Table I. Refined EXAFS Parameters for $BrOBrO_3$[a]

shell	occupation	distance/Å	Debye–Waller factor, $2\sigma^2$
Br=O	1.5	1.605 (3)	0.0108 (3)
Br—O	1	1.862 (2)	0.0029 (3)
Br···Br	1	3.053 (2)	0.0088 (2)
E_0 = 12.6 (3) eV, FI[b] = 0.40, R^b = 24.7			

[a] Standard deviations in parentheses; errors arising from data collection and analysis are estimated to be ±1.0–1.5% in well-defined shells (Corker, J. M.; Evans, J.; Leach, H.; Levason, W. *J. Chem. Soc., Chem. Commun.* **1989**, 181–183. [b] As defined in ref 3.

products suggests that Br(VII) is present in the BrO_2 and that a possible formulation is bromine perbromate (bromine(I) bromate(VII)), $BrOBrO_3$.

Raman spectroscopy[11] of the freshly deposited yellow solid at 77 K showed prominent bands at 35 (vs, br), 46 (vs), 453 (s), 582 (s), 594 (m), 842 (s), and 856 (m) cm^{-1}; of particular note are the vibrations between 500 and 600 cm^{-1}, assignable to the Br–O–Br bridge. Our Raman spectrum thus differs significantly from that of "BrO_2" obtained by ozonization of bromine in solution,[6,12] which shows no evidence for such a bridge (our spectra also showed weaker, variable features corresponding to those reported[6]). We believe that the two materials have different structures.

Bromine K-edge EXAFS data were obtained in the transmission mode[13] from samples deposited on a thin aluminum window.[14] Data reduction and curve fitting were performed as previously described,[3] and the unsmoothed, background-subtracted EXAFS spectrum and corresponding Fourier transform are shown in Figure 1 together with the best fit simulated curves. The refined parameters are shown in Table I. Three distinct shells are observed corresponding to terminal Br–O, bridging Br–O, and nonbonded Br···Br distances at 1.61 (2), 1.86 (2), and 3.05 (3) Å, respectively, concomitant with the proposed structure. No evidence of residual Br_2, d(Br–Br) = 2.28 Å, was found. The terminal Br–O distance compares with that in perbromate (1.61 Å (av)[8]) indicative of Br^{VII}–O bonds, while the bridging Br–O bond length and the nonbonded Br···Br distance correspond closely to the distances in Br_2O,[3] in keeping with the bridged species proposed. A Br–O–Br angle of 110 ± 3° may be calculated by triangulation.

We have thus shown that the yellow "BrO_2" obtained by high-voltage discharge of Br_2/O_2 mixtures is structurally bromine perbromate, an analogue of the known $ClOClO_3$[15] and $BrOClO_3$.[16] An investigation of the reaction chemistry of this new oxide will be reported in due course. Further studies to establish the structure of the yellow product obtained from Br_2 and O_3 in solution[6] are also planned.

Acknowledgment. We thank the SRFC for support and the director of the Daresbury Laboratory for the provision of facilities.

Registry No. $BrOBrO_3$, 141438-65-5; Br^-, 24959-67-9; BrO^-, 14380-62-2; BrO_3^-, 15541-45-4; BrO_4^-, 16474-32-1.

(11) Raman spectra were obtained on a Coderg T 800 with a krypton ion laser operating at 647.1 nm.

(12) In ref 6, major features were reported at 205 (vs) ν(Br–Br), 861 (m), 878 (s), 882 (sh), 910 (s), and 919 (s) cm^{-1} ν(Br–O), in addition to weaker bends and deformations. The absence of any features in the Br–O–Br bridging region should be noted.

(13) Bromine K-edge EXAFS data were measured on beam line 9.2 at the Daresbury Synchrotron Radiation Source, operating at 2 GeV and with an average beam current of 150 mA. A double crystal Si(220) monochromator was utilized, and the spectra were calibrated to the Au L_{II} edge (13.731 keV) of a 10-μm gold foil.

(14) The sample was prepared as described in ref 7, but utilizing a glass cell fitted with 75-μm Kapton outer windows and a central cold-finger fitted with a copper block attached to a glass dewar with a graded seal. The aluminum window was then bolted to the bottom of the copper block and cooled by liquid nitrogen in the dewar. The imperfect thermal contact led to a base temperature of ca. −160 °C at the window.

