(m, 2, CH_2), 4.53 (m, 1, CH), 6.90–7.50 (m, 4, arom); ORD (*c* 0.3, MeOH) $[\phi]_{700}$ −76°, $[\phi]_{589}$ −110°, $[\phi]_{355}$ −259° (tr), $[\phi]_{296}$ +200° (pk), $[\phi]_{255}$ −1875° (tr), $[\phi]_{229}$ +12,499° (pk), $[\phi]_{218}$ −53,746° (pk); CD (*c* 0.01 *M*, MeOH) $[\theta]_{290}$ 0, $[\theta]_{288}$ +750, $[\theta]_{270}$ +1850, $[\theta]_{236}$ −650, $[\theta]_{223}$ +40,000, $[\theta]_{207}$ −32,500. *Anal.* ($C_{14}H_{16}N_2O_2 \cdot HCl$) C, H, N.

(−)-(1*S*,3*S*)-6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (2c) and (+)-(1*R*,3*S*)-6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (3c). A solution of 30 g (0.136 mol) of L-5-hydroxytryptophan and 39.2 g (0.89 mol) of CH_3CHO (freshly distilled) in 370 ml of 0.005 *N* H_2SO_4 was stirred under N_2 at room temperature overnight. The solids were collected and crystallized from 1.5 l. of water to give 17.8 g (52%) of 2c: mp 277–279°; [α]D −86.1°; nmr (MeOD + DCl) δ 1.80 (d, 3, *J* = 7 Hz, *Me*CH), 3.10 (m, 1, CH_2), 4.25 (m, 1, *CH*COOH), 4.72 (q, 1, MeC*H*); ORD (*c* 0.15, 1:1 MeOH–0.1 *N* HCl) $[\phi]_{700}$ −145°, $[\phi]_{589}$ −212°, $[\phi]_{316}$ −1754° (tr), $[\phi]_{294}$ +319° (pk), $[\phi]_{265}$ −1754° (tr), $[\phi]_{234}$ +7177° (pk), $[\phi]_{219}$ −45,456° (tr); CD (*c* 0.0063 *M*, 1:1 MeOH–0.1 *N* HCl) $[\phi]_{335}$ 0, $[\theta]_{306}$ −1532, $[\theta]_{279}$ +957, $[\theta]_{252}$ +415, $[\theta]_{227}$ +26,803. *Anal.* ($C_{13}H_{14}N_2O_3 \cdot 0.3H_2O$) C, H, N.

The above aqueous mother liquors obtained upon crystallization of 2c were concentrated to 100 ml, cooled, and filtered. The filtrate was evaporated to 35 ml; the resulting crystals were filtered and recrystallized from H_2O to give 950 mg (3.6%) of 3c: mp 249–250°; [α]D +5.92°; nmr (MeOD + DCl) δ 1.74 (d, 3, *J* = 7 Hz, *Me*CH), 3.30 (m, 2, CH_2), 4.60 (dd, 1, *J* = 6 and 9 Hz, CH_2 *CH*), 4.95 (q, 1, *J* = 7 Hz, MeC*H*), 6.76 (dd, 1, *J* = 2.5 and 8.5 Hz, arom), 6.90 (d, 1, *J* = 2.5 Hz, arom), 7.21 (d, 1, *J* = 8.5 Hz, arom); ORD (*c* 0.26, 1:1 MeOH–0.1 *N* HCl) $[\phi]_{700}$ +8°, $[\phi]_{589}$ +11.7°, $[\phi]_{340}$ −27° (tr), $[\phi]_{314}$ +225°, $[\phi]_{280}$ −4498° (tr), $[\phi]_{250}$ +1499° (pk), $[\phi]_{237}$ +500° (tr), $[\phi]_{220}$ +44,983° (pk); CD (*c* 0.01 *M*, 1:1 MeOH–0.1 *N* HCl) $[\theta]_{325}$ 0, $[\theta]_{305}$ +1000, $[\theta]_{265}$ −5950, $[\theta]_{230}$ −21,000, $[\theta]_{210}$ +35,000. *Anal.* ($C_{13}H_{14}N_2O_3 \cdot 0.75H_2O$) C, H, N.

