0.5 h an equal volume of petroleum ether was added, and the crystalline product was collected to give 21.22 g (mp 128–130 °C, lit.[21] mp 131–132 °C).

Biochemical Assay. Inhibition of binding of 1 nM N^6-[^{3}H]cyclohexyladenosine was assayed as described.[22] Inhibition of binding by a range of concentrations of each adenosine analogue was assessed in triplicate for at least two separate experiments, and the IC_{50} values were estimated graphically for each experiment.

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Registry No. **1**, 23589-16-4; **2**, 29204-54-4; **3**, 29204-77-1; **4**, 96760-57-5; **5a**, 96760-58-6; **5b**, 96760-59-7; **5c**, 96760-60-0; **5d**, 96760-61-1; **5e**, 96760-62-2; **5f**, 96760-63-3; **5g**, 96760-64-4; **5h**, 96760-65-5; **6**, 96760-66-6; **7a**, 96760-67-7; **7b**, 96760-68-8; **8**, 96760-69-9; **9a**, 96760-70-2; **9b**, 96760-71-3; **9c**, 96760-72-4; **10**, 5399-87-1; **11**, 1197-55-3; **12**, 96760-73-5; **13a**, 74-89-5; **13b**, 459-73-4; **13c**, 95-53-4; **13d**, 108-44-1; **13e**, 106-49-0; **13f**, 455-14-1; **13g**, 89545-73-3; **13h**, 96760-74-6; **14**, 83528-16-9; **15a**, 14464-29-0; ethylenediamine, 107-15-3; *N*-succinimidyl-*d*-biotin, 35013-72-0; *p*-nitro-L-phenylalanine methyl ester hydrochloride, 17193-40-7; trifluoroacetic anhydride, 407-25-0; *N*-hydroxysuccinimide, 6066-82-6; acetic anhydride, 108-24-7.

New Cysteamine (2-Chloroethyl)nitrosoureas. Synthesis and Preliminary Antitumor Results

J. C. Madelmont,*† D. Godeneche,† D. Parry,† J. Duprat,‡ J. L. Chabard,§ R. Plagne,§ G. Mathe,∥ and G. Meyniel†

INSERM U 71, B.P. 184, 63005 Clermont-Ferrand, Cedex, Faculté de Médecine, B.P. 38, 28, 63001 Clermont-Ferrand, Cedex, Faculté de Pharmacie, B.P. 38, 28, 63001 Clermont-Ferrand, Cedex, I.C.I.G. Hôpital Paul Brousse, 16bis, Avenue Paul-Vaillant Couturier, 94800 Villejuif, Cedex, France. *Received June 4, 1984*

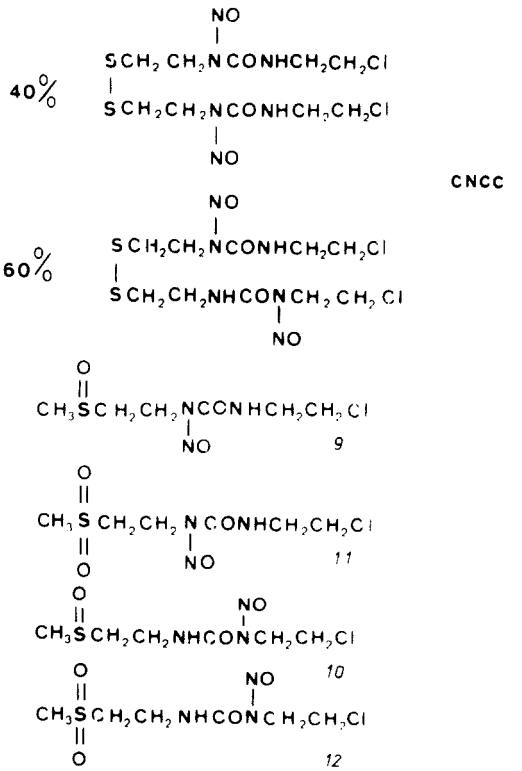
Three chemical pathways were used for the synthesis of four new N'-(2-chloroethyl)-N-[2-(methylsulfinyl)ethyl]- and N'-(2-chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N- or -N'-nitrosoureas. These compounds are plasma metabolites of CNCC, a promising antineoplastic (2-chloroethyl)nitrosourea. Preliminary antitumor evaluation was performed against L1210 leukemia implanted intraperitoneally in mice. Among these compounds, two of them exhibited a greater antitumor activity compared to that of the parent mixture.

