 1240–1246. (b) Schabacher, K.; Zeeck, A. *Tetrahedron Lett.* **1973**, 2691–2694. (c) Gyimesi, J.; Mehasfalvi-Vajna, Zs.; Horváth, Gy. *J. Antibiot.* **1978**, *31*, 626–627.

(21) After we submitted this paper, a tetramic acid from a marine sponge was reported. Gunasekara, S. P.; Gunasekera, M.; McCarthy, P. *J. Org. Chem.* **1991**, *56*, 4830–4833.

measurements. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Supplementary Material Available: 1D and 2D NMR spectra for **1** and **2** (13 pages). Ordering information is given on any current masthead page.

Automerization of Naphthalene. New Evidence Consistent with the Intermediacy of Benzofulvene[1]

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We first reported the thermal interconversion of [α-^{13}C]-naphthalene (**1**α) and [β-^{13}C]naphthalene (**1**β) in 1977[2] and have subsequently observed the 1,2-scrambling of carbon atoms at high temperatures in a variety of other aromatic hydrocarbons,[3-8] as well as in benzene-$^{13}C_2$.[9] Considerable evidence suggests that the mechanism of these "automerization" reactions and related thermal rearrangements of benzenoid hydrocarbons involves reversible contraction of a benzene nucleus to a five-membered-ring intermediate.[7,10,11] Herein we present new evidence that deposes the previously proposed carbene **2** as a candidate for that intermediate in favor of benzofulvene (**3**); Scheme I depicts plausible pathways to **3** via ring contractions of carbenes **4** and **5**.

R. F. C. Brown et al. have reported that flash vacuum pyrolysis (FVP) of benzofulvene gives naphthalene as the exclusive product.[12] We have now repeated this reaction using isotopically labeled benzofulvene, enriched to 99% ^{13}C in the methylene group (**3**).[13] At 900 °C/10^{-3} Torr, labeled benzofulvene **3** yields [α-^{13}C]naphthalene (**1**α) and [β-^{13}C]naphthalene (**1**β) in a ratio of 21:79 ± 3.[14,15] Control experiments (repyrolysis of the labeled naphthalene mixture) confirm that **1**α and **1**β do not interconvert (≤3%) under these conditions.[16]

We believe that the disparate product distribution in this experiment argues strongly against carbene **2** as an intermediate *on the pathway from benzofulvene to naphthalene*. Our reasoning is as follows: At 900 °C, a kinetically controlled product ratio of 21:79 requires a difference in free energy of activation ($\Delta\Delta G^{\ddagger}$) of ca. 3.1 kcal/mol between two competing pathways.[15] If carbene **2** were the intermediate in the aromatization of benzofulvene (Scheme I), then these two competing pathways would be the aryl shift (**2** → **1**α) and the vinyl shift (**2** → **1**β).[17] Such rearrangements of carbenes, however, are extremely exothermic reactions with very low energy barriers (Figure 1A)[18] and should be characterized by quite early (carbene-like) transition states.[19] Given two equally exothermic pathways (**2** → **1**α vs **1**β),[20] both with very low energy barriers, it seems highly unlikely that they could differ in $\Delta G^{\ddagger}$ by as much as 3.1 kcal/mol. Thus, carbene **2** appears improbable as an intermediate *on the pathway from benzofulvene to naphthalene*.

Brown et al. originally proposed an alternate pathway for the aromatization of benzofulvene via carbene **4** (Scheme 1).[12] This mechanism would certainly account for the minor product (**1**α) we obtain from labeled benzofulvene **3**, and the major product (**1**β) could presumably arise by an analogous pathway via carbene **5**. It would be reasonable to assume that the initial ring expansions, rather than the subsequent hydrogen shifts, represent the rate-limiting steps on these two competing pathways.

This proposal has the virtue that the product-determining branch point in the mechanism involves highly endothermic reactions with very high energy barriers (Figure 1B) that should be characterized by quite late (carbene-like) transition states.[19] These two competing pathways, which lead to different carbenes, could more easily differ in $\Delta G^{\ddagger}$ by 3.1 kcal/mol. Indeed, Dewar and Merz place carbene **5** lower in energy than carbene **4** by 3.4 kcal/mol on the basis of MNDO calculations.[21] This calculated difference in energy not only qualitatively predicts the preferential formation of **1**β from **3** via carbene **5** but even agrees quantitatively with the product ratio we observe.

