Chart I

	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
1:	Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-$^{\alpha}$Gln-Phol
2:	Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-$^{\alpha}$Gln-Phol
3:	Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Ala-Phol
4:	Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Phol
5:	Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phol
6:	Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Pro-Aib-Pro-Phol
7(ZIA):	Ac-Trp-Ile-Glu-Iva-Val-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
8(ZIB):	Ac-Trp-Val-Glu-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
9(ZIB'):	Ac-Trp-Ile-Glu-Aib-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
10(ZIC):	Ac-Trp-Ile-Glu-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
11(ZIIA):	Ac-Trp-Ile-Gln-Aib-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
12(ZIIB):	Ac-Trp-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
13(ZII-1):	Ac-Trp-Ile-Gln-Aib-Val-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
14(ZII-2):	Ac-Trp-Ile-Gln-Aib-Ile-Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
15(ZII-3):	Ac-Trp-Val-Gln-Aib-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
16(ZII-4):	Ac-Trp-Ile-Gln-Iva-Val-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol
17(ZII-5):	Ac-Trp-Ile-Gln-Iva-Ile-Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol

component, zervamicin IIA, can be deduced from the FAB mass spectrum, which indicates that the Iva at position 4 of zervamicin IIB is replaced by Aib (Scheme I).

Partial hydrolysis of zervamicin IIB in trifluoroacetic acid, a procedure we earlier showed to cleave selectively Aib–Hyp and Aib–Pro bonds,[4-7,9] yielded several oligopeptides which were separated by HPLC (methanol–water–acetic acid, 70:29:1) and identified by GC/MS of the derivatized total acid hydrolyzate. The oligopeptides included the nonapeptide Ac-Trp-Ile(Leu)-Gln-Iva-Ile(Leu)-Thr-Aib-Leu(Ile)-Aib (containing no Hyp), the dodecapeptide Ac-Trp-Ile(Leu)-Gln-Iva-Ile(Leu)-Thr-Aib-Leu-(Ile)-Aib-Hyp-Gln-Aib (containing 1 Hyp), and the tetradecapeptide Ac-Trp-Ile(Leu)-Gln-Iva-Ile(Leu)-Thr-Aib-Leu(Ile)-Aib-Hyp-Gln-Aib-Hyp-Aib (containing 2 Hyp's). The dipeptide fragments Hyp-Aib and Pro-Phol and the tripeptide Hyp-Gln-Aib were also detected as major components of the partial hydrolyzate by GC/MS following derivatization.[4] These results confirm the location of Hyp at positions 10 and 13, Pro at 15, Gln at 11, and Aib at 9, 12, and 14. Partial hydrolysis of zervamicin IIB in 8.0 N hydrochloric acid–methanol also proved useful, since the tetrapeptide Thr-Aib-Leu(Ile)-Aib was identified by GC/MS of the derivatized hydrolyzate, confirming the proposed sequence at positions 6–9.

The remaining problem, concerning the location of the Ile and Leu residues in these peptides, was solved by studying several minor components of the zervamicin complexes. Hydrolysis, derivatization, and GC/MS indicated the amino acid compositions shown in Table I, and FABMS gave molecular ions and characteristic ions for several amino acids as well as fragment ions indicating isomorphous replacement, as shown in Table II. Particularly significant are the amino acid compositions of zervamicins IB and II-3 in which Val replaces one Ile residue of zervamicins IC, IIA, and IIB (Table I) and which can be shown from FAB fragment ions (Table II) to be the amino acid at position 2, i.e., Ile should be at position 2 in zervamicins IC, IIA, and IIB. Similarly, in zervamicins IA, II-1, and II-4 Val replaces an Ile of zervamicins IC, IIA, and IIB (Table I) which should be at position 5 (Table II), while in zervamicins II-2 and II-5 Val replaces a Leu residue of zervamicins IC, IIA, and IIB (Table I) which should be at position 8 (Table II).

