Cyclic Urea Nucleosides. Cytidine Deaminase Activity as a Function of Aglycon Ring Size

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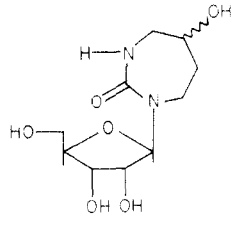
Five β-D-ribofuranosyl cyclic urea nucleosides (14–18), ranging in ring size from five to eight membered, were synthesized and evaluated as cytidine deaminase (CDA) inhibitors. The precursor protected nucleosides (9–13) were prepared by a condensation procedure utilizing persilylated ureas with a halo sugar under the specific catalytic activity of a $HgO/HgBr_2$ mixture which provided exclusively the β-anomers. Catalytic hydrogenation of known 1-(2,3,5-tri-*O*-benzoyl-β-ribofuranosyl)-1,2-dihydropyrimidin-2-one (19) afforded nucleoside 10 identical with that obtained by the mercury-catalyzed condensation procedure. CDA activity varies significantly with the ring size of the urea aglycon and reaches its maximum level for the seven-membered analogues 16 and 17. The unexpected high potency of nucleoside 17 ($K_i = 2.5 \times 10^{-8}$ M, human liver enzyme) is reported. This compound represents the most potent inhibitor of human liver CDA yet discovered.

In our preliminary communication regarding the synthesis and cytidine deaminase (CDA) inhibitory activity of 1-β-D-ribofuranosyl-5-hydroxyperhydro-1,3-diazepin-2-one (1), the relationship between aglycon ring size and



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CDA inhibition was briefly discussed.[1] Approximately one order of magnitude difference in binding affinity was observed between six- and seven-membered unsubstituted urea nucleosides. The compound with the seven-membered aglycon was the more potent with an activity level very similar to that of tetrahydrouridine (THU).[1]

It appears peculiar that the enzyme should bind better to the inhibitor having the seven-membered aglycon rather that to the inhibitor having the same ring size as the substrate. However, this behavior also has been observed for the inhibition of adenosine deaminase (ADA).[2] It was therefore of interest to determine how the stepwise variation in aglycon methylene units would affect the binding affinity for these inhibitors. As the ring size increases, there is an increasingly distorted conformation presented to the enzyme. If there is, indeed, a greater enzyme affinity for a distorted inhibitor, which optimizes the complementarity of the enzyme–inhibitor (EI) complex, it is necessary to define the limits of this distortion in terms of the aglycon's ring size for CDA. This goal also presented the opportunity to test the general applicability of the nucleosidation reaction, originally developed for compound 1, to a family of cyclic ureas (4–8). The synthesis, characterization, and CDA inhibitory activity of these cyclic urea nucleosides are the subject of the present study.

Chemistry. Very few examples for the condensation of nonaromatic heterocycles with sugar derivatives exist in the literature. The condensation of silylated 5,6-dihydro-5-methyl-*s*-triazine-2,4(1*H*,3*H*)-dione with a protected deoxyribosyl chloride[3] and that of various cyclic lactim ethers with either 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride or 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl acetate (2), reported as unpublished results,[4] represent, perhaps, the only two pertinent examples. These two nucleosidations were successfully catalyzed by $SnCl_4$ following a methodology similar to that developed earlier by Vorbrüggen for the condensation of aromatic heterocycles with sugar derivatives.[5] Our attempts to condense persilylated cyclic ureas with the sugars 2 or 3 by this procedure failed, however. This failure could possibly result from (1) the exclusive formation of *N,N'*-bis(trimethylsilyl)urea structures which would be unreactive toward the halogenated sugar or the 1,2-*O*-acyloxonium ion intermediate or (2) the formation of a very stable complex between $SnCl_4$ and the nucleophilically reactive *O,N*-bis(trimethylsilyl)ureas, leading to an unreactive nondissociable species.

The last possibility was discarded because of the lack of success in isolating any product after attempting the reaction with solvents of different polarity (i.e., benzene, dichloroethane, and acetonitrile).

