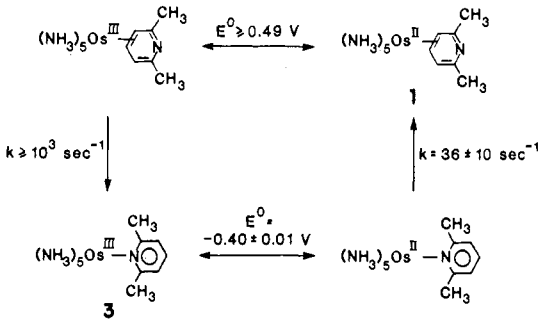
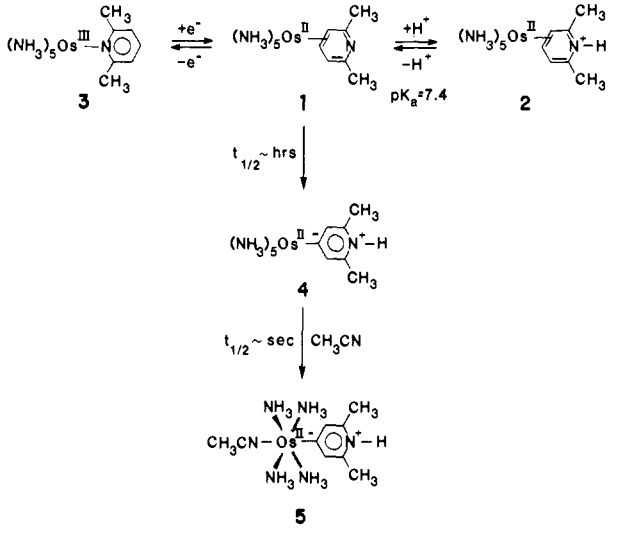
Scheme I



Scheme II



chemically irreversible wave is present at −0.45 V, attributed to the reduction of a new species, **3**. Compound **3** can be generated by chemical oxidation of **1** and persists in DME solution for several hours. Reduction of **3** regenerates **1** quantitatively, suggesting a reversible redox-induced linkage isomerization. Given that $E°$ for the 3+/2+ couples of the pyridine and 3-picoline complexes under the same conditions is approximately −0.45 V,[10] we postulate that **3** is a nitrogen-bound 2,6-lutidine complex of Os(III).

From data obtained at fast scan rates (ν = 10–100 V/s), certain of the relevant dynamic parameters could be determined[11] and are summarized in Scheme I. Attempts to synthesize **3** directly from $(NH_3)_5Os(TFMS)_3$ and 2,6-lutidine failed.

Over a period of hours, **1** rearranges intramolecularly in solution to a new product, **4**, which can be isolated as a deep green solid[12] (Scheme II). A cyclic voltammogram of **4** shows a reversible wave at −0.50 V. A ^{1}H NMR spectrum of this solid shows resonances at 9.92 (s, 1 H, b), 6.95 (s, 2 H), 4.16 (s, 3 H, b), 3.70 (s, 12 H, b), and 1.80 (s, 6 H) ppm, suggesting metal coordination at the para position of a lutidine tautomer, formally a lutidinium ylide. The ^{13}C NMR spectrum[13] of **4** shows singlets at 19.2, 136.8, 141.0 ppm, and a weak resonance at 210.2 ppm, which we attribute to the para carbon.

The ring nitrogen in **4** is not deprotonated by excess proton sponge (pK_a = 12.4) in acetone, though exchange is rapid in the presence of D_2O. An increase by 7 units in the pK_a of pyrazinium ion occurs upon coordination to pentaammineosmium(II), a striking effect attributable to back-bonding.[14] A similar enhancement in the basicity of the ring nitrogen would be expected for the isoelectronic, pentaammineosmium(II) ylide complex **4**.

