N-(4-Biphenyl)-N-(2-diethylaminoethyl)-mandelamide Hydrochloride. A Potent Local Anesthetic for Use with Sulfhydryl Inhibitors for Cancer Therapy[1]

FRANCES E. KNOCK

Department of Surgery, Presbyterian-St. Luke's Hospital, Chicago, Illinois, and Surgical Service, Veterans' Administration Hospital, Hines, Illinois

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Selected sulfhydryl inhibitors have regressed a variety of human cancers without injury to hematologic status and wound healing or with actual improvement in hematologic status in some patients.[2,3] Rapid acting sulfhydryl inhibitors of the arsenoso and iodoacetyl type can cause much pain on intravenous infusion or intratumor injection, so that potent local anesthetics for concomitant use are at times needed. For this purpose, N-(4-biphenylyl)-N-(2-diethylaminoethyl)mandelamide hydrochloride (I) has been clinically useful. In sensitivity tests against a variety of animal and human cancer cells, it can significantly potentiate the activity of clinically useful sulfhydryl inhibitors.[4]

Biological Activity.—By intradermal wheal tests in animals and humans, activity of I is about ten times as great as that of procaine, about five times as great as that of lidocaine. A 0.2% solution of I is approximately equivalent in activity to 1.0% lidocaine. Toxicity of I closely approximates that of lidocaine on intravenous injection in mice and exceeds somewhat that of procaine.

In the guinea pig sciatic nerve block test, 0.25 ml of a 1.0% solution of I placed directly on the isolated nerve produced mean duration of activity of 27 ± 11.2 hr, by contrast with minutes for other commonly used anesthetics. Anticholinergic activity of I is very weak. The *in vitro* atropine ratio is 0.00008. There is no apparent effect on acetylcholine in cardiovascular–respiratory tests. The curaremimetic activity of I is very low, the curare ratio in the frog rectus test being 0.15. The compound is inactive in the electroshock and pentylenetetrazole anticonvulsant tests.

Clinical trial with 30 patients has shown that I is not suitable for use in standard nerve block anesthesia because of low diffusibility and tendency to cause irritation in high concentrations.

For direct intratumor injection with sulfhydryl inhibitors, however, for which sulfhydryl inhibitor and I are dissolved together in the same solution and injected simultaneously, low diffusibility has presented no clinical problems while high potency and relatively low toxicity of I offer significant advantages over other available local anesthetics.

Experimental Section[5]

N-(4-Biphenylyl)-N-(2-diethylaminoethyl)mandelamide.—A 10% excess of acetylmandelyl chloride[6] in a twofold volume of dry benzene was slowly added to a solution of N-(4-biphenylyl) N′,N′-diethylethylenediamine[7] in a twofold volume of dry benzene, with good stirring and cooling to keep the temperature below 20°. Reaction was completed by refluxing for 1 hr. After cooling, a fourfold volume of ligroin (bp 66–75°) was added to complete separation of the crude product. Yield of crude product, mp 175–179°, was 97%. The product was purified by solution in 50% alcohol–acetone and precipitation with ether, to give a product of mp 185–186°. The acetyl group was removed by refluxing for 1 hr with a 10% excess of NaOH in 50% aqueous ethanol. The product was evaporated to dryness, taken up in ether, and washed several times with water, and the ether was evaporated to give the viscous, pale yellow base. This was dissolved in five times its weight of absolute methanol, and the stoichiometric quantity of 9.0 *N* solution of dry HCl in absolute methanol was added. The clear colorless solution was diluted to incipient cloudiness with absolute ether and allowed to stand at 5° for 24 hr. The precipitate was redissolved in absolute methanol and the precipitation with absolute ether was repeated. After drying the precipitate for 24 hr at 70°, the yield of product melting at 169–170° was 90%. The last trace of solvent was removed by drying over P_2O_5 *in vacuo* at 78° for 24 hr to give a hygroscopic product melting at 176–177°.

