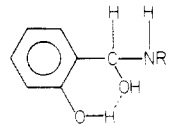
ing will affect the observed rate adversity.

The dehydration of the carbinolamine derived from the reaction of phenylhydrazine and *o*-hydroxybenzaldehyde, observed from pH 3.5 to 6, occurs with specific-acid catalysis but without general-acid catalysis by carboxylic acids, while general-acid catalysis by carboxylic acids has been observed in the dehydration of other carbinolamines.[8] It is possible to explain the absence of general-acid catalysis of the dehydration by an intramolecular acid catalysis by hydrogen bond.



Acknowledgment. The authors are indebted to Dr. Eugene H. Cordes for helpful comments concerning this work.

Registry No. *o*-Hydroxybenzaldehyde, 90-02-8; phenylhydrazine, 100-63-0; *o*-hydroxybenzaldehyde phenylhydrazone, 614-65-3.

Supplementary Material Available: Table I, determination of values of pK_a for *o*-hydroxybenzaldehyde at 25.0 °C in 20% aqueous ethanol and an ionic strength of 0.50 (1 page). Ordering information is given on any current masthead page.

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(±)-[*methyl*-^{3}H and -^{2}H]Mianserin. Participants in a Dramatic Instance of HPLC Isotopic Fractionation[1]

Crist N. Filer,* Robert Fazio, and David G. Ahern

Ligands Group, New England Nuclear, Boston, Massachusetts 02118

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One functionality common to a number of valuable central nervous system (CNS) drugs both naturally occurring and synthetic is the *N*-methyl group. The availability of C^3H_3I at high specific activity (40–90 Ci/mmol) has allowed the preparation of *N*-[^{3}H]methyl CNS drugs at correspondingly high specific activity from accessible nor precursors for receptor binding studies.[2] In this way, we have prepared [*methyl*-^{3}H]oxymorphone (**1**), [*methyl*-^{3}H]morphine (**2**), [*methyl*-^{3}H]dihydromorphine (**3**), and [*methyl*-^{3}H]LSD (**4**) at high specific activity.[3] Although the identity of these substances has been conclusively demonstrated by spectroscopic (UV, ^{3}H NMR) evidence and by in vitro receptor binding assay, we have occasionally noted differences in their HPLC behavior as compared to cold standard. In particular, **2**–**4** have been observed to elute later than their respective cold standards on microporasil HPLC and we have attributed this behavior to isotopic fractionation.[4] To document the often intriguing HPLC behavior of these *N*-[^{3}H]methyl CNS drugs and thereby facilitate their identification, we now report the most dramatic instance of this phenomenon that we have yet observed.

(±)-Mianserin (**5a**) is an *N*-methyl tetracyclic antidepressant with affinity for both the serotonin and histamine receptors.[5] By use of either CNBr or vinyl chloroformate,[6]

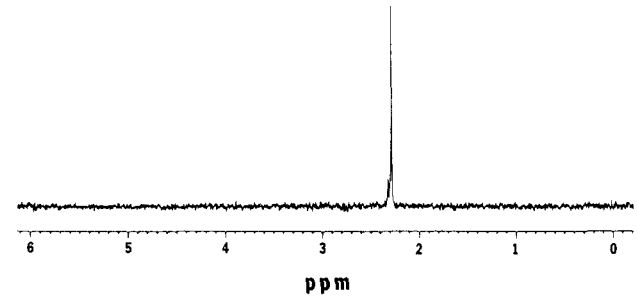


Figure 1. ^{3}H NMR spectrum of [*methyl*-^{3}H]mianserin (**5c**) in $CDCl_3$. Chemical shift values are in parts per million downfield from internal $(CH_3)_4Si$.

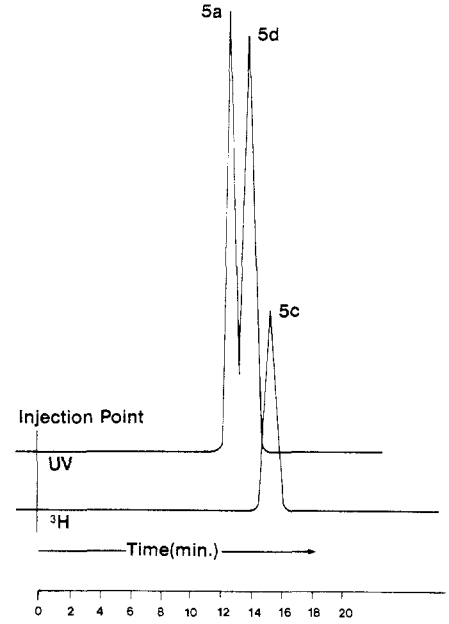
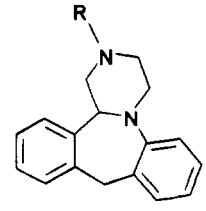


Figure 2. HPLC trace of compounds **5a**, **5d**, and **5c** coinjected on microporasil eluted at 1 mL/min with CH_2Cl_2–CH_3OH (98:2) and monitored by simultaneous UV (254 nm) and ^{3}H detection.

