NMT toward the hydroxylated derivatives was found to be essentially identical with that displayed by NMT toward the parent compounds, thus confirming that the enhanced activity of the hydroxylated analogues resulted from favorable interactions in the region that we sought to investigate. The preliminary conclusion based on these results is that the active site in the vicinity of the side-chain terminus is hydrophilic in character, and added hydrophilic character in this region would supplement the binding of inhibitors to the active site. Efforts to carry out a more detailed characterization of this region and to assess its potential utility for inhibitor design are continuing.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus that had been calibrated with known compounds. Combustion analyses were performed on a Hewlett-Packard 185B CHN Analyzer at the University of Kansas. IR spectra were recorded on a Beckmann IR-33 spectrophotometer and NMR spectra on a Varian T-60 spectrometer with Me_4Si as an internal standard. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter at 589 nm. The following compounds were purchased from Sigma Chemical Co. and used directly: **6a,c** and **7a,b**. Compound **6b** was prepared from phenylalanine by the method of Seki et al.[10] Butyrophenone, 1-phenyl-2-butanone, and 1,3-diphenylacetone required for the preparation of **4**, **1**, and **3**, respectively, were purchased from Aldrich Chemical Co. α-Cyclohexylacetophenone was prepared as described by Dodds et al.[11] Unless specified as absolute, EtOH and ethanol refer to 95% ethanol.

1-Phenyl-2-pentanone. 1-Phenyl-2-pentanol (1.27 g, 7.83 mmol) in 50 mL of hexane was treated with 15.0 g of pyridinium chlorochromate on alumina[12] at room temperature for 1 h. Filtration and evaporation left 1.09 g (87%) of the desired ketone, which was suitable for use in the next step: IR (film) 1718 cm^{-1}; NMR ($CDCl_3$) δ 7.23 (s, 5, arom), 3.60 (s, 2, benzylic), 2.36 (t, J = 7 Hz, 2, $COCH_2$), 1.50 (m, 2, CH_2CH_2), 0.80 (t, J = 7 Hz, 3, CH_3).

Leuckardt Reaction. Synthesis of 1-Phenyl-2-aminopentane (2). The procedure of Ingersol et al.[13] was employed. Ammonium formate (9.0 g, 0.143 mol) was heated in a flask equipped with a 10-cm Vigreux column and distillation head to 185 °C and maintained at that temperature for 2 h. The clear distillate that was collected during this period was returned to the pot (after cooling), and 0.86 g (5.3 mmol) of 1-phenyl-2-pentanone was added. The heterogeneous mixture was heated with stirring to a final temperature of 165 °C, which was maintained for 3 h. Over this period the contents of the pot became homogeneous. After the mixture was cooled, 20 mL of 6 N HCl was added, and the mixture was heated at reflux for 16 h. The cooled solution was diluted with 25 mL of H_2O and made basic by the careful addition of KOH pellets. Extraction with ether (3 × 30 mL), followed by drying over K_2CO_3 and evaporation, left 0.81 g of a yellow oil, which was distilled (bp 72 °C, 0.3 mm) to yield 0.77 g (88%) of free amine **2**: IR (film) 3370, 3310 (NH_2) cm^{-1}; NMR ($CDCl_3$) δ 7.23 (br s, 5, arom), 2.61 (t, J = 4.5 Hz, 2, benzylic), 2.35 (d, J = 4.5 Hz, 1, $CHNH_2$), 1.36 (br m, 4, CH_2CH_2), 0.98 (s, 2, D_2O exchangeable, NH_2), 0.88 ppm (t, 3, CH_3).

The same procedure was used for compounds **1** and **3-5**: yield (distilled product): **1**, 62%; **3**, 20%; **4**, 27%; **5**, 57.7%.

