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ACKNOWLEDGMENTS

All polymers and the model compound were provided by Dr. J. Heller of SRI International, Menlo Park, CA 94304. This work was supported by INTER$_x$ Research Corp., Merck Sharp & Dohme Research Laboratories.

Synthesis and *In Vitro* Antiviral Activity of 3′-*O*-Acyl Derivatives of 5′-Amino-5′-deoxythymidine: Potential Prodrugs for Topical Application

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Received April 20, 1983, from the *Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT 06510.* Accepted for publication September 19, 1983.

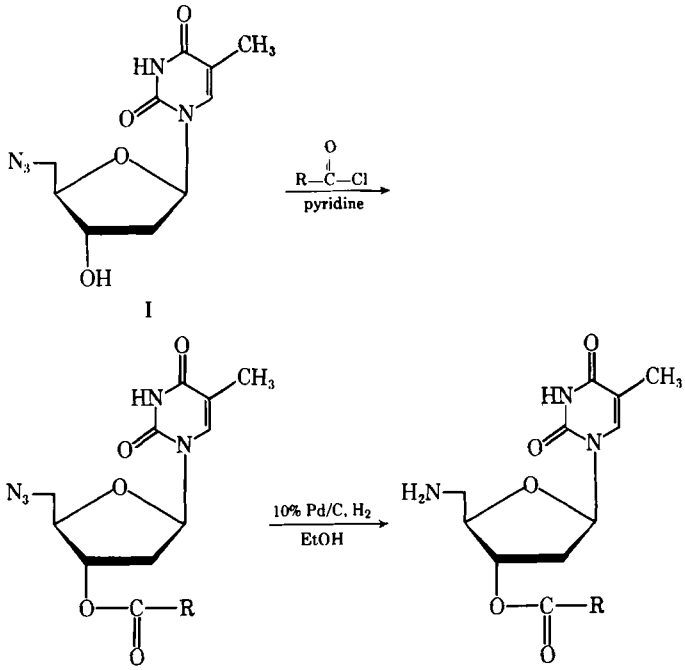
Abstract □ A series of 3′-*O*-acyl derivatives of 5′-amino-5′-deoxythymidine (5′-NH_2-TdR) (IIIa–j) was synthesized by acylation of 5′-azido-5′-deoxythymidine (I). The resulting acetoxy azides were reduced by catalytic hydrogenation to give the corresponding amines. The antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro*, the aqueous solubilities, and the octanol–water partition coefficients of these compounds were determined. All these derivatives have shown potency against HSV-1 virus similar to the parent compound, except the aromatic and highly branched aliphatic esters, IIIi and IIIj, which are less active. Because of their markedly improved lipophilicity, these compounds are believed to penetrate the biological membranes more easily, and thus, would be more effective for the topical treatment of cutaneous herpes virus infections.

Keyphrases □ 3′-*O*-Acyl derivatives of 5′-amino-5′-deoxythymidine—synthesis, antiviral activity □ Prodrugs—potential, topical application. 3′-*O*-acyl derivatives of 5′-amino-5′-deoxythymidine

The 5′-amino analogue of thymidine (5′-NH_2-TdR) has demonstrated potent antiviral activity against herpes simplex virus type 1 (HSV-1) in the complete absence of toxicity to the uninfected host Vero cells in culture (1, 2). This compound was therapeutically effective in the topical therapy of herpetic keratouveitis in rabbits, and systemic administration into the neonatal mouse revealed no adverse effect *in vivo* or by the histopathological examination (3). Chen *et al.* (4) have recently found that 5-NH_2-TdR was a substrate for the HSV-1-encoded thymidine kinase but was not a substrate for the cellular thymidine kinase. The complete lack of toxicity of 5′-NH_2-TdR to the uninfected Vero cells is probably due to the lack of the herpes simplex virus-induced thymidine kinase which is required for activation (4, 5).

Recently, Baker *et al.* (6) have reported the synthesis of a series of 5′-*O*-acyl derivatives of 9-β-D-arabinofuranosyladenine (vidarabine, ara-A). These compounds were designed as prodrugs for vidarabine and have demonstrated better lipophilicity, and thus, the potential for improved membrane transport over vidarabine. Among these compounds, the 5′-*O*-valeryl derivative has shown a marked increase in both lipophilicity and antiviral activity compared with that of vidarabine.