Table I. Field-Desorption Mass Spectral Data for 5c

m/e	% of base peak	species
264	8.8	cold mianserin
266	1.5	[*N*-C^{1}H$_2$^{3}H$_1$]mianserin
268	10.3	[*N*-C^{1}H$_1$^{3}H$_2$]mianserin
270	79.4	[*N*-C^{3}H$_3$]mianserin



5a R = CH_3
5b R = H
5c R = C^3H_3
5d R = C^2H_3

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(4) Klein, P. D. In "Advances In Chromatography"; Giddings, J. D., Keller, R. A., Eds.; Marcel Dekker: New York, 1966; Vol. 3, p 3.

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(±)-normianserin (**5b**) was easily prepared in 30% yield from **5a**. Samples of **5b** obtained by either N-demethylation procedure were identical with one another (TLC, HPLC, ^1H NMR ($CDCl_3$) and, by treatment with CH_3I, could be reconverted back to **5a** whose cochromatography (TLC, HPLC) and ^1H NMR ($CDCl_3$) were identical with those of authentic **5a**. Treatment of **5b** with C^3H_3I afforded radiochemically pure (±)-[*methyl*-^3H]mianserin (**5c**) in 36% radiochemical yield, exhibiting cochromatography (TLC) with **5a** and a UV (EtOH) spectrum that was superimposable on that of **5a**. Also, the ^3H NMR ($CDCl_3$) (Figure 1) and field desorption mass spectra[7] (Table I) of the free base of **5c** and the in vitro receptor binding assay of **5c** at the serotonin and histamine receptors[8] were consonant with its proposed structure. Remarkably, however, **5c** did not coelute with **5a** when coinjected on microporasil HPLC (Figure 2) but had a significantly longer retention time.

To prove that the anomalous HPLC retention time of **5c** with respect to that of **5a** was due to isotopic fractionation, the preparation of (±)-[*methyl*-^2H]mianserin (**5d**) and a study of its HPLC behavior were undertaken. Therefore, compound **5b** was treated with C^2H_3I to afford pure **5d** in 31% yield. Compound **5d** cochromatographed with **5a** on TLC and gave a UV (EtOH) spectrum superimposable on that of **5a**. It also displayed ^1H and ^2H NMR ($CDCl_3$) spectra and a high-resolution mass spectrum in agreement with its proposed structure. However, in harmony with what would be expected from the standpoint of isotopic fractionation, when coinjected on microporasil HPLC (Figure 2), **5d** eluted after **5a** and before **5c**.[9] We believe that **5c** and **5d** do indeed possess their proposed structures and that their anomalous HPLC behavior is a dramatic instance of isotopic fractionation. These observations indicate that by HPLC **5c** behaves as if it were more polar (on microporasil, later eluting) than its cold counterpart **5a**. Apparently **5d**, eluting between **5a** and **5c** on microporasil HPLC, is of intermediate polarity.

HPLC cochromatography is increasingly relied on to establish the identity and purity of radiochemicals. Our observations on the relative HPLC retention times of compounds **5a**, **5c**, and **5d** will serve to clarify the interpretation of the HPLC behavior of other high specific activity *N*-[^3H]methyl CNS drugs.