(2-Chloroethyl)nitrosoureas, a class of very active antitumor agents, are of interest for the treatment of glioblastoma, Hodgkin's disease, non-Hodgkin's lymphoma, bronchial carcinoma, and gastric cancers.[1-5] To date the most widely used in cancer chemotherapy are BCNU, CCNU, and MeCCNU. Among the several compounds of this important series, the more effective agents chlorozotocin, ACNU, MeCCNU, and RFCNU have undergone phase I–II clinical trials.[6] They were selected on the basis of their wide spectrum of activity. CNCC (Chart I), a recently developed (2-chloroethyl)nitrosourea,[6-10] shows a very promising antineoplastic activity in several experimental animal tumor models.[6,7,10]

In vivo metabolic studies of CNCC[11,12] allowed us to identify active plasma metabolites in different animal species (mouse, rat, rabbit) and in man. These biotransformation products originate in the breakdown of the disulfur bridge with subsequent methylation and oxidation of the sulfur atom. Structure analysis of the four isolated metabolites listed in Chart I shows that they could be antitumor agents. To test this hypothesis we have synthesized **9–12** (Chart I) and evaluated their antitumor activity. This paper describes the synthesis and the preliminary biological results of antitumor testing against L_{1210} in mice.[13]

Chemistry. The four compounds were prepared by several routes. Scheme I leads to the mixture of sulfoxides and sulfones. The urea **1** is easily prepared by reacting 2-chloroethyl isocyanate with 2-(methylthio)ethylamine in diethyl ether. Conventional nitrosation of **1** gives dominance to the formation of the N-nitroso-N'-(2-chloroethyl) compound **2**. Oxidation with H_2O_2 in acetone or formic acid yields a mixture of nitroso sulfoxides and sulfones.

Chart I



† INSERM U 71.
‡ Faculté de Médecine.
§ Faculté de Pharmacie.
∥ I.C.I.G. Hôpital Paul Brousse.

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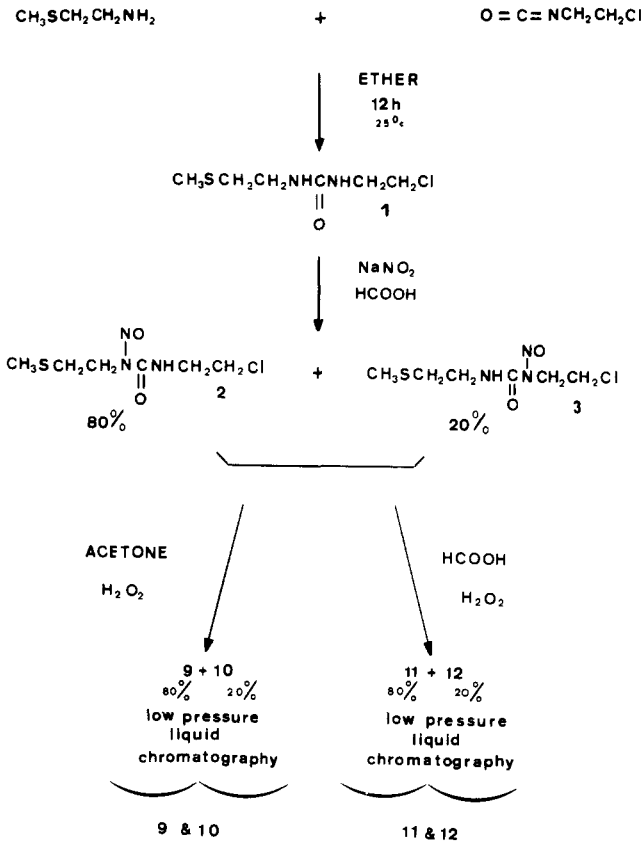
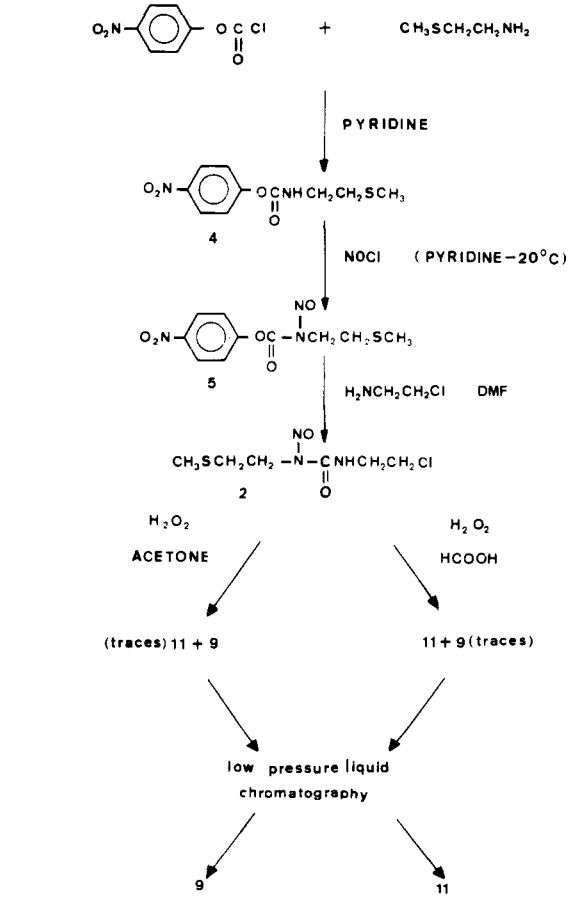
Scheme I