Recently, Hettinger *et al.* (7) have reported that both 5-iodo-2′-deoxyuridine (idoxuridine, IUdR) and 5-iodo-3′,5′-di-*O*-acetyl-2′-deoxyuridine (Ac_2IDU), an *O*-acetyl derivative of IUdR, were effective against keratitis. However, Ac_2IDU was significantly more effective than the placebo 1 d sooner than was idoxuridine. The greater lipid solubility of the Ac_2IDU that resulted in greater epithelial penetration could account for this difference. Based on these findings, a series of 3′-*O*-acyl derivatives of 5′-NH_2-TdR has been synthesized. Preliminary studies indicated that all these compounds retain the same degree of antiviral activity in comparison with the parent compound, except the 3′-*O*-*tert*-butylacetyl and 3′-*O*-benzoyl esters, IIIi and IIIj, which were found to be less active. The lipophilicities of these derivatives increased



Scheme I

0022-3549/84/1100-1568$01.00/0

Table I—Physical Data for 5′-*O*-Acyl Derivatives of 5′-Amino-5′-deoxythymidine

Compound	Solubility, mg/mL[a]	ε Value		Partition Coefficient (P)[d]	log P
		Buffer[b]	1-Octanol[c]		
5′-NH_2-TdR	57.5	10,146	7674	0.00886	−2.05
IIIa	339.1	9,630	8971	0.0319	−1.49
IIIb	88.8	9,504	8154	0.0323	−1.49
IIIc	433.7	8,884	8347	0.0645	−1.19
IIId	17.2	9,629	9036	0.268	−0.57
IIIe	6.9	9,546	9053	0.504	−0.29
IIIf	28.7	8,622	8797	1.87	0.27
IIIg	131.1	8,996	9674	2.21	0.34
IIIh	174.7	8,953	7683	0.148	−0.83
IIIi	407.8	8,994	8742	0.333	−0.48
IIIj	3.0	11,293	8861	0.357	−0.45

[a] Determined in 0.1 M phosphate buffer (pH 7.0). [b] At λ_{max} 265 in 0.1 M phosphate buffer (pH 7.0). [c] At λ_{max} 265 in 1-octanol. [d] P = $[solute]_{1\text{-octanol}}/[solute]_{phosphate\ buffer}$.

markedly with the increase of the carbon-chain length, the 3′-*O*-octanoyl ester IIIg showing the highest lipophilicity. Because of their increased lipophilicity, these prodrugs might penetrate the biological membranes and the lipoidal stratum corneum more readily (8), and therefore, would be more suitable and effective for topical application for treatment of cutaneous virus infections.

RESULTS AND DISCUSSION

Chemistry—A series of 3′-*O*-acyl derivatives of 5′-amino-5′-deoxythymidine (5′-NH_2-TdR) with various chain lengths (IIIa-i) and aromatic substituents (IIIj) were synthesized as outlined in Scheme I. Treatment of 5′-azido-5′-deoxythymidine (I) (2) with the corresponding acyl chloride in pyridine at 4°C for 24 h gave the 5′-azido-3′-*O*-acyl compounds IIa-j. Catalytic (10% palladium on charcoal) hydrogenation of IIa-j in EtOH afforded the desired 5′-amino-3′-*O*-acyl derivatives IIIa-j. These compounds were characterized by ^{1}H-NMR spectroscopy and elemental analysis. The ^{1}H-NMR spectra (Me_2SO-d_6) showed a downfield shift for the 3′-methinyl protons in all of these compounds in comparison with 5′-amino-5′-deoxythymidine, except the highly branched 3′-*O*-*tert*-butylacetyl ester IIIi. The downfield shift probably is caused by the deshielding effect of the 3′-*O*-acyl function. The C-1′, C-2′, C-4′, and C-5′ protons in the sugar moiety, and the C-6 proton in the pyrimidine base ring in IIIi, shifted upfield relative to that of the parent compound. This upfield shift appears to be the result of the shielding effect exerted by the highly branched *tert*-butylacetyl substituent in the 3′-*O*-acyl group.

