eluting shortly before benzene.[13] When this product is absorbed in isohexane and treated with methanolic HCl, the expected quantities of bicyclic ethers are obtained. The ultraviolet absorption spectrum of the isohexane solution exhibits no maximum above 2100 A but has a broad shoulder between 2200 and 2300 A ($\epsilon \sim 2500$). The known substituted benzvalenes have absorption maxima at 2320 A (ϵ 2500)[2] and 2350 A (ϵ 3500).[3] The ultraviolet absorption decreases with a half-time of about 10 days at room temperature; analysis by gas chromatography indicates that benzene is formed in an amount equivalent to the loss of photoproduct.

The 100-Mc nmr spectrum[14] of the product, shown in Figure 1, has three resonances of equal area: an unsymmetrical triplet (1.5- and 1.7-cps couplings) at τ 4.05, a symmetrical triplet (1.5-cps couplings) at τ 6.47, and a quintet (ΣJ = 6.2 cps) at τ 8.16. By decoupling at τ 8.16 both triplets are collapsed to narrow singlets. We can conceive of only two benzene isomers compatible with these results: benzvalene and 3-methylenetricyclo[2.1.0.0^{2,5}]pentane ("criss-cross fulvene"). Only benzvalene is in accord with the facile rearomatization of the photoproduct and its methanolysis to derivatives of bicyclo[3.1.0]hexene. Once the resonance at τ 4.05 is assigned to olefinic protons 3 and 4 in the benzvalene structure, it follows from symmetry considerations that the resonances at τ 6.47 and 8.16 must be assigned to protons 1,6 and 2,5, respectively.

A major problem in this work has been the achievement of concentrations of benzvalene high enough for nmr spectroscopy and preparative gas chromatography. The limiting concentration attainable by irradiation of liquid benzene at room temperature is only 0.01%. It increases with temperature, but is still less than 0.05% at 60°. Enrichment of the solutions by fractional freezing is practical, but we prefer to take advantage of the higher conversions which can be reached by irradiating solutions of the benzene in saturated hydrocarbons.[16] Use of hydrocarbon solvents which are either nonvolatile or more volatile than benzene permits the isolation of these more concentrated solutions by vacuum distillation. We have obtained[15] 1% solutions of benzvalene by irradiating dilute solutions of benzene in hexadecane at 65°.[17]

(13) A 5 ft × 0.25 in. stainless steel column, packed with Chromosorb G coated with 5% didecyl phthalate and 1.25% triethanolamine, was used at room temperature with a helium flow of 100 cc/min. The retention volume of the product relative to benzene was 0.80. On columns coated with didecyl phthalate alone or with hexadecane, the product eluted very close to fulvene[6] and appeared to undergo some decomposition. On polar Ucon in a 12-ft copper column at 50° the product appeared to be completely converted to fulvene.

(14) Spectra were taken on a Varian HA-100 spectrometer (benzene lock); we thank Miss Gail Norman for these measurements. The sample, a 1% solution of benzvalene in benzene, was prepared as described in ref 15. The only other peaks observed in the spectrum were those of the benzene ^{13}C satellites and of the small amount of fulvene present.

(15) A solution of 0.2 ml of benzene in 25 ml of hexadecane, contained in a tube fitted with a Vycor 7910 well (light path 1.5 mm), was swept with N_2 and irradiated (6 min, 10^{21} quanta) at 65° with a mercury resonance lamp, Nester and Faust NFUV-300, operated at 2500 v. The benzene removed from the solvent on the vacuum line at room temperature contained 1% benzvalene and 0.1% fulvene, with only traces of other detectable contaminants.

(16) The limiting conversions increase steadily with dilution of the benzene, over at least a 100-fold range. It is interesting that fulvene formation is also increased by temperature and dilution and that similar limiting concentrations are reached. Fulvene is initially formed at a slower rate than benzvalene, in accord with the suggestion[10] that the latter is a precursor. When one quantum has been absorbed per molecule of benzene, the concentration of benzvalene has reached its limit, but that of fulvene is still low.

(17) NOTE ADDED IN PROOF. If the higher field nmr resonances were assigned in the reverse fashion, as suggested by a referee, the olefinic protons would have no couplings to adjacent or allylic cyclopropyl protons, in contrast to results found[3] for tri-*t*-butylbenzvalene.