(15) Schack, C. J.; Pilipovich, D. *Inorg. Chem.* **1970**, *9*, 1387–1390.

(16) Schack, C. J.; Christie, K. O.; Pilipovich, D.; Wilson, R. D. *Inorg. Chem.* **1971**, *10*, 1078–1080.

Efficient Catalysis of a Redox Reaction by an Artificial Enzyme

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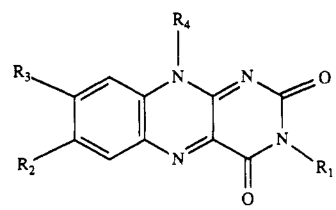
Received February 26, 1992
Revised Manuscript Received April 6, 1992

Models of flavoenzymes[1] have been used with limited success to illustrate the importance of the binding process in enzymatic catalysis.[2,3] The highest acceleration factor for an artificial flavoenzyme over riboflavin reported[2] so far is 29. We now report an acceleration factor of 6.5×10^2 for the oxidation of *p*-*tert*-butylbenzyl alcohol by a system that we recently synthesized.[4] We further demonstrate that binding of the substrate to the artificial enzyme plays an important role in these rate accelerations.

The oxidation reaction of several substituted benzyl alcohols to their corresponding aldehydes, catalyzed by 2-[(7α-*O*-10-methyl-7-isoalloxazino)methyl]-β-cyclodextrin (**1**) and by riboflavin (**2**) under photochemical conditions at low pH,[5] was investigated.[6] The reactions catalyzed by **1** were found to be



1 R_1=R_3=H, R_4=CH_3, R_2= 2-(*O*-methylene)-β-cyclodextrin

2 R_1=H, R_2=R_3=CH_3, R_4=ribityl

3 R_1=R_3=H, R_2=R_4=CH_3

4 R_1=R_4=CH_3, R_2,R_3=1',4',7',10',13',16'-hexaoxacyclooctadec-2'-ene (18-crown-6)

5 R_1=H, R_2=CH_3, R_4=ribityl, R_3=6-(*S*-methylene)-α-Cyclodextrin

considerably faster[6b] than those catalyzed by either **2** or 7,10-dimethylflavin[7] (**3**). For example, the **1**-catalyzed oxidation of *p*-*tert*-butylbenzyl alcohol is complete within 2.5 h, whereas the same reaction catalyzed by **2** or **3** is very slow.[6b] It is observed that **2** decomposes under photochemical conditions and can exhibit up to only 7 turnovers, whereas the artificial enzyme is more stable[8] and can exhibit more than 100 turnovers under these conditions. The initial oxidation rates of various substituted benzyl alcohols, by the artificial enzyme and riboflavin, are given in Table I. The structural similarity of the flavin moiety in **1** and **3** suggests that the change in the oxidation potentials caused by the substituents on flavin is not responsible for the rate acceleration exhibited by **1** over **3** or **2**. The oxidation rate of *p*-*tert*-butylbenzyl alcohol

(1) Ror a review of flavoenzymes and their mechanism of action, see: (a) *Chemistry and Biochemistry of Flavoenzymes*; Müller, F., Ed.; CRC Press, Inc.: Boston, 1991; Vol. I. (b) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979; Chapters 10–12.

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(4) Rong, D.; Ye, H.; Boehlow, T. R.; D'Souza, V. T. *J. Org. Chem.* **1992**, *57*, 163–7.

(5) For an explanation for the use of low pH, $HClO_4$ and other experimental conditions, see: Fukuzumi, S.; Tanii, K.; Tanaka, T. *J. Chem. Soc., Chem. Commun.* **1989**, 816.

(6) (a) A reaction mixture consisting of aqueous solutions of substituted benzyl alcohols (5.0×10^{-4} M), flavin (5.0×10^{-5} M), and $HClO_4$ (0.037 M, pH 1.7 remains constant throughout the reaction) was irradiated at 360 nm $< \lambda <$ 440 nm, and the reaction was monitored for appearance of the corresponding aldehydes by HPLC. (b) See supplementary material for details.

(7) Kumar, V.; Woode, K. A.; Bryan, R. F.; Averill, B. A. *J. Am. Chem. Soc.* **1986**, *108*, 492.

(8) (a) The stability of the flavin moiety of the artificial enzyme under photochemical conditions, brought about by its structure, offers an advantage to these systems over other flavin derivatives. (b) For a discussion of the structure, see: Tong, W.; Ye, H.; Rong, D.; D'Souza, V. T. *J. Comput. Chem.* **1992**, *13*, 614.