(−)-(1*S*,3*S*)-1,2-Dimethyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (2d). In a manner similar to the procedure for 1b, 1.6 g (6.38 mmol) of 2c was N-methylated to give 1.5 g (84%) of 2d: mp 268°; [α]D −27.9°; nmr (DMSO-d_6) δ 1.58 (d, 3, *J* = 6.5 Hz, *Me*CH), 2.52 (s, 3, MeN), 2.90 (m, 2, CH_2), 3.72 (m, 1, *CH*CH_2), 4.39 (q, 1, *J* = 6.5 Hz, MeC*H*), 6.58 (dd, 1, *J* = 2 and 8 Hz, arom), 6.76 (d, 1, J_{meta} = 2 Hz, arom), 7.11 (d, 1, J_{ortho} = 8 Hz, arom); ORD (*c* 0.19, 1:1 MeOH–0.1 *N* HCl) $[\phi]_{700}$ −59°, $[\phi]_{589}$ −75°, $[\phi]_{331}$ −140° (pk), $[\phi]_{316}$ −350° (tr), $[\phi]_{285}$ +2097° (pk), $[\phi]_{259}$ −1538° (tr), $[\phi]_{233}$ +9785° (pk), $[\phi]_{219}$ −36,344° (tr); CD (*c* 0.007 *M*, 1:1 MeOH–0.1 *N* HCl) $[\theta]_{480}$ 0, $[\theta]_{460}$ +7, $[\theta]_{368}$ +25, $[\theta]_{305}$ −978, $[\theta]_{275}$ +3494, $[\theta]_{226}$ +23,414. *Anal.* ($C_{14}H_{16}N_2O_3 \cdot 0.5H_2O$) C, H, N.

Acknowledgments. We wish to thank the following members of our Physical Chemistry Department (Director, Dr. R. P. W. Scott): Dr. F. Scheidl for the microanalyses, Dr. T. Williams for the nmr spectra, and Dr. V. Toome for the ORD and CD spectra. We are particularly grateful to Mr. P. Heslin for technical assistance.

References

(1) S. Teitel, J. O'Brien, and A. Brossi, *J. Med. Chem.*, 15, 845 (1972) (paper 2).
(2) W. M. Whaley and T. R. Govindachari, *Org. React.*, 6, 151 (1951).
(3) D. B. Calne and M. Sandler, *Nature (London)*, 226, 21 (1970); V. E. Davis and M. J. Walsh, *Science*, 167, 1005 (1970); G. Cohen and M. Collins, *ibid.*, 167, 1749 (1970); T. L. Sourkes, *Nature (London)*, 229, 413 (1970).
(4) A. Brossi, A. Focella, and S. Teitel, *Helv. Chim. Acta*, 55, 15 (1972).
(5) V. Erspamer, *Fortschr. Arzneimittelforsch.*, 3, 179 (1961).
(6) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 113, 759 (1936).
(7) S. Yamada and H. Akimoto, *Tetrahedron Lett.*, 3105 (1969).
(8) S. Yamada, M. Konda, and T. Shioiri, *ibid.*, 2215 (1972).
(9) E. Siegmund, R. Cadmus, and G. Lu, *Proc. Soc. Exp. Biol. Med.*, 95, 729 (1957).
(10) N. W. Dunham and T. S. Miya, *J. Amer. Pharm. Ass., Sci. Ed.*, 46, 208 (1957).
(11) L. O. Randall and J. J. Selitto, *Arch. Int. Pharmacodyn*, 111, 409 (1957).