Compound **4** is unstable with respect to loss of trans NH_3, a feature observed for certain other carbon-bound pentaammine systems.[15] Upon dissolution in acetonitrile, **4** reacts rapidly to form a new product, **5** (Scheme II). A ^{1}H NMR spectrum of **5** confirms the trans coordination of the nitrile ligand.[16] In general, **4** readily reacts with a variety of ligands[17] to yield the corresponding trans substituted tetraammine complexes quantitatively.

Though the preparations and measurements outlined above were performed in rigorously dried nonaqueous solvents, it is noteworthy that once formed **1** resists attack by water and O_2. NMR evidence suggests that in D_2O the rearrangement of **1** to the carbon-bound species takes place over a period of hours.[18]

We have observed analogous carbon–hydrogen bond activation in certain η^2-cationic pyridines,[19] though this reaction has not been detected in any of the many other pentaammineosmium(II) η^2-arenes which have been studied.[1,6] The enhanced reactivity in the heterocyclic systems is in marked contrast to the pattern observed for electrophilic aromatic substitution in the free ligands.

Acknowledgment. We thank W. D. Harman for his insightful suggestions. Support of this work by National Institutes of Health Grant GMI3638-21 is also gratefully acknowledged.

(9) (a) All redox potentials versus NHE. (b) Electrochemical conditions unless otherwise specified: 0.5 M NaTFMS in DME; ν = 200 mV/s; Pt° working electrode; $Fe(Cp)_2^+/Fe(Cp)_2$ internal reference.

(10) Unpublished results.

(11) (a) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*; John Wiley and Sons: New York, 1980; pp 453, 454. (b) Measurements in Scheme I taken in acetone saturated with NaTFMS assuming reversible electron transfer in the range ν = 20–200 mV/s.

(12) Preparation of **4**: Solutions of **1**[4] are allowed to stand for 3 h. Addition of Et_2O (15 mL) causes precipitation of the product which can be purified from acetone and Et_2O.

(13) Proton decoupled; triflate resonance at 123 ppm (q).

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(16) ^{1}H NMR spectrum of **5**: 10.71 (1 H, b), 7.17 (2 H), 3.47 (12 H, b), 2.88 (3 H), 1.98 (6 H) ppm.

(17) E.g., DMSO, pyridine, benzonitrile, isonicotinamide.

(18) Ligand resonances appear with time at 6.53 and 1.63 ppm (ratio 1:3) concomitant with the disappearance of **1**.

(19) Work on pyridinium, *N*-methylpyridinium, 2,6-lutidinium, and *N*-methyl-4-picolinium complexes will be reported separately.

Biosynthesis of Ansatrienin. Nonincorporation of Shikimic Acid into the mC_7N Unit and Stereochemistry of Its Conversion to the Cyclohexanecarboxylic Acid Moiety

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Received September 4, 1987

Recent studies on the biosynthesis of ansatrienin A (mycotrienin I) (**1**), a metabolite of *Streptomyces collinus*[1,2] and *S. rishiriensis*,[3-5] have shown that this antibiotic contains two structural moieties originating from the shikimate pathway of aromatic biosynthesis. The cyclohexanecarboxylic acid moiety attached via an alanine residue to the ansa ring is derived intact from the seven carbon atoms of shikimic acid, possibly via 1-cyclohexenecarboxylic acid and 1,4-cyclohexadienecarboxylic acid.[6] The mC_7N unit, representing the benzoquinone ring with the attached nitrogen and C-17, originates specifically from 3-amino-5-hydroxybenzoic acid (AHBA),[6] itself derived via the shikimate pathway.[7-20] However, the exact mode of formation

