pressure. The residue was treated with 10% aqueous $NaHCO_3$ and then extracted with ether. The organic layer was washed with 10% HCl and the aqueous layer was then made basic with 10% NaOH. The oil that separated was again extracted with ether. The organic layer was washed with water and dried ($Na_2SO_4$). Upon removal of the solvent a white solid was obtained, which was recrystallized from ether to yield 600 mg of III.

**Procedure B. 4-[Bis(2-hydroxyethyl)amino]-3-methoxy-1,3-5-estratrien-17β-ol (V).**—A solution of 4-[bis(2-hydroxyethyl)-amino]-3-methoxy-1,3,5-estratrien-17-one (III) (1.4 g) in methanol (50 ml) was treated with excess $NaBH_4$ (2.0 g), stirred for 1 hr at room temperature, and poured into aqueous $NaHCO_3$. The solution was extracted with ether and the organic layer was washed and dried ($Na_2SO_4$). The solvent was removed and the solid residue was recrystallized from methanol to yield 700 mg of V.

**Procedure C. 4-[Bis(2-chloroethyl)amino]-3-methoxy-1,3,5-estratrien-17-one (IV).**—A mixture of III (500 mg) and $POCl_3$ (10 ml) was heated on a steam bath for 2 hr. The excess $POCl_3$ was removed under reduced pressure and the remaining oil was dissolved in ether–benzene. The solution was washed with dilute HCl and then water. The organic layer was dried ($Na_2SO_4$), and the solvent was removed. The residue was dissolved in benzene (5 ml) and absorbed on a column of silica gel G (50 g). After removal of an oily side product by eluting with benzene, IV was eluted with ether–benzene (1:1) as an oil (300 mg), which would not crystallize. This reaction failed with $SOCl_2$.

Treatment of the triol (IX) with $POCl_3$ gave a compound that could not be identified. Acid shifted the ultraviolet absorption from 290 to 282–288 mμ. No hydroxyl absorption appeared in the infrared spectrum. The nmr spectrum showed the aromatic protons as singlets τ 2.3 and 3.17. The 3-methoxy and 13-methyl protons appeared where they did in the starting material. No other protons could be identified. *Anal.* Found: C, 43.23; H, 6.66; N, 2.28; Cl, 17.60; P, 7.6. It is conceivable that this material could be a dimer or polymer.

**Acknowledgments.**—We are indebted to H. Cheng for his help in the preparation of the starting materials and to the Cancer Chemotherapy National Service Center, National Institutes of Health, U. S. Public Health Service, Bethesda, Md., for the biological data.

## Some Alkylating Derivatives of Nicotinic Acid. Synthesis and Antineoplastic Activities

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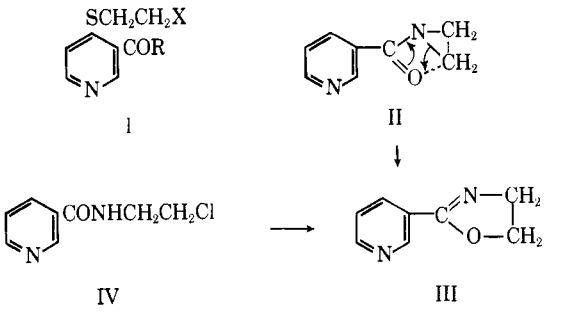
4-Substituted nicotinamides might act as stereospecific inhibitors of the glycolytic process by which many cancer cells derive an appreciable proportion of their energy requirements.[1,2] More effective and potentially irreversible antagonists may be produced by incorporating a chemically reactive grouping into the 4 substituent. The preparation of such derivatives containing alkylating groups is now described.

The preparation of 4-(2-bromoethylthio)nicotinic acid (I, R = OH; X = Br), isolated as its hydrobromide, has already been described.[2] The corresponding amide (I, R = $NH_2$; X = Br) has now been prepared by the action of ammonia on the mixed anhydride formed from the acid (I, R = OH; X = Br) and isobutyl chloroformate.