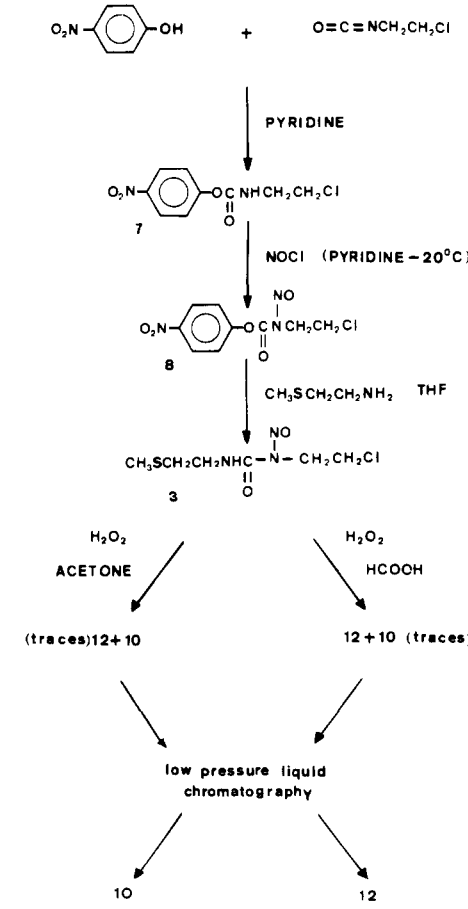


Scheme II



Separation of each isomer is achieved by low-pressure liquid chromatography on a silica column. Two other procedures were developed to obtain selective nitrosation.

Scheme III



As shown in Scheme II, 4-nitrophenyl chloroformate is reacted with 2-(methylthio)ethylamine to yield the carbamate **4**. Nitrosated carbamate **5** can be obtained from conventional nitrosation with nitrosyl chloride in pyridine. The reaction of 2-(chloroethyl)amine with **5** gives the [2-(methylthio)ethyl][2-chloroethyl]nitrosourea **2**. Oxidation in acetone or formic acid leads to either the sulfoxide **9** as the major product and the sulfone **11** as a byproduct or the sulfone **11** as the main compound and the sulfoxide **9** as a byproduct. Separation is achieved by low-pressure liquid chromatography on silica column.

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Table I. Comparative Antitumor Activity against L1210 Leukemia[a]

drug	dose, μmol/kg	T/C × 100 (survivors/6)[b] ip	iv
CNCC	25	250 (0)	
	50	∞[c] (6)	
	75	∞ (6)	
	95	∞ (3)	
	120	125 (0)	
9/10 (70%/30%)	40	300 (2)	170 (0)
	80	∞ (6)	270 (0)
	125	∞ (6)	∞ (6)
	165	∞ (5)	∞ (6)
	210	295 (0)	∞ (3)
11/12 (70%/30%)	40	190 (0)	190 (0)
	80	∞ (5)	230 (0)
	115	∞ (6)	∞ (6)
	155	∞ (6)	∞ (6)
	195	∞ (5)	∞ (5)
	235	410 (0)	290 (0)
9	40–250		NS[d]
10	20		190 (0)
	40		∞ (6)
	60		∞ (4)
	80		190 (0)
11	40–250		NS
12	20		200 (0)
	40		∞ (6)
	60		∞ (6)
	80		300 (1)
	120		190 (0)

[a] Mice were intraperitoneally implanted with 10^5 L1210 cells on day 0. Dose schedule on days 1, 5, 9. [b] Median survival time of treated groups/median survival time of control group. [c] 50–100% of survivors at day 90. [d] T/C × 100 < 125.