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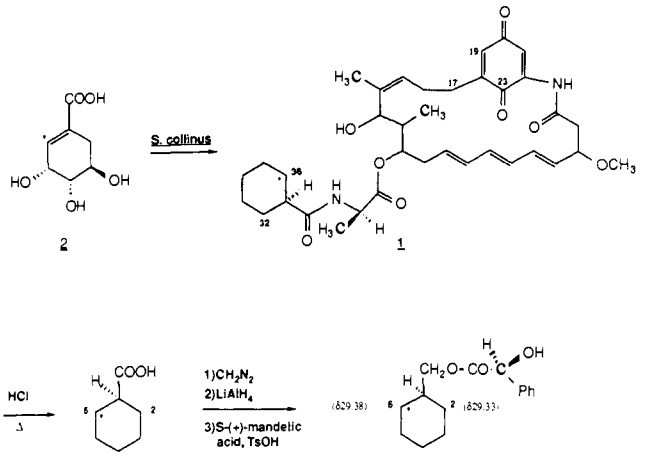
Scheme I. Stereochemical Fate of D-(−)-[2-^{13}C]Shikimic Acid (**2**) in the Conversion to the Cyclohexylcarbonyl Moiety of Ansatrienin (**1**)



of AHBA is not clear; neither shikimate nor dehydroquinate are incorporated into AHBA-derived mC_7N units. This suggests that either shikimic acid is not able to penetrate to the site of synthesis or the formation of AHBA branches off from the main shikimate pathway at a very early stage.

To shed more light on this question and on the formation of the cyclohexanecarboxylic acid moiety, we fed D-(−)-[2-^{13}C]-shikimic acid (50 mg/L) to 40 100-mL cultures of *S. collinus* Tü 1892 24 h after inoculation.[6] The precursor (99 atom% ^{13}C) was synthesized in 18% yield from D-[1-^{13}C]mannose by the method of Fleet and co-workers.[21] The cultures were harvested 48 h later, and ansatrienin A (175 mg/L) was isolated as previously described. ^{13}C NMR analysis of this sample showed a single enhanced signal indicating about 4.8% ^{13}C enrichment at C-32 or C-36. No enrichment was detectable at C-19 or C-23, the two positions in the mC_7N unit that could be labeled if shikimate were incorporated into that part of the molecule. This experiment proves unequivocally that the nonincorporation of shikimate into the mC_7N unit is not due to impermeability of the cells to this compound. Hence the branchpoint for AHBA formation must be earlier in the shikimate pathway, possibly at the stage of a 4-amino analogue of the first intermediate, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP), as proposed by Hornemann and co-workers.[14] We further propose that amino-DAHP is formed by a variant of the DAHP synthase reaction in which erythrose 4-phosphate is modified by Schiff's base formation with ammonia, generated by hydrolysis of the amide bond of glutamine, prior to condensation with phosphoenolpyruvate. The amide nitrogen of glutamine has been identified as the source of the nitrogen of the mC_7N unit in rifamycin.[22]