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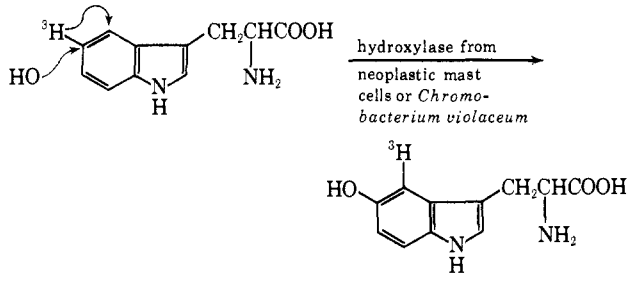
Selective Exchange of Nuclear Protons in Hydroxyindoles

Sir:

The enzymatic hydroxylation of tryptophan-5-^{3}H proceeds with over 90% retention of radioactivity and



leads to 5-hydroxytryptophan-4-^{3}H.[1] This intramolecular shift during enzymatic hydroxylations has made necessary an investigation of the lability of tritium or deuterium in hydroxyindoles and their mechanism of tritiation, deuteration, and detritiation.

As the survey in Table I shows, the deuteration of hydroxyindoles under controlled conditions[2] is selective, as the nmr data indicate.[3]

5-Hydroxyindole, bufotenine, serotonin, and 5-hydroxytryptophan contain a characteristic peak for the 4-proton, a *meta*-coupled doublet (AB system) at 6.86–6.95 which disappeared after exchange with deuterium oxide, while the doublet of doublets (ABC system), belonging to the 6-proton, collapsed to an *ortho*-coupled doublet (BC system). Mass spectral data confirmed the *incorporation of one deuteron*, except in the case of the unsubstituted 5-hydroxyindole which also exchanged the 3-proton in accordance with previous observations.[4-6]

4-, 5-, 6-, or 7-hydroxyindoles partially exchange (70–100%) the 3-proton for deuterium after 16 hr at 60° in a much slower reaction than the exchange of the 4-proton in 5-hydroxyindole, which was complete in less than 3 hr. 6-Hydroxyindole exchanged the 3- and 7-protons (70–80%), while the 5-proton was stable (Table I).

The isomeric 4- and 7-hydroxyindoles exchanged only the 3-proton. Melatonin, a representative 5-methoxy-

(1) J. Renson, J. Daly, H. Weissbach, B. Witkop, and S. Udenfriend, *Biochem. Biophys. Res. Commun.*, **5**, 504 (1966).

(2) The indole (0.2 mmole) was heated (60°) in a sealed tube under nitrogen with D_2O or T_2O, dimethylformamide (0.3 ml), and trimethylamine (20 μl) for 16 hr. Excess water or ethanol was added, the solution was treated with Norite and concentrated to dryness *in vacuo*, and the product was analyzed by mass spectrometry, nmr spectroscopy, and paper or thin-layer chromatography.

(3) *Cf.* L. A. Cohen, J. W. Daly, H. Kny, and B. Witkop, *J. Am. Chem. Soc.*, **82**, 2184 (1960).

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(5) R. L. Hinman and E. B. Whipple, *J. Am. Chem. Soc.*, **84**, 2534 (1962).

(6) R. A. Heacock, O. Hutzinger, B. D. Scott, J. W. Daly, and B. Witkop, *ibid.*, **85**, 1825 (1963).

Table I. Exchange of Aromatic Protons of Hydroxyindoles with Deuterium Oxide

Compound	Nuclear magnetic resonance spectral data[a] 4-H	5-H	6-H	7-H	2-H	3-H
4-Hydroxyindole[b]	....	6.40 d	6.85 m	6.85 m	6.95 d	6.50 d
4-Hydroxyindole-3-^{2}H	....	6.38 d	6.82 m	6.80 m	7.02 s	....
5-Hydroxyindole[b]	6.95 d	....	6.68 dd	7.20 d	7.11 d	6.26
5-Hydroxyindole-3,4-^{2}H	....	....	6.68 d	7.18 d	7.14 s	....
6-Hydroxyindole[b]	7.35 d	6.62 dd	....	6.75 d	6.81 d	6.30
6-Hydroxyindole-3,7-^{2}H	7.35 d	6.60 d	....	(80%)	6.92 s	....
7-Hydroxyindole[b]	6.68 dd	6.98 dd	6.46 dd	....	7.08 d	6.33 d
7-Hydroxyindole-3-^{2}H	6.59 dd	6.93 dd	6.48 dd	....	7.13 s	....
Serotonin[c]	6.86 d	....	6.66 dd	7.16 d	7.02 s	....
Serotonin-4^{2}H	....	....	6.68 d	7.17 d	7.03 s	....
Bufotenine[c]	6.90 d	....	6.65 dd	7.13 d	7.00 s	....
Bufotenine-4-^{2}H	....	....	6.67 d	7.15 d	7.00 d	....
5-Hydroxytryptophan[c]	6.95 d	....	6.70 dd	7.18 d	7.08 s	....
5-Hydroxytryptophan-4-^{2}H	....	....	6.70 d	7.20 d	7.08 s	....
Melatonin[b]	7.02 d	....	6.70 dd	7.24 d	6.98 s	....