An alternative well-known route[14] can be followed to synthesize the activated carbamate **7** (Scheme III). The nitroso carbamate **8** is obtained in a high yield by reacting **7** with nitrosyl chloride in pyridine. Reaction of **8** with 2-(methylthio)ethylamine gives the [2-(methylthio)-ethyl][2-chloroethyl]nitrosourea **3** in quantitative yield. The sulfoxide **10** and the sulfone **12** are obtained and separated in the same way as in procedure II.

Identification. The structure of each compound was assigned according to the different chemical routes followed for the synthesis of the authentic isomers and on the basis of the spectrometric data (NMR and mass spectrometry). The 60-MHz proton NMR spectra shows that the chemical shifts of the C-1 and C-1′ protons are in good agreement with the position of the nitroso group on the corresponding nitrogen atom. Mass spectra were performed via both electron impact and chemical ionization. The chemical-ionization process allows us to find the molecular ion for each isomer. For sulfoxide **9** and sulfone **11**, electron impact shows the two following main fragments: m/z 136 and 152. Masses of these fragments are in good agreement with the nitroso group position. For the two other isomers (sulfoxide **10** and sulfone **12**) the main fragments are respectively m/z 134 and 150. The presence of these two fragments can be explained by a cleavage of the molecule between the carbonyl and the N'-NO group.

Biological Results and Discussion

In Table I, preliminary data are presented that show a comparison of the relative activities of CNCC and compounds **9–12** against L_{1210} leukemia implanted intraperitoneally in mice. In order to compare the antitumor potencies of the parent drug mixture with those of its metabolites, we first have administered to mice mixtures of **9/10** and **11/12** in the same proportions as those expected from the cleavage of the disulfur bridge of CNCC (Chart I). Thus, animals have been intraperitoneally or intravenously treated with a 70%:30% mixture of either isomers **9/10** or **11/12**. The lack of aqueous solubility of CNCC does not allow us to test its oncostatic effect by the intravenous route. The mixtures **9/10** and **11/12** administered intraperitoneally show a very good effectiveness at doses ranging from 80 to 165 μmol/kg for mixture **9/10** and 80 to 195 mol/kg for mixture **11/12**. The mixtures display a maximal efficient dose interval larger than that of CNCC (50–95 μmol/kg). Furthermore, the number of survivals at day 90 is greater when mice are treated with either one of the two mixtures than with CNCC.

After intravenous administration, the mixture **9/10** shows a very good oncostatic effect at 125 and 165 μmol/kg doses, and as after intraperitoneal injection, the maximal efficient dose interval is better for the mixture **11/12**, the active doses ranging from 115 to 195 μmol/kg. The comparative antitumor activity of each isomer intravenously administered to mice bearing L_{1210} leukemia is listed in Table I. Compounds **10** and **12** display a high antitumor effectiveness at doses ranging from 40 to 60 μmol/kg. In contrast, isomers **9** and **11** do not exhibit a significant oncostatic effect in this tumor system.

In summary, these data show that among the four metabolites of CNCC, two of them (**10** and **12**) are novel active anticancer agents in mice. The finding that compounds **9** and **10** are ineffective in curing mice bearing L_{1210} leukemia is not unexpected. Indeed, according to literature reports on the "in vitro" and "in vivo" degradation of the (2-chloroethyl)nitrosoureas,[15-18] it is very likely that the decomposition of compounds **9** and **11** leads to the formation of monoalkylating entities such as $CH_3SO_nCH_2CH_2N{=}NOH$, while the same degradation pathway applied to compounds **10** and **12** give rise to the formation of the very reactive bialkylating entity, namely, the (2-chloroethyl)diazonium intermediate $ClCH_2CH_2N{=}NOH$. If the bulk of the antitumor activity of this class of compounds should be due to lethal DNA cross-linking, the lack of activity of isomers **9** and **11** would be predictable. This observation is based on the fact that their chemical degradation produces monoalkylating derivatives inactive as antitumor agents.

Experimental Section

All melting points were determined on a Kofler apparatus and were uncorrected. IR spectra were determined with a Perkin-Elmer 398 spectrophotometer. ^1H NMR spectra were recorded on a JEOL PMX 60 spectrometer with Me_4Si as internal standard. Chemical shifts are given as δ values. Chemical-ionization and electron-impact mass spectra were obtained with a Hewlett Packard Model 5385 computer system. Elemental analyses are within ±0.4% of the theoretical values. TLC was performed on silica gel Si 60 F 254 glass plates. Low-pressure liquid chromatography was performed on a silica gel (70–230 mesh) column.