A Convenient, General Procedure for Preparing Specifically [^{3}H]-Labeled Amines. Synthesis of [^3H]-Meperidine Hydrochloride

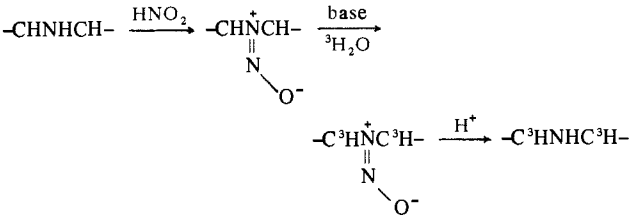
P. S. Portoghese* and D. L. Larson

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received November 30, 1972

In connection with a study on the distribution and metabolism of N-alkyl homologs of normeperidine (1), we wished to prepare 1 with ^{3}H localized exclusively in the piperidine ring. The ready supply of **1** prompted us to explore a potentially efficient and inexpensive, general method of *specifically* labeling amines with isotopic hydrogen. Since it is often the case that the labeling procedure is the most time-consuming operation in drug metabolism studies, we thought the development of such a method would simplify the task of labeling and make available specifically labeled compounds which are not easily accessible by other routes. In this report we describe a procedure which allows the protons α to a secondary amine function to be exchanged with isotopic hydrogen in relatively high yield.

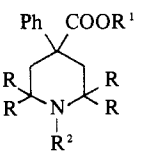
It has been reported[1-3] that the *N*-nitroso group is capable of stabilizing a carbanion at the α position and, thus, facilitating proton exchange under basic conditions. This suggested a method whereby **1** and other secondary amines could readily be labeled. Accordingly, the general labeling procedure (Scheme I) involves conversion of a secondary amine to

Scheme I



its *N*-nitroso derivative and subjecting this intermediate to base-catalyzed exchange in ^3H_2O. The radiolabeled *N*-nitroso derivative then would be denitrosated to the desired amine under acidic conditions.

The specificity of isotopic hydrogen incorporation by this procedure was determined by studying the exchange of D for ^{1}H in **3**. Intermediate **3** was obtained in an overall yield of 78% by conversion of **1** to its nitroso derivative **2** followed by ester hydrolysis. Although **3** was isolated prior to the ex-



1, R = H; R^1 = Et; R^2 = H
2, R = H; R^1 = Et; R^2 = NO
3, R = R^1 = H; R^2 = NO
4, R = D; R^1 = H; R^2 = NO
5, R = D; R^1 = Et; R^2 = H

change reaction for the purpose of characterization, in practice no isolation is required.

Exchange was accomplished by exposure of **3** to 3–6 *M* NaOD in D_2O at 95°. The progress of the exchange was monitored by observing the disappearance of the pmr signals for the α protons at δ 3.2–5.0. With 3 *M* NaOD virtually

complete exchange took place after 10 hr. Heating the mixture appears to be necessary, as no significant amount of exchange was noted after standing 16 hr at 25°.

Upon treatment of the sodium salt of 4 with $SOCl_2$ in ethanol, both esterification and denitrosation took place to afford normeperidine-*d* hydrochloride (5 · HCl). Denitrosation is due to the action of HCl generated in the reaction mixture, as cleavage also could be effected with ethanolic HCl. It was found that addition of urea greatly facilitated denitrosation by trapping NO^+ generated in this reaction.[4]

Mass spectral analysis of 5 showed it to contain 66.5% D_4, 25.3% D_3, 4.4% D_2, and 1.7% D, the balance (2.1%) being undeuterated. The pmr spectrum of 5 exhibited two doublets (J_{gem} = 14 Hz) at δ 2.35 and 2.76 which are due to the axial and equatorial protons at C-3 and C-5. This is consistent with the α positions being the sites of exchange and is in marked contrast with the pmr spectrum of the undeuterated compound 1 which exhibits an envelope absorption in the δ 2.2–3.7 region.

Radiolabeled 3 was prepared by a similar procedure using 3 *M* NaO^3H in ^3H_2O (250 mCi). This intermediate was not isolated but, subsequent to denitrosation–esterification, was converted by the Leuckart reaction to [^3H]-meperidine.