As noted at the outset, zervamicin II shows considerably reduced membrane pore-forming ability and considerably enhanced antibacterial activity compared to alamethicins, antiamoebins, and emerimicins III and IV. Comparing the sequences of the zervamicins with those of **1**–**6** (and most particularly of **5** and **6**), there are obvious regions of close similarity of zervamicins with the antiamoebins (amino acids 7–16), as well as regions of difference (amino acids 1–6). Interchange of the nonpolar amino acids Aib, Iva, Leu, and Ile is not regarded as of primary importance nor is chain shortening, as in emerimicins III and IV (**3** and **4**). Which of the major replacements (Trp-Phe, Thr-Gly, Gln-Aib) is (are) responsible for these major alterations of bioactivity will be the subject of future reports. It should be noted, however, that all of the replacements are in the direction of forming a more polar N terminus in the zervamicin antibiotics (vs. **3**–**6**). The present communication shows that relatively minor variations in amino acid composition and sequence have profound effects and that these and related antibiotics can probably be studied optimally by FABMS combined with the previously employed[4-9] GC/MS techniques.

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Hydrogenation on the Hindered Face of *syn*-Sesquinorbornene Photosensitized by Acetone

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In addition to unusual steric effects in the reactions of syn[1] and anti[2] isomers of sesquinorbornene, it has recently been found by X-ray crystallography[3a] that two derivatives of *syn*-sesquinorbornene (**1**) show a hingelike bending of the double bond with a dihedral angle of 162–164° between the plane of carbon atoms 4a, 8a, 1, 4 and that of atoms 4a, 8a, 5, 8. Careful examination of the intermolecular interactions suggested no way in which the deformation could be due to forces between molecules within the crystal.[3]

(1) Paquette, L. A.; Carr, R. V. C; Böhm, M. C.; Gleiter, R. *J. Am. Chem. Soc.* **1980**, *102*, 1186, 7218.
(2) Bartlett, P. D.; Blakeney, A. J.; Kimura, M.; Watson, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 1383.
(3) (a) Watson, W. H.; Galloy, J.; Bartlett, P. D.; Roof, A. A. M. *J. Am. Chem. Soc.* **1981**, *103*, 2022. (b) Pinkerton, A. A.; Schwarzenbach, D.; Stibbard, J. H.; Carrupt, P.-A.; Vogel, P. *J. Am. Chem. Soc.* **1981**, *103*, 2095.

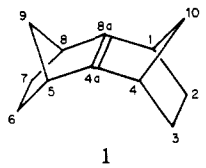
0002-7863/81/1503-6520$01.25/0

Table I. Photosensitized Reactions of Sesquinorbornene

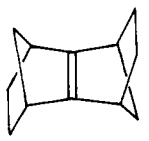
expt	sesquinorbornene isomer, M	solvent	sensitizer[b]	T, °C	irradn time, h	% reaction	products	yield, %
1	anti (0.05)	acetone	acetone	20	6	100	**5a**	54[a]
							5b	11[a]
2	syn (0.04)	acetone	acetone	15	2	100	**3b**	55[a]
							4b	3.1
							4a	25[a]
							3a	2.2
3	syn (0.125)	acetone-d_6	acetone-d_6	15	6	85	**3b**-d_6	50
							4a-d_2	20
							3a-d_2	1
							4b-d_6	5
							epoxide	2
4	syn (0.016)	acetone + O_2	acetone	10	2.5	5	no **3** or **4**	

[a] Isolated yield; others by VPC. [b] Irradiation of 1 in the absence of sensitizer in cyclohexane for 6 h or in benzene for 45 h produced no **3a** or **4a**.

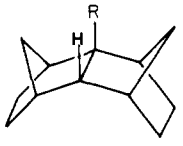
Chart I



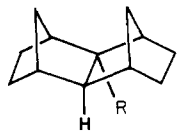
1



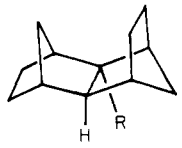
2



3a, R = H
b, R = CH_3COCH_2



4a, R = H
b, R = CH_3COCH_2



5a, R = H
b, R = CH_3COCH_2

We now find that in photochemical reaction with acetone the sesquinorbornenes, like norbornene,[4-11] yield acetonyl addition products and hydrogenation products, but with *syn*-sesquinorbornene the hydrogen addition occurs mainly on the endo face of the double bond, which has not been seen to be attacked in any thermal reaction.