In relation to the first and most likely possibility, it is generally accepted that cyclic amides are preferentially N-silylated unless the *O*-silyl form is sufficiently stabilized by the development of extended conjugation or heteroaromaticity.[6] It is expected, therefore, that nonaromatic cyclic ureas would behave similarly, providing only the unreactive *N,N'*-bis(trimethylsilyl)ated form. Experiments by Klebe et al. are in agreement with such reasoning and suggest, after variable-temperature NMR studies, that the single trimethylsilyl absorption of bis(trimethylsilyl)-substituted ethyleneurea (4), *N,N'*-dimethylurea, and *N,N'*-diphenylurea is consistent with the *N,N'*-bis(trimethylsilyl)urea structure.[7]

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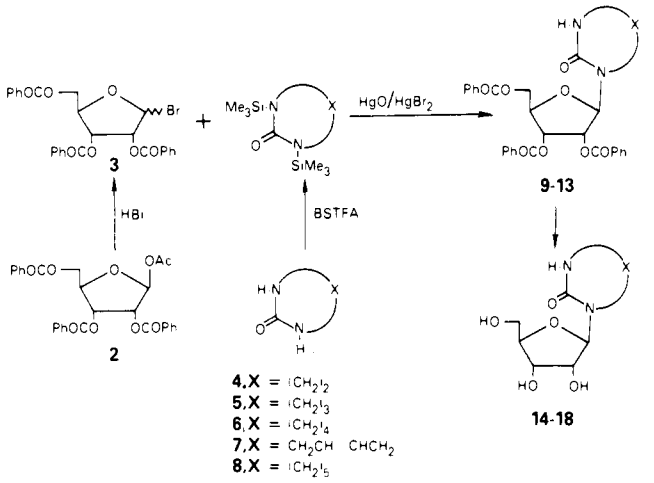
Scheme I



In cyclic ureas where extensive conjugation is possible, ^{13}C NMR reveals a downfield shift of 5.23 to 5.26 ppm of the carbonyl carbon signal that has been associated with the presence of the O-silylated form.[8,9] However, in our series, using ethyleneurea (**4**) as an example, the downfield shift of the carbonyl carbon was only of 1.8 ppm after silylation. On the contrary, the methylene carbons experienced an upfield shift of 2.81 ppm which may be due to the neighboring effect of the trimethylsilyl groups. Finally, the appearance of the spectrum with only three carbon signals is consistent with a symmetrical *N,N'*-bis(trimethylsilyl)ated species.

Assuming then that the first possibility was correct, we searched for catalysts that would react with the persilylated urea promoting the interconversion of the *N,N'*-bis(trimethylsilyl) form to the reactive *O,N*-bis(trimethylsilyl) form. In our hands, a nearly equimolar mixture of HgO and $HgBr_2$ appears to catalyze best the reaction to produce a single nucleoside in yields varying between 42 and 76% (Scheme I). Other mercury catalysts, such as $HgBr_2$ or $Hg(CN)_2$ used alone, failed to promote the reaction between persilylated cyclic ureas and **3**. When HgO was used alone, however, the reaction proceeded smoothly, but the product obtained corresponded to the O-nucleoside (or glycoside). This unstable intermediate was isolated, silylated, and converted to the N-nucleoside in the exclusive presence of $HgBr_2$. Finally, the combination of $HgO/HgBr_2$ was used as the standard catalytic mixture for these condensations. The mechanistic role of HgO and other catalysts capable of inducing these reactions will be the subject of a separate publication. As anticipated, product formation was not observed (TLC) in the uncatalyzed reaction in which the urea was expected to be exclusively N-silylated. It is interesting to compare these results with those of Wittenburg, who obtained acceptable yields of nucleoside products in the uncatalyzed reactions between sugar halides and silylated pyrimidines in refluxing benzene.[10] Under those conditions the silylated pyrimidines probably exist as the more stable heteroaromatic O-silylated species.[6]

Another key feature of the $HgO/HgBr_2$ catalyzed reaction was the isolation of the single β-anomeric product after simple column chromatography. Aside from some unreacted sugar, no other product was visualized on TLC analysis of the reaction mixtures. Based on mechanistic