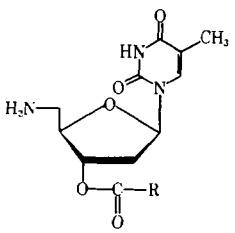
The water solubility and partition coefficient (P) of IIIa-j were determined by UV spectroscopy (6, 9, 10), and the data are summarized in Table I. The aqueous solubilities of the short-chain (R = 1-3 carbons) esters, IIIa-c, increase 6-, 1.5-, and 7.5-fold, respectively, over that of the parent compound, 5′-amino-5′-deoxythymidine (5′-NH_2-TdR). As the carbon-chain length increases to five-seven carbon atoms (IIId-f), the aqueous solubilities decrease. In this series, the 3′-*O*-hexanoyl derivative IIIe is the least soluble in water, with a solubility of 6.9 mg/mL. Unexpectedly, the water solubility of the eight-carbon chain ester, IIIg, increased to 131.1 mg/mL, which is ~2 times greater than that of the parent compound (57.5 mg/mL). Among these 3′-*O*-acyl derivatives, the 3′-*O*-butyryl ester IIIc and the 3′-*O*-benzoyl ester IIIj possess the highest and the lowest water solubilities, with values of 433.7 mg/mL and 3.0 mg/mL, respectively. The highly branched 3′-*O*-*tert*-butylacetyl ester IIIi, has the second highest water solubility (407.8 mg/mL).

The lipophilicities of these 3′-*O*-acyl derivatives (IIIa-j) were determined *via* a 1-octanol–water (0.1 M phosphate buffer) distribution measured in terms of partition coefficient (P). As expected, the lipophilicities of these compounds increase commensurately with the increase of the carbon-chain length. The 3′-*O*-octanoyl ester IIIg has the greatest lipophilicity, as reflected by the P value which approaches 2.21. Compound IIIg was found to be ~250 times more lipid soluble than the parent compound (5′-NH_2-TdR, P = 0.00886). The highly branched 3′-*O*-*tert*-butylacetyl and the aromatic substituted 3′-*O*-benzoyl derivatives, IIIi and IIIj, are also quite lipid soluble, with P values of 0.333 and 0.357, respectively.

Biological Activity—The antiviral activity of these compounds against herpes simplex virus type 1 (HSV-1) was determined. The effect of these compounds on the replication of the uninfected host Vero cells was also investigated. The results are listed in Table II. At 400 μM, IIIa-h show significant antiviral activity by inhibition of the formation of infectious HSV-1 virions, the inhibition ranging from 95% for IIIa to 99% for IIIe. The aromatic substituted 3′-*O*-benzoyl derivative IIIj is less active than the aliphatic straight-chain esters, and the highly branched *tert*-butylacetyl ester IIIi demonstrates little antiviral activity.

All compounds, except the long-chain esters IIIf and IIIg, show little or no cytotoxicity to the host Vero cells at the test concentrations. However, IIIf and IIIg were found to be somewhat cytotoxic against the Vero cells at 400 and 200 μM, respectively. It is worthwhile to acknowledge that among these compounds the valeryl ester IIId and the hexanoyl ester IIIc have shown significant antiviral activity and favorable lipophilicity with no apparent cytotoxicity to the host.

Table II—Effect of Various 5′-Amino-3′-*O*-acyl-thymidine Derivatives on the Replication of Herpes Simplex Virus Type 1 and Vero Cells *In Vitro*[a]



Compound	R	Conc., μM	Percent Inhibition	
			HSV-1	Vero Cells
IIIa	CH_3	400	95	None
IIIb	CH_2CH_3	400	97	None
IIIc	$CH_2CH_2CH_3$	400	97	20
IIId	$CH_2(CH_2)_2CH_3$	400	98	19
IIIe	$CH_2(CH_2)_3CH_3$	200	99	None
		400	99.6	25
IIIf	$CH_2(CH_2)_4CH_3$	400	99.2	66
IIIg	$CH_2(CH_2)_5CH_3$	200	96	83
IIIh	$CH(CH_3)_2$	400	96	40
IIIi	$C(CH_3)_3$	400	19	.....
IIIj	C_6H_5	400	62	–
5′-Amino-5-deoxythymidine		100	92	None
		400	99	None

[a] Assays were carried out in triplicate with appropriate controls. Virus titer in absence of compound was 2.1×10^7 pfu.