Table I lists the results of a series of these photoreactions, performed under irradiation in Pyrex with the unfiltered light of a 450-W medium-pressure Hanovia arc lamp for the times indicated. The major products were characterized by ^1H and ^{13}C NMR, infrared, and mass spectrometry. Compound **3a** (Chart I) was identical with the thermal reaction product from **1** and diimide, whose structure and configuration were established by Paquette and co-workers.[1] Compound **4a**, shown to be isomeric with **3a** by its mass spectrum, has the same symmetry as **3a** (only four peaks in the ^{13}C NMR spectrum), which unambiguously establishes its structure and configuration. Of the pair of acetonyl adducts **3b** and **4b**, **3b** was the exclusive product of acetone addition initiated by di-*tert*-butyl diperoxyoxalate, placing this isomer in the family of thermal products which have an unbroken record of exo,exo orientation. Compound **4b**, present in too small an amount for complete characterization, was shown to be an isomer of **3b** by its mass spectrum. Compounds **5a** and **5b**, having no stereoisomers, are fully characterized by their mass and ^{13}C NMR spectra (7 peaks for **5a** and 15 peaks for **5b**). In addition to the products in Table I, the presence of 2,5-hexanedione was established by mass spectrometry, confirming the involvement of acetonyl radicals in the addition reactions.

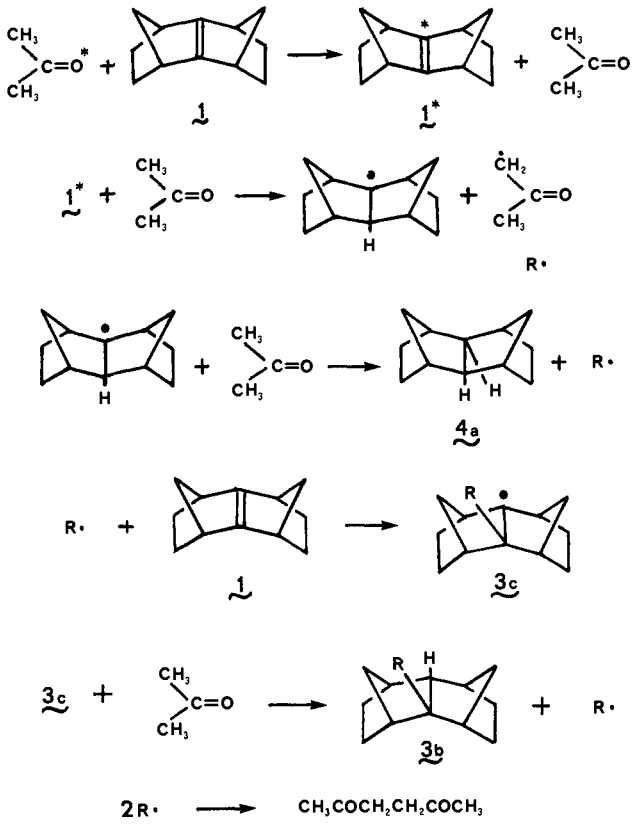
A rational account of these reactions must explain the following features: (1) Thermal additions to *syn*-sesquinorbornene lead exclusively to reaction on the exo face (between the methylene bridges). (2) The light-initiated acetone addition to the double bond occurs predominantly exo. (3) The hydrogenation accompanying acetone addition occurs overwhelmingly (as much as 20:1) on the hindered endo face of the double bond. (4) The photochemical additions and hydrogenations alike are inhibited by molecular oxygen.

Preferred exo attack on **1** is to be expected in any case from the lesser hindrance imposed by the methylene compared to the ethylene bridges. The observation of the bend in the double bond of ground-state *syn*-sesquinorbornene, however,[3] shows that the exo stereoselectivity is the result not only of the greater openness that would exist with a planar double-bond system but of an actual spreading apart (to about 4 Å) of H(9en) and H(10en), with the accompanying inevitable partial rehybridization at the double bond, resulting in greater electron density on the exo than on the endo face. What happens in the excited state to bring about this emphatic reversal in selectivity? Mere increased energy content in the excited state would not do it; there has to be a specific geometrical change in the excited state to produce the strong endo preference or indeed even to make endo addition possible.