The ^3H_2O was recovered from the reaction mixture and possessed sufficient activity to warrant its use in another isotopic exchange reaction.

In summary, the results of this study indicate that the labeling procedure is useful for localizing isotopic hydrogen α to an amine function. The specificity of the reaction and the nonlability of isotopic hydrogen in the α position of amines offers a distinct advantage over random labeling procedures. The facility and inexpensiveness of the method make it possible to prepare labeled amines which are otherwise obtainable only by more laborious procedures.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. Microanalyses were performed by M-H.W Laboratories, Garden City, Mich. Glc analysis was carried out on a Varian 2100 instrument equipped with a flame ionization detector and a 0.25 × 72 in. glass column packed with 3% OV-17 on Chromosorb W (80–100 mesh) using N_2 carrier gas. Nmr spectra were obtained in $CDCl_3$ or D_2O with a Varian A-60D spectrometer using TMS or DDS as internal standards. Mass spectra were obtained on a Hitachi RMU 6 spectrometer.

N-Nitrosonormeperidine (2). A stirred solution of 1 · HCl (5.0 g, 0.0186 mol) in pH 4, acetate buffer (10 *M*, 200 ml) was maintained at 95° and treated dropwise over a 3-hr period with $NaNO_2$ (25 g) in water (50 ml). After the reaction mixture was cooled, it was extracted with $CHCl_3$ and washed successively with solutions of saturated NaCl and Na_2CO_3 (10%), and the $CHCl_3$ extract was dried ($MgSO_4$). Removal of the solvent *in vacuo* afforded 4.8 g (98%) of 2: mp 38–40°; ir (neat) 1725 (C=O), 1425 (N=O), 980 cm^{-1} (NN). *Anal.* ($C_{14}H_{18}N_2O_3$) C, H, N.

N-Nitrosonormeperidinic Acid (3). Intermediate 2 (4.8 g, 0.0182 mol) was dissolved in an ethanolic solution of 0.6 *N* KOH (200 ml) and the mixture refluxed for 2.5 hr. The solvent was removed *in vacuo* and the solid was dissolved in H_2O. Acidification (10% HCl) afforded a precipitate which was collected by filtration, washed (H_2O), and twice crystallized (EtOH) to yield 3.4 g (80%) of 3: mp 185–186°; ir (KBr) 3200–2500 (H-bonded OH), 1725 (acid C=O), 1425 (N=O), and 985 cm^{-1} (NN). *Anal.* ($C_{12}H_{14}N_2O_3$) C, H, N.

Normeperidine-*d* (5). A 0.5-ml glass reaction vessel containing 0.25 ml of 6 *M* NaOD (prepared from 230 mg of Na_2O and 0.5 ml of D_2O) and 75 mg (0.32 mmol) of 3 was shaken over a steam bath for 9 hr. The contents of the vessel were frozen and lypholyzed (0.5 mm). Dry EtOH (3 ml) was added and $SOCl_2$ (0.7 ml) was dropped into the mixture which was cooled (ice bath) and continuously agitated. Urea (190 mg, 3.2 mmol) was then added and the reaction mixture was refluxed for 3 hr. The mixture then was diluted with H_2O (10 ml) and the EtOH was partially removed *in vacuo*. The residual acidic solution was extracted (Et_2O), made basic (10% Na_2CO_3), and partitioned into $CHCl_3$. The combined $CHCl_3$ extracts were washed with saturated NaCl and dried ($MgSO_4$), and the solvent was removed and replaced with Et_2O. Addition of ethereal HCl afforded normeperidine-*d* HCl (50 mg, 60%), mp 129–131°, which was recrystallized (EtOH–Et_2O) and dried *in vacuo*. Tlc comparisons with authentic material corresponded [mass spectrum *m/e* (M^+, rel intensity) 233 (3.3), 234 (2.6), 235 (9.9), 236 (35.0), 237 (100) (nondeuterated material *m/e* (M^+, rel intensity) 232 (10), 233 (100), 234 (10))] with mol % deuterium incorporation: 2.2, nondeuterated; 1.7, monodeuterated; 6.6, dideuterated; 23.2, trideuterated; 66.3, tetradeuterated. Nmr ($CDCl_3$): δ 1.18 (t, 3, CH_3), 2.35 and 2.76 (d, J = 14 Hz, ~3.5, CH_2CD_2), 4.15 (q, 2, OCH_2).