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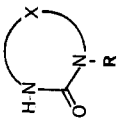
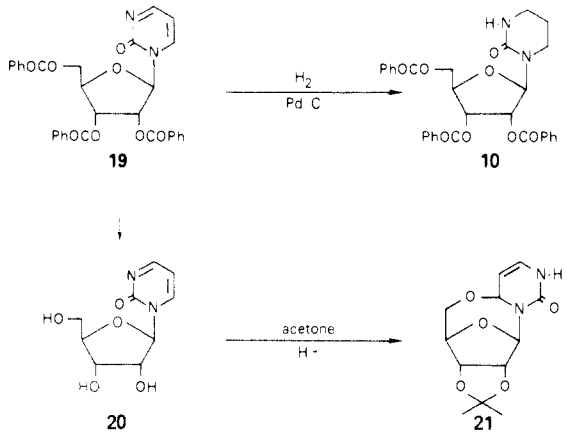
Table I. Physical, Spectral, and Biological Properties of Protected and Free Cyclic Urea Nucleosides



no.	R[a]	X	mp,[b] °C	R_f[c]	^1H NMR, δ (H-1')	MS, *m/z* (M^+·)[d]	$[\alpha]^{23}_D$,[e] deg	formula	anal.[f]	yield, %	K_i, M: mouse kidney	K_i, M: human liver
9	A	$(CH_2)_2$	67–74	0.31	6.00 (d, J = 7 Hz)			$C_{29}H_{26}N_2O_8$	C, H, N	49		
10	A	$(CH_2)_3$	149–151[g]	0.16	6.40 (m, 6-Hz wide)			$C_{30}H_{28}N_2O_8$	*g*	76		
11	A	$(CH_2)_4$	59–61	0.51	6.10 (d, J = 6 Hz)			$C_{31}H_{30}N_2O_8$	C, H, N	46		
12	A	$CH_2CH{=}CHCH_2$	63–64	0.40	6.05 (d, J = 6 Hz)			$C_{31}H_{28}N_2O_8$	C, H, N, H_2O	60		
13	A	$(CH_2)_5$	72–76	0.46	6.30 (d, J = 7 Hz)			$C_{32}H_{32}N_2O_8$	C, H, N	42		
14	B	$(CH_2)_2$	101–104[h]		5.40 (m, 6-Hz wide)	506	−34	$C_8H_{14}N_2O_5$	C, H, N, H_2O	83	2×10^{-4}	3×10^{-4}
15	B	$(CH_2)_3$	65–68		5.80 (d, J = 6 Hz)	520	−43	$C_9H_{16}N_2O_5$	*i*	86	4×10^{-6}	2.8×10^{-6}
16	B	$(CH_2)_4$	165–166[j]		5.40 (d, J = 6 Hz)	534	−138	$C_{10}H_{18}N_2O_5$	C, H, N	82	3×10^{-7}	4×10^{-7}
17	B	$CH_2CH{=}CHCH_2$	132–135[j]		5.45 (m, 6-Hz wide)	532	−127	$C_{10}H_{16}N_2O_5$	C, H, N, H_2O	85	7×10^{-8}	2.5×10^{-8}
18	B	$(CH_2)_5$	72–75		5.70 (d, J = 6 Hz)	533[k]	−33	$C_{11}H_{20}N_2O_5$	C, H, N, H_2O	79	3.4×10^{-5}	1.5×10^{-5}

[a] A = 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl; B = β-D-ribofuranosyl. [b] Most of the products were obtained as foams. Whenever possible they were recrystallized from the solvent indicated. [c] Silica gel, ethyl acetate–hexane (3:2). [d] Tetrakis(trimethylsilyl) derivatives. [e] *c* 0.1, H_2O. [f] All analyses gave results within ±0.4% of the calculated values. [g] Recrystallized from EtOH, lit. mp 143–145 °C.[31] [h] Recrystallized from $CHCl_3$–MeOH. [i] Reference 32. [j] Recrystallized from $CHCl_3$–MeOH–Et_2O. [k] This peak corresponds to the M^+· – CH_3 fragment.