Although we must not underrate the subtlety of all the effects that make up the character of the double bond, we would point out here that a simple hypothesis of the steric demand of occupied bonding and antibonding orbitals will give a statisfactory general account of the present photochemistry. A bonding π orbital exerts a space demand which is as great midway between the carbon atoms as at the ends. The double bond of **1** in the ground state therefore exerts a steric force on H(9en) and H(10en) which may be the chief origin of the distortion seen in the *syn*-sesquinorbornene ring system. In the excited state an electron is promoted to an antibonding π orbital, which has minimum electron density at the middle and maximum above and below the carbon atoms. The balance of forces in the excited state is therefore such as to push harder on the four endo hydrogen atoms of the ethylene bridges relative to the ground state. If we were to assume that this gave the excited-state double bond a bend in the opposite

(4) Sauers, R. R.; Schinski, W.; Mason, M. M. *Tetrahedron Lett.* **1967**, 4763.
(5) For a series of ketones reacting photochemically with norbornene, the competition between oxetane formation and energy transfer depends critically on the energy level of the ketone triplet. See: Arnold, D. R. *Adv. Photochem.* **1968**, *6*, 301–423. Saltiel, J.; Neuberger, K. R.; Wrighton, M. *J. Am. Chem. Soc.* **1969**, *91*, 3658. Reusch, W. *J. Org. Chem.* **1962**, *27*, 1882.
(6) Kropp, P. J. *J. Am. Chem. Soc.* **1967**, *89*, 3650.
(7) Scharf, H. D. *Fortschr. Chem. Forsch.* **1968**, *11*, 216.
(8) Arnold, D. R.; Abraitys, V. Y. *Mol. Photochem.* **1970**, *2*, 27.
(9) Schroeter, S. H. *Mol. Photochem.* **1972**, *4*, 473.
(10) Cristol, S. J.; Micheli, R. P.; Lee, G. A.; Rodgers, J. E. *J. Org. Chem.* **1975**, *40*, 2179.
(11) Paulson, D. R.; Murray, A. S.; Fornoret, E. J. *J. Org. Chem.* **1978**, *43*, 2010.

Scheme I



direction from that in the ground state,[12] we should conclude that products arising from a free excited olefin molecule would be predominantly endo, while those arising from a ground-state initiated sequence would be exo. In the following scheme, it is postulated that excited triplet acetone transfers its energy to *syn*-sesquinorbornene[5,13] and that this excited olefin, in two successive captures of hydrogen from the solvent, is converted into the *endo*-dihydride **4a**. The acetonyl radicals formed in this process propagate a chain reaction with ground state **1**, leading to the exo adduct **3b**. The termination step of this chain reaction forms the coupling product 2,5-hexanedione.

The light intensity delivered to our samples was calibrated by running the photoelimination of propylene from valerophenone, of known quantum yield,[14] in two degassed solvents in the same apparatus as the acetone reaction with **1**. For an initial concentration of **1** of 0.046 M in acetone solvent, the quantum yields of *endo*-dihydride **4a** and *exo*-acetonyl adduct **3b** were 0.76 and 1.25, respectively. The latter quantum yield was more than doubled when the initial concentration of **1** was 0.092 M.

A number of aspects of this reaction are under active investigation, including the effects of a number of variables on the product distribution, the study of other sensitizers, and the structures of some minor products.

The acetonyl adduct **3b** reacts slowly on irradiation in acetone, yielding several new products, chiefly one with 2 mass units less than **3b** (15.5% in 16 h). The presence of 5% of **4a** is evidently a sequel to a Norrish Type II cleavage of the starting material. Although this and other processes are not included in Scheme I, the scheme accounts broadly for the results, including the fact that the adduct **3b** increases in importance, while **4a** and the dione decrease, with increasing initial concentration of *syn*-sesquinorbornene. It is inherent in this mechanism that (as observed) neither the **a** nor the **b** products are formed in the presence of the triplet quencher, O_2, or in the absence of ketone. The 2% of epoxide formed in experiment 3 of Table I suggests incomplete degassing, and also that excited acetone which escapes quenching can sensitize a little epoxidation in competition with the processes of Scheme I.

The following ^{13}C NMR spectra are definitive for identification of the key products: **1**, 151.45, 50.16, 42.75, 25.21; **2**, 153.86, 54.51, 41.39, 26.57; **3a**: 47.95, 46.98, 41.39, 25.27; **3b**, 208.76, 58.02, 55.88, 50.68, 46.72, 46.07, 41.58, 31.58, 25.73, 25.27; **4a**, 53.67, 40.22, 35.22, 30.86; **5a**, 49.96, 41.97, 41.12, 36.06, 34.11, 31.12, 24.23; **5b**, 208.17, 57.69, 53.86, 50.15, 46.84, 41.84, 40.47, 39.63, 36.97, 36.64, 30.66, 29.82, 27.80, 24.81 (two peaks). Chemical shifts are in ppm downfield from Me_4Si.