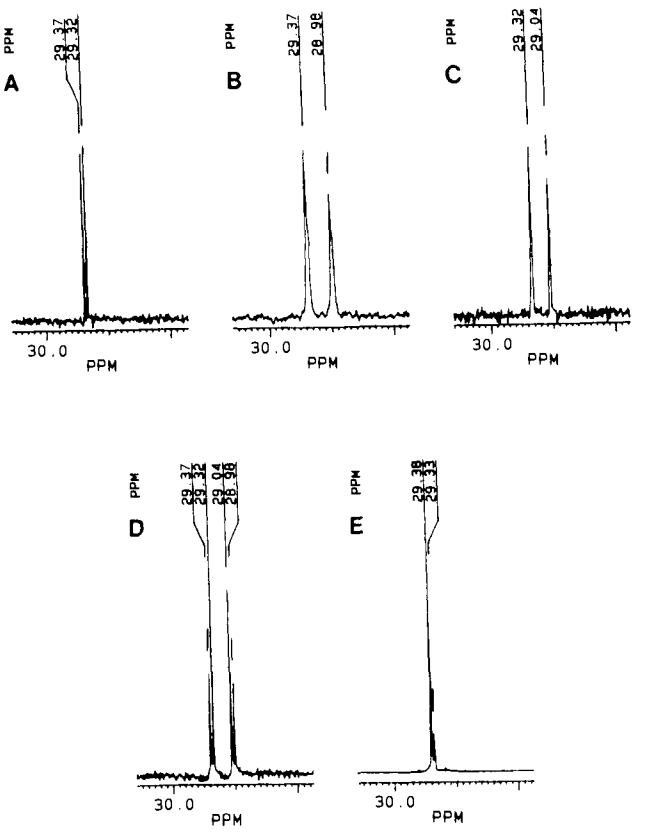


Figure 1. ^{13}C NMR spectra of cyclohexylcarbinyl mandelate, signals for C-2 and C-6 of the cyclohexane ring: (a) unlabeled cyclohexylcarbinyl (*S*)-(+)-mandelate; (B) (1*R*,2*R*)-[2-$^2H_1$]cyclohexylcarbinyl (*S*)-(+)-mandelate; (C) (1*R*,2*R*)-[2-$^2H_1$]cyclohexylcarbinyl (*R*)-(−)-mandelate; (D) 1:2 mixture of samples B + C; (E) [^{13}C]cyclohexylcarbinyl (*S*)-(+)-mandelate from **1** biosynthesized from [2-^{13}C]shikimic acid. Spectra A and E are ^{1}H broad band decoupled; B, C and D are ^{1}H,^{2}H broad band decoupled.

To determine if D-[2-^{13}C]shikimic acid was processed stereospecifically to label only C-32 or C-36 in the cyclohexanecarboxylic acid moiety, the ^{13}C-labeled **1** was hydrolyzed, and the cyclohexanecarboxylic acid was esterified and reduced to cyclohexylcarbinol (Scheme I). The latter was then esterified with *S*-(+)-mandelic acid.[23] The ^{13}C NMR spectrum of the corresponding unlabeled compound showed two signals at δ 29.38 and 29.33 ppm for C-2/C-6 of the cyclohexane ring; in the derivative obtained from the labeled **1** only the signal at δ 29.38 ppm was enhanced (Figure 1). Hence, shikimic acid is indeed processed stereospecifically in the conversion to cyclohexanecarboxylic acid.

The absolute stereochemistry of this process was determined by synthesizing an authentic sample of (1*R*,2*R*)-[2-$^2H_1$]cyclohexylcarbinol by the route shown in Scheme II. Fermenting baker's yeast reduction of 2-carbethoxycyclohexanone gave ethyl (1*R*,2*S*)-2-hydroxycyclohexanecarboxylate,[24,25] which was reduced to the diol with $LiAlH_4$. Deuterium was then introduced by stereospecific displacement of the secondary alcohol function, as its tosylate, with $LiEt_3B^2H_1$.[26] Aliquots of the (1*R*,2*R*)-[2-$^2H_1$]cyclohexylcarbinol were esterified with *S*-(+)- and *R*-(−)-mandelic acid,[23] and the broad band [^{1}H,^{2}H]decoupled ^{13}C NMR spectra of the samples were recorded (Figure 1). Each spectrum showed for the C-2/C-6 pair of carbons one unshifted and one deuterium shifted (0.34 ppm upfield ^{2}H isotope shift) signal. The chemical shift difference between the unshifted and the shifted signal was 0.39 ppm ($\Delta^2H + \Delta\delta_{C\text{-}2/C\text{-}6}$) in the *S*-(+)-mandelate

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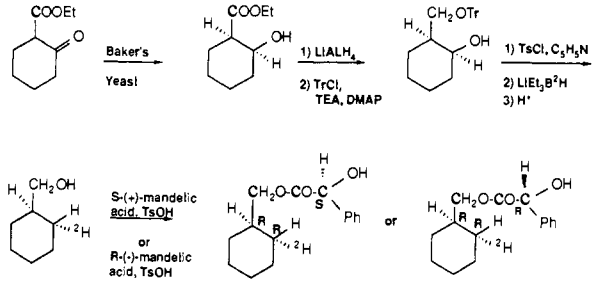
(26) Small amounts of other stereoisomers (<15%) present in the original hydoxy ester were removed in subsequent steps by crystallization to give final product which was optically pure as judged by NMR (>95% ee).