EXPERIMENTAL SECTION[1]

General Procedure for the Synthesis of IIIa–j—Compound I (10.0 g, 0.037 mol) was dissolved in 100 mL of pyridine at room temperature. The solution was cooled in an ice-bath, and, with stirring, 0.045 mol of the appropriate acyl chloride was added dropwise *via* an addition funnel. The mixture was stirred at 4°C for 24 h, and then 8 mL of water was added. The solvent was evaporated under reduced pressure below 30°C. The glassy residue was dissolved

[1] Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography was performed on EM Silica gel 60 F_{254} sheets (0.2 mm). The UV spectra were recorded on a Beckman-25 spectrophotometer, and the NMR spectra were taken on a Varian T-60 spectrometer at 60 MHz, using Me_4Si as internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

Table III—Characteristics of 5′-*O*-Acyl Derivatives of 5′-Amino-5′-deoxythymidine

Compound	Formula[a]	Description	Yield, %[b]	Melting Point, °C
IIIa	$C_{12}H_{17}N_3O_5$	white powder	62	137–138
IIIb	$C_{13}H_{19}N_3O_5$	white powder	32	141–142
IIIc	$C_{14}H_{21}N_3O_5$	white powder	30	228–231 (dec.)
IIId	$C_{15}H_{23}N_3O_5$	white powder	36	162–163
IIIe	$C_{16}H_{25}N_3O_5$	white powder	28	210–215 (dec.)
IIIf	$C_{17}H_{27}N_3O_5$	white powder	29	213–214 (dec.)
IIIg	$C_{18}H_{29}N_3O_5$	white needles	22	220–221 (dec.)
IIIh	$C_{14}H_{21}N_3O_5$	white needles	20	240–242 (dec.)
IIIi	$C_{16}H_{25}N_3O_5$	white needles	25	235–236 (dec.)
IIIj	$C_{17}H_{19}N_3O_5$	white needles	22	234–235 (dec.)

[a] All compounds were analyzed for C, H, and N; all values were within ±0.4% of the theoretical value. [b] Yield calculations are based on 5′-azido-5′-deoxythymidine (I).

in 200 mL of $CHCl_3$ and the organic phase was washed with H_2O, saturated $NaHCO_3$ solution, and H_2O. The solution was then clarified with amorphous carbon[2], filtered, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give the corresponding 5′-azido-3′-*O*-acyl derivatives IIa–j.

Solutions of IIa–j in 100 mL of EtOH were hydrogenated at 50 psi at room temperature in the presence of 10% palladium on charcoal (0.5 g) for 2 h. After filtration, the filtrates were concentrated to afford glassy residues which were crystallized from EtOH–Et_2O to give the 5′-amino-3′-*O*-acyl derivatives IIIa–j. The physical data of these compounds are listed in Tables I and III.

Determination of Solubility for IIIa–j—Each compound (10 mg) was stirred overnight at room temperature (21°C) in 10 mL of 0.1 M phosphate buffer. Excess compound was removed by filtration through a sintered glass funnel, and the concentration of the solute was determined by UV spectroscopy using the epsilon (ϵ) value, which was previously determined in the phosphate buffer.

Determination of Partition Coefficients for IIIa–j—Solutions (10 mL) of each compound in 0.1 M phosphate buffer having a UV absorbance of 1.0–1.7 of the λ_{max} were vigorously stirred at room temperature (21°C) for 2 h with 10 mL of 1-octanol. The solutions were then transferred to a 60-mL separatory funnel, and the layers were separated. The concentration of the solutes in aqueous phosphate buffer and in 1-octanol at equilibrium were determined by the UV spectroscopic technique, and the partition coefficient of each compound was calculated as $P = [\text{solute}]_{\text{1-octanol}}/[\text{solute}]_{\text{water}}$.

^{1}H-NMR Data (Me_2SO-d_6)—*5′-Amino-5′-deoxythymidine*—δ 1.80 (s, 3, 5-CH_3), 2.15 (t, 2, 2′-H), 2.77 (br d, 2, 5′-H), 3.70 (m, 1, 4′-H), 4.25 (m, 1, 3′-H), 4.78 (br s, 2, 5′-NH_2, D_2O exchangeable), 6.17 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), and 7.62 ppm (s, 1, 6-H).

