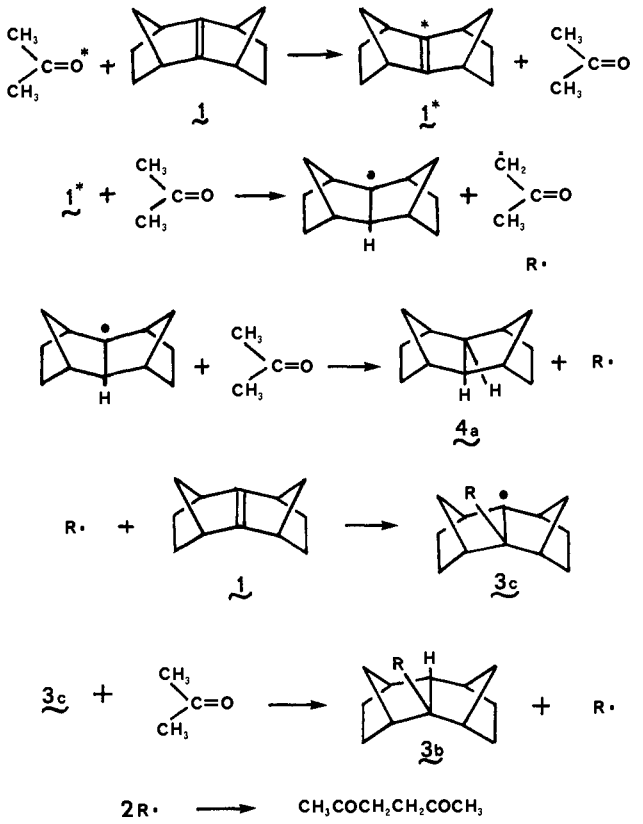
Scheme I



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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Received June 8, 1981

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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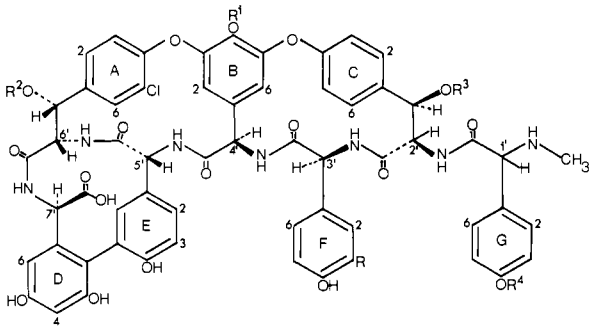
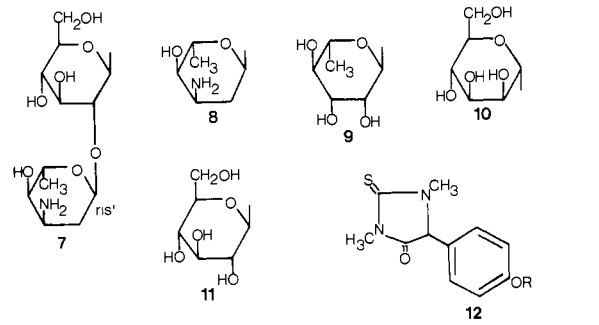
Scheme I



1. α-Avoparcin: R=H; R^1=O-2-α-(ristosaminyl)-O-β-D-glucose (7); R^2=O-α-ristosamine (8); R^3=O-α-D-mannose (10); R^4=O-α-L-rhamnose (9).
2. β-Avoparcin: R=Cl; R^1, R^2, R^3, R^4 same as α.
3. Desristosaminyl α and β-avoparcin; R=H and Cl; R^1=O-β-D-glucose (11); R^2, R^3, R^4 same as α.
4. β-Avoparcin-CDP-I; R=Cl; R^1=R^4=H; R^2=O-α-ristosamine (8); R^3=O-α-D-mannose (10).
5. Mannosyl-β-avoparcin aglycone: R=Cl; R^1=R^2=R^4=H; R^3=O-α-D-mannose (10).
6. Desmannosyl-β-avoparcin: R=Cl; R^1=O-2-α-(ristosaminyl)-O-β-D-glucose (7); R^2=O-α-ristosamine (8); R^3=H; R^4=O-α-L-rhamnose (9).



process which leads to a mixture of unchanged antibiotic and most significantly epiavoparcin, a diastereomer which is 1–2 orders of magnitude less active than β-avoparcin against gram-positive organisms.