Scheme II



considerations, one would expect the products to have the β configuration. However, NMR analysis of the observed coupling constants for the anomeric protons was not considered definitive enough for the complete assignment of absolute configuration (Table I). We have previously suggested that the potent CDA inhibitory activity observed for the corresponding deblocked nucleosides was strong supportive evidence for the β configuration of these nucleosides.[1] Now we present chemical evidence in support of this observation. As indicated in Scheme II, compound **10** was obtained by the catalytic hydrogenation of the known nucleoside **19**.[11] This material was identical in all respects with the compound (**10**) isolated from the $HgO/HgBr_2$ catalyzed condensation between **3** and **5** (Scheme I). The β configuration of nucleosides **19** and **20** had been established previously on spectral grounds,[11-18] and we have also shown that the deblocked nucleoside **20**, likewise, interacted strongly with CDA.[19] Finally, we were able to show that nucleoside **20** underwent a smooth transformation to the O^6-5′-cyclonucleoside **21**, a reaction that is only possible in the β configuration (Scheme II). This reaction is the result of an acid-catalyzed intramolecular "hydration" with participation of the 5′-hydroxy group of the furanose ring. The formation of **21** is probably facilitated by the rigidity and skewing imposed on the furanose ring by the acetonide group,[20,21] since this intramolecular reaction was not observed with **20**. Compound **21** was isolated as a single isomer (TLC), but the absolute configuration at C-6 is not known. All these data characterize nucleosides **19**, **20**, and **10** as the β-anomers. By analogy, the configuration of nucleosides **9–13** was assigned as β, since the condensation conditions were identical, yielding a single product in each case.

The series of compounds made by this apparently general procedure is presented in Table I. The deblocked nucleosides were obtained after treatment with a saturated solution of ammonia in MeOH and isolated by standard procedures.

Biological Results

All the final deprotected nucleosides were tested as CDA (EC 3.5.4.5) inhibitors against the mouse kidney enzyme following the experimental procedure described earlier[19] (see Table I). The original observation that the seven-membered nucleoside **16** was an order of magnitude more powerful than the six-membered nucleoside **15** against mouse kidney CDA was confirmed in studies with the human liver enzyme. It appears that seven is the optimum ring size for this family of nucleosides, since the eight-membered ring nucleoside (**18**) is two orders of magnitude less tightly bound than its seven-membered analogue. Previous observations with some 5-substituted cytidine derivatives as substrates indicate that CDA appears to be quite steric sensitive with respect to the size of the 5-substituent.[22,23] Therefore, it is possible that the bulkier eight-membered aglycon projects its extra methylene unit into a region equivalent to that of the 5-substituent in the cytidine substrates and this prevents a close association in the EI complex. Reducing the ring size of the aglycon below six is even more deleterious for strong EI complex formation, and the K_i of compound **14** (2×10^{-4} M), the inhibitor with a five-membered aglycon, becomes greater than the K_m for the substrate, cytidine (5×10^{-5} M).

We have also reported that one of the diastereoisomers of **1** is the most potent inhibitor of CDA known.[1] To explain such potent activity, it was postulated that the tetrahedral configuration at C-5 with the hydroxyl group in the appropriate orientation allows **1** to behave as a transition-state inhibitor of the enzyme. Since it appeared that a tetrahedral carbon at C-5 was required, it was surprising to find that the unsaturated compound, **17**, acted as a very potent inhibitor of mouse kidney CDA. In addition, compound **17** appears slightly more potent than **1** ($K_i = 4 \times 10^{-8}$ M) against the human liver enzyme. It is possible that **17** is hydrated by the enzyme to the most active diastereoisomer of **1**. This hypothesis is presently under investigation and is based on the fact that the double bond in this ring system is very reactive toward addition reactions under very mild conditions.[24] The possibility was examined that compound **17**, with a potentially reactive double bond, is a slowly associating tight-binding inhibitor of CDA, as defined by Cha and exemplified by coformycin in the adenosine deaminase system.[25] We evaluated the effect of preincubation of **17** with both the mouse kidney and human liver enzyme as described by Agarwal and his colleagues for adenosine deaminase inhibitors.[2] No difference was detected in the degree of inhibition produced by **17** with either enzyme whether the compound was preincubated with the enzyme or not. This observation implies that the inhibition observed represents rapid establishment of steady-state conditions when **17** interacts with the enzyme. This interpretation is consistent with preliminary kinetic results that indicate **17** to behave formally as a competitive inhibitor of both enzymes studied.

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In summary, compound 17 represents the most potent inhibitor of human liver CDA known to date. Coupled with the fact that its synthesis is a great deal simpler than that of 1, compound 17 is an excellent candidate for drug combination studies where CDA activity is to be blocked.