0002-7863/92/1514-5470$03.00/0

Table I. Initial Rates for Oxidation of Substituted Benzyl Alcohols by Flavins[a]

R^b	initial rates ν (M min^{-1}) × 10^7 1	2	ν_1/ν_2 ×10^{-1}	E_{HOMO} (eV)[c]
CH_3	24 ± 1	0.46 ± 0.01	5.3	-8.78
tert-butyl	24 ± 5	0.19 ± 0.02	13	-8.95
Cl	2.1 ± 0.2	0.14 ± 0.01	1.5	-9.11
H	0.62 ± 0.08	0.14 ± 0.02	0.45	-9.16

[a] [Substrate] = 0.5 mM, [flavin] = 0.05 mM, in water containing 0.037 M $HClO_4$, pH = 1.7, at 25 °C. [b] Substituent at the para position of benzyl alcohol. [c] Calculated using MINDO/3 in AMPAC.

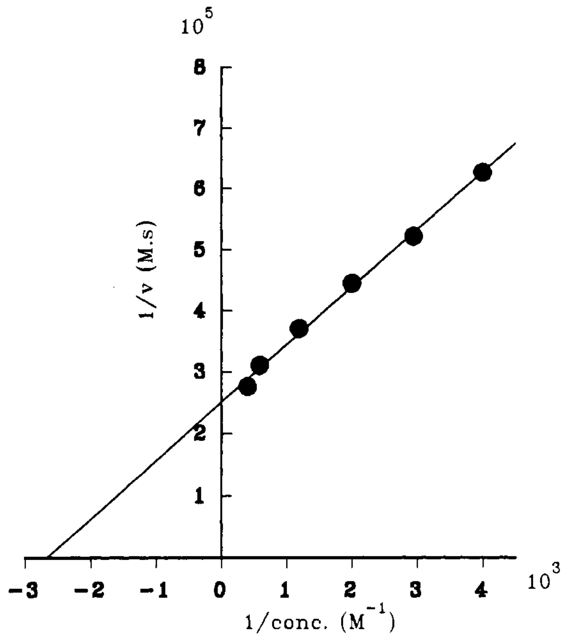

Figure 1. Double reciprocal plot for oxidation of *p-tert*-butylbenzyl alcohol by the artificial enzyme **1**: [Fl] = 5.00 × 10^{-5} M, [$HClO_4$] = 0.037 M, pH = 1.7, light intensity = 0.25 mW/cm^2. K_a = 2768 ± 77 M^{-1}; k_{cat} = 1.4 ± 0.04 × 10^{-3} s^{-1}.

by **2** decreases in the presence of β-cyclodextrin,[6b] demonstrating the importance of covalent attachment of the catalytic site to the binding site.

Reduced flavins are known[1b] to be reoxidized by air with a half-life of less than 1 s. Assuming a fast rate of reoxidation of the reduced form of **1**, the oxidation of benzyl alcohol can be proposed as the rate-determining step in the catalytic cycle.[9] Reactions catalyzed by the artificial enzyme are expected to follow an enzymatic reaction scheme whereas reactions of riboflavin should follow second-order kinetics.[10] A plot of the initial rates vs substrate concentration,[6b,11] for the oxidation of *p-tert*-butylbenzyl alcohol catalyzed by riboflavin, gives a straight line with zero intercept.[6b] This indicates a first-order dependence on the substrate. A similar plot for the same reaction catalyzed by the artificial enzyme shows saturation kinetics. Lineweaver–Burk treatment of these data gives an excellent fit (Figure 1), which suggests that the artificial enzyme is similar to real enzymes and binds the substrate prior to the reaction. Assuming that the dissociation of the cyclodextrin–substrate complex is much faster than the turnover step, the Michaelis–Menton constant (K_m) obtained from Figure 1 represents the dissociation constant[12] for the complex. The enzyme efficiency (k_{cat}/K_m) is an apparent

(9) (a) Fukuzumi, S.; Tanaka, T. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: New York, 1988; Part C, p 671. (b) Fukuzumi, S.; Tanii, K. *Chem. Lett.* **1989**, 35.

(10) Fukuzumi, S.; Tanii, K.; Tanaka, T. *J. Chem. Soc., Chem. Commun.* **1989**, 816.

