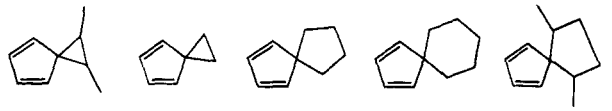
is estimated at 50–56 kcal/mol.[12] Suppression of the radical chain reaction by incorporating the dialkyl substituents in a ring can be seen in the spiroheptadienes **3**[13] and **4**.[14] Both of these compounds give reactions characteristic of cyclopropanes: loss of ring stereochemistry for **3**[13] and H migration for **4**.[14] Spiro nona- and decadienes **5** and **6** both contain virtually strain-free fused alicyclic rings, and give reaction



3 4 5 6 7

products and rates which are sensible only in terms of a concerted reaction. The activation energies are lower (35 and 45 kcal/mol for **5** and **6**) than any estimated bond homolysis step by a substantial amount.[15] The most cogent argument for a concerted path is provided by the observation of a high stereosopecificity in the (1,5) sigmatropic shift of *cis*- and *trans*-**7**.[16]

In summary, the thermal chemistry of 5,5-disubstituted cyclopentadienes now provides examples or radical, diradical, and concerted rearrangements. Future experiments and interpretations must be designed with some attention to these possibilities.

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M. Robert Willcott III,*[17] I. M. Rathburn III[18]
Department of Chemistry, University of Houston
Houston, Texas 77004
Received April 28, 1973

Stereospecificity of Proton Uptake in the Enzymatic Conversion of Sphinganine 1-Phosphate to Ethanolamine 1-Phosphate[1]

Sir:

Sphingolipid bases are major constituents of a variety of biologically important classes of compounds including ceramides, sphingomyelins, gangliosides, cerebrosides, and other glycosphingolipids. The enzymatic degradation of one of the sphingolipid bases, sphinganine (I, C_{18}-dihydrosphingosine, Figure 1), has been shown to be initiated by an ATP-dependent phosphorylation to yield sphinganine 1-phosphate (II).[2,3] Stoffel, *et al.*,[4,5] have reported the degradation of II involves conversion to palmitaldehyde and ethanolamine 1-phosphate (III). Recent results from the same laboratory indicate that the enzyme responsible for this catalysis (sphinganine 1-phosphate lyase) is specific for the D-*erythro*-(2*S*,3*R*) isomer of II.[6] We have directed our attention to the following questions. First, is a hydrogen atom from the solvent incorporated into III in the enzymatic degradation of II, and, second, if this is the case, is the incorporation of the hydrogen atom also stereospecific?

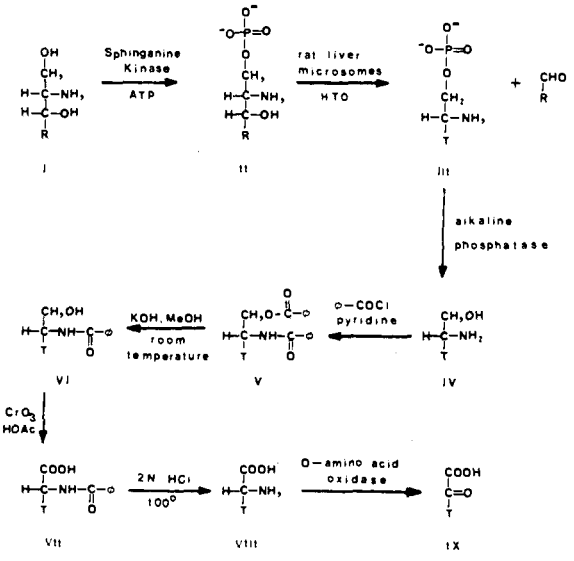

Figure 1. Enzymatic degradation of sphinganine. Stereospecificity in the conversion of sphinganine 1-phosphate to ethanolamine 1-phosphate (R = $C_{15}H_{31}$).

Accordingly, we have incubated II (2.5 μmol, prepared by chemical synthesis[7]) with rat liver microsomes in the presence of HTO (2 Ci).[8] ^{3}H-Labeled III was isolated by chromatography on a Dowex-1-formate column, preparative paper chromatography, and rechromatography on a Dowex-1-formate column. Identity and radiopurity of the labeled III was established by paper radiochromatography and gas–liquid radiochromatography (of the trimethylsilylated product). In two separate incubations, the recovery of labeled III was 2.76×10^5 and 3.24×10^5 cpm. Treatment of [^{3}H]-III with alkaline phosphatase[9] gave [^{3}H]ethanolamine (IV) (~44% yield) which was purified by preparative paper chromatography and its identity and radiopurity was established by paper and thin-layer radiochromatography. Treatment of [^{3}H]-IV with benzoyl chloride in pyridine gave (~77% yield) [^{3}H]-*N*,*O*-dibenzoylethanolamine (V) which was isolated by silicic acid column chromatography and whose radiopurity was established by thin-layer radiochromatography. [^{3}H]-V was converted to [^{3}H]-*N*-benzoylethanolamine (VI) by mild alkaline hydrolysis and the

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(9) Sigma Chemical Co., Type IV.

product was isolated (~70% yield) by silicic acid column chromatography. The radiopurity of VI was established by thin-layer radiochromatography. [^{3}H]-VI, upon oxidation with CrO_3 in 90% acetic acid, gave [^{3}H]-*N*-benzoylglycine (VII) (~40% yield) which was purified by DEAE cellulose acetate column chromatography and showed a single radioactive component upon thin-layer radiochromatographic analysis. Cocrystallization with authentic VII from acetone–hexane showed no significant change in specific radioactivity.[10] [^{3}H]-VI, upon acid hydrolysis,[11] gave [^{3}H]glycine (VIII) which was purified by chromatography on a Dowex-1-acetate column (~98% yield) and whose radiopurity was established by paper and thin-layer radiochromatography.