***N'*-(2-Chloroethyl)-*N*-[2-(methylthio)ethyl]urea (1) (Scheme I).** An equimolecular mixture of 2-(methylthio)-ethylamine (4.55 g, 50 mmol) and 2-chloroethyl isocyanate (5.27 g, 50 mmol) in ethyl ether is stirred for 12 h at 25 °C. The white crystalline precipitate is filtered off and washed with 10 mL of

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ethyl ether. The product can be used without further purification: yield (7.86 g, 80%): mp 74 °C; TLC (eluent 5% EtOH/$CHCl_3$) R_f 0.5; IR (KBr) ν 3320 (NH), 2900–3000 (CH), 1630 (CO), 1570–1520 (NHCO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06 (s, 3, CH_3S), 2.60 (t, 2, SCH_2), 3.36 (m, 2, SCH_2CH_2), 3.4–3.6 (m, 4, $NHCH_2CH_2Cl$), 5.23–5.9 (m, 2, NH). Anal. ($C_6H_{13}ClN_2OS$) C, H, N.

N'-(2-Chloroethyl)-N-[2-(methylthio)ethyl]-N-nitrosourea (2) and N'-(2-Chloroethyl)-N-[2-(methylthio)ethyl]-N'-nitrosourea (3) (Scheme I). The urea 1 (7.86 g, 40 mmol) is dissolved in a mixture of formic acid (70 mL) and water (30 mL). To the cooled solution (0 °C) is added sodium nitrite (27.6 g, 400 mmol) at such a rate that the temperature never increases more than 5 °C. The solution is then stirred for 1 h at 0 °C. The reaction mixture is poured into ice–water (150 mL) and extracted with $CHCl_3$ (5 × 50 mL). The organic layer is dried on sodium sulfate. Chloroform is distilled under reduced pressure and the residual oil isolated. The crude product (95.96% purity) can either be used without purification or purified by low-pressure liquid chromatography (eluent CH_2Cl_2); yield 8.12 g, 90%. This isomeric mixture checked by HPLC shows a 80/20 ratio between **2** and **3**.

N'-(2-Chloroethyl)-N-[2-(methylsulfinyl)ethyl]-N-nitrosourea (9) and N'-(2-Chloroethyl)-N-[2-(methylsulfinyl)ethyl]-N'-nitrosourea (10) (Scheme I). The isomeric mixture **2** and **3** (3.38 g, 15 mmol) is dissolved in 30 mL of acetone. Hydrogen peroxide (15 mL; H_2O, 110%) is added with stirring to the cooled solution of the above nitrosoureas. The colorless solution is stirred for 1 h at 0 °C and poured into ice water (70 mL). The reaction mixture is then quickly extracted with chloroform (4 × 50 mL). The organic layer is quickly dried over sodium sulfate and chloroform is distilled under vacuum. Residues are purified on a silica column. Elution performed with a gradient of ethanol in chloroform (0–2%) allowed the separation of **9** (1.81 g, 7.5 mmol), **9** and **10** (0.96 g, 4 mmol), and **10** (0.24 g, 1 mmol).

Compound **9**: mp 73 °C; TLC (eluent EtOH/$CHCl_3$, 5%) R_f 0.30; IR (KBr) ν 3320 (NH), 2900–3000 (CH), 1710 (CO), 1520 (NHCO), 1480 (NNO), 1030 (SO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3, CH_3S), 2.80 (t, 2, $SCH_2CH_2N(NO)$), 3.60–3.90 (m, 4, $NHCH_2CH_2Cl$), 4.20 (t, 2, $SCH_2CH_2N(NO)$), 7.60–8 (m, 1, $NHCH_2CH_2Cl$); chemical-ionization mass spectrum, 241–243; electron-impact mass spectrum, m/z 136 $(CH_3SOCH_2CH_2N{=}NOH)^{+\cdot}$, 105.107 $(CONCH_2CH_2Cl)^+$, 92 [136 – (NNO)]$^{+\cdot}$, 78 $(CH_3SOHCH_2)^{+\cdot}$, 64 $(CH_3SOH)^{+\cdot}$. Anal. ($C_6H_{12}ClN_3O_3S$) C, H, N.