Catalytic Hydrogenation of 6b,c. The method of Leithe was followed.[14] Compound **6b** [308 mg; $[\alpha]^{22}_D$ 20.8° (c 1.77, EtOH), lit.[10] $[\alpha]^{23}_D$ 24.1° (EtOH)] was dissolved in 30 mL of 95% EtOH, to which 0.5 mL of concentrated HCl had been added, in a Parr shaker flask. PtO_2 (40 mg) was added and hydrogenation commenced at 50 psi for 18 h at room temperature. Filtration of the catalyst and evaporation of the solvent left 380 mg of a white residue, which contained no aromatic protons by NMR analysis (D_2O). The residue was recrystallized from ethanol/ethyl acetate to yield **8b**·HCl, mp 152-154 °C (phase transition to clear gel at 135 °C): $[\alpha]^{20}_D$ -2.24° (c 2.99, H_2O). Anal. ($C_9H_{19}NO$·HCl) C, H, N. Compound **6c** was reduced as above to yield **8c**·HCl: mp 151-154 °C (phase transition at 135 °C), $[\alpha]^{21}_D$ +2.05° (c 4.87, H_2O).

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(10) Seki, H.; Koga, K.; Matsuo, H.; Ohki, S.; Matsuo, I.; Yamada, S. *Chem. Pharm. Bull.* **1965**, *13*, 995.
(11) Dodds, E. C.; Lawson, W.; Williams, P. C. *Proc. R. Soc. London, Ser. B* **1944**, *132*, 119.
(12) Cheng, Y.-S.; Liu, W.-L.; Chen, S. *Synthesis* **1980**, 223.
(13) Ingersol, A. W.; Brown, J. H.; Kim, C. K.; Beauchamp, W. D.; Jennings, G. *J. Am. Chem. Soc.* **1936**, *58*, 1808.
(14) Leithe, W. *Chem. Ber.* **1932**, *65*, 660.
(15) Dey, B. B.; Ramanathan, V. S. *Proc. Natl. Inst. Sci. India* **1943**, *9*, 193.
(16) Patwardhan, M. V.; Phalnikar, N. L.; Bhide, B. V. *J. Univ. Bombay, Sect. A, Part 5* **1950**, *No. 27*, 22.
(17) Ghislandi, V.; Conte, U. *Farmaco, Ed. Sci.* **1968**, *23*, 1022.

Importance of the Aromatic Ring in Adrenergic Amines. 8. 2-(Aminomethyl)-*trans*-2-decalols as Inhibitors of Norepinephrine *N*-Methyltransferase[1a]

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In an effort to determine the surface appearance of the hydrophobic ring binding region of the norepinephrine *N*-methyltransferase active site, we employed some *trans*-decalin analogues of 1-(aminomethyl)cycloundecanol (**1**), a potent NMT inhibitor. These analogues [axial and equatorial 2-(aminomethyl)-*trans*-2-decalol, **2** and **3**] closely resemble a low energy "crown" conformation of **1**. Both compounds were as potent as **1** at inhibiting NMT (K_i = 3.6, 5.6, and 3.8 μM for **1**, **2**, and **3**, respectively), indicating that this conformation is most likely adopted within the active site in order to optimize contact with a flat hydrophobic area. None of the compounds showed significant substrate activity for NMT, a fact that is consistent with our proposed active site binding model.

Interest in developing more potent and specific inhibitors of norepinephrine *N*-methyltransferase (NMT,[2] EC 2.1.1.28) as potential pharmacological tools for the selective modulation of brain epinephrine prompted us to begin an investigation of the active site of this enzyme, using se-

(1) (a) Taken from the Ph.D. Dissertation presented to the Graduate School of the University of Kansas by M.F.R., 1982. (b) NIH Predoctoral Trainee (Grant GM 07775).

(2) This enzyme has also been referred to as phenylethanolamine *N*-methyltransferase (PNMT).