Many critical ^{1}H NMR assignments for β-avoparcin (**2**) are given in Table I. These chemical shift assignments were made on the basis of extensive spin-decoupling and nuclear Overhauser experiments (NOE's) and the effect of pH. Of great help, especially with regard to the anomeric proton signals, was the availability of the spectra of compounds **3**–**6** which differ from **1** and **2** only in the number of sugars attached to the aglycon.[7] We were also helped by the work of Kalman and Williams on vancomycin and ristocetin.[2,3] The attachment of a chlorine to either the C- or the N-terminal β-hydroxytyrosine moieties is considered first. The ^{1}H NMR spectra of **1** and **2** show a broad meta-coupled singlet at δ 7.73 which we have assigned to A_6. This is based on its chemical shift value which compares well with that of the identically situated proton on the C-terminal side of the triphenyl unit in vancomycin.[2] Moreover, the chemical shift of this proton is pH independent, and thus we conclude that it is well removed from the basic N-terminal phenylsarcosine moiety. In contrast, proton C_6, the counterpart of A_6 on the N-terminal side, resonates at δ 7.61 in the spectrum of **2** as an ortho- and meta-coupled broad doublet (J = 8.8 and 1.5 Hz). This assignment is based on the low field chemical shift of the corresponding proton in ristocetin[3] which has no aromatic chlorines and contains similar phenylglycine units on this side of the molecule. Proton C_6 is markedly pH dependent and undergoes a downfield shift of 0.22 ppm on acidification and, therefore, must be proximal to the *N*-methyl grouping. thus, on the basis of these arguments, the placement of the chlorine on the triphenyl unit is as shown.

Table I. Proton Chemical Shift[a] Assignments (δ) for β-Avoparcin Together with Certain Corresponding Assignments from the Literature for Ristocetin and Vancomycin[2,3,11]

	chemical shift, δ (J, Hz)		
proton	β-avoparcin	ristocetin	vancomycin
N-CH_3	2.22 s		
1′	4.08 s	4.83	3.69
6′	4.33 d (12, α-NH)	4.38 (12)	4.20 (12)
7′	4.39 d (5, α-NH)	4.55 (5)	4.41 (5)
5′	4.63 d (4.5, α-NH)	4.73 (6.5)	4.49 (5)
Ris H_1	4.78 s		
Man H_1	4.93 s		
2′	5.05 d (4.4)	5.09 (5)	4.86 (5)
A_{bz}	5.11 s	5.17	5.19
B_2	5.25 s	5.37	5.21
3′	5.32 d (10, α-NH	5.25 (10)	4.41 (7)
Rha H_1	5.34 br s		
C_{bz}	5.40 d (4.4)	5.19 (5)	5.19 (5)
Ris′ H_1	5.47 br s		
4′	5.65 d (7.5, α-NH)	5.65 (8.2)	5.70 (8)
Glu H_1	5.65 d (8)	5.28 (7.8)	5.35 (8)
B_6	5.75 s	5.85	5.63
D_6	6.29 d (2.2)	6.32 (2)	6.30 (2)
D_4	6.42 d (2.2)	6.85 (2)	6.44 (2)
E_3	6.71 d (8)	6.77 (8)	6.73 (8)
F_5	6.80 d (8)		
E_2	6.88 dd (8, 2)	6.84 (8, 2)	6.78 (8, 1)
F_6	6.83 dd (8, 2)		
$G_{3,5}$	6.95 dd (9, 1.5)		
F_2	7.04 d (1.5)		
$G_{2,6}$	7.22 dd (9, 1.5)		
C_6	7.61 dd (9, 1.5)	7.86 (8)	7.43 (5)
A_6	7.73 br s	7.44	7.87

[a] 270-MHz ^{1}H NMR spectra in Me_2SO-d_6 at concentrations of ca. 15 mM at 75 °C.

Evidence for placing the benzylic ristosamine on the C-terminal side was obtained by the observation of negative NOE's on A_6 (ca. 20%) and the A-ring benzylic proton, A_{bz} (ca. 15%), by irradiation at δ 4.78, the chemical shift of H_1 of the benzylic ristosamine in the spectrum of **2**. Likewise, irradiation of the resonance at δ 4.93, the chemical shift of the mannose H_1, resulted in a negative NOE (ca. 20%) at the resonance of the C-ring benzylic proton, C_{bz}. In addition, the mannose anomeric proton signal is pH dependent as addition of deuteriotrifluoroacetic acid to the NMR sample resulted in a small but significant downfield shift of ca. 0.15 ppm. Thus, the glycosidation pattern of the benzylic hydroxyls is as shown.