Compound **10**: mp 107 °C; TLC (silica; eluent EtOH/$CHCl_3$, 5%) R_f 0.25; IR (KBr) ν 3320 (NH), 2900–3000 (CH), 1700 (CO), 1525 (NHCO), 1480 (NNO), 1045 (SO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3, CH_3S), 3.00 (t, 2, SCH_2CH_2NH), 3.40 (t, 2, $N(NO)CH_2CH_2Cl$), 3.86 (m, 2, SCH_2CH_2NH), 4.10 (t, 2, $N(NO)CH_2CH_2Cl$), 7.40–7.60 (m, 1, SCH_2CH_2NH); chemical-ionization mass spectrum, 241.243; electron-impact mass spectrum, m/z 134 $(CH_3SOCH_2NHCO)^+$, 91 $(CH_3SOCH_2CH_2)^+$, 63.65 $(CH_2CH_2Cl)^+$, 64 $(CH_3SOH)^{+\cdot}$. Anal. ($C_6H_{12}ClN_3O_3S$) C, H, N.

N'-(2-Chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N-nitrosourea (11) and N'-(2-Chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N'-nitrosourea (12) (Scheme I). A procedure nearly identical with that for making **9** and **10** is used for conversion of the mixture **2** and **3** into **11** and **12**. Formic acid has to be used as solvent and the temperature is kept controlled at from 0 to 40 °C for 10 min. Starting with **2** and **3** (3.38 g, 15 mmol), the residue can be separated by low-pressure liquid chromatography on the silica column. Elution performed with a gradient of ethanol in chloroform (0–1%) allowed the separation of **11** (1.93 g, 7.5 mmol), **11** and **12** (1.03 g, 4 mmol), and **12** (0.26 g, 1 mmol).

Compound **11**: mp 72.76 °C; TLC (eluent EtOH/$CHCl_3$, 5%) R_f 0.55; IR (KBr) ν 3300 (NH), 2900–3000 (CH), 1710 (CO), 1520 (NHCO), 1485 (NNO), 1130 (SO_2) cm^{-1}; ^1H NMR $CDCl_3$ δ 2.93 (s, 3, CH_3S), 3.10 (t, 2, SCH_2CH_2), 3.66–3.90 (m, 4, $NHCH_2CH_2Cl$), 4.20 (m, 2, $SCH_2CH_2N(NO)$), 7.20–7.33 (m, 1, $NHCH_2CH_2Cl$); chemical-ionization, mass spectrum, 257.259; electron-impact mass spectrum, m/z 152 $(CH_3SO_2CH_2CH_2N{=}NOH)^{+\cdot}$, 105.107 $(CONCH_2CH_2Cl)^+$, 108 [m/z 136 – (NNO)]$^{+\cdot}$, 94 $(CH_3SO_2HCH_2)^{+\cdot}$, 80 $(CH_3SO_2H)^{+\cdot}$. Anal. ($C_6H_{12}ClN_3O_4S$) C, H, N.

Compound **12**: mp 94.97 °C; TLC (eluent EtOH/$CHCl_3$, 5%) R_f 0.50; IR KBr ν 3350 (NH), 2910–3000 (CH), 1715 (CO), 1525 (NHCO), 1420 (NNO), 1125 (SO_2) cm^{-1}; ^1H NMR $CDCl_3$ δ 3.00 (s, CH_3S), 3.26–3.66 (m, 4, SCH_2CH_2NH and $N(NO)CH_2CH_2Cl$), 3.83–4.23 (m, 4, SCH_2CH_2NH and $N(NO)CH_2CH_2Cl$), 7.33–7.66 (m, 1, CH_2CH_2NH); chemical-ionization mass spectrum, 257.259; electron-impact mass spectrum, m/z 150 $(CH_3SO_2CH_2CH_2NHCO)^+$, 107 $(CH_3SO_2CH_2CH_2)^+$, 80 $(CH_3SO_2H)^{+\cdot}$, 63.65 $(CH_2CH_2Cl)^+$. Anal. ($C_6H_{12}ClN_3O_4S$) C, H, N.

4-Nitrophenyl [2-(Methylthio)ethyl]carbamate (4) (Scheme II). 4-Nitrophenyl chloroformate (20.1 g, 100 mmol) is reacted for 12 h with 2-(methylthio)ethylamine (9.1 g, 100 mmol) in pyridine (100 mL) at 25 °C. The reaction mixture is poured into ice water (300 mL), and the resulting precipitate is filtered off, washed with cold water, and dried in the desiccator over phosphorus pentoxide: yield 15.36 g (60%); ^1H NMR ($CDCl_3$) δ 2.10 (s, 3, SCH_3), 2.66 (t, 2, CH_2CH_2S), 3.23–3.66 (m, 2, $NHCH_2CH_2$), 5.40–5.83 (m, 1, NH), 7.06–8.33 (m, 4, C_6H_4). Anal. ($C_{10}H_{12}N_2O_4S$) C, H, N.