Compound IIIa—δ 1.80 (s, 3, 5-CH_3), 2.11 (s, 3, $COCH_3$), 2.21–2.53 (m, 2, 2′-H), 2.83 (br d, 2, 5′-H), 3.88 (m, 1, 4′-H), 4.63 (br s, 2, 5′-NH_2, D_2O exchangeable), 5.26 (m, 1, 3′-H), 6.18 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), and 7.78 ppm (s, 1, 6-H).

Compound IIIb—δ 1.02 (t, 3, C—CH_3), 1.79 (s, 3, 5-CH_3), 2.02–2.62 (m, 4, 2′-H and -$COCH_2$-C), 2.77 (br d, 2, 5′-H), 3.82 (m, 1, 4′-H), 4.59 (br s, 2, 5′-NH_2, D_2O exchangeable), 5.22 (m, 1, 3′-H), 6.12 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), and 7.72 ppm (s, 1, 6-H).

Compound IIIc—δ 0.92 (t, 3, C—CH_3), 1.62 (m, 2, C-CH_2-C), 1.82 (s, 3, 5-CH_3), 2.11–2.61 (m, 4, 2′-H and -$COCH_2$-C), 3.12 (br d, 2, 5′-H), 4.16 (m, 1, 4′-H), 5.21 (m, 1, 3′-H), 6.16 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), 7.68 (s, 1, 6-H), and 8.48 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIId—δ 0.95 (m, 3, C-CH_3), 1.10–1.70 [m, 4, C-$(CH_2)_2$-C], 1.80 (s, 3, 5-CH_3), 2.38 (t, 4, 2′-H and -$COCH_2$-C), 3.14 (br d, 2, 5′-H), 4.12 (m, 1, 4′-H), 5.19 (m, 1, 3′-H), 6.11 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), 7.68 (s, 1, 6-H), and 7.90 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIIe—δ 0.90 (m, 3, C-CH_3), 1.04–1.59 [m, 6, C-$(CH_2)_3$-C], 1.74 (s, 3, 5-CH_3), 2.32 (m, 4, 2′-H and -$COCH_2$-C), 2.80 (br d, 2, 5′-H), 3.84 (m, 1, 4′-H), 4.61 (br s, 2, 5′-NH_2, D_2O exchangeable), 5.20 (m, 1, 3′-H), 6.10 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), and 7.70 ppm (s, 1, 6-H).

Compound IIIf—δ 0.90 (m, 3, C-CH_3), 1.10–1.68 [m, 8, C-$(CH_2)_4$-C], 1.80 (s, 3, 5-CH_3), 2.39 (br t, 4, 2′-H and -$COCH_2$-C), 3.20 (br d, 2, 5′-H), 4.20 (m, 1, 4′-H), 5.26 (m, 1, 3′-H), 6.09 (t, 1, $J_{1',2'}$ = 6.5 Hz, 1′-H), 7.79 (s, 1, 6-H), and 8.59 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIIg—δ 0.88 (m, 3, C-CH_3), 1.02–1.68 [m, 10, C-$(CH_2)_5$-C], 1.80 (s, 3, 5-CH_3), 2.34 (br t, 4, 2′-H and -$COCH_2$-C), 3.18 (br d, 2, 5′-H), 4.16 (m, 1, 4′-H), 5.22 (m, 1, 3′-H), 6.13 (t, 1, $J_{1',2'}$ = 7.5 Hz, 1′-H), 7.72 (s, 1, 6-H), and 8.52 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIIh—δ 1.05 (s, 3, C-CH_3), 1.17 (s, 3, C-CH_3), 1.83 (s, 3, 5-CH_3), 2.25–3.85 (m, 3, 2′-H and -$COCH<$), 3.17 (br d, 2, 5′-H), 4.18 (m, 1, 4′-H), 5.20 (m, 1, 3′-H), 6.12 (t, 1, $J_{1',2'}$ = 6.5 Hz, 1′-H), 7.70 (s, 1, 6-H), and 8.55 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIIi—δ 0.82 [s, 9, C-$(CH_3)_3$], 1.26 (s, 3, 5-CH_3), 1.02–1.70 (m, 4, 2′-H and -$COCH_2$-C, overlapping with 5-CH_3), 2.20 (br d, 2, 5′-H), 3.20 (m, 1, 4′-H), 4.22 (m, 1, 3′-H), 5.18 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), 6.72 (s, 1, 6-H), and 7.48 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Compound IIIj—δ 1.84 (s, 3, 5-CH_3), 2.55 (m, 2, 2′-H), 3.32 (m, 2, 5′-H), 4.40 (m, 1, 4′-H), 5.52 (m, 1, 3′-H), 6.30 (t, 1, $J_{1',2'}$ = 7 Hz, 1′-H), 7.32–8.20 (m, 6, 6-H and -C_6H_5), and 8.42 ppm (br s, 2, 5′-NH_2, D_2O exchangeable).