(11) The UV absorbance was used to ensure that substrate concentration does not decrease over time because of its insolubility.

(12) Reference 1b, p 66.

Table II. A Comparison of the Catalytic Activity of Artificial Redox Enzymes

enzyme	k_{cat} (s^{-1})	K_a (M^{-1}) × 10^3	$k_{cat}K_a$ (M^{-1} s^{-1})	k_2 (M^{-1} s^{-1})	acc fact.[a] × 10^{-2}
1[b]	1.4 × 10^{-3}	2.8	3.8	5.8 × 10^{-3}	6.5
4[c]	3.7 × 10^{-5}	9.9	0.36	1.3 × 10^{-2}	0.29
5[d]	0.5	2.5	1.3 × 10^3	1.2 × 10^{2e}	0.11

[a] Acceleration factor is calculated by the ratio of the two second-order rate constants $k_{cat}K_a$ and k_2. [b] This work, oxidation of *p-tert*-butylbenzyl alcohol, error limits in k_{cat} and K_m ±2.8%. [c] From ref 2, oxidation of 1-(1-hexyl)-1,4-dihydronicotinamide. [d] From ref 3, oxidation of N^3-dodecyl-1-[*p*-(ammoniomethyl)benzyl]-1,4-dihydronicotinamide. [e] pH 7.0, 25 °C.

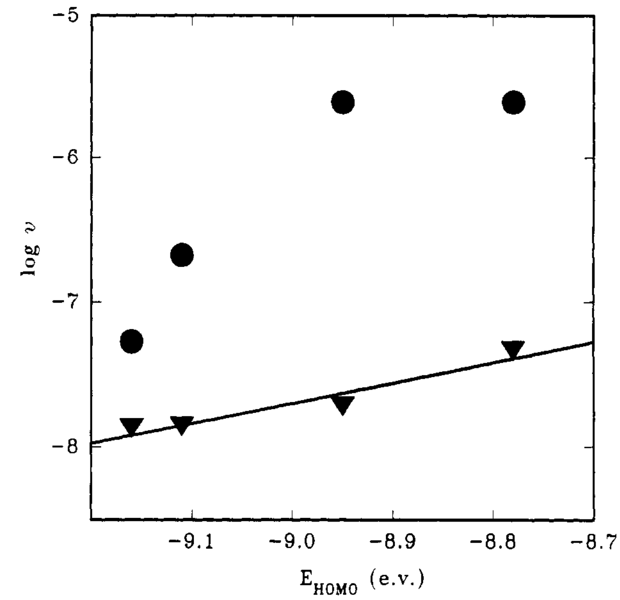

Figure 2. Correlation between oxidation rates of substituted benzyl alcohols by **1** (●) and **2** (▼) and their HOMO levels: [substrate] = 0.5 mM, [flavin] = 0.05 mM, in water containing 0.037 M $HClO_4$, pH = 1.7, at 25 °C, light intensity = 0.25 mW/cm^2. The HOMO levels were calculated using MINDO/3 in AMPAC.

second-order rate constant[13] which can be used to assess the rate enhancement by enzyme-like catalysts through molecular recognition.[2] The turnover constant (k_{cat}), the association constant ($K_a = 1/K_m$), and the enzyme efficiency (k_{cat}/K_m) for this system are given in Table II.

A rate-determining one-electron-transfer mechanism has been proposed for protonated flavin catalyzed photooxidation of benzyl alcohols in acidic medium.[9] Thus, it can be envisioned that the substrate-binding process by the artificial enzyme aligns the HOMO of the substrate with the SOMO (singly occupied molecular orbital) of the excited flavin moiety and facilitates the electron-transfer reaction. A plot of the log of initial oxidation rates vs the HOMO levels of the substrates catalyzed by **2** is linear, and the corresponding plot for catalysis by **1** is nonlinear (Figure 2). The substituents on the phenyl group that enhance its binding to the cyclodextrin cavity[14] (e.g., *tert*-butyl and methyl) seem to accelerate the rate of the reaction to a greater extent than other substituents, suggesting that the binding plays an important role in these reactions.

It is interesting to compare this artificial enzyme with previously published nonproteinic enzyme models (Table II): flavo-crown ether[2] (**4**) and 6-(8α-*S*-riboflavo)-α-cyclodextrin[3] (**5**). While the binding constants (K_a) for all these systems are in the same range, the turnover number (k_{cat}) for **1** is higher than that for **4**. The turnover number for **5** is high because the bimolecular rate

(13) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1984; Chapter 3.