Experimental Section

Materials. All the cyclic ureas used in this study were prepared by known literature procedures: trimethyleneurea (5),[26] tetramethyleneurea (6),[27] pentamethyleneurea (8),[27] and 1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (7).[28,29] The yields and physical properties of the products isolated agreed with all the reported values. The five-membered urea 4 (2-imidazolidone) as well as the rest of the organic chemical reagents were purchased either from Aldrich Chemical Co. or from Pfanstiehl Laboratories, Inc. Mercuric oxide (red) and mercuric bromide were purchased from Fisher Scientific Co.

Chemical Methods. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 141 polarimeter. Proton NMR spectra were determined on Varian T-60 or HA-100D instruments. Chemical shifts are given as δ values with reference to Me_4Si or deuterated sodium 3-(trimethylsilyl)propionate (TSP). ^{13}C NMR spectra were recorded on a Varian FT-80A spectrometer. The spectra were run at room temperature with all values referenced to ^{13}C of added dioxane, which was 66.67 ppm relative to Me_4Si. Elemental analyses were carried out by the NIAMDD, NIH, and by Galbraith Laboratories, Inc., Knoxville, TN. Low-resolution electron-impact mass spectra were obtained on a DuPont 21-492B gas chromatograph–mass spectrometer (GC/MS) system interfaced to a VG 2040 data system. Samples were introduced either by direct probe or via a Varian 2740 GC (trimethylsilyl derivatives) coupled to the mass spectrometer by a single-stage glass jet separator. Columns for chromatography were packed with silica gel (Bio-Sil A, 200–400 mesh, Bio-Rad Laboratories) and eluted with mixtures of ethyl acetate-hexane. Preparative HPLC was performed on a Waters instrument prep LC/system 500A.

2,3,5-Tri-*O*-benzoyl-D-ribosyl Bromide (3). This material was prepared according to the procedure of Stevens et al.[30] It was isolated as a clear yellow syrup and used without further purification for the condensation reactions with persilylated cyclic ureas.

1,3,4,7-Tetrahydro-2*H*-1,3-diazepin-2-one (7).[28] New Method of Synthesis. *cis*-1,4-Diphthalimido-2-butene[28] (34.6 g, 0.1 mol) suspended in 700 mL of ethanol was refluxed under a nitrogen atmosphere. To the refluxing mixture was added hydrazine hydrate (10 mL, 0.7 mol), mixed with 10 mL of water, over a period of 20 min. After 3 h, carbonyl sulfide was bubbled into the refluxing mixture for 4 h. The reaction mixture was cooled and after 0.5 h filtered. The filtrate was evaporated to dryness and triturated with 150 mL of a methylene chloride–methanol (9:1) solution. The solid material was discarded, and the solution was passed through a dry silica column (25 × 300 mm) and eluted with the same solvent mixture. All the fractions containing the product were combined and evaporated. The solid residue obtained was washed with methylene chloride and dried to give 3.45 g (30%) of a pure product, mp 187–190 °C, identical with that obtained previously.[28] The compound moves as a single spot on a silica plate with the same solvent system. The spots were visualized by an alkaline solution of $KMnO_4$.

General Procedure for the Condensation Reaction. To a suspension of the cyclic urea (1–5 mmol) stirred in dry acetonitrile (distilled over P_2O_5) at room temperature was added excess bis(trimethylsilyl)trifluoroacetamide (BSTFA) in a ratio of 1 mL to 1 mmol of heterocycle. The mixture was stirred for 2 h at room temperature, and the excess reagents were removed in vacuo to leave a clear oil. The NMR spectra revealed complete absence of NH signals. The oily persilylated cyclic urea was dissolved in 2–10 mL of dry benzene and added rapidly into a refluxing mixture of HgO and $HgBr_2$ (400 mg each/1 mmol of cyclic urea) in dry benzene (25–125 mL). After 10 min, a solution of 3 (1.24 mmol/1 mmol of cyclic urea) in benzene (5–25 mL) was rapidly added and refluxing continued for 18 h. After cooling, the mixture was filtered through a bed of Celite, and the filter cake was washed with ethyl acetate. The combined organic filtrate and washings were extracted with solutions of saturated $NaHCO_3$ and water and then dried over anhydrous $MgSO_4$. The solution was then concentrated to ca. 5 mL and applied to a silica gel column. Elution of the column with ethyl acetate–hexane (3:2) provided the desired nucleosides as colorless foams (Table I).