Biological Test Procedures—The antiviral activities of various compounds listed in Table I were determined. Vero cells were grown to confluency in 25-cm^2 flasks using Dulbecco's medium supplemented with 10% fetal calf serum. The cells were then infected with Herpes simplex virus type 1 (CL-101)[3] at a multiplicity of infection (MOI) of 20. After a 1-h absorption period at 37°C, the viral inoculum was removed, and the flask was washed once with phosphate-buffered saline. The test compounds indicated in Table I were dissolved in Dulbecco's medium supplemented with serum and then added to the flask. The infected cultures were incubated at 37°C for 40 h and then frozen until virus titrations were performed. Virus was released by freezing and thawing the media–cell suspension one time. The cell lysates were diluted directly, and the virus yield was assayed by plaque formation on Vero cells. The number of plaque-forming units (pfu) of virus in the drug-treated cultures relative to that found in the drug-free condition was determined and expressed as percent inhibition in Table III.

The cytotoxicity of the various test compounds on the uninfected host Vero cells was determined (Table III). Vero cells in Dulbecco's medium (2.5 mL) supplemented with 10% fetal calf serum were added to eight 25-cm^2 flasks at a concentration equivalent to 0.1 confluency for each compound under assay. After incubation at 37°C in 5% CO_2–95% air for 1 d, the test compound, dissolved in 2.5 mL of the above growth medium, was added and two flasks were harvested immediately by decanting the medium, washing once with 5 mL of buffered saline, and then incubating at 37°C for 15 min with a 5-mL solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure were generally in clumps and were dispersed by repeated forceful pipetting of the suspension against the surface of the flask. To 1 mL of the well-dispersed cell suspension, 0.2 mL of trypan blue solution was added, and the number of cells was counted using a hemocytometer. Each day for the next 3 d, two of the remaining flasks were harvested in the aforementioned manner for determination of cell number.

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ACKNOWLEDGMENT

This research was supported by PHS Grant EY022168 11 and PHS Grant CA-05262 from the National Cancer Institute, Department of Health and Human Services. The author expresses his appreciation to Diane Mozdziesz and J. J. Lee for their excellent technical assistance.

Determination of Orally Coadministered Nadolol and Its Deuterated Analogue in Human Serum and Urine by Gas Chromatography with Selected-Ion Monitoring Mass Spectrometry

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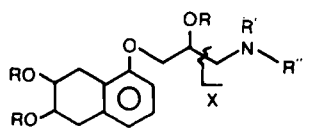
Received April 4, 1983, from the *Departments of *Analytical Research and [‡]Clinical Pharmacology, The Squibb Institute for Medical Research, [§]New Brunswick, NJ 08903* and [¶]*Princeton, NJ 08540.* Accepted for publication September 23, 1983.

Abstract □ A cartridge serum and urine extraction procedure of the β-adrenergic antagonist, nadolol, employing a cross-linked styrene-divinyl benzene macroreticular resin is described. Samples were analyzed as the silylated derivative by gas chromatography–mass spectrometry (GC–MS) using selected-ion monitoring. When nadolol was orally coadministered with its deuterated analogue, relative bioavailability could be demonstrated with six or fewer subjects. Employing a base-deactivated GC phase, the limit of detection is 1 ng and 0.5 ng/mL of serum for nadolol and the deuterated analogue, respectively. For levels of <10 ng/mL, the respective coefficients of variation are 4 and 2%. For concentrations of >10 ng/mL, the *CV* is 1% for nadolol and nadolol-d_9.