Anal. Calcd for $C_{26}H_{31}N_2O_2Cl$: C, 71.15; H, 7.07; N, 6.39. Found: C, 71.16; H, 7.24; N, 6.22.

(1) This investigation was supported by grants from E. R. Squibb and Sons and the Knock Research Foundation.

(2) F. E. Knock, *Perspectives Biol. Med.*, **10**, 310 (1967).

(3) F. E. Knock, *J. Am. Geriat. Soc.*, **15**, 41 (1967).

(4) F. E. Knock, "Anticancer Agents," Charles C Thomas, Publisher, Springfield, Ill., 1967, p 218.

(5) Melting points were taken in capillary tubes by means of a stirred oil bath and are corrected. Bioassays were performed by Merck Sharp and Dohme, Rahway, N. J. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(6) F. K. Thayer, "Organic Syntheses," Coll. Vol. I, A. H. Blatt, Ed., 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1941, p 12.

(7) L. Bauer, J. Cymerman, and W. J. Sheldon, *J. Chem. Soc.*, 2342 (1951).

Sulfate Esters of 5-Iododeoxyuridine and 5-Iododeoxycytidine[1]

PAULINE K. CHANG, LOUIS J. SCIARINI, AND JOHN W. CRAMER

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

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In view of the reported biological activities of the sulfate esters of several nucleosides,[2-6] and because of interest in halogenated pyrimidine deoxyribonucleosides[7-9] and deoxyribonucleotides[8,10] in this laborae

TABLE I
SULFATION OF IUdR AND ICdR

Product	Yield, mole %: $HOSO_2Cl$, r[a] 1.6	$HOSO_2Cl$, r 2.5	PST, r 1.6	PST, r 2.5	R_f[b]
IUdR 3′-sulfate (A)	16	15	12	7	0.38
IUdR 5′-sulfate (B)	40	33	27	25	0.31
IUdR disulfate (C)	12	40	5	59	0.18
ICdR 3′-sulfate (A′)	4	11	9	8	0.45
ICdR 5′-sulfate (B′)	78	49	23	32	0.37
ICdR disulfate (C′)	12	28	5	57	0.23

[a] r = sulfating agent/nucleoside. [b] Solvent system: isobutyric acid–H_2O–concentrated NH_4OH (66:33:1).

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(2) F. Egami and K. Yagi, *J. Biochem.* (Tokyo), **43**, 153 (1956).

(3) M. Niwa, S. Higuchi, and F. Egami, *ibid.*, **45**, 89 (1958).

(4) J. Arnold and T. D. Price, *Federation Proc.*, **22**, 292 (1963).

(5) P. W. Wigler and H. U. Choi, *J. Am. Chem. Soc.*, **86**, 1636 (1964).

(6) G. Kowollik and P. Langen, *Ber.*, **99**, 2725 (1966).

(7) P. K. Chang and A. D. Welch, *Biochem. Pharmacol.*, **6**, 50 (1961).

(8) P. K. Chang and A. D. Welch, *J. Med. Chem.*, **6**, 428 (1963).

(9) W. H. Prusoff, *Cancer Res.*, **23**, 1246 (1963).

(10) W. H. Prusoff, Y. S. Bakhle, and L. Sekely, *Ann. N. Y. Acad. Sci.*, **130**, 135 (1965).

TABLE II
FAILURE TO INHIBIT GROWTH OF P815Y CELLS BY SULFATE ESTERS OF IUdR OR ICdR

	Growth inhib, %[a]					
	Drug conen, *M*					
Compd	1×10^{-6}	4×10^{-6}	1×10^{-5}	4×10^{-5}	1×10^{-4}	4×10^{-4}
IUdR	64	79	87	92	92	..
IUdRP (5′)	23	39	47	..	..	..
IUdRS (5′)	0	0	0	0	0	0
IUdRS (3′)	0	0	0	0	0	0
IUdRSS (3′,5′)	13	13	9	20	13	14
ICdR	10	46	64	84	85	86
ICdRP (5′)	..	..	53	67	..	..
ICdRS (5′)	0	0	0	0	0	0
ICdRS (3′)	0	0	0	0	0	..
ICdRSS (3′,5′)	12	17	13	15	22	26