General Procedure for the Removal of the Benzoyl Protective Groups. To a solution of the protected nucleoside in a few milliliters of chloroform was added a saturated solution of ammonia in methanol (50 mL/0.5 g of protected nucleoside), and the resulting mixture was kept at room temperature for 1 day in a pressure bottle. The solvent was evaporated and the residue was extracted with water. The aqueous solution was extracted with chloroform (5 times) to afford a clear aqueous layer, which was subsequently lyophilized to afford the corresponding deblocked nucleosides. The final products were either recrystallized or relyophilized after treatment of an aqueous solution with activated carbon (Table I).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (19). This material was obtained by the method of Vorbrüggen et al.[11] in yields of ca. 70%, mp 152–154 °C (EtOH) (lit.[11] mp 155–158).

1-β-D-Ribofuranosyl-1,2-dihydropyrimidin-2-one (20). This well-known compound [14-18] was obtained after treatment of 19 with a solution of saturated methanolic ammonia at 4 °C for 24 h. It was recrystallized with difficulty from absolute ethanol to yield a very hygroscopic material. A nonhygroscopic compound was obtained after preparative HPLC purification using one reverse-phase column (Prepak 500/C-18) and elution with water at 100 mL/min. A white nonhygroscopic foam was obtained after lyophilization (62%): NMR (D_2O) δ 8.50 (m, 2), 6.70 (dd, 1, J = 7 Hz, J' = 5 Hz), 5.75 (d, 1, $J \simeq$ 1 Hz, H-1′), 4.20 (m, 3), 3.95 (m, 2). The remainder of the physical properties were identical with those reported earlier.[14-18]

1-(2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-*O*^{6},5′-cyclo-1,2,3,6-tetrahydropyrimidin-2-one (21). A suspension of 20 (5 g, 22 mmol) in 400 mL of acetone was stirred for 1 h with 20 g of *p*-toluenesulfonic acid monohydrate. The solution was partially reduced to dryness and then treated with a saturated solution of $NaHCO_3$. Exhaustive chloroform extraction of the mixture (4 times) afforded, after drying (Mg_2SO_4) and evaporation of the solvent, a yellow oil which solidified as a foam under high vacuum. The product was purified on a silica column using ethyl acetate to yield an amorphous powder: mp 104–106 °C; NMR ($CDCl_3$) δ 8.50 (d, 1, J = 5 Hz, NH, D_2O exchanged), 6.25 (dd, 1, J = 8 Hz, J' = 5 Hz, H-4), 5.80 (s, 1, H-1′), 5.50 (d, 1, J = 3 Hz, H-6), 4.60 (m, 4, H-2′, H-3′, H-4′, H-5), 3.80 (br s, 2, H-5′, H-5′a), 1.50 (s, 3, CH_3), 1.30 (s, 3, CH_3). Anal. ($C_{12}H_{16}N_2O_5$) C, H, N.

Catalytic Hydrogenation of Nucleoside 19 to 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)hexahydropyrimidin-2-one (10). A solution of 19 (0.3 g, 0.55 mmol) in a mixture of chloroform and ethanol was reduced at 40 psi of hydrogen in the presence of 0.1 g of 10% Pd/C catalyst for 5 h. Removal of the catalyst and evaporation of the solvent yielded a colorless solid, which was recrystallized from ethanol to yield needles, mp 149–151 °C (lit.[31] mp 143–145 °C) identical in all spectral and chromatographic behavior with the compound obtained from the nucleosidation of 5 with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (3).

Biological Methods. Mouse kidney cytidine deaminase was isolated and partially purified from mouse kidney acetone powder