The stability of the label of [^{3}H]-IV through all of the reactions and procedures utilized in the conversion of [^{3}H]-IV through [^{3}H]-VIII was established in independent experiments.[12]

The [^{3}H]glycine derived from the [^{3}H]ethanolamine 1-phosphate formed enzymatically and [2*RS*-^{3}H]glycine derived from [2*RS*-^{3}H]ethanolamine[12] were each mixed with [1-^{14}C]glycine and incubated with D-amino acid oxidase,[13] an enzyme which, in the catalysis of the conversion of glycine to glyoxylic acid, specifically removes the hydrogen in the *S* configuration at carbon atom 2 of glycine.[14] Glycine and glyoxylic acid (IX) were isolated by Dowex-1-OH column chromatography and paper chromatography. The ratios of ^{3}H/^{14}C in the glycine and glyoxylic acid are presented in Table I.

Table I. Enzymatic Conversion of Glycine to Glyoxylic Acid[a]

Substrate	^{3}H/^{14}C Ratios in glycine and glyoxylic acid recovered after incubation	
	Glycine	Glyoxylic acid
[2-^{3}H,1-^{14}C]Glycine (derived from [^{3}H]-ethanolamine 1-phosphate)		
Experiment 1		0.97
Experiment 2	1.06	0.88
[2*RS*-^{3}H,1-^{14}C]Glycine (derived from [2*RS*-^{3}H]ethanolamine)		
Experiment 1		0.51
Experiment 2 (no enzyme)	0.97	

[a] For ease of comparison, the ratios presented in this table have been calculated in reference to an assigned value of unity in the substrate, *i.e.*, measured ^{3}H/^{14}C ratio in compounds after incubation divided by the measured ^{3}H/^{14}C ratio of the substrate.

The glyoxylic acid derived from the [2*RS*-^{3}H, 1-^{14}C] glycine showed the expected ^{3}H/^{14}C ratio of ~0.5. The glyoxylic acid derived from the labeled glycine (obtained from the [^{3}H]ethanolamine 1-phosphate) showed ratios of 0.88 and 0.97, indicating that the configuration of the labeled hydrogen in the glycine, and hence in the [^{3}H]-ethanolamine 1-phosphate, is *R*. The ^{3}H/^{14}C ratio in the glycine recovered after incubation was essentially unchanged indicating the absence of a significant isotope effect under the conditions studied.

Thus, the enzyme-catalyzed conversion of (2*S*,3*R*)-sphinganine 1-phosphate to ethanolamine 1-phosphate involves the stereospecific incorporation of one atom of solvent hydrogen at carbon atom 2 of the latter compound.

(10) Specific activities: initial, 33.9 ± 0.6 cpm/mg; after one recrystallization, 34.9 ± 0.5 cpm/mg; after two recrystallizations, 32.6 ± 0.3 cpm/mg.

(11) 2 *N* HCl, 100°, 2 hr.

(12) [2*RS*-^{3}H]-IV, Amersham/Searle Co., (292 cpm/μmol) was converted successively, by the reactions outlined above, to [^{3}H]-V (288 cpm/μmol), [^{3}H]-VI (297 cpm/μmol), [^{3}H]-VII (279 cpm/μmol), and [^{3}H]-VIII (288 cpm/μmol).

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T. Akino, T. Shimojo, Y. Miura, G. J. Schroepfer, Jr.*
Departments of Biochemistry and Chemistry
University of Illinois, Urbana, Illinois 61801
and Rice University, Houston, Texas 77001*
Received November 2, 1973

Reactivity Characteristics of Cytochrome *c*(III) Adduced from Its Reduction by Hexaammineruthenium(II) Ion

Sir:

Extensive interest in the redox properties of mammalian cytochrome *c*, a particularly well-studied component of the mitochondrial respiratory system,[1-3] has been buttressed by X-ray determinations of the oxidized and reduced structures of this[4] and a related[5] protein in the crystalline state. Recent kinetic studies indicate that one pathway for the homogeneous reduction of horse heart cytochrome c^{III} by simple reagents in aqueous solution can be governed by a rate-limiting event ($k \sim 30$–60 sec^{-1}) within the protein, which has been plausibly interpreted as involving the opening of the heme crevice and/or substitution on the iron center, followed by a rapid redox conversion.[6] The suggestion has been advanced that the physiological reduction of cytochrome *c* might be related mechanistically to this event.[6b]

While some physiological evidence is consistent with this suggestion,[6b] recent results with improved models are suggestive of a considerably more rapid physiological reduction of cytochrome *c*.[7] It seems possible that the observed rate-limiting event might be accelerated *in vivo*, *e.g.*, through the assistance of heme crevice opening by the reductase. Alternatively, reduction could occur *via* what has been called[6] a "remote" pathway, a description which is apparently intended to include all possible reactions at the periphery of the protein whether they occur by simple outer-sphere, electrostatic complex outer-sphere,[2] or substitution mechanisms. In this communication we report evi-

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