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REGIO-CONTROLLED HYDROGEN-DEUTERIUM EXCHANGE OF BIOLOGICALLY IMPORTANT INDOLES UNDER UV IRRADIATION[# 1]

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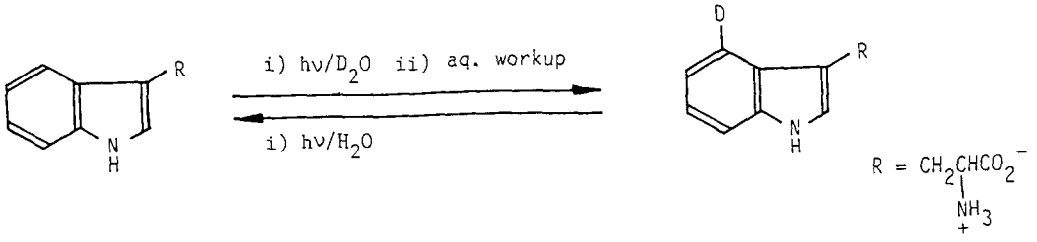
Summary: Photochemical hydrogen-deuterium exchange reaction of biologically important indoles is reported. The regioselectivity of the photodeuteration was found to be controlled by the ammonium group of the side chain.

A convenient method for regioselective isotopic labeling of biologically important indoles under mild conditions is highly desirable for the use of the labeled indoles in physiological and endocrinological studies.[2,3] Deuterated or tritiated trifluoroacetic acid has been used for labeling of tryptophan (Trp), either free or incorporated in a protein.[4] However, the labeling is not regioselective and all five hydrogen atoms of the indole ring are exchangeable.[4] We recently observed a highly efficient (Φ = 0.14) and regioselective incorporation of the deuterium into the C-4 position of Trp upon irradiation in D_2O at neutral pD and demonstrated that a similar photosubstitution occurring in normal light water constitutes a major path of nonradiative decay of singlet excited Trp.[5] We now wish to report that such photosubstitution is applicable to deuterium labeling of a variety of indoles including biologically important indoles. Notable features of the present method are as follows: (i) the regioselectivity can be controlled by the ammonium group of the side chain, and (ii) labeled indoles, once formed, are readily converted to unlabeled species by irradiation in normal light water.[5]

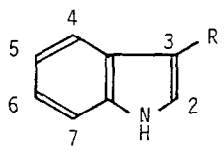


[#] Dedicated to Professor Harry H. Wasserman on the occasion of his 65th anniversary.

As shown in Table I, irradiation of a degassed solution of tryptamine hydrochloride (2, 1 mg) in a 1:4 mixture of CH_3OD-D_2O (5 mL) with Pyrex-filtered light from a 400-W high-pressure mercury lamp resulted in a highly regioselective incorporation of the deuterium into the C-4 position, whereas less selective deuteration at C-4 was observed on irradiation of 3-(3-indolyl)propylamine hydrochloride (3) under the same conditions.[6] By contrast, irradiation of N^b-protected indole 4 or 5 in CH_3OD-D_2O containing a small amount of DCl resulted in a non-selective deuteration at the C-2, C-4 and C-7 positions of the indole ring. In the absence of DCl the photosubstitution of 4 and 5 did not proceed under these conditions. Irradiation of indole-3-acetic acid (6) and 3-indolepropionic acid (7) in D_2O also led to non-selective deuteration.

Table I

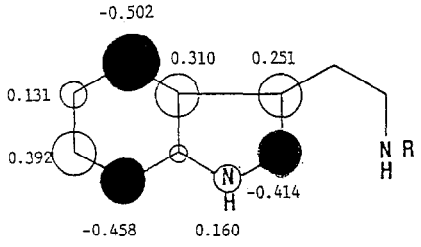
compound R	D incorporation (%)[a] C-2	C-4	C-7	C-4 selectivity C-4/ C-2 + C-7
1; $-CH_2\underset{\underset{NH_3^+}{|}}{C}HCO_2^-$ [b]	7	96	8	6.4
2; $-CH_2CH_2NH_3Cl$ [c]	10	95	9	5.0
3; $-(CH_2)_3NH_3Cl$ [c]	28	65	9	1.8
4; $-CH_2\underset{\underset{NHCO_2Me}{|}}{C}HCO_2Me$ [d]	10	22	10	1.1
5; $-CH_2CH_2NHAc$ [d]	63	65	47	0.6
6; $-CH_2CO_2H$ [c]	17	19	0	1.1
7; $-CH_2CH_2CO_2H$ [c]	24	16	4	0.6



[a]Determined by ^1H NMR (400 MHz). [b]In D_2O. [c]In CH_3OD-D_2O (1:4).
[d]In CD_3OD (1 mL) containing DCl (5 μL).