Experimental Section

General Methods. Evaporations were carried out on a Büchi rotary evaporator in vacuo at bath temperatures below 40 °C. TLC was performed on Analtech 5 × 15 cm, 250 μm (analytical), and 20 × 20 cm, 1000 μm (preparative), silica gel GF coated glass plates. Common solvent combinations were S_1 (CH_3OH–acetone, 1:1), S_2 (CH_3OH–EtOAc–cyclohexane–NH_4OH, 3:14:3:1), S_3 (CH_3OH–2 N NH_4OH, 9:1), and S_4 (CH_3OH–PhH–H_2O, 7:1:1). Autoradiography was performed at 0 °C after TLC plates were sprayed with PPO (New England Nuclear) and exposed to Eastman Kodak SB-5 film. TLC plates were also scanned for activity by using a Packard 7201 scanner. Analytical HPLC was run on a Waters instrument, using a microporasil column eluted with either S_5 (CH_2Cl_2–CH_3OH, 92:8) or S_6 (CH_2Cl_2–CH_3OH, 98:2). Peak detection was performed simultaneously by a Waters 440 UV detector at 254 nm and a liquid scintillation flow monitor. UV spectra were measured on a Beckman Model 25 spectrophotometer. The ^1H, ^2H, and ^3H NMR spectra were obtained on a Bruker WP 200-MHz instrument. Chemical shift values are expressed in parts per million downfield from internal $(CH_3)_4Si$. The high-resolution mass spectra were performed by Shrader Analytical Laboratories, Detroit, MI.

(±)-Normianserin (5b). To a stirred solution of **5a** free base (Organon, 88 mg, 0.33 mmol) in 4 mL of $CHCl_3$ was added 64 mg (0.60 mmol) of CNBr in 2 mL of $CHCl_3$. The reaction was heated under nitrogen for 2 h at 60 °C. After this time, it was cooled and concentrated to a volume of 0.3 mL and purified on two 1000-μm silica gel TLC plates eluted with S_1. The main band (R_f 0.9) was visualized by UV, scraped off, and eluted with EtOH. Solvent evaporation yielded 113 mg (92%) of *N*-cyanomianserin. To a solution of this intermediate in 3 mL of H_2O–HOAc (1:2) was added 700 mg of zinc dust and the mixture was heated under nitrogen at 60 °C for 3 h. After this time, the reaction was cooled, filtered, and diluted with 10 mL of 1% aqueous tartaric acid. It was then extracted with two 10-mL portions of $CHCl_3$. The $CHCl_3$ solution was concentrated to 0.3 mL and purified on two 1000-μm silica gel plates eluted with S_2. The main band (R_f 0.5) was visualized by UV, scraped off, and eluted with $CHCl_3$. Solvent evaporation yielded 25 mg (30% yield) of **5b** as an oil that was homogeneous on TLC (silica gel, S_2) and HPLC (microporasil, S_5); ^1H NMR ($CDCl_3$) δ 7.23–6.80 (m, 8), 4.85 (d, 1, J = 12.94 Hz), 3.95 (dd, 1, J = 3.91, 9.03 Hz), 3.30–3.00 (m, 7). The NCH_3 resonance at δ 2.29 was conspicuously absent; exact mass calcd for $C_{17}H_{18}N_2$ (M^+) 250.1468, found 250.1488.

Compound **5b** prepared by the vinyl chloroformate method[6] cochromatographed with (silica gel TLC, S_2; microporasil HPLC, S_5) and displayed a ^1H NMR ($CDCl_3$) spectrum identical with **5b** prepared by CNBr treatment of **5a**.

(±)-[*methyl*-^3H]Mianserin (5c). A solution of 7 mg (0.024 mmol) of **5b**, 5 mg of $NaHCO_3$ in 2 mL of CH_3OH, and 4.56 Ci (0.08 mmol at 57 Ci/mmol) of C^3H_3I (New England Nuclear) was heated at 60 °C in a sealed vessel for 1.5 h. After this time, excess solvent was evaporated and the crude concentrated reaction was purified by preparative TLC on two 500-μm silica gel plates eluted with S_3. Authentic **5a** was allowed to elute side by side with **5c** to facilitate the location of **5c** on the TLC plates. The main radioactive band corresponding to **5c** (R_f 0.5) was visualized by UV, scraped off, and eluted with EtOH. The total activity contained in the EtOH eluent was 503 mCi (a 36% radiochemical yield based on **5b**). TLC (silica gel, S_2, S_3, S_4) of **5c** underspotted with **5a** indicated that its radiochemical purity was 99%, and in these TLC systems, **5c** cochromatographed with **5a**. Compound **5c** was also found to be 99% radiochemically pure by HPLC (microporasil, S_6). The UV (EtOH) spectrum of **5c** was completely superimposable on that of **5a**, and the specific activity of **5c** was determined to be 57.7 Ci/mmol by UV spectroscopy (282 nm (ε 1930) for **5a**). For the ^3H NMR of **5c** see Figure 1 and for the field-desorption mass spectral data for **5c** see Table I.