[a] P815Y cells were assayed in culture against the derivatives of IUdR or ICdR given above by determining the increase in cell number over the inoculum (20,000 cells/ml) after a 48-hr period of incubation (four cell generations) in the presence or absence of drug. The differences in cell numbers between the drug-treated cultures and the control were expressed as percentages of the control count.

tory, we have prepared the sulfate esters of 5-iododeoxyuridine (IUdR) and 5-iododeoxycytidine (ICdR).

IUdR and ICdR were easily esterified by the use of either chlorosulfonic acid or pyridine–sulfur trioxide[11] (PST), yielding a mixture of the 3′-sulfate, 5′-sulfate, and 3′,5′-disulfate derivatives. It appears that PST is preferred as the esterifying agent only when the disulfate is the desired product (Table I). The monosulfate isomers were identified by the exclusive synthesis of the 3′-sulfate *via* the 5′-trityl intermediate, while the method of preparation of IUdR 5′-sulfate *via* the 3′-acetate intermediate, which led to the formation of the two monosulfate isomers and the disulfate, indicated the lability of the 3′-acetate bond under the conditions of sulfation. Because of the instability of the 3′,5′-disulfates in ion-exchange chromatography systems, the products were separated by paper chromatography.

The sulfate esters were assayed for their potential as growth inhibitors against the P815Y murine mastocytoma cell grown in tissue culture. The technique for culture of this cell line have been described previously.[12] It is clear that none of the sulfate esters were inhibitory of growth in the concentrations tested, *e.g.*, in the range of 1×10^{-6} to 4×10^{-4} *M* (Table II). In contrast, the parent nucleosides, IUDR or ICDR, were fully active as inhibitors within this range. Also, the corresponding monophosphate esters showed activity. Failure of the sulfate esters to inhibit may indicate a lack of transport of the compounds into the cell, or, if they are transported, they may fail to inhibit enzymes or compete with normal substrates of enzymes intracellularly. Removal of the sulfate group by sulfatase action within the cell would yield the active nucleoside. An inhibitory activity of these sulfate esters against such cells might thus be anticipated and may yet prove of value in treating specific types of tumors or even virus-infected cells having elevated sulfatase levels.

Experimental Section[13]

Reaction of Chlorosulfonic Acid with Either 5-Iododeoxyuridine or 5-Iododeoxycytidine.—To a vigorously stirred mixture of 5-iododeoxyuridine[14] or 5-iododeoxycytidine[7] (2.5 mmoles) in dry pyridine (35 ml) was added a solution of chlorosulfonic acid (450 mg, 4 mmoles) in $CHCl_3$ (1.3 ml) dropwise at 0° over a period of 20 min. After stirring at room temperature for 24 hr, the reaction was terminated by the addition of 30 ml of H_2O. The mixture, after concentration to dryness *in vacuo* at 40°, was dissolved in methanol (30 ml) and again concentrated to dryness *in vacuo* at 40°; this operation was repeated five times in order to remove most of the pyridine. The residue was taken up in H_2O (35 ml) and 1-ml aliquots of the solution were applied to Whatman 3 MM paper (16 × 55 cm). The chromatogram was developed in a descending manner for 18 hr with isobutyric acid–H_2O–concentrated NH_4OH (66:33:1). The fractions of the isomeric 3′- and 5′-monosulfates and the disulfate were eluted with water and converted to the corresponding salts by passing the concentrated eluates through a column of Dowex 50 (K^+ or Ba^{2+} form). Their respective R_f values, ultraviolet spectra, and infrared spectra were identical with those of the same esters obtained by the PST method.

In a separate experiment, in order to increase the yield of the disulfate, the amount of cholorosulfonic acid was increased from 4 mmoles to 6.5 mmoles (760 mg, in 2.6 ml of $CHCl_3$, r ($HOSO_2Cl$/nucleoside) = 2.5, and the reaction was carried out in the same manner as was described above (Table I).

