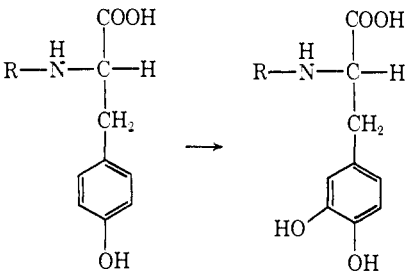
Microbiological Synthesis of L-3,4-Dihydroxyphenylalanine

Sir:

Victims of Parkinson's disease have been shown to respond to the experimental drug L-3,4-dihydroxyphenylalanine (L-dopa), when administered in relatively high doses.[1] This observation aroused our interest in devising an efficient synthesis of L-dopa in anticipation of the enormous therapeutic need for this compound. We herein record a facile microbiological method for the preparation of L-dopa from L-tyrosine.

A priori, the obvious approach to the problem would be to find a suitable microorganism, capable of converting L-tyrosine to L-dopa efficiently. Unfortunately, a survey of the literature reveals that microorganisms in general decompose L-tyrosine readily to yield *p*-hydroxyphenylpyruvic,[2] *p*-coumaric,[3] or homogentisic[4] acids. Although protocatechuic acid[3] and catechol[5] were also identified as metabolites of L-tyrosine, no L-dopa was detectable, suggesting that deamination of L-tyrosine may be the first degradative reaction proceeding at a rapid rate.

On the other hand, if deamination and aromatic hydroxylation reactions can occur independently, it should then be possible to selectively inhibit deaminase activity by the introduction of suitable N-blocking groups, resulting in the accumulation of the desired N-substituted L-dopa derivatives. Added advantages of N-substituted tyrosines as substrates are their increased solubility and their inertness to the action of racemases. To verify this assumption, N-carbobenzoxy (N-CBZ), N-formyl, and N-*t*-butoxycarbonyl (*t*-BOC) derivatives of L-tyrosine were prepared and incubated with microorganisms. It was found that the following microorganisms were capable of catalyzing the desired transformations: *Aspergillus ochraceus, Penicillium duclauxi, Gliocladium deliquescens, Stemphylium solani, Scopulariopsis constantini, Memnoniella echinata, Trichoderma viride, Corynespora cassicola, Fusarium solani, Stysanus fimetarius*, etc. These observations indicate that this reaction is widespread among fungi.



R = CHO; *t*-BOC, CBZ

In a model experiment, 1.5 g of N-formyl-L-tyrosine was exposed to *Gliocladium deliquescens* in 50 ml of soybean–dextrose medium. L-Ascorbic acid (900 mg) was added intermittently in five portions to the flask. After 44 hr, the reaction was terminated by acidification, followed by 1-butanol extraction. The formyl group was removed by exposure to 5 *N* HCl for 8 hr at room temperature, and the resulting mixture of L-tyrosine and L-dopa was then separated by chromatography on a Dowex-50-4X (200–400 mesh) H^+ form column. Elution of the column with 0.75 *N* HCl afforded 25.3% L-dopa,[6] $[\alpha]^{25}D$ −11° (*c* 3.7, 4% HCl), and 57% of unreacted L-tyrosine.

In a similar fashion, N-CBZ- and N-*t*-BOC-tyrosine derivatives were transformed by *Aspergillus ochraceus* into their corresponding L-dopa derivatives in about 30% yield.

The aforementioned microorganisms are also capable of converting N-substituted D-tyrosine derivatives into their corresponding D-dopa products. Also, to obtain optimum yields of dopa, it is imperative to add L-ascorbic acid to the fermentation to prevent melanin formation. These properties closely resemble those of polyphenol oxidases from plants.[7]

The microbial synthesis herein described is simple and utilizes the inexpensive starting material L-tyrosine. In our opinion, this constitutes one of the most economical processes to date for the preparation of L-dopa.