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constant (k_2) for this reaction, catalyzed by riboflavin, is the highest among all these systems. The efficient electron transfer reported[3] for **5** is a property of the reactive substrate used in the reaction rather than the advantage gained by binding of the substrate to the artificial enzyme. The highest accelerator factor (6.5×10^2) exhibited by **1** over riboflavin[15] can be attributed to an effective flavin-substrate geometry within the enzyme-substrate complex, and these important geometric considerations are discussed elsewhere.[8b]

The artificial redox enzyme investigated herein exemplifies two of the advantages that artificial enzymes can offer to a reaction. (1) It converts a sluggish reaction, which cannot be completely catalyzed by flavin, into an efficient reaction. (2) It can benefit from reaction conditions (photochemical in this case) that are not commonly used by real enzymes.

Acknowledgment. We gratefully acknowledge the financial support from Mallinckrodt Specialty Chemicals Company, the Missouri Research Assistance Act, the donors of the Petroleum Research Fund, administrated by the American Chemical Society, and the University of Missouri-St. Louis.

Supplementary Material Available: Experimental details for the oxidation of *p-tert*-butylbenzyl alcohol (8 pages). Ordering information is given on any current masthead page.

(15) The redox properties and absorption characteristics of **1** and **2** differ slightly, and the contribution to the rate acceleration is assumed to be not significant. Ye, H.; Rong, D.; Tong, W.; D'Souza, V. T. *J. Chem. Soc., Perkin Trans. 2*, manuscript submitted.

Biosynthesis of 6β-Hydroxytropine in *Datura stramonium*: Nonregiospecific Incorporation of [1,2-$^{13}C_2$]Acetate†

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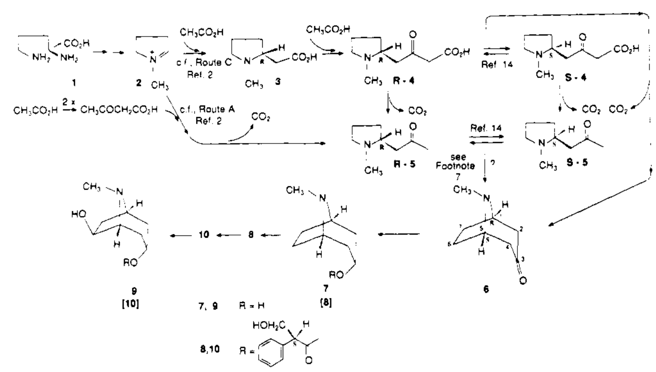
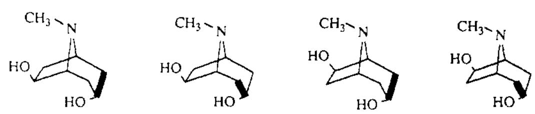
Received March 2, 1992

Recent investigations of the formation of the acetate-derived segment of cocaine[1] and of *N*-methylpelletierine[2] provide evidence for unexpected biochemical diversity in biosynthetic processes leading from one and the same substrate to analogous fragments in structurally related natural products.

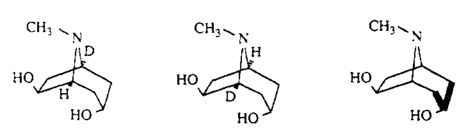
In further exploration of this theme we have investigated the entry of [1,2-$^{13}C_2$]acetate into the C_3 bridge of 6β-hydroxytropine (= 3α,6β-dihydroxytropane = 3-*endo*, 6-*exo*-3,6-dihydroxytropane) (**9**) in *Datura stramonium*. It has been inferred from tracer experiments that the ring skeleton arises from ornithine[3] (**1**) and two acetate units,[4] that *N*-methyl-Δ^1-pyrrolinium ion[5] (**2**) and hygrine[6,7] (**5**) are intermediates, and that the entry of the hydroxy group into the ornithine-derived ring of tropine (**7**) takes place late in the biosynthetic process[8,9] (Scheme I).[10] By analogy with the findings in *N*-methylpelletierine and cocaine, incorporation of sodium [1,2-$^{13}C_2$]acetate (49% $^{13}C_2$, 1 g, in 40 mL of water)[2] into 6β-hydroxytropine (**9**) was anticipated to lead to a product showing either one or the other of the two labeling patterns, **9A** or **9B**. Unexpectedly, a different result was obtained: the ^{13}C NMR spectrum (125 MHz, 104 000 scans) of the 6β-hydroxytropine that was isolated[11] (5 mg in 0.6 mL of $CHCl_3$; % enrichment: C-2, 0.38%; C-3, 0.76%; C-4, 0.42%) showed that the product consisted of a mixture of **9A** and **9B**,[12] equimolar within the limits of determination (δ 27.8 C-2 (d), 30.5 C-4 (d), 74.5 C-3 (d) ppm, $J_{2,3} = J_{3,4} = 35$ Hz). Such an outcome can arise from one of several variations in the entry of the side chain into the *N*-methyl-Δ^1-pyrrolinium ion (**2**) and the further elaboration of the intermediates, so generated, into tropine (**7**) and 6β-hydroxytropine (**9**). The experiment with [1,2-$^{13}C_2$]acetate cannot distinguish among these alternatives.