Reaction of Pyridine–Sulfur Trioxide and 5-Iododeoxyuridine or 5-Iododeoxycytidine.—A mixture of 5-iododeoxyuridine or 5-iododeoxycytidine (2 mmoles), pyridine–sulfur trioxide[15] (5 mmoles), and dry pyridine (30 ml) was stirred at 28° for 48 hr. The reaction was terminated with H_2O (30 ml) and the procedure for the chlorosulfonic acid reaction was followed to yield the 3′,5′-disulfate as the major product (Table I). The analytical samples were recrystallized from the appropriate solvents (Table III).

5′-O-Trityl-5-iododeoxyuridine.—A solution of 5-iododeoxyuridine (7.1 g, 20 mmoles) and trityl chloride (9 g, 32 mmoles) in dry pyridine (130 ml), after standing in a stoppered flask at room temperature for 7 days, was poured into ice–water (500 ml) with stirring and the mixture was concentrated *in vacuo* at 40°. The residue was dissolved in methanol (125 ml) and the solution again was poured slowly into ice–water (1250 ml) with stirring. Crude 5′-O-trityl-5-iododeoxyuridine separated after standing at 0° for 3 hr. It was washed several times (CCl_4) dried (P_2O_5), and recrystallized from acetone and benzene (7.8 g, 66%). The analytical sample was recrytallized from ethyl acetate and petroleum ether (bp 30–60°): mp 208°, $\lambda_{max}^{95\% \text{ EtOH}}$ 285 mμ (ε 6500).

Anal. Calcd for $C_{28}H_{25}IN_2O_5$: C, 56.38; H, 4.22; I, 21.20; N, 4.68. Found: C, 56.41; H, 4.54; I, 21.20; N, 4.37.

5′-O-Trityl-5-iododeoxycytidine.—5-Iododeoxycytidine was substituted for 5-iododeoxyuridine and the procedure used in the preparation of 5′-O-trityl-5-iododeoxyuridine was followed to produce 5′-O-trityl-5-iododeoxycytidine. The crude product was recrystallized with tetrahydrofuran and H_2O (6.5 g, 56%). The analytical sample was recrystallized from dimethyl sulfoxide–ethanol–H_2O; mp 189°, $\lambda_{max}^{95\% \text{ EtOH}}$ 295 mμ (ε 5350).

(11) P. Baumgarten, *Ber.*, **59**, 1166 (1926).

(12) G. A. Fischer and A. C. Sartorelli, *Methods Med. Res.*, **10**, 247 (1964).

(13) Melting points were determined in a capillary tube in a copper block and have been corrected. Ultraviolet spectra were measured on a Model 505 Bausch & Lomb Spectronic recording spectrophotometer. Microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.

(14) W. H. Prusoff, *Biochim. Biophys. Acta*, **32**, 295 (1959).

(15) Prepared by the method of H. H. Sisler and L. F. Audrieth, *Inorg. Syn.*, **2**, 173 (1946).

TABLE III

Compd	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	I, % Calcd	I, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found	Recrystn solvent	Uv data $\lambda_{max}^{0.1\ N\ HCl}$, mμ (ε)
IUdR 3′-sulfate	$C_9H_{10}IKN_2O_8S$[a]	22.89	22.69	2.13	2.35	26.87	26.63	5.93	5.68	6.79	7.00	H_2O–EtOH	286 (6830)
IUdR 5′-sulfate	$C_9H_{10}IKN_2O_8S$	22.89	22.85	2.13	2.30	26.87	27.18	5.93	6.15	6.79	6.84	H_2O–EtOH	288 (7015)
IUdR disulfate	$C_9H_9IK_2N_2O_{11}S_2$	18.31	18.02	1.54	1.88	21.49	20.96	4.74	4.70	10.86	10.97	H_2O–EtOH	288 (7019)
ICdR 3′-sulfate	$(C_9H_{11}IN_3O_7S)_2Ba$[a]	21.58	21.73	2.21	2.49	25.34	25.21	8.40	8.18	6.40	6.20	H_2O	308 (8130)
ICdR 5′-sulfate	$(C_9H_{11}IN_3O_7S)_2Ba$	21.58	21.34	2.21	2.37	25.34	25.08	8.40	8.18	6.40	6.29	H_2O–EtOH	308 (7890)
ICdR disulfate	$C_9H_{10}IK_2N_3O_{10}S_2$	18.34	18.49	1.70	2.12	21.53	20.85	7.13	7.05	10.88	10.57	H_2O	308 (8570)