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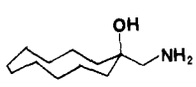
Table I. Inhibition Constants (K_i) and Substrate Activities of *trans*-Decalin Analogues of 1-(Aminomethyl)cycloundecanol (1) with Respect to NMT

no.	K_i ± SEM, μM	K_m ± SEM, μM	V_{max}[a]	$V_{max}/K_m \times 100$
1	3.56 ± 0.6	6.8 ± 1.3	0.04	0.59
2	5.63 ± 0.5	4.7 ± 0.5	0.03	0.64
3	3.80 ± 0.4	5.7 ± 0.7	0.024	0.42

[a] Units of V_{max} are nmoles of product formed per milligram of protein per minute.

lected analogues of the substrate phenylethanolamine. We recently reported on the ability of a number of fully saturated ring analogues to inhibit NMT;[3] among the most potent of these was 1-(aminomethyl)cycloundecanol (1),



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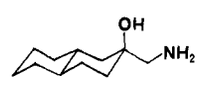

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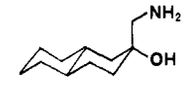

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which was found to have an IC_{50} value of 8 μM. We have proposed that compounds such as 1 interact with a large hydrophobic region within the active site that is adjacent to or overlapping with the site normally occupied by the aromatic ring of the physiological substrate. This hydrophobic area is capable of accommodating large ring structures, such as the cycloundecyl ring, and appears to have dimensions of at least 6–7 × 2–3 Å, which were determined by considering the activity of a series of aliphatic amines.[3] All of the compounds used to determine these dimensions were, like 1, capable of conformationally adapting to bind to a nonuniform surface. Since this hydrophobic area could be a major consideration in the future design of more specific inhibitors of NMT, knowledge of the general appearance of this area would be of value. The fact that 2-amino-1-(2-phenanthryl)ethanol has been reported to be an effective alternate substrate inhibitor of NMT[4] is consistent with the presence of a large, flat area near the ring binding site; however, evidence that the active-site orientations may be different for aromatic and nonaromatic substrates[5] makes it difficult to extrapolate this assumption to the hydrophobic region.

To ascertain the conformational requirements of the hydrophobic area, we have evaluated two *trans*-decalin analogues of 1 for their ability to bind to NMT. The *trans*-decalins were chosen because of their similar size to the cycloundecyl ring of 1 and because of the conformational definition not found in 1. In addition, the *trans*-decalin ring is a close approximation to an overall planar and low-energy "crown" conformation of the cycloundecyl ring. By comparing the ability of the *trans*-decalin analogues to bind to NMT, we should be able to determine if restricting the conformation of the ring in this manner is beneficial or detrimental for interaction with the hydrophobic region. By examining both the axial and equatorial side-chain analogues, we should also be able to obtain some information regarding the orientation of the aminomethyl side chain of 1 within the active site. In this report, we describe the activities found for two *trans*-decalin analogues of 1 (compounds 2 and 3, Table I) as inhibitors of NMT and present our conclusions based on these activities.

Chemistry. Both 2 and 3 have been reported in the literature.[6] We found it convenient to prepare 2 by an alternate procedure by the addition of Me_3SiCN to *trans*-2-decalone, followed by hydride reduction. A mixture of 2 and 3 was expected from this reaction; however, we were unable to detect any 3 in the product obtained. Identification of the isolated product as the equatorial isomer 2 was accomplished by examination of the NMR spectrum in trifluoroacetic acid, as described by Carlson and Behn.[6]

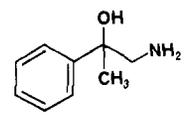
Biochemistry. The radiochemical assay employed in this study uses partially purified bovine adrenal NMT and has been detailed elsewhere.[7] For the determination of K_i values, phenylethanolamine was used as the variable substrate. All of the compounds were measured for substrate activity as well, although we previously reported that 1 was a poor substrate.[8]

Results and Discussion

The kinetic constants found for 1–3 are presented in Table I. Both 2 and 3 were found to be potent inhibitors of NMT, with K_i values very close to the value for 1. These results suggest that the planar appearance of 2 and 3 is a close approximation of the conformation assumed by 1 when bound to the hydrophobic region of the active site, which in turn requires that the region be overall relatively flat in order to allow maximum interaction. We have previously found that a more bulky hydrophobic ethanolamine substrate, 2-(1-adamantyl)-2-hydroxyethylamine, is a relatively poor ligand for the NMT active site;[3] this can be rationalized based on the results presented here in that only one face of the adamantane skeleton can interact with the flat binding site, with the rest of the skeletal bulk being unable to contribute to binding.