(1) G. C. Cotzias, P. S. Papavasiliou, and R. Gellene, *New Engl. J. Med.*, **280**, 337 (1969).
(2) T. Tanaka, *Bull. Pharm. Res. Inst.* (Osaka), No. 74, 1 (1968).
(3) K. Moore, P. V. SubbaRao, and G. H. N. Towers, *Biochem. J.*, **106**, 507 (1968).
(4) L. M. Utkin, *Biokhimiya*, **15**, 330 (1950).
(5) S. Hamasaki, *Kumamoto Med. J.*, **21**, 122 (1968).
(6) Identification of L-dopa was made by comparison of its infrared spectrum with that of an authentic specimen. In essence, this represents a 54% yield (divide actual yield by the fraction of substrate disappeared).
(7) C. R. Dawson and R. J. Kagee, *Methods Enzymol.*, 2, 817 (1955).

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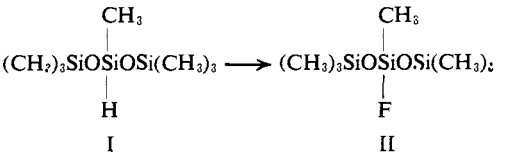
Hydride–Fluoride Conversions in Organosiloxane Chains. 3-Fluoroheptamethyltrisiloxane

Sir:

We wish to report the synthesis and characterization of 3-fluoroheptamethyltrisiloxane (II). So far as we are



able to determine, II is the first example of a linear organosiloxane molecule bearing a (–RSiF–) chain unit which has been isolated and characterized. (Chain Si–F bonds occur in the inorganic siloxanes such as octafluorotrisiloxane and the reported $(SiO_{1.5}F)_n$ structure.[1] Linkage between silicon and fluorine occurs also in the simple triesters of monofluoroorthosilicic acid, the most closely related compound being the reported fluorotris(triphenylsiloxy)silane.[2])

(1) D. R. Secrist and J. D. Mackenzie, *J. Polymer Sci., Part B*, 4, 537 (1966).
(2) V. S. Chugunov, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1059 (1956).

The 3-hydride precursor[3] I was dissolved to the extent of 10% by volume in 50% acetone–50% benzene and treated with increments of purified AgF at room temperature. A smooth reaction at the solid–liquid interface evolved hydrogen and deposited metallic silver, leaving II as the only product in solution. Isolation of II involved the steps: centrifugation and subsequent filtration of the supernatant liquid through Millipore filters; removal of acetone–benzene solvent; and gas–liquid chromatographic separation of the product from any residual solvent. Yields of II uniformly exceeded 95% with the only detectable contaminant being unreacted I.

II is a colorless, volatile liquid possessing a distinctive but not unpleasant odor. The boiling point is 148° at 754.5 mm, which may be compared with the corresponding values for I (141°) and octamethyltrisiloxane (153°). *Anal.* Calcd: C, 34.96; H, 8.80; F, 7.90. Found: C, 32.50; H, 8.47; F, 7.81. The appreciable volatility of II affected the C–H analyses, but not the F analysis in sealed gelatin capsules.

The reaction was monitored throughout by direct measurements of proton magnetic resonance (pmr). The pmr spectrum[4] of I in benzene consists of the hydride quartet centered at 2.21 ppm and the 3-methyl doublet centered at 7.05 ppm with $J_{H-CH_3} = 1.6$ Hz, and the terminal methyl singlet at 7.03 ppm. (In acetone or benzene, the 3-methyl doublet is partially merged with the terminal methyl absorption; in CCl_4, the doublet appears farther upfield and is clearly observed.) As AgF was added to I in acetone–benzene, the hydride resonance signal diminished until it was no longer detected. The original 3-methyl doublet was progressively replaced by a second 3-methyl doublet of larger splitting.

The pmr spectrum of II in benzene solution consists of the terminal methyl singlet at 7.08 ppm and the 3-methyl doublet centered at 7.12 ppm with $J_{F-CH_3} = 4$ Hz. The ^{19}F magnetic resonance spectrum[5] of II shows only the expected quartet, centered 9.8 ppm downfield from the CH_3SiF_3 quartet, with a splitting of 4 Hz.