The non-selective deuteration observed with 4-7 is most simply explained in terms of LUMO-controlled deuteron attack on the C-2, C-4 and C-7 positions of the singlet excited indole rings as typically illustrated in 8[7], followed by proton loss, whereas regioselective C-4 deuteration observed with 1-3 is consistent with an intramolecular proton-transfer process from the side chain ammonium group to the C-4 position of the singlet excited indole ring as suggested previously.[5] Irradiation of 1-methyltryptophan (9) in D_2O gave 4-deuterated 1-methyltryptophan regioselectively.[5] It is interesting to note here that NOE-FID difference spectrum of the 400 MHz ^1H NMR of 9 in D_2O exhibited an enhanced signal of the C-4 proton (δ 7.64) together with those of the C-2 and β-methylene protons by saturation of the α-methine proton (δ 4.44) of the side chain, apparently indicating an appreciable contribution of a folded conformation, 9, in its ground state.

LUMO coefficients of 5



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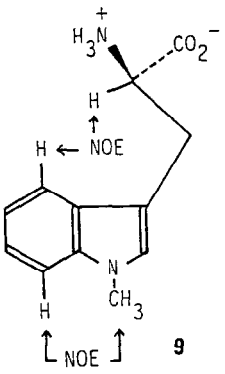

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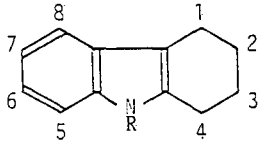
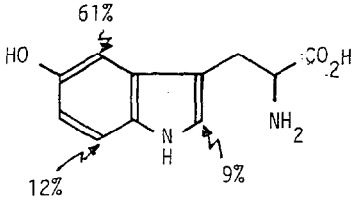
In order to further prove the intramolecular proton-transfer mechanism, we examined the photodeuteration of N-substituted 1,2,3,4-tetrahydrocarbazoles (Table II). Irradiation of 10 gave a mixute of C-5 and C-8 deuterated products with C-8 deuteration predominating, whereas irradiation of 11 and 12 bearing ammonium side chains resulted in a regiospecific deuteration at their C-5 positions. Thus, the regioselectivity is reversed by introducing ethylamino or propylamono side chain at the N-1 position. By contrast, irradiation of 13 or 14 which possess a longer alkyl ammonium side chain, sufficient to reach the C-8 position, resulted in a non-selective deuteration at C-5 and C-8 positions.

Table II

compound R		D incorporation (%)[a] C-5	C-8
	10; $-(CH_2)_2CO_2Me$[b]	7	30
	11; $-(CH_2)_2NH_3Cl$[c]	83	0
	12; $-(CH_2)_3NH_3Cl$[c]	45	0
	13; $-(CH_2)_5NH_3Cl$[c]	14	11
	14; $-(CH_2)_7NH_3Cl$[c]	23	31

[a]Determined by ^1H NMR (400 MHz). [b]In CH_3OD-phosphate buffer (pD 6) (1:2). [c]In CH_3OD-D_2O (1:4).

The present photosubstitution is also applicable to photolabeling of biologically important indoles. When free 5-hydroxytryptophan (15) and serotonin hydrochloride (16) were irradiated in D_2O with Pyrex-filtered light, the deuterium was incorporated mainly into the C-4 positions with minor deuteration at the C-2 and C-7 positions. Likewise, irradiation of Gly-Leu-Trp (17) in CD_3OD containing a small amount of DCl resulted in a regioselective deuteration at the C-4 position of the Trp moiety.



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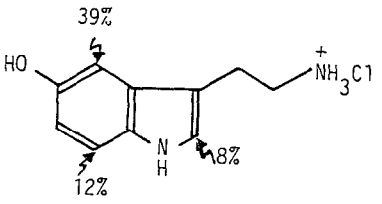

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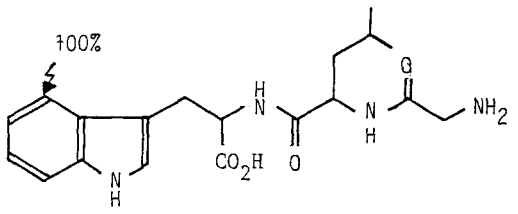

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This photochemical method may be used for specific labeling of Trp residues of polypeptides and proteins with deuterium or tritium.[8]

References and Notes

1. Photoinduced reactions. 165
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3. P. Marche, J. -P., Girma, J. -L. Morgat and P. Fromageot, Eur. J. Biochem. 50, 375 (1975).
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   (b) B. Bak, J. Led and E. J. Pederson, Ibid. 23, 305 (1969).
   (c) L. A. Holt, B. Milligan and D. F. Rivett, Biochem. 10, 3559 (1971).
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5. I. Saito, H. Sugiyama, A. Yamamoto, S. Muramatsu and T. Matsuura, J. Am. Chem. Soc. 106, 4286 (1984).
6. After evaporation of the photolysate, the D content was determined by careful integration of the decreased signals (e.g., C-4 H of 2 at δ 7.70) in the 400 MHz ^1H NMR. Assignment of the deuterated positions was made on the basis of chemical shifts of their ^1H and ^{13}C NMR. The assignment was further confirmed by ^1H NMR NOE techniques for 9 and 11.
7. The LUMO coefficients were calculated by Dr. K. Yamaguchi, Osaka University, using the INDO method.
8. Labeling of Trp residue of certain proteins by irradiation in tritiated water has been reported: L. A. Holt and B. Milligan, Biochem. Biophys. Acta 264, 432 (1972).

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