Acknowledgment. We thank the Robert A. Welch Foundation, the National Science Foundation, and the National Institutes of Health for support of this work.

(12) We have no experimental model of what the selectivity of a *planar syn*-sesquinorbornene double bond would be, but molecular models strongly suggest that actual reversal of the ground-state bend is necessary to produce greater reactivity on the endo face than on the exo.

(13) Such energy transfer has been observed intramolecularly also: Cargill, R.; Damewood, J.; Cooper, H. *J. Am. Chem. Soc.* **1966**, *88*, 1330. Cf.: Engel, P. S.; Ziffer, H. *Tetrahedron Lett.* **1969**, 5181.

(14) Wagner, P. J. *J. Am. Chem. Soc.* **1967**, *89*, 5898.

Avoparcin and Epiavoparcin

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Avoparcin[1] is a commercially important animal feed antibiotic and consists primarily of two closely related glycopeptides, α and β, which are structurally related to vancomycin[2] and ristocetin.[3] Recently we published spectral and degradative evidence which defined the overall structures of these principal components.[4] We present here 270-MHz ^1H NMR studies which have resolved the site of attachment of the chlorine to the triphenyl diether and the orientation of the benzylic sugars in terms of the complete structures **1** and **2** (Scheme I) for α- and β-avoparcin, respectively.[5,6] In addition, we describe an important equilibration

(1) Avoparcin is marketed in Europe under the tradename Avotan.

(2) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature (London)* **1978**, *271*, 223.

(3) (a) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 897. (b) Williams, D. H.; Rajananda, V.; Bojesen, G.; Williamson, M. P. *J. Chem. Soc., Chem. Commun.* **1979**, 906. (c) Sztaricskai, F.; Harris, C. M.; Neszmelyi, A.; Harris, T. M. *J. Am. Chem. Soc.* **1980**, *102*, 7093. (d) Sztaricskai, F.; Neszmelyi, A.; Bognar, R. *Tetrahedron Lett.* **1980**, 2983.

(4) McGahren, W. J.; Martin, J. H.; Morton, G. O.; Hargreaves, R. T.; Leese, R. A.; Lovell, F. M.; Ellestad, G. A.; O'Brien, E.; Holker, J. S. E. *J. Am. Chem. Soc.* **1980**, *102*, 1671.

(5) The avoparcin components used in this work were prepared by extensive preparative HPLC. A Waters Associates (Milford, Mass.) Prep LC System/500 instrument was used with Prep Pak-500/C_{18} cartridges for the solid support. Antibiotic mixtures were adsorbed on the column from a buffer solution consisting of 2.5% acetic acid, 0.08 M ammoniun hydroxide, and 0.01 M sodium heptanesulfonate. Elution was carried out with the same buffer in the presence of 13–17% acetonitrile. The elution was monitored by UV detection at 254 nm. Analytical HPLC was carried out on a Waters Associates C_{18} μ-Bondapak column with the same system as above except that the concentration of acetonitrile was 11.8%.

(6) For the 270-MHz ^1H NMR experiments it was necessary to lyophilize repeatedly D_2O solutions (pH ca. 4) of the various purified preparations. Although this treatment readily exchanged the hydroxyl, phenolic, and amino hydrogens, the amide nitrogen protons were only partially exchanged so that some α-C*H*-*H*NCO couplings could still be observed. The addition of deuteriotrifluoroacetic acid to the NMR sample did of course result in the almost complete exchange of the amide hydrogens as evidenced by the dramatic sharpening of the α-CH signals. Under the conditions in which the spectra were obtained (Me_2SO-d_6, 70 °C, ca. 15 mM), the *N*-methyl grouping is predominantly in the neutral (uncharged) form as evidenced by the downfield shift of the *N*-methyl signal (ca. 0.4 ppm) on acidification. The chemical shifts of protons proximal to the two ristosamine amino groups are not shifted on acidification in Me_2SO-d_6 at "pH 4.0", indicating that the amino sugars are protonated under these conditions. Electrophoretic experiments on **2** at various pH's indicated the isoelectric point to be between 6.5 and 7.0.

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