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(prepared with acetone only), obtained from Sigma Chemical Co., St. Louis, MO. The powder (3 g) was extracted at 55–60 °C for 5 min with pH 8.0 phosphate buffer (0.05 M), and the extract was filtered through a Nalgene filter unit (0.45-μm grid membrane) to yield a clear yellow filtrate. The filtrate was fractionated with ammonium sulfate, essentially as described by Wentworth and Wolfenden,[33] and the active ammonium sulfate fraction was dissolved in 2.0 mL of phosphate buffer (0.05 M; pH 7.0). The K_m for deamination of cytidine using this preparation was found to be 5×10^{-5} M, in good agreement with the value of 7×10^{-5} M reported previously by Tomchick et al.[22] for mouse kidney cytidine deaminase. Human liver was obtained at autopsy, and cytidine deaminase was isolated and purified as previously described;[33] suitable enzyme preparations could be obtained from liver samples frozen at −20 °C. Cytidine deaminase was measured by following the decrease in absorbance at 282 nm (290 nm for crude preparations) that characterizes the conversion of cytidine to uridine;[34] all assays were performed at pH 7.0 (phosphate buffer) and 37 °C with substrate (cytidine) at 1×10^{-4} M. Spectroscopic determinations were carried out with a Beckman Model 34 kinetic spectrophotometric system with the recorder set for full-scale deflection in the range 0.0 to 0.1 absorbance unit. Candidate inhibitors were incubated routinely with the enzyme for 2 min prior to initiation of the enzymatic reaction by addition of cytidine. Studies to evaluate whether inhibition by 17 involved slow establishment of a steady state were carried out using a 10-min preincubation of enzyme with 17 prior to the additin of cytidine.

Acknowledgment. The authors thank Dr. James A. Kelley (NIH) for mass spectral measurements and Dr. L. V. Feyns, USP, for the ^{13}C NMR data. Studies at the University of Vermont were supported, in part, by grants from the National Cancer Institute (CA-24543 and CM-97278).

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Mammalian Metabolism of Phencyclidine

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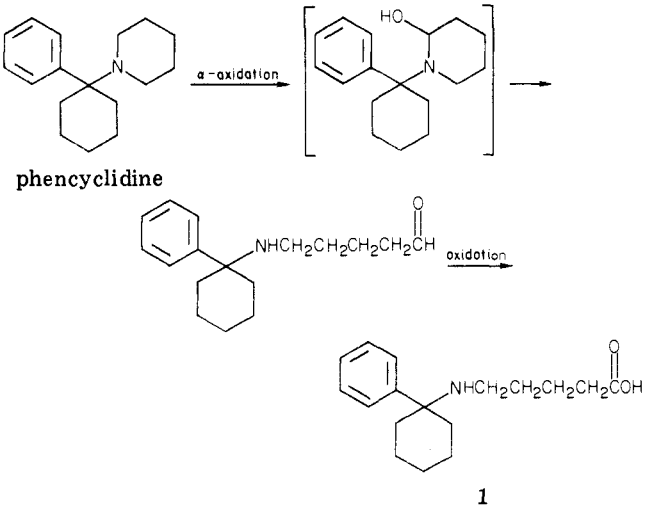
Received October 10, 1980

In dogs, the major metabolite of phencyclidine was found to be 5-[*N*-(1′-phenylcyclohexyl)amino]pentanoic acid (1). The γ-aminobutyric acid like metabolite was also pharmacologically evaluated to determine if the purported GABA-ergic mediated effects of phencyclidine on locomotor activity might be attributed to the metabolite. Preliminary pharmacological evaluation of 1 and its methyl ester indicates that the metabolite has little, if any, association with the effect of phencyclidine on locomotor activity.

Phencyclidine has become a widely used drug of abuse that exhibits a diverse pharmacological profile, and frequently the onset of these effects is observed long after the blood and brain levels have reached maximum values. In some cases the pharmacological effects appear to be biphasic in nature. In man, acute intoxication is characterized by hypotension, but the blood pressure may rise above normal 2 or 3 days later.[1] A schizophreniform condition appears in a small number of the subjects and the psychosis may persist for several weeks.[2-5] These observations suggest that the delayed effects of phencyclidine may be related to the production of a metabolite of phencyclidine.

In studies using the rhesus monkey and ^{14}C-labeled phencyclidine, 60% of the total activity appeared in the urine in 12 h and only trace quantities of the unchanged drug was detected.[6] Though the half-life of the drug is short (2.36 h, monkey; 2.86 h, dog),[7] only trace levels of the drug and low levels of metabolites have been detected in the urine. In man and dog, the major metabolite has been tentatively identified by GC–mass spectroscopy as 4-phenyl-4-piperidinocyclohexanol, and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine was observed as a minor metabolite.[8-10] Only very small quantities of the metabolite where both the cyclohexyl and piperidyl rings are hy-

Scheme I



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