[a] The analytical sample was prepared from the 3′-sulfate obtained *via* the 5′-trityl intermediate.

Anal. Calcd for $C_{28}H_{55}IN_3O_4$: C, 56.47; H, 4.40; I, 21.35; N, 7.06. Found: C, 56.54; H, 4.49; I, 21.20; N, 6.89.

5-Iododeoxyuridine 3′-Sulfate.—A mixture of 5′-trityl-5-iododeoxyuridine (1.2 g, 2 mmoles) and pyridine–sulfur trioxide (0.476 g, 2.8 mmoles) in dry pyridine (8 ml) was stirred at 28° for 48 hr. The reaction was terminated with H_2O (40 ml) and allowed to stand overnight at 0°. The mixture, after concentration to dryness *in vacuo* at 40°, was dissolved in methanol (20 ml) and again concentrated to dryness *in vacuo* at 40°; this operation was repeated several times. The powdery residue was dissolved in 80% aqueous acetic acid (30 ml) and the solution was stirred for 2 hr at 45°. The solvent was removed *in vacuo* at 40° and the residue was taken up in H_2O (30 ml). 5-Iododeoxyuridine 3′-sulfate (1.6 mmoles, 78%), separated by paper chromatography in isobutyric acid–H_2O–concentrated NH_4OH (66:33:1), was converted to the potassium salt *via* Dowex 50 (K^+ form). Its infrared spectrum, R_f, and ultraviolet spectra were identical with those of the potassium salt of fraction A (Table I).

5-Iododeoxycytidine 3′-Sulfate.—5′-O-Trityl-5-iododeoxycytidine was treated with PST, according to the procedure for 5-iododeoxyuridine 3′-sulfate, to give 5-iododeoxycytidine 3′-sulfate in 82% yield. Its barium salt had the same R_f value, as well as infrared and ultraviolet spectra, as the barium salt of fraction A′ (Table I).

3′-O-Acetyl-5-iododeoxyuridine.—To a cooled solution of 5′-O-trityl-5-iododeoxyuridine (4.8 g, 8 mmoles) in dry pyridine (40 ml) was added acetic anhydride (1.8 ml) at 0° with stirring. The solution, after stirring an additional 24 hr at room temperature, was poured into ice–water (15 ml) and the solvent was removed *in vacuo* at 40°. The residue was dissolved in 80% aqueous acetic acid (100 ml); after stirring at 45° for 54 hr, the solution was stored at 4° for 12 hr and the separated triphenylcarbinol was removed by filtration. The filtrate was concentrated to dryness *in vacuo* at 40° to yield the crude 3′-O-acetyl derivative (2.5 g), which was washed (CCl_4) and recrystallized from methanol and petroleum ether (bp 30–60°). The analytical sample was recrystallized from ethyl acetate and petroleum ether; mp 196°, $\lambda_{max}^{95\%\ EtOH}$ 283 mμ (7600).

Anal. Calcd for $C_{11}H_{13}IN_2O_6$: C, 33.35; H, 3.31; I, 32.03; N, 7.06. Found: C, 33.09; H, 3.52; I, 32.15; N, 7.25.