Keyphrases □ Nadolol—human serum and urine, GC–MS, selected-ion monitoring, nadolol-d_9 □ GC–MS—selected-ion monitoring, nadolol, human serum and urine, nadolol-d_9

Nadolol (I) is a nonselective β-adrenergic antagonist recommended for the treatment of hypertension and angina pectoris (1, 2). Dreyfuss *et al.* have determined bioavailability parameters of orally and intravenously administered [^{14}C]-nadolol (3, 4) in mildly hypertensive humans. Nadolol is partially and variably absorbed (1), like other β blockers, but it is eliminated without metabolism. With oral administration, the pharmacokinetic profile of nadolol is described by an open two-compartment model with respective absorption, distribution, and elimination half-lives of ~1, 1.5, and 17 h (3, 4).

A gas chromatography–selected-ion monitoring mass spectrometry (GC–SIM MS) determination of nadolol serum levels was previously reported (5). Carlin *et al.* (6) have recently presented an elegant application of GC–SIM MS to the measurement of low levels of the β-blocker timolol and its deuterated analogue in a coadministration bioavailability evaluation. Coadministration bioavailability studies for other drug types have been reported by Strong *et al.* (7), Wolen *et al.* (8), d'A. Heck *et al.* (9), Walle and Walle (10), Alkalay *et al.* (11), Murphy and Sullivan (12), and most recently by Cohen *et al.* (13). The extraction and isolation procedure (5) has been simplified for more effective sample processing by GC–SIM MS (13). The bioavailability of two 80-mg formulations of nadolol (I) given to six subjects, administered either as a tablet or solution along with a concomitant 80-mg solution of nonadeuterated nadolol (II) is presented.



		R	R'	R''	X EI	MH$^+$ PCI
Nadolol	I	H	H	$C(CH_3)_3$		
Deuterated nadolol	II	H	H	$C(CD_3)_3$		
Internal reference	III	H	CH_3	$C(CH_3)_3$		
	IV	$Si(CH_3)_3$	H	$C(CH_3)_3$	86	526
	V	$Si(CH_3)_3$	H	$C(CD_3)_3$	95	535
	VI	$Si(CH_3)_3$	CH_3	$C(CH_3)_3$	100	540

EXPERIMENTAL SECTION

Reagents—Nadolol (I)[1] and its *N*-methyl derivative (III)[1] were characterized, pharmaceutical-grade materials (14). The structures and purity were confirmed by elemental analyses, ^{1}H-NMR, and MS (15) and by GC–MS after silylation. The nonadeuterated nadolol (II) was prepared from the

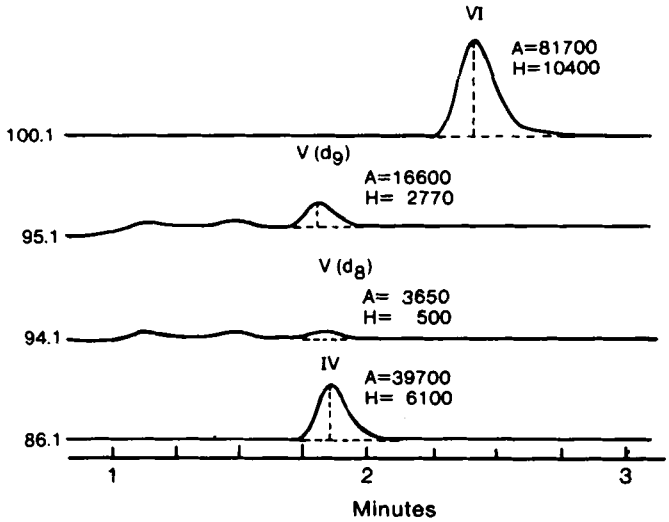


Figure 1—*EI–SIM of a typical processed serum extract containing 133, 83.4, and 250 ng/mL of I, II, and III, respectively, measured as their trimethylsilylated derivatives, IV, V, and VI.*

[1] Nadolol (CAS 42200-33-9), *N*-methyl derivative (CAS 67247-47-6); E. R. Squibb & Sons, Princeton, N.J. Deuterium-labeled *tert*-butylamine; Merck, Sharp and Dohme of Canada.

0022-3549/84/1100-1571$01.00/0