Scheme II. Synthesis of (1*R*,2*R*)-[2-$^2H_1$]Cyclohexylcarbinol *R*-(−)- and (*S*)-(+)-Mandelate Ester



derivative and 0.29 ppm ($\Delta^2H - \Delta\delta_{C\text{-}2/C\text{-}6}$) in the *R*-(−)-mandelate ester, indicating that in the *S*-(+)-mandelate ester the lower field (δ 29.38 ppm) signal originates from C-6 (the *pro*-S carbon) and the higher field signal (δ 29.33 ppm) from C-2 (*pro*-*R* carbon). The assignment is most easily deduced from the signal pattern of a 2:1 mixture of the *R*-(−) and the *S*-(+) esters [Figure 1D].

The stereochemical assignment shows that C-2 of shikimate gives rise to C-6 of the cyclohexanecarboxylic acid moiety of **1**. This finding agrees with the steric course of cyclohexane ring formation in the biosynthesis of ω-cyclohexyl fatty acids deduced by Okuda and co-workers.[27] While this result has been interpreted to indicate a direct double bond reduction of shikimic acid on the *re* face,[27] the observed stereochemical outcome may well be the result of a much more complex sequence of events. This possibility is suggested by the observation that D-(−)-[6-$^2H_1$]shikimic acid (98% ^2H, 6*R*:6*S* 2:1)[28] fed to *S. collinus* (50 mg/L) gave no detectable (<0.1%) deuterium incorporation into **1**, indicating complete loss of both methylene hydrogens of **2** in the conversion.

Acknowledgment. We are indebted to Prof. S. Okuda, Prof. A. Kawaguchi, and Dr. J. Furukawa, University of Tokyo, for apprising us of their work prior to publication and to the National Institutes of Health for financial support (Grant AI 20264).

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(28) Synthesized by the route of Fleet et al.[21] by using NaB^2H_4 in the reduction of the aldehyde function derived from C-5 of mannose.

Divergent Photochemistry of 2,4-Di-*tert*-butylacetophenone and -benzophenone

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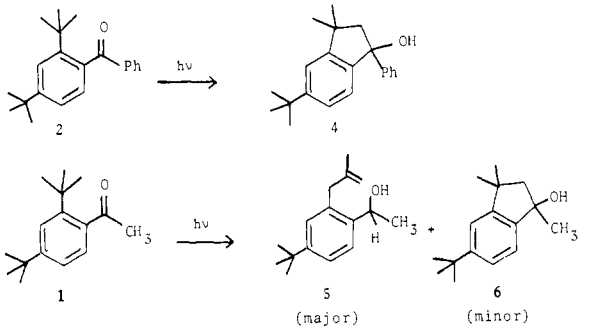
Received August 17, 1987

We have already reported that *o*-alkoxyacetophenones and benzophenones behave quite differently photochemically and ascribed the differences to differing rotational freedom in the 1,5-biradical intermediates formed from the ketone triplets.[1] We have now found an even more dramatic contrast between the photobehavior of *o*-*tert*-butylacetophenones and benzophenones that appears to verify the importance of rotational freedom in determining product ratios in the reactions of biradicals with benzylic centers.

Both 2,4-di-*tert*-butylacetophenone (**1**) and 2,4-di-*tert*-butylbenzophenone (**2**) were prepared by Friedel–Crafts acylation of 1,4-di-*tert*-butylbenzene. Their structures were determined by X-ray crystallography.[2] They share an important structural feature with *o*-*tert*-butylbenzophenone (**3**),[3] namely a 68–70° dihedral angle between the dibutylphenyl ring and the carbonyl.