(±)-[*methyl*-^2H]Mianserin (5d). A solution of 15 mg (0.052 mmol) of **5b** and 5 mg of $NaHCO_3$ in 2 mL of CH_3OH with 6.5 μL (0.105 mmol) of C^2H_3I (Aldrich 99+% D, 17, 603-6) was allowed to stir at 30 °C for 2 h. After this time, another 5 μL of C^2H_3I was added and the reaction was allowed to stir at 30 °C for another 1 h. The reaction was then concentrated to 0.3 mL and purified by preparative TLC on one 1000-μm silica gel plate eluted with S_3. The main band was visualized by UV (R_f 0.5), scraped off, and eluted with EtOH. Solvent evaporation yielded 5 mg (31% yield) of **5d** as an oil that was chromatographically homogeneous on TLC (silica gel, S_3, S_4) and HPLC (microporasil, S_5). On TLC, **5d** cochromatographed with **5a** and the UV (EtOH) of **5d** was superimposable on that of **5a**. Other spectral data for **5d** now follow: ^1H NMR ($CDCl_3$)[10] δ 7.25–6.80 (m, 8) 4.85 (d, 1, J = 12.70

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(8) Personal communication with Professor S. H. Snyder (Johns Hopkins).

(9) Figure 2 is an accurate representation of an actual HPLC trace for **5a**, **5d**, and **5c**. It should be pointed out that, due to recorder pen (UV, ^3H) offset and the fact that the UV detector is in tandem with but upstream from the radioactivity detector, the actual peak separation of **5d** and **5c** is somewhat smaller than that displayed in Figure 2. However, on occasion we have injected on HPLC essentially equivalent mass amounts of **5a**, **5d**, and **5c** and have observed *by UV detection* clear (although not baseline) separation of **5c** eluting after **5d**.

(10) The ^1H NMR ($CDCl_3$) spectrum of **5d** is essentially identical (except for the missing *N*-methyl group) with that of **5a**. For a discussion of the ^1H NMR spectrum of **5a** see: Ramon, C. Thèse d'Université, Paris, 1969.

Hz), 4.05 (dd, 1, J = 2.69, 10.01 Hz), 3.45–3.20 (m, 3), 3.00–2.80 (m, 2), 2.50–2.30 (m, 2); ^{2}H NMR ($CHCl_3$) δ 2.33 (s, NC^2H_3); exact mass calcd for $C_{18}{}^2H_3{}^1H_{17}N_2$ (M^+) 267.1813, found 267.1806.

Acknowledgment. We thank Dr. R. M. Koenig (Organon) for a generous gift of **5a**. We gratefully acknowledge the technical assistance of L. Todisco (NEN) and R. Nugent (NEN) in the conversion of **5b** to **5c** and the help of Dr. P. Srinivasan (NEN) and L. Thomas (NEN) in obtaining the ^{3}H NMR for **5c** and the ^{2}H NMR spectrum for **5d**. We also thank Professor S. H. Snyder (Johns Hopkins) for performing receptor binding experiments on **5c** and Professor H.-R. Schulten (University of Bonn) for obtaining the field-desorption mass spectrum of **5c**. Finally we also gratefully acknowledge the technical assistance of M. Tutunjian (NEN) and R. Wellman (NEN) in performing the HPLC experiments on **5a**, **5c**, and **5d**.

Registry No. (±)-**5a**, 76612-54-9; (±)-**5b**, 77862-25-0; (±)-**5c**, 77862-26-1; (±)-**5d**, 77862-27-2.

1,4-Dihydroxy-2,5-dioxopiperazines from Activated *N*-Hydroxy Amino Acids

Jacobus D. M. Herscheid, J. Hans Colstee, and Harry C. J. Ottenheijm*

Department of Organic Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

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With the characterization[1] of the mold metabolite aspergillic acid as a cyclic hydroxamic acid and the demonstration[2] of the in vivo N-hydroxylation of amides, interest in oxidized peptide bonds [C(O)N(OH)] continues to grow. It is now recognized that such hydroxamic acids occur frequently in nature, serving as growth factors, antibiotics, and microbial pigments.[3] In addition, they may play a role in the biosynthesis of microbial metabolites.[4] Such considerations led us to undertake the synthesis of 1,4-dihydroxy-2,5-dioxopiperazines (**9**) in our study of the biosynthesis of gliotoxin.[5]