Comparison of the mass spectra of I (calculated molecular weight = 222.510) and II (calculated molecular weight = 240.500) also demonstrates the fluoride-for-hydride substitution. The parent ion of I is observed, and peaks at *m/e* 221 and 207 indicate fragmentation by the competing loss of either H or CH_3, respectively. The parent ion of II is not observed, but peaks at *m/e* 221 (weak) and 225 (intense) indicate fragmentation by the competing loss of either F or CH_3, respectively. Other differences of 18 mass units between fluoride and hydrogen occur at *m/e* 209 and 193 in II *vs.* respective lines at *m/e* 191 and 175 in I. Both compounds show the intense line of the terminal fragment $(CH_3)_3Si^+$ at *m/e* 73, and a basically similar fragmentation pattern.

The infrared and Raman spectra of I and II were obtained and compared. Apart from the conspicuous absence of the strong Si–H stretching frequency at $\Delta\bar{\nu} = 2150$ cm^{-1}, II possessed a Raman spectrum virtually identical with that of I. For the infrared spectra also, the principal difference was the presence or absence of the Si–H stretching frequency at 2150 cm^{-1}, the remainder of each spectrum being highly similar. However, II did show new shoulders at 1270 and 1120 cm^{-1}, as well as two new peaks at 875 and 795 cm^{-1}, when compared with I. There were also some apparent differences in the 600–250-cm^{-1} region. Because such a comparison involves loss of Si–H as well as gain of Si–F, we can only list the above frequencies as likely candidates for Si–F vibrational modes.

We have found that II, in pure form, is inert to either air or moisture for at least 2 months, and appears to be equally stable in neutral or slightly basic media. However, in acid media the fluoride migrates to carbon, forming a C–F bond and leaving behind a Si–H bond at the 3 position. This reversion of Si–F to Si–H at the chain silicon position occurs readily in acetone solutions containing a trace of either HF or HCl. The above process may be observed directly by pmr, or by infrared measurements after removal of acetone solvent. Spectra obtained by either technique are complex, indicating a mixture of several different fluoro derivatives. Fluorine magnetic resonance applied to such mixtures shows several sets of lines. Presumably, AgF treatment followed by fluoride migration under controlled conditions could be employed in repetitive sequence for extensive fluorination at both carbon and silicon.

As the customary synthesis of linear organosiloxanes involves the hydrolysis of organosilicon chlorides,[6] the acidic environment favors such migration of fluoride. For this reason we question the reported presence of chain Si–F bonds in compounds prepared by hydrolysis of organofluorosilicon chlorides.[7,8]

(3) Obtained through the courtesy of A. N. Pines, Silicones Laboratories, Union Carbide Corp., New York, N. Y.

(4) A Varian HA-100 was employed; the listed chemical shifts are upfield from the benzene signal (≡0).

(5) Measured with a modified Varian HR-60 operating at 56.4 MHz. We are indebted to Dr. Rolf B. Johannesen for the ^{19}F data.

(6) R. J. H. Voorhoeve, "Organohalosilanes. Precursors to Silicones," American Elsevier Publishing Co., New York, N. Y., 1967.

(7) British Patent 627,800 (1949); *Chem. Abstr.*, 44, 4023 (1950).

(8) M. S. Cohen and D. Graftstein, U. S. Patent 2,981,746 (1961).

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Sodium Borohydride Reduction of Oxymercury Compounds

Sir:

Reduction of 3-acetoxy-5-norbornen-2-ylmercuric chloride or 5-acetoxy-3-nortricyclylmercuric chloride, prepared by the method of Pande and Winstein,[1] gives three acetates: 2-*exo*-acetoxynorborn-5-ene (**1**), 7-*anti*-acetoxynorborn-2-ene (**2**), and 3-acetoxynortricyclene (**3**) in the ratio 6:34:60 (±3%), respectively. The acetates were isolated in high yield (98 and 90%, respectively from the two reductions), separated by preparative gas chromatography,[2] and characterized by comparison with authentic samples prepared by unambiguous synthetic routes.[3] Ir, nmr, mass spectra, and glpc reten-

(1) K. Pande and S. Winstein, *Tetrahedron Lett.*, 3393 (1964).

(2) A Wilkins Autoprep was used for preparative chromatography with a 2-m column packed with DEGS (20% w/w) on Chromosorb W, 30–60 mesh, at 110°.

(3) The 2-*exo*-acetoxy compound (1) was prepared by the method of S. J. Cristol, T. C. Morrill, and R. A. Sanchez, *J. Amer. Chem. Soc.*,