A comparison (Table I) of the chemical shift values and splitting constants of the α-CH and benzylic proton signals of the peptide backbone for avoparcin with those of vancomycin and ristocetin shows good agreement for the common structural moieties and indicates the same relative stereochemistry at these positions except for the N-terminal moiety in avoparcin, which remains uncertain (see below).[8] The *cis*-amide linkage between the fifth and sixth amino acid residues is dictated by the strong negative NOE of

(7) The mannose H_1 signal at δ 4.93 in the spectra of compounds **1**–**5** is absent in that of desmannosyl-β-avoparcin (**6**). The signals attributed to H_1 of glucose and the H_1 of the ristosamine attached to it (ris′ in the structure), as well as the rhamnose H_1, are missing in the spectrum of β-avoparcin–CDP-I (**4**). The rhamnose H_1 signal is assigned on the basis of its chemical shift in the spectrum of the Edman degradation product **12** [R = *O*-α-L-rhamnose (**9**)].

(8) The circular dichroism curve of β-avoparcin (**2**) consists of a relatively symmetrical Cotton effect at 287 nm (Δε −4.5) and two intense Cotton effects of opposite sign at 230 nm (Δε +26) and 210 nm (Δε −76) which are undoubtedly primarily due to the biphenyl moiety.[9] The curve is qualitatively very similar to that of vancomycin[9] and thus suggests that the absolute stereochemistry of the avoparcin antibiotics is as depicted.[2,3] This implies that ring E is orthogonal to ring D with the E_{2-4} carbons below the plane of the paper. The spectrum of **4** is identical with that of **2** except that the two opposite dichroic bands in the far UV are more symmetrical: 287 nm (Δε −5.7), 230 nm (Δε +47), 210 nm (Δε −68). The absence of the phenolic rhamnoside glycosidic linkage in **4** perhaps accounts for this. The CD curves for avoparcin were recorded in water with a Jasco J-40 spectropolarimeter at concentrations of 0.013 and 0.13 mg/mL. We thank Professor K. Nakanishi of Columbia University for these curves.

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about 50% between the 5′- and 6′-methine protons similar to that observed in NOE experiments on vancomycin and ristocetin.[3] Such a relationship was unequivocally demonstrated by the results of the X-ray analysis of vancomycin CDP-I.[2]

The stereochemistry of the glycosidic linkages is as indicated on the basis of a combination of anomeric proton couplings and $^1J(^{13}CH_1)$ values.[10] The glucosidic linkage is β ($J_{1,2}$ = 7.5 Hz) as in vancomycin and ristocetin. The rhamnose glycosidic linkage is α based on the rhamnose C_5 shift of 70.1 Hz and the $^1J(^{13}CH_1)$ of 171 Hz in the ^{13}C NMR of **12** [R = *O*-α-L-rhamnose (**9**)]. An α linkage is also indicated for the two ristosaminyl glycosidic bonds since both anomeric proton signals are broad singlets which preclude an $H_{1,2}$ axial–axial orientation. The mannose linkage can be discerned as α based on the $^1J(^{13}CH_1)$ of 170 Hz in the fully coupled spectrum of 5.

An important feature of the avoparcin chemistry is that aqueous solutions of α- and β-avoparcin lose antibacterial activity when they are heated at 80 °C for 16 h in the pH range 5–8. When monitored by analytical HPLC, an equilibrium mixture is observed consisting of ca. 30% of starting antibiotic and 70% of a diastereomer which we call epiavoparcin and which has a longer retention time in the HPLC profile. Equilibration of purified epi-β-avoparcin yields the same ratio of β-avoparcin and epi-β-avoparcin as observed above. It is especially noteworthy that epi-β-avoparcin is 10–100-fold less active against gram-positive bacteria than avoparcin. We believe this transformation is best explained by an epimerization at the α-methine (1′) of the N-terminal phenylsarcosine subunit. This could prevent effective binding to mucopeptide precursor molecules involved in bacterial cell wall biosynthesis, thus resulting in the greatly reduced activity.[11]