The acid (I, R = OH; X = Br) was readily obtained by the action of concentrated hydrobromic acid on the hydroxy acid (I, R = X = OH) but the hydroxy acid was recovered unchanged after prolonged heating with concentrated HCl. When heated with thionyl chloride the hydroxy acid gave an unstable product, presumably the hydrochloride of the acid chloride (I, R = X = Cl), which afforded 4-(2-chloroethylthio)nicotinic acid (I, R = OH; X = Cl), methyl 4-(2-chloroethylthio)nicotinate (I, R = OMe; X = Cl), and 4-(2-chloroethylthio)nicotinamide (I, R = $NH_2$; X = Cl) on treatment with HCl, methanol, and methanolic ammonia, respectively.




Many difunctional alkylating agents have greater carcinostatic activity than the corresponding monofunctional analogs.[3] The acid (I, R = H; X = Cl) could be converted into a novel type of difunctional alkylating agent, having two different alkylating groups, by the preparation of its ethylenimide (I, R = N=$(CH_2)_2$; X = Cl).

As a model for this synthesis the preparation of N-nicotinoylethylenimine (II) was examined. The action of nicotinoyl chloride on ethylenimine has been reported to give II[4] but the product has not been adequately characterized. When this preparation was repeated, only 2-(3-pyridyl)-2-oxazoline[4] (III), formed by internal alkylation of the initial product, was obtained. Ethylenimides can dimerize to give piperazines but the preparation of N,N′-dinicotinoylpiperazine by an unambiguous synthesis showed that this had not occurred.

Nicotinoylethylenimine (II) was prepared by condensing nicotinic acid with ethylenimine in the presence of dicyclohexylcarbodiimide, and N-4-(2-chloroethylthio)nicotinoylethylenimine (I, R = N=$(CH_2)_2$; X = Cl) was similarly obtained from the acid (I, R = OH; X = Cl).

**Biological Data.**—The results of screening tests against the Walker 256 tumor[5] and the lymphoid leukemia L1210[6] are given in Tables I and II. Only moderate activity (*ca.* 50% inhibition) at tolerated doses against the Walker tumor was shown by the hydrobromide of the acid (I, R = OH; X = Br) and nicotinoylethylenimine (II). Significant activity against the L1210 leukemia was shown only by the chloro acid (I, R = OH; X = Cl) at the maximum tolerated dose.

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(5) The protocol for this carcinostatic assay is given by T. A. Connors, B. C. V. Mitchley, V. M. Rosenaner, and W. C. J. Ross, *Biochem. Pharm.*, **13**, 395 (1964).
(6) The protocol for this assay is esentially that given in *Cancer Chemotherapy Rept.*, **1**, 42 (1959); the $C_{57}$/DBA2 hybrid strain of mouse was used as host.

TABLE I

SCREENING AGAINST WALKER 256 (SUBCUTANEOUS) TUMOR

| Compd 1 R | X | Vehicle | Dose, mg/kg ip | No. of daily doses[a] | Survivors | C/T ratio[b] |
|---|---|---|---|---|---|---|
| OH | Cl | Na salt in water | 100 | 7 | 3/3 | 1.2 |
| | | | 250 | 7 | 1/3 | *d* |
| OMe | Cl | Arachis oil | 100 | 7 | 3/3 | 1.1 |
| | | | 250 | 7 | 2/3 | 1.1 |
| $NH_2$ | Cl | ... | 100 | 6 | 3/3 | 1 |
| | | | 250 | 6 | 1/3 | 2 |
| OH | Br[c] | Dimethyl sulfoxide | 1.5 | 7 | 3/3 | 2 |
| | | | 3.0 | 7 | 3/3 | 1.3 |
| | | | 6.0 | 7 | 3/3 | 1.8 |
| $NH_2$ | Br | Arachis oil | 50 | 6 | 3/3 | 1.2 |
| | | | 125 | 6 | 2/3 | 1 |
| $NH_2$ | Br[c] | ... | 50 | 6 | 3/3 | 1.1 |
| | | | 125 | 6 | 1/3 | 1.4 |
| $N{-}(CH_2)_2$ | Cl | 10% acetone–arachis oil | 20 | 1 | 3/3 | 1.1 |
| | | | 50 | 1 | 3/3 | 1.3 |
| Nicotinoylethyleneimine | | 10% ethanol–arachis oil | 20 | 1 | 3/3 | 1.5 |
| | | | 50 | 1 | 3/3 | 1.9 |
| | | | 125 | 1 | 0/3 | ... |