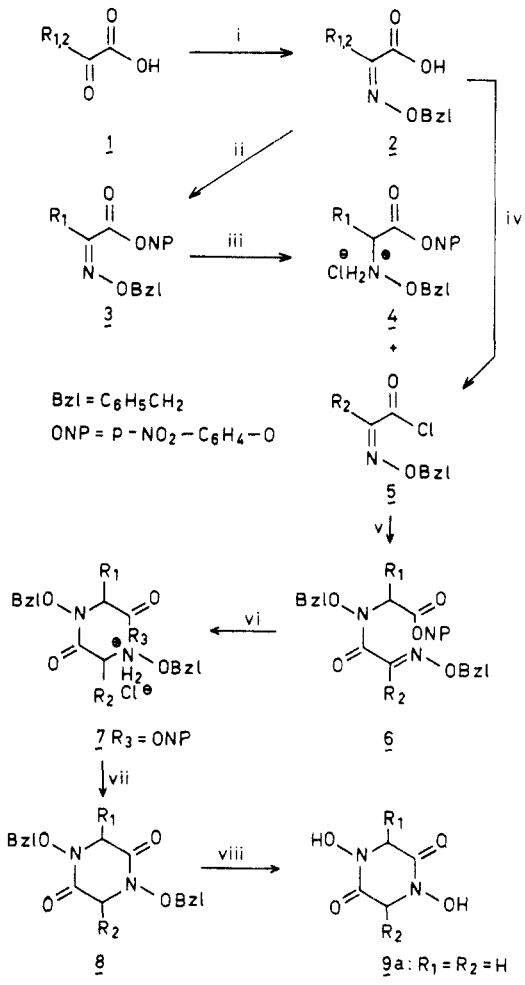
Since the only synthesis of *N,N'*-dihydroxydioxopiperazines (**9**) reported to date[6] is rather inefficient, we first set out to devise an efficient, generally applicable scheme for these compounds. Conventionally, dioxopiperazines are prepared by ring closure of the corresponding dipeptide alkyl esters. This approach fails, however, in the synthesis of the title compounds as the ethyl esters of *N,N'*-bis(benzyloxy)dipeptides **7** (R_3 = OC_2H_5) resisted all attempts to produce **8** by ring closure. This failure can be rationalized by assuming that the N(H) benzyloxy group in **7** (R_3 = OC_2H_5) causes increased steric hindrance and decreased nucleophilicity compared to the corresponding amine. The conversion **7** → **8** thus seemed to require an activated carboxylic acid derivative of **7**. Indeed, we found that ring closure could be achieved by neutralizing a solution of the *p*-nitrophenyl ester **7** with pyridine, giving **8** in fair to good yields (see Table I).

Table I. *N,N'*-Dibenzyloxydioxopiperazines **8**[a]

8	% yield from 4	mp, °C (CH_2Cl_2/hexane)	formula
a, R_1 = H, R_2 = H	61	217-218	$C_{18}H_{18}N_2O_4$
b, R_1 = H, R_2 = CH_3	60	137-138	$C_{19}H_{20}N_2O_4$
c, R_1 = CH_3, R_2 = H	35		
d, R_1 = CH_3, R_2 = CH_3	44	170-171	$C_{20}H_{22}N_2O_4$
e, R_1 = *i*-C_3H_7, R_2 = H	21	114-115	$C_{21}H_{24}N_2O_4$
f, R_1 = *i*-C_3H_7, R_2 = CH_3	29	141-142	$C_{22}H_{26}N_2O_4$

[a] Satisfactory analyses (±0.3 for C, H, N) were reported for all compounds in the table.

Scheme I[a]



[a] i, $C_6H_5CH_2ONH_2$·HCl; ii, *p*-$NO_2C_6H_4OH$, DCC, AcOEt; iii, $(CH_3)_3N{\cdot}BH_3$, HCl, Et_2O; iv, $SOCl_2$, toluene; v, pyridine, CH_2Cl_2; vi, $(CH_3)_3N{\cdot}BH_3$, HCl, dioxane; vii, pyridine, CH_2Cl_2; viii, Pd/C, H_2, dioxane.

The precursor, active ester **7**, could easily be prepared since we found that our conditions[7] for the reduction of the α-oximino carboxylic acid derivatives **3** and **6** are, fortunately, compatible with the presence of a *p*-nitrophenyl ester. Thus, on reduction of **3** with $(CH_3)_3N{\cdot}BH_3$ in anhydrous ether, saturated with HCl, compound **4** precipitated from the reaction mixture as a crystalline solid

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