If benzofulvene isomerizes to naphthalene via the six-membered-ring carbenes **4** and **5**, as we now propose, rather than via the indenyl carbene **2**, then a strong case can be made that the automerization of naphthalene is also more likely to proceed via **4**, **5**, and benzofulvene rather than via simple ring contraction to **2**. A crucial element of this argument is the postulate that the transition state between **2** and **3** lie lower in energy than the transition states separating **2** from either **1**α or **1**β. This seems reasonable, since 1,2-hydrogen shifts in carbenes almost always occur more readily than 1,2-carbon shifts.[18] Furthermore, Kjell and Sheridan have actually observed that carbene **2**, when generated in a frozen matrix at low temperatures, rearranges exclusively to benzofulvene and gives no naphthalene.[22] Conse-

† Visiting scholar from Sharif University of Technology, Tehran, Iran.
‡ Undergraduate summer research student from Northwestern University.

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(13) Benzofulvene enriched to 99% ^{13}C in the methylene group (**3**) was synthesized by (a) quenching the lithium salt of indene in ether with $^{13}CO_2$ generated from 99%-^{13}C-enriched $BaCO_3$, (b) esterification of the resulting indenecarboxylic acid ($SOCl_2/CHCl_3$, then EtOH/THF), (c) reduction of the ester with $LiAlH_4/AlCl_3/Et_2O$, and (d) dehydration of the indenylmethanol in benzene at 10 °C with methanesulfonyl chloride (1.0 equiv) and triethylamine (9 equiv). The indene derivatives in this synthetic sequence are known compounds in their unlabeled form: see ref 12 and citations therein.
(14) Flash vacuum pyrolyses were conducted in a commercially available Trahanovsky pyrolysis apparatus purchased from Kontes, Inc., Vineland, NJ 08360. Polymerization of **3** in the sample chamber was suppressed by matrix-isolating the material in frozen benzene at 0 °C; a portion of the benzene dimerizes to biphenyl in the pyrolysis tube. The pyrolysate was doubly sublimed prior to quantitative ^{13}C NMR analysis; the appropriate correction was made for unequal α and β ^{13}C signal intensities in the NMR spectrum of unenriched naphthalene.
(15) The 900 °C temperature we report here is the thermocouple reading inside the oven at the midpoint of, but external to, the pyrolysis tube.
(16) The automerization of naphthalene has a half-life of approximately 2 s at 1035 °C in a nitrogen flow system.[2]
(17) Cyclization of carbene **2** to naphthvalene would not account for formation of **1**α.
(18) Tomioka, H.; Ueda, H.; Kondo, S.; Izawa, Y. *J. Am. Chem. Soc.* **1980**, *102*, 7817-7818. Stevens, I. D. R.; Liu, M. T. H.; Soundararajan, N.; Paike, N. *Tetrahedron Lett.* **1989**, *30*, 481-484. Moss, R. A.; Ho, G.-J. *Tetrahedron Lett.* **1990**, *31*, 1225-1228. Moss, R. A.; Ho, G.-J. *J. Am. Chem. Soc.* **1990**, *112*, 5642-5644. Evanseck, J. D.; Houk, K. N. *J. Am. Chem. Soc.* **1990**, *112*, 9148-9156 and references cited therein.
(19) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334. For a modern discussion of the Hammond postulate, see: Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*, 3rd ed., Plenum Press: New York, 1990; Part A, pp 211-215.
(20) The thermodynamic product ratio **1**α:**1**β is essentially 50:50.[2]
(21) Dewar, M. J. S.; Merz, K. M., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 5146-5153. The tricyclic tetraenes proposed by these authors as intermediates separating benzofulvene from carbenes **4** and **5** not only are superfluous but also are incompatible with the experimental observation that naphthalene (with a greater total resonance energy) automerizes significantly more readily than does benzene.[9]

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