Scheme I





9 A **9 B** **9 C** **9 D**



9 E **9 F** **9 G**

Firstly, introduction of the side chain into **2** might take place stereospecifically and concurrently by both the "pelletierine mechanism"[2] (analogous to route A in ref 2) (**2** → → **5**, Scheme I) and the "cocaine mechanism"[1] (analogous to route C in ref 2) (**2** → **3** → **4**, Scheme I), and the intermediates between **2** and **6** maintain their chirality.

The result of a second experiment, with sodium [1,2,3,4-$^{13}C_4$]acetoacetate (49% $^{13}C_4$, 1 g, in 40 mL of water)[2] as the substrate, disposes of any scheme that implicates the "pelletierine" mechanism: the ^{13}C NMR spectrum of the sample of 6β-hydroxytropine from this experiment (7 mg in 0.6 mL of $CHCl_3$; % enrichment: C-2/C-4, 0.7%; C-3, 1.4%) showed the presence of a doublet (J = 34 Hz) in each of the signals due to C-2 and

† This paper is dedicated to the memory of Professor Edward Leete, who died in February 1992 after a long and courageous battle with cancer.

(1) Leete, E.; Kim, S. H. *J. Am. Chem. Soc.* **1988**, *110*, 2976.

(2) Hemscheidt, T.; Spenser, I. D. *J. Am. Chem. Soc.* **1990**, *112*, 6360.

(3) Leete, E.; Marion, L.; Spenser, I. D. *Nature* **1954**, *174*, 650. Leete, E. *J. Am. Chem. Soc.* **1962**, *84*, 55; *Tetrahedron Lett.* **1964**, 1619.

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(5) Leete, E. *J. Am. Chem. Soc.* **1967**, *89*, 7081.

(6) McGaw, B. A.; Woolley, J. G. *Phytochemistry* **1978**, *17*, 257.

(7) A few months before his death E. Leete informed us of recent results in his laboratory that threw doubt on the intermediacy of hygrine (**5**) in the biosynthesis of tropine. In the light of this finding, Scheme I shows the intermediacy of (*R*)-**5** ⇌ (*S*)-**5** as doubtful (?) and indicates the formation of tropinone (**6**) directly from *N*-methylpyrrolidineacetoacetate ((*R*)-**4** ⇌ (*S*)-**4** (by dehydrogenation and ring closure accompanied by decarboxylation).

(8) Hashimoto, T.; Yamada, Y. *Plant Physiol.* **1986**, *81*, 619.

(9) Hashimoto, T.; Yamada, Y. *Eur. J. Biochem.* **1987**, *164*, 277.

(10) For a recent review, see: Leete, E. *Planta Med.* **1990**, *56*, 339.

(11) The crude alkaloid mixture obtained by conventional methods was hydrolyzed with methanolic ammonia (10% v/v) for 2 days at room temperature, and 6β-hydroxytropine was separated from other alkaloids by chromatography on silica gel and elution with chloroform/methanol/0.880 ammonia (85:14:1 followed by 65:34:1).

(12) A recently reported independent investigation of the incorporation of [1,2-$^{13}C_2$]acetate into the 6β-hydroxytropine moiety of 6β-hydroxyhyoscyamine in *Hyoscyamus albus* gave an analogous result.[13]

(13) Sankawa, U.; Noguchi, H.; Hashimoto, T.; Yamada, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2066.

(14) C.f. Leete, E. *Planta Med.* **1979**, *36*, 97.

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