[a] Starting on the day after implantation. [b] Weight of tumors in controls/weight of tumors in treated rats. [c] As hydrobromide. [d] No tumor in the sole survivor.

TABLE II

SCREENING AGAINST LYMPHOID LEUKEMIA L1210

| Compd 1 R | X | Vehicle | Dose, mg/kg ip | No. of daily doses[a] | T/C ratio[b] | Approx $LD_{50}$ (mouse),[c] mg/kg |
|---|---|---|---|---|---|---|
| OH | Cl | Na salt in water | 12.5 | 10 | 98 | 50 |
| | | | 25 | 10 | 100 | |
| | | | 50 | 10 | 143 | |
| OMe | Cl | Arachis oil | 50 | 10 | 112 | 200 |
| | | | 100 | 9 | 67 | |
| | | | 200 | 6 | 58 | |
| $NH_2$ | Cl | ... | 35 | 10 | 95 | 140 |
| | | | 70 | 10 | 103 | |
| | | | 140 | 10 | 115 | |
| OH | Br[d] | ... | 3.5 | 9 | 85 | 14 |
| | | | 7 | 9 | 104 | |
| | | | 14 | 9 | 100 | |
| $NH_2$ | Br | ... | 35 | 5 | 105 | 140 |
| | | | 70 | 5 | 100 | |
| | | | 140 | 5 | 63 | |
| $NH_2$ | Br[d] | ... | 35 | 5 | 98 | 140 |
| | | | 71 | 5 | 98 | |
| | | | 140 | 5 | 50 | |
| $N{-}(CH_2)_2$ | Cl | 10% acetone–arachis oil | 10 | 1 | 96 | 40 |
| | | | 20 | 1 | 105 | |
| | | | 40 | 1 | 74 | |
| Nicotinoylethyleneimine | | 10% ethanol–arachis oil | 7 | 1 | 112 | 28 |
| | | | 14 | 1 | 81 | |
| | | | 28 | 1 | 90 | |

[a] Starting on the day after inoculation. [b] Average survival time of treated mice/average survival time of controls × 100. [c] The approximate $LD_{50}$'s cited were determined for noninoculated mice. [d] As hydrobromide.

## Experimental Section[7]

**4-(2-Chloroethylthio)nicotinic Acid.**—4-(2-Hydroxyethylthio)-nicotinic acid[2] (10 g) and $SOCl_2$ (40 ml) were heated under reflux for 2 hr. Addition of dry benzene (200 ml) and then petroleum ether (bp 40–60°, 200 ml) to the cooled solution caused the separation of fine needles (product A) which was collected by rapid filtration, washed with petroleum ether, and stored in a vacuum desiccator.

Product A was added in small portions to 37% HCl (50 ml, *d* 1.19) kept at 0° and the solution was evaporated to dryness under reduced pressure at 30–40°. Water (60 ml) was added and the solid, mp 171–173°, which proved to be the hydrochloride of 4-(2-chloroethylthio)nicotinic acid, was filtered off.

*Anal.* Calcd for $C_8H_8ClNO_2S \cdot HCl$: C, 37.8; H, 3.6; Cl, 27.9; N, 5.5; S, 12.6. Found: C, 37.7; H, 3.5; Cl, 27.3; N, 5.0; S, 12.2.

When an aqueous solution of this hydrochloride was adjusted to pH 4 by the addition of aqueous NaOH, the free acid was precipitated, yield 8 g. 4-(2-Chloroethylthio)nicotinic acid formed flattened needles, mp 178–180° dec, from glycol dimethyl ether. This material is a monohydrate: titration equiv, 235; calcd, 235.7. The analytical specimen was dried at 100° (20 mm) for 5 hr.