It was somewhat surprising that compounds 2 and 3 were found to be nearly equivalent in inhibitory activity, when one considers that the side-chain orientation of these compounds is quite different. This could be interpreted to mean that the predominant binding forces that secure these compounds occur with the hydrophobic ring, relegating the amino group to a minor contributor in this regard; however, it is also possible that the amine binding site is so located that it is accessible to both 2 and 3. The latter possibility would simply require the amine binding site to be slightly (1–2 Å) out of the plane of the hydrophobic region, in a position which apparently is slightly more accessible to the amino group of 3.

The substrate parameters for compounds 1–3 are also presented in Table I. Compound 1 had previously been shown to be a very poor substrate for NMT in terms of both V_{max} and V_{max}/K_m ratio, and the *trans*-decalin analogues 2 and 3 were found to be almost identical with 1 in substrate activity. It was reported earlier by Fuller et al.[9] that phenylethanolamine derivatives such as 4, in



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(3) Vincek, W. C.; Aldrich, C. S.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.* **1981**, *24*, 7.
(4) Fuller, R. W.; Roush, B. W. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *17*, 727.
(5) Rafferty, M. F.; Wilson, D. S.; Monn, J. A.; Krass, P.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.*, under Articles in this issue.
(6) Carlson, R. G.; Behn, N. S. *J. Org. Chem.* **1968**, *33*, 2069.
(7) Grunewald, G. L.; Borchardt, R. T.; Rafferty, M. F.; Krass, P. *Mol. Pharmacol.* **1981**, *20*, 377.
(8) Grunewald, G. L.; Vincek, W. C.; Davis, D. P.; Borchardt, R. T. in "Catecholamines: Basic and Clinical Frontiers"; Usdin, E.; Kopin, I. J.; Barchas, J., Eds.; Pergamon Press: New York, 1979; Vol. 1, p 189.

which the hydroxy-bearing side-chain carbon is fully substituted, were unacceptable as substrates for NMT; since the compounds in this study are also fully substituted at the equivalent side-chain carbon, this may account for the weak activities found in this study. However, recent results from our laboratory[5] also suggest an alternative explanation that is more directly related to the hydrophobic nature of the compounds in Table I. Evidence suggests that more hydrophobic, nonaromatic analogues of phenylethanolamine are displaced deeper into the hydrophobic ring binding region than are aromatic substrates, such that the side-chain hydroxy loses contact with its corresponding binding site. Since the hydroxy group interaction is known to be important for substrate activity with NMT,[10] this loss of binding interaction results in a loss in substrate activity and also in stereoselectivity for the substrate. It is therefore conceivable that compounds **1–3** are also bound so deep within the hydrophobic binding region that the side-chain hydroxy is unable to contact its binding site; thus, methylation of these compounds cannot occur to any significant extent.

In summary, we have found that the *trans*-decalin analogues **2** and **3** of a known alicyclic inhibitor (**1**) of NMT demonstrated equivalent inhibitory potency. These results suggest that the hydrophobic region of the ring binding site of NMT is a relatively flat area, which best accommodates structures having an overall planar shape or which are able to assume such a shape. The amino group binding site is indicated by the activity of these compounds to lie slightly out of the plane of the hydrophobic region, in an area which is accessed by two rather distinct side-chain orientations. These results will be applied to further investigations of the NMT active site.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus calibrated with known compounds. NMR spectra were recorded on a Varian A-60A spectrometer in trifluoroacetic acid, with Me_4Si as the internal reference. Combustion analyses were performed on a Hewlett-Packard 185B CHN Analyzer. IR spectra were recorded on a Beckman IR 33 spectrophotometer. β-Decalone was purchased from Aldrich Chemical Co., and cyanotrimethylsilane was obtained from Petrarch Chemical Co. The axial isomer **3** was generously provided to us by Dr. R. G. Carlson, University of Kansas.