[^3H]-Meperidine Hydrochloride. Intermediate 3 (75 mg, 0.32 mmol) was mixed with 0.25 g of ^3H_2O (250 mCi) and 50 mg of NaOMe in a 0.5-ml glass vessel which then was sealed and heated in a steam bath for 10 hr. The reaction mixture was frozen and the ^3H_2O was removed *in vacuo* (0.5 mm) and collected in a Dry Ice trap. The residue was diluted with unlabeled 3 (225 mg), treated successively with $SOCl_2$ (1 ml), anhydrous EtOH (3 ml), and urea (190 mg), and then refluxed (1 hr). The reaction mixture was then treated with 37% CH_2O (2.5 ml) and 88% HCOOH (0.9 ml) and heated on a steam bath for 4 hr. The solvent was removed *in vacuo* and the residue dissolved in H_2O and extracted with Et_2O. The aqueous layer was basified (10% Na_2CO_3) and extracted (Et_2O). The Et_2O extract was washed with saturated NaCl, decolorized, and dried ($MgSO_4$). The ethereal solution was made acidic with ethanolic HCl and the precipitate crystallized three times (EtOH–Et_2O) to give 103 mg (30%) of [^3H]-meperidine HCl: mp 183–184°; specific activity 0.54 mCi/mmol. Chemical and isotopic purity were confirmed by ir and tlc radioassay.

Acknowledgment. We wish to thank Dr. F. C. Nachod of the Sterling-Winthrop Research Institute for providing us with a generous supply of normeperidine hydrochloride.

References

(1) L. K. Keefer and C. H. Fodor, *J. Amer. Chem. Soc.*, 92, 5747 (1970).
(2) W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron*, 26, 5137 (1970).
(3) R. R. Fraser and Y. Y. Wigfield, *Tetrahedron Lett.*, 2515 (1971).
(4) P. A. S. Smith and R. N. Loeppky, *J. Amer. Chem. Soc.*, 89, 1147 (1967).

Anticancer Compounds. Further Analogs of 1-(4-Dimethylaminobenzylidene)indene†,‡

Carl T. Bahner,* Norma H. Bingham, David H. Brotherton, Jane Cline, Dan Darby, Harry D. Harmon, Lydia M. Rives, Bill L. Stump, and Stuart L. Watson, Jr.

Department of Chemistry, Carson-Newman College, Jefferson City, Tennessee 37760. *Received January 5, 1972*

Several years ago 1-(4-dimethylaminobenzylidene)indene (1) was prepared as an analog of 4-(4-dimethylaminostyryl)-quinoline[1] (2). Tests by Haddow, Everett, and Mitchley against the subcutaneous Walker 256 tumor by the single dose method showed that 1 was as effective as 2 in this test and that 1 was far less toxic than 2, so that the therapeutic ratio was much more favorable. Further tests in other laboratories showed that 1 was very effective also against the established intramuscular Walker 256 tumor and against Lymphoma 8[2,§] but not against Leukemia 1210. We have reported syntheses and test results on a number of variations

†This investigation was supported by Public Health Service Research Grants No. CA-03717-09-13 from the National Cancer Institute and by the Hamblen County Cancer Association.

‡Part of this material was presented at the Southeastern Regional Meeting of the American Chemical Society in Richmond, Va., Nov 6, 1969, and part at the meeting in Nashville, Tenn., Nov 5, 1971.

§R. M. Folk, private communication, Battelle Memorial Institute.