Irradiation of **2** in several solvents produces only indanol **4** in a reaction completely analogous to that of **3**. The quantum yield varies from 0.03 (hexane) to 1.0 (methanol). Triplet lifetimes were measured by flash kinetics[4] and are 1.7 ns in toluene and 3.3 ns in methanol at 25°, some 3 times longer than for **3**. The reaction is readily quenched by conjugated dienes, with $k_q\tau$ values of 6.5 in hexane and 20 in methanol, corresponding to k_q values of 4–6 × 10^9 M^{-1} s^{-1}, a very normal range.[5]

Irradiation of **1** in several solvents produces primarily an internal redox product **5**,[6] with only 5–10% of indanol **6**[7] as a byproduct. Total quantum yields are only 0.02–0.05 in benzene and methanol. Triplet lifetimes are 25 ns in toluene and 130 ns in methanol.[8] The reaction is barely quenchable in hydrocarbon solvents, and k_q for 2,4-hexadiene in methanol is only 2 × 10^8 M^{-1} s^{-1}. The same value was determined by measuring sensitized yields of triplet 1-methylnaphthalene.[9]

It remains to be explained why triplets **1** and **2** undergo triplet energy transfer at such different rates and why they give such different products. We believe that both differences arise from triplet **1** having a considerably smaller dihedral angle between the carbonyl and the dibutylbenzene ring than is the case for its ground state and for all states of **2** and **3**. The solvent effect on reactivity indicates that the lowest triplet of **1** is π,π^*,[10,11] as expected from the known effect of dimethyl substitution.[10] π conjugation is far more important in excited states than in ground states,[12] so it is expected that the carbonyl of **1** would conjugate more with the benzene ring in the triplet than in the ground state. In **2** and **3**, the other benzene ring is available for conjugation with the carbonyl, so that the o-*tert*-butyl group remains rotated away from the carbonyl in the triplet state. The slow triplet energy transfer from triplet **1** presumably reflects the in-plane o-*tert*-butyl

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(6) 2-Methyl-3-(2-(1-hydroxyethyl)-4-*tert*-butylphenyl)propene was isolated by chromatography on silica gel: IR (CCl_4) 3610, 3080, 2975, 2940, 2915, 2880, 1635, 1010, 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (d, 1 H, J = 8.2 Hz), 7.29 (d of d, 1 H, J = 8.2, 2.2 Hz), 7.13 (d, 1 H, J = 2.2 Hz), 5.08 (quartet, 1 H, J = 6.4 Hz, $CH(CH_3)$–OH), 4.82 (s, 1 H, vinyl), 4.48 (s, 1 H, vinyl), 3.38 (s, 2 H, CH_2), 1.76 (s, 3 H, C=C–CH_3), 1.72 (s, 1 H, exchangeable OH), 1.45 (d, 3 H, J = 6.4 Hz, CH(OH)–CH_3), 1.31 (s, 9 H, *t*-Bu); MS, *m/e* 232, 217, 199, 157, 143, 91, 77, 57 (100).

(7) 1,3,3-Trimethyl-5-*tert*-butyl-1-indanol isolated by chromatography on silica gel: ^1H NMR ($CDCl_3$) δ 7.13–7.48 (m, aromatic), 2.07 (d, 2 H, J = 1.55 Hz (collapsed AB quartet), CH_2), 1.35 (s, 9 H, *t*-Bu), 1.32 (s, 3 H, CH_3), 1.29 (s, 3 H, CH_3), 1.25 (s, 3 H, CH_3); MS, *m/e* 214, 199 (100), 184, 158, 143, 128, 57.

(8) This change corresponds to the sixfold decrease in the rate constant for γ-hydrogen abstraction by triplet 2,4-dimethylvalerophenone in *tert*-butyl alcohol compared to benzene.[10]

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