2(*e*)-(Aminomethyl)-*trans*-2-decalol (2). The method of Evans et al.[11] was used. Into a flame-dried, N_2-purged flask was placed 3.27 g of Me_3SiCN (33 mmol) and 60 mg of ZnI_2 catalyst. The mixture was cooled to 0 °C, and 5.0 g of β-decalone (33 mmol) was added dropwise via syringe with stirring. The mixture was allowed to warm slowly to room temperature, and the progress of the reaction was monitored by IR spectroscopy for residual C=O stretching frequency at 1712 cm^{-1}. Complete disappearance of this band occurred within 30 min; a weak nitrile band was detected (2230 cm^{-1}). The pot contents were drawn into a syringe and added dropwise to a slurry of 3.0 g of $LiAlH_4$ in dry ether at a rate of addition that maintained a gentle reflux. After the addition was complete, the reaction was allowed to stir at room temperature for 16 h. After quenching the excess $LiAlH_4$ with 3 mL of H_2O, followed by 5 mL of 1 N NaOH, the reaction was filtered and the filtrate was extracted with ether (3 × 25 mL). The pooled ether layers were dried (K_2CO_3) and evaporated, leaving a white residue, which was recrystallized from ether to yield 4.45 g (72%) of white needles, mp 90–93 °C. NMR analysis (trifluoroacetic acid) showed the appearance of a quartet at 3.49 ppm, which was assigned to the side-chain methylene. It was reported that the chemical shift of this methylene of the equatorial isomer (**2**) was 3.53 ppm; the signal for the axial isomer appeared at 3.29 ppm.[6] No signals could be detected downfield from 3.49 ppm, confirming the absence of any of the axial isomer **3**. Further purification of the product was achieved by sublimation (75 °C, 0.1 mm), to yield a white solid, mp 90 °C (lit.[6] mp 92 °C). Anal. ($C_{11}H_{21}NO$) C, H, N.

Acknowledgment. Financial support for this study was provided by NIH Research Grants HL 21887 and HL 24093.

(9) Fuller, R. W.; Molloy, B. B.; Day, W. A.; Roush, B. W.; Marsh, M. M. *J. Med. Chem.* **1973**, *16*, 101.
(10) Axelrod, J. *J. Biol. Chem.* **1962**, *237*, 1657.
(11) Evans, D. A.; Carroll, G. L.; Truesdale, L. K. *J. Org. Chem.* **1974**, *39*, 914.

Mechanism of Action of 5-Nitro-2′-deoxyuridine

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Results are described that demonsrate that the mechansim of action of the potent cytotoxic agent 5-nitro-2′-deoxyuridine (NO_2dUrd) involves thymidine (dThd) kinase catalyzed formation of 5-nitro-2′-deoxyuridylate (NO_2dUMP) and subsequent potent inhibition of thymidylate (dTMP) synthetase by this compound. The evidence for this is as follows: (a) cells lacking dThd kinase are not inhibited by high concentrations of NO_2dUrd; (b) the drug has no effect on dThd or uridine (Urd) incorporation into nucleic acids but prevents incorporation of deoxyuridine (dUrd); (c) growth inhibition is reversed by dThd but not by dUrd; (d) NO_2dUrd causes changes in deoxynucleoside triphosphate pool sizes which are characteristic of specific inhibition of dTMP synthetase; (e) cells treated with [^{3}H]NO_2dUrd possess macromolecular bound [^{3}H]NO_2dUMP, which has properties characteristic of the NO_2dUMP–dTMP synthetase complex. Treatment of L1210 leukemic mice at 400 mg/kg daily for 6 days gave only a 33% increase in life span, probably because of its rapid degradation to the inactive nitrouracil.

Thymidylate synthetase (EC 2.1.1.45) catalyzes the conversion of 2′-deoxyuridylate (dUMP) and methylenetetrahydrofolate to dTMP and dihydrofolate. Because of its central role in the de novo synthesis of dTMP, this enzyme has been the target of a number of inhibitors with potential chemotherapeutic utility.[2] dTMP synthetase is a target for drugs such as 5-fluorouracil and 5-fluoro-2′-deoxyuridine (FdUrd); metabolism of these compounds

(1) Present address: Department of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.
(2) Santi, D. V. *J. Med. Chem.* **1980**, *23*, 103. Danenberg, P. V. *Biochim. Biophys. Acta* **1977**, *473*, 73.

0022-2623/82/1825-1252$01.25/0