Potential Carcinostatic Agents. I. Derivatives and Analogs of 1-(2-Hydroxyethyl)-3-(4-tolyl)urea

HAROLD G. NELSON, FREDERIC J. SHELTON, AND WILLIAM H. WETZEL

Reichhold Chemicals, Inc., Pacific Northwest Division, P.O. Box 1482, Tacoma, Washington 98401

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During the course of a program for synthesizing a group of substituted ureas designed as potential herbicides to be evaluated in our greenhouse, we also submitted these to the Cancer Chemotherapy National Service Center for antitumor screening. One of these, the title compound, showed some activity against Sarcoma 180 in mice and it was decided to synthesize a number of derivatives and analogs of this compound, not already reported and tested,[1] for similar screening. One of these derivatives, 1-(2-chloroethyl)-1-nitroso-3-(4-tolyl)urea, was particularly active against L1210 lymphoid leukemia in two types of mice. Table I lists these substances and their physical properties. They were made by literature procedures, as outlined for typical cases in the Experimental Section.

Biological Data.—Although several of these compounds passed stage I or stage II in the Lewis lung carcinoma test, they did not have confirmed activity. 1-(2-Hydroxyethyl)-3-(4-tolyl)urea had confirmed activity against Sarcoma 180, but was inactive in the lymphoid leukemia (L1210) test. 1-(2-Hydroxyethyl)-1-nitroso-3-(4-tolyl)urea showed slight activity against L1210 (T/C = 100–125% in the multiple-dose assay). However, 1-(2-chloroethyl)-1-nitroso-3-(4-tolyl)urea had considerable activity (L1210); see Table II.

Experimental Section[2]

Method A. 1-(2-Hydroxyethyl)-3-(4-tolyl)urea.—A stirred suspension of 2-aminoethanol (6 g, 0.98 mole) in toluene (100 ml) was treated at room temperature with *p*-tolyl isocyanate (13 g, 0.098 mole). After stirring for 4 hr the product was collected on a filter and washed with toluene and then with ligroin (bp 60–70°).

Method B. 1-(2-Chloroethyl)-3-(4-tolyl)urea.—A stirred solution of *p*-toluidine (10.5 g, 0.098 mole) in toluene (100 ml) was treated with 2-chloroethyl isocyanate (10 g, 0.098 mole) at room temperature. After stirring for 2 hr the product was collected on a filter and washed with toluene and then ligroin. The product was recrystallized from acetonitrile.

Method C.[1b] **1-(2-Chloroethyl)-1-nitroso-3-(4-tolyl)urea.**—A stirred solution of 1-(2-chloroethyl)-3-(4-tolyl) urea (7 g, 0.033 mole) in 300 ml of 98% formic acid (5–7°) was treated with dry $NaNO_2$ (6 g, 0.09 mole) in small portions over a period of 1.5 hr. The solution was stirred for an additional hour (0–5°) and 175 ml of ice water was then added slowly. The mixture was stirred an additional 30 min at 0°. The light yellow crystalline product was collected on a filter, washed with cold water, and dried (P_2O_5, under vacuum).

Method D. 1-(2-Iodoethyl)-3-(4-tolyl)urea.—1-(2-Chloroethyl)-3-(4-tolyl)urea (6 g, 0.06 mole) was refluxed with anhydrous NaI[3] (9 g, 0.06 mole) in 50 ml of dry acetone for 24 hr ($CaCl_2$ drying tube). The solution was then filtered hot to remove any salts and the filtrate was diluted with 500 ml of cold water. The white precipitate that formed was collected on a filter, washed with water, and dried. The product was recrystallized from acetonitrile.

Method E. 1-(2-Bromoethyl)-3-(4-tolyl)urea.—1-(2-Hydroxyethyl)-3-(4-tolyl)urea (26 g, 0.144 mole) was added slowly with stirring to 40 ml of PBr_3 at room temperature. After the addition, the reaction mixture was heated on a hot-water bath and stirred for 3 hr. During this time, an orange suspension formed.

(1) (a) For example, T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963); (b) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *ibid.*, **9**, 892 (1966).

(2) Melting points were taken on a Fisher-Johns block and are corrected to standards.

(3) The NaI was heated strongly (~300°) on a hot plate just prior to use.