4-Nitrophenyl [2-(Methylthio)ethyl]nitrosocarbamate (5) (Scheme II). Compound 4 (10.24 g, 50 mmol) dissolved in pyridine (50 mL) is treated with 6 mL of nitrosyl chloride at –20 °C for 4 h. The reaction mixture is poured into ice water (150 mL) and extracted by chloroform (3 × 50 mL). The organic layer is washed with a cold solution of hydrochloric acid (3 N). Chloroform is quickly dried and evaporated under reduced pressure. The oily residue (yield 11.4 g, 80%) can be used without further purification for the following step. **5**: ^1H NMR ($CDCl_3$) δ 2.10 (s, 3, SCH_3), 3.56 (t, 2, $CH_2CH_2SCH_3$), 4.0 (t, 2, $CH_3SCH_2CH_2N(NO)$), 7.33–8.40 (m, 4, C_6H_4). Anal. ($C_{10}H_{11}N_3O_5S$) C, H. N.

N'-(2-Chloroethyl)-N-[2-(methylthio)ethyl]-N-nitrosourea (2) (Scheme II). To a cooled (0.5 °C) solution of **5** (5.7 g, 20 mmol in 25 mL of dimethylformamide is added dropwise a solution of 2-chloroethylamine hydrochloride (2.3 g, 20 mmol) and triethylamine (2.02 g, 20 mmol) in 25 mL of dimethylformamide. After 1 h of stirring at room temperature, dimethylformamide is distilled under reduced pressure (5 × 10^{-2} torr) and the residue is purified by low-pressure liquid chromatography on a silica column. Elution is performed with dichloromethane. The product is an oil: yield 3.16 g (70%); TLC (eluent $CHCl_3$) R_f 0.40; IR KBr ν 3350 (NH), 2900–3000 (CH), 1710 (CO), 1520 (NHCO), 1480 (NNO) cm^{-1}; ^1H NMR $CDCl_3$ δ 2.10 (s, 3, CH_3), 2.46 (t, 2, $CH_2CH_2N(NO)$), 3.66–3.83 (m, 4, $NHCH_2CH_2Cl$), 4.00 (t, 2, $SCH_2CH_2N(NO)$), 7–7.60 (m, 1, $NHCH_2Cl$). Anal. ($C_6H_{12}ClN_3O_2S$) C, H, N.

N'-(2-Chloroethyl)-N-[2-(methylsulfinyl)ethyl]-N-nitrosourea (9) and N'-(2-Chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N-nitrosourea (11) (Scheme II). A procedure identical with that for making **9** + **10** and **11** + **12** was used. With use of **2** (2.25 g, 10 mmol) in acetone as the starting material, **9** (1.56 g, 65%) was obtained with some amount of **11** (0.13 g, 5%), while in formic aciid 1.67 g (68%) of **11** was obtained with some amount of **9** (0.15 g, 6%). ^1H NMR and mass spectra are identical with those obtained in Scheme I.

4-Nitrophenyl (2-Chloroethyl)nitrosocarbamate (8) (Scheme III). This compound was prepared in quantitative yield by a well-known procedure.[14]

N'-(2-Chloroethyl)-N-[2-(methylthio)ethyl]-N'-nitrosourea (3) (Scheme III). To a cooled solution of **8** (5.47 g, 20 mmol) in tetrahydrofuran (30 mL) is added dropwise 1.82 g (20 mM) of 2-(methylthio)ethylamine with stirring. After 1 h of stirring at 25 °C, the solvent is evaporated under reduced pressure. The residue is purified by low-pressure liquid chromatography on a silica column. Elution is performed by dichloromethane. The product is an oil: yield 4.06 g (90%); TLC (eluent $CHCl_3$) R_f 0.40; IR (KBr) ν 3350 (NH), 2900–3000 (CH), 1715 (CO), 1520 (NHCO), 1480 (NNO); ^1H NMR ($CDCl_3$) δ 2.13 (s, 3, CH_3S), 2.75 (t, 2, CH_2CH_2NH), 3.43 (m, 2, $N(NO)CH_2CH_2Cl$), 3.70 (m, 2, SCH_2CH_2NH), 4.10 (t, 2, $N(NO)CH_2CH_2Cl$), 7–7.50 (m, 1, SCH_2CH_2NH). Anal. ($C_6H_{12}ClN_3O_2S$) C, H, N.