[a] Values are expressed in δ units (parts per million). Symbols s, d, dd, and m stand for singlet, doublet, a doublet of doublets, and an unresolved multiplet, respectively. [b] Solvent deuteriochloroform. [c] Solvent deuterium oxide.

indole, did not exchange under these conditions, nor did phenol itself.

The 4-^{3}H derivative of bufotenine, required for biosynthetic studies,[7] was prepared in this manner (New England Nuclear Corp.) and was purified by preparative paper chromatography (Whatman No. 1, 1-butanol–1.0 *N* ammonium hydroxide, 22:5). The 4-^{3}H proton was quite labile under acidic and basic conditions and formed tritiated water which was then isolated in a cold trap of a lyophilization apparatus.[8] Passage of a solution of bufotenine-4-^{3}H through a short Dowex 50 column at pH 4, an IRC-50 column at pH 6.5, or a deactivated charcoal column[9] resulted, respectively, in the loss of activity, as THO, of 50, 15, and 8%. Treatment for 5 min at 0° with 0.4 *N* perchloric acid or 1% trichloroacetic acid gave losses of 16 and 5%. Treatment with trimethylamine, water, and dimethylformamide at 60° (see above) resulted in complete loss of tritium as THO during 2–3 hr. Exchange reactions of bufotenine and 5-hydroxytryptophan with D_2O were followed by nmr spectroscopy. In 1.0 *N* DCl $t_{1/2}$ was <5 min, whereas in 1.0 *N* NaOD $t_{1/2}$ was approximately 7 hr. Under such mild conditions phenol, catechol, and hydroquinone do not exchange.[10,11] Acid catalysis causes complete exchange of both the 4- and 6-protons: 5-hydroxytryptophan on heating with 1.0 *N* DCl at 50° for 1 hr is converted quantitatively to 5-hydroxytryptophan-4,6-$^2H_2$.

Analogously 5-hydroxytryptophan-4-^{3}H was prepared and compared with the radioactive 5-hydroxytryptophan resulting from tryptophan-5-^{3}H by the action of hydroxylase from neoplastic mast cells from mice.[1] Both the synthetic and the enzymatic product lost their tritium at the same rate either on incubation at 50° for 60 min in 10% trichloroacetic acid or on digestion with 0.8 *N* NH_4OH.[1]

This intramolecular shift of tritium or deuterium during enzymatic hydroxylation of indoles from the 5 position to the 4 position is analogous to the 4→3 shifts of ^{2}H and ^{3}H in *para*-substituted phenyl derivatives, such as phenylalanine-4$^{3(2)}$H,[12,13] acetanilide-4$^{3(2)}$H,[14] and even of chlorine in 4-chlorophenylalanine.[15]

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(15) G. Guroff, K. Kondo, and J. Daly, *Biochem. Biophys. Res. Commun.*, **25**, 622 (1966).

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Orientation and Motion of Vinyl Radicals in Neon Matrices

Sir:

We have observed the epr spectrum of vinyl radicals produced by vapor-phase pyrolysis of the iodide and trapped near 4°K in a neon matrix under conditions which have been shown to produce preferentially oriented samples.[1,2] The spectrum obtained can be accommodated with the isotropic parameters from liquid-phase measurements[3] and with dipolar couplings in model systems,[4] provided the following assumptions are made: (1) highly oriented samples are produced in which the carbon–carbon axis is aligned perpendicular to the surface of the flat sapphire rod on which the matrix is deposited; (2) motional averaging of the epr spectrum occurs about the axis of preferential alignment; and (3) the β-hydrogens are magnetically equivalent on the time scale of these measurements (10^{-8} sec).

Spectra obtained for two mutually perpendicular orientations of the sample in the magnetic field are shown in Figure 1. The line shapes and their dependence on sample orientation are more characteristic of single crystals than of powders, indicative of the high degree of alignment. In each of the extreme orientations shown, the spectrum consists essentially of a triplet of doublets, with second-order splitting of the innermost doublet members resolved. The relative simplicity of the spectrum requires an axially symmetric

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