The structure of epi-β-avoparcin is based on the following chemical and spectral evidence. The isomeric nature of epi-β-avoparcin with β-avoparcin was shown by a molecular weight determination using ^{252}Cf-plasma desorption mass spectroscopy.[15] This gave a strong quasi-molecular ion $(M + Na)^+$ at 1965 ± 2 identical to that observed for β-avoparcin. The IR and UV spectra were also identical with those of β-avoparcin. The specific rotation, however, was slightly higher (−110°) compared to the original −94°. The 270-MHz ^1H NMR spectrum of epi-β-avoparcin is generally the same as for β but with some slight chemical shift differences in the aromatic and peptide methine patterns. Most significantly, proton C_6 now resonates on the downfield side of A_6 (δ 7.73 in both α and β) at δ 7.84 from 7.61 and undergoes an upfield shift on protonation. This is exactly opposite to the situation with α- and β-avoparcin and suggests a change in C_6's relationship to the *N*-methyl grouping. Although the chemical shift of methine 1′ (a sharp singlet at δ 4.09) was unchanged, that of methine 2′ was shifted downfield to δ 5.19 from 5.05. The resonance of the remaining peptide methine protons are unchanged from those in β, suggesting a difference only in the N-terminal side of the molecule. This is supported by the slight upfield shift (ca. 0.1 ppm) of the phenylsarcosine aromatic proton doublets $G_{3,5}$ and $G_{2,6}$ to δ 6.87 and 7.15, respectively. There were no changes in the α-CH–NH coupling constants of any of the peptide methine protons, thus ruling out any inversion of an α-CH adjacent to an NHCO grouping. The lability of the 1′-methine proton of avoparcin has been amply demonstrated by several experiments. By subjecting CDP-I avoparcin to a very mild first stage Edman reaction (pH 8.0, 1 h, room temperature, and no acid treatment) two optically inactive thiohydantoins of (*p*-hydroxyphenyl)sarcosine were isolated.[16] One was the normally expected product while the other contained a benzylic hydroxyl group instead of the α-methine proton.[4] In addition when pure β-avoparcin was epimerized in D_2O and the resultant two products isolated, the 270-MHz ^1H NMR spectrum of each lacked the 1′ signal at δ 4.08, confirming complete exchange with deuterium at this position. The partially deuterated β-avoparcin from this experiment exhibited normal optical and biological properties. No other peptide methine protons exchanged. There was some sharpening of the α-CH signals due to further exchange of amide NH's. The aromatic hydrogens on ring D exchanged completely and those ortho to phenolic hydroxyls on rings E and F only partially.

The epimerization at the terminal methine center occurs under identical conditions for all the known avoparcin components. In other words, for each avoparcin component (total isolated to date is 5) there is a corresponding epiavoparcin. Details on all these minor components as well as their epimerized counterparts will be published in a full paper.

Acknowledgment. We thank Professor K. Nakanishi of Columbia University for helpful discussions and P. Demou of Yale University for the 270-MHz ^1H NMR spectral determinations. We also express our thanks to N. Kuck and associates for the microbiological assays.

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(11) Avoparcin is a bacterial cell wall inhibitor[12] as are other members[13,14] of this class of antibiotics which act by binding to mucopeptide precursor molecules which contain the terminal D-Ala-D-Ala fragment.

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(15) Courtesy of Professor R. D. McFarlane of Texas A & M University.

(16) Using the identical Edman conditions, D(−)-(*p*-hydroxyphenyl)glycine gave an optically active thiohydantoin: $[\alpha]^{25}D$ −166° (*c* 0.89, MeOH). This result suggests that the *N*-methyl grouping exerts a labilizing effect on this center in avoparcin. At this point we are uncertain as to the effect of pH on the epimerization since below 5 and above 8 the complexity of the product mixture makes analysis difficult. Avoparcin CDP-I has been isolated from acid hydrolysis (0.14 N HCl) of avoparcin, and it is not epimerized. Of course, this does not rule out the formation of an epimer under these conditions but we have not isolated it.

Optically Active Radical Complexes, ESR–HPLC–OR Study of the Carbonyl(quinone)rhenium–(+)- and (−)-DIOP Radical Complexes

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Received June 1, 1981

For many years it has been a constant challenge to this laboratory to produce and isolate optically active radicals or radical complexes for ESR, optical, and chemical studies. Reactions which involve optically active radical intermediates have been of interest for many years. The configuration of an optically active radical intermediate is often inferred from the product analysis[1-4] or by observations of different optical rotation values for the solution containing both the presumed optically active radical and the parent optically active reactant.[5] With organic radicals, two factors contribute to the difficulty of direct detection of optical activity. The first one is the chemical reactivity of the radicals, and the second one is the probable loss of chirality in the derived carbon radicals due to the change in the tetrahedral symmetry. However, among many organometallic radical reactions induction of optical activity can occur in labile adducts of radical complexes, as compared to the well-known cases of induced optical activity in paramagnetic complexes.[6] Numerous paramagnetic metal

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