N'-(2-Chloroethyl)-N-[2-(methylsulfinyl)ethyl]-N'-nitrosourea (10) and N'-(2-Chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N'-nitrosourea (12) (Scheme III). A procedure identical with that for making **9** + **10** and **11** + **12** was followed. With use of **3** (2.25 g, 10 mmol) in acetone as the starting material,

1.69 g (70%) of **10** was obtained with **12** (0.13 g, 5%) as a by-product, while in formic acid 1.80 g (70%) of **12** with some amount of **10** (0.05 g, ~2%) was isolated. ^{1}H NMR and mass spectra are identical with those obtained in Scheme I.

Antitumor Evaluation. F_1 hybrid ($DBA_2 \times C$ 57 BL 6) mice (23–25 g) were ip inoculated with 10^5 L_{1210} cells on day 0. The compounds **9**/**12** as aqueous solution and CNCC as suspension in olive oil were intraperitoneally or intravenously administered on days 1, 5, and 9. The average median survival times were measured for each group. The control animals survived 10.0 days on average. The long term survivors in treated groups (90.0 days) were recorded.

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In the Search for New Anticancer Drugs. 13. Phosphonic and Phosphinic Analogues of Ornithine

George Sosnovsky,*† Jan Lukszo,† Enrico Gravela,‡ and Maria Franca Zuretti‡

Department of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201, and Istituto di Patologia Generale, Universita di Torino, Torino 10125, Italy. *Received December 17, 1984*

Phosphonic (**4a,b**) and phosphinic (**5a–d**) analogues of ornithine were synthesized and evaluated for their inhibitory activity against ornithine decarboxylase and against the lymphocytic leukemia P388. The title compounds possess a low degree of inhibition against rat liver ornithine decarboxylase as compared to α-(difluoromethyl)ornithine. Thus, compounds **4a** and **5a** inhibit by 40% the ornithine decarboxylase activity at a 5 mM concentration. The other derivatives are less potent. Compounds **4a**, **4b**, **5b**, and **5d** are inactive against P388 tumor in CD_2F_1 mice at doses of 50 and 150 mg/kg.

In the past 20 years, the polyamines putrescine, spermidine, and spermine have been extensively investigated because of their involvement in cell growth.[1-4] The elevated levels of polyamines in cancer patients have been associated with increased cell proliferations in rapidly growing tissues.[5] Elevated levels of ornithine decarboxylase and/or of putrescine, spermidine, and spermine have been correlated with the rate of proliferation of cancer cells.[6] On the basis of these results it was proposed to use the polyamines as sensitive cancer markers[7] that could be conveniently monitored by specific assays. It can also be hypothesized that by administering suitable inhibitors of ornithine decarboxylase the proliferation of cancer cells may be retarded, and hence a successful chemotherapy might be developed. The most effective inhibitors of polyamine formation that have been synthesized to date belong to various structural analogues of ornithine (**1a**) and methylglyoxal hydrazones such as α-(difluoromethyl)ornithine (α-DFMO, **1c**),[8-10] α-methylornithine (**1b**),[9,11] 2-hydrazino-5-aminopentanoic acid,[12] DL-2-hydrazino-2-methyl-5-aminopentanoic acid,[13] *N*-(5-phosphopyridoxyl)ornithine,[14] DL-(*E*)-2,5-diamino-3-pentenoic acid,[15] and methylglyoxal bis(guanylhydrazone), MGBG (**2**).[16,17] The most widely investigated of these inhibitors are α-DFMO and MGBG. Specifically, the administration of either α-DFMO[8,9] or MGBG[16,17] to rapidly proliferating cells retards their further growth. The data obtained with cell cultures indicated that α-DFMO exerts a cytostatic rather than a cytocidal effect.[18] In vivo experiments with α-DFMO using L1210 leukemia bearing mice produced moderate prolongation of their survival time.[19-21] α-DFMO has also shown activity against solid tumors such as murine mammary sarcoma,[20,22] glioma,[23] rat hepatoma,[20] and gliosarcoma.[23] Clinical trials with α-DFMO[24] and MGBG[25] apparently have not fulfilled the high expectation. Therefore, the search for new inhibitors is continuing, and in the present investigation a somewhat

† University of Wisconsin—Milwaukee.
‡ Universita di Torino.

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