*Anal.* Calcd for $C_8H_8ClNO_2S$: C, 44.1; H, 3.7; Cl, 16.3; N, 6.4; S, 14.7. Found: C, 44.4; H, 4.0; Cl, 16.3; N, 6.5; S, 14.5.

**Methyl 4-(2-Chloroethylthio)nicotinate.**—Product A (from 10 g of the nicotinic acid) was added portionwise to methanol (200 ml) kept at 0° and then the solvent was removed under reduced pressure. The residue was suspended in water (50 ml) and excess anhydrous $Na_2CO_3$ was added. The mass was extracted with four 200-ml portions of ether and the dried ($Na_2SO_4$) extract was concentrated to a small volume; addition of petroleum ether (bp 40–60°) caused the separation of the methyl ester, mp 67–69°, as long needles, yield 9.6 g.

*Anal.* Calcd for $C_9H_{10}ClNO_2S$: C, 46.7; H, 4.4; Cl, 15.3; N, 6.0; S, 13.8. Found: C, 46.4; H, 4.7; Cl, 15.2; N, 6.1; S, 13.8.

**4-(2-Chloroethylthio)nicotinamide.**—Product A (from 10 g of the nicotinic acid) was added in small portions to rapidly stirred saturated methanolic $NH_3$ (100 ml) kept at −30°. The excess solvent was removed under reduced pressure at 30° and the residue was extracted with hot benzene (300 ml). On cooling, the benzene extract yielded 6 g of product, mp 101–104°, as long flattened needles.

*Anal.* Calcd for $C_8H_9ClN_2OS$: C, 44.3; H, 4.2; Cl, 16.3; N, 12.9; S, 14.8. Found: C, 44.1; H, 4.1; Cl, 16.5; N, 13.2; S, 14.8.

The benzene mother liquors contained the methyl ester which could be separated from the amide by passing the solution through a column of activated alumina. Benzene eluted the ester, mp 67°, 3.5 g, and subsequent elution with 10% methanol in chloroform removed a small quantity of the amide.

**4-(2-Bromoethylthio)nicotinamide.**—4-(2-Bromoethylthio)-3-carboxypyridinium bromide[2] (10 g) was suspended in ice-cold water (50 ml) and sufficient 1 *N* NaOH to give a clear solution was added. Addition of an excess of glacial acetic acid then gave a precipitate which was rapidly filtered off and washed successively with water, ether, and acetone. After drying ($CaCl_2$, vacuum desiccator) for 2 days the acid (6 g) was dissolved in tetrahydrofuran (THF, 150 ml) containing triethylamine (2.31 g). Isobutyl chloroformate (3.12 ml) was added slowly to the cooled (0°), stirred solution during 0.75 hr. After keeping at 0° for 1 hr dry $NH_3$ was passed in for 3 hr keeping the temperature below 5°. The precipitate was removed by filtration through Hyflo filter aid and washed with THF (100 ml). The combined filtrates were evaporated under reduced pressure at 30°. On covering the residual oil with benzene, a solid (3.5 g) was obtained and crystallized by solution in dry $CHCl_3$ followed by gradual addition of benzene: prismatic needles, mp 101–103°.

*Anal.* Calcd for $C_8H_9BrN_2OS$: C, 36.8; H, 3.5; Br, 30.6; N, 10.7; S, 12.3. Found: C, 36.9; H, 3.6; Br, 30.6; N, 10.4; S, 12.1.

Addition of concentrated HBr (1 ml) in methanol (10 ml) to a solution of the amide (1 g) in methanol (10 ml) caused the separation of the hydrobromide as diamond shaped lozenges, mp 238–239°.

*Anal.* Calcd for $C_8H_9BrN_2OS \cdot HBr$: C, 28.1; H, 2.9; Br, 46.7; N, 8.2; S, 9.4. Found: C, 28.6; H, 3.1; Br, 47.2; N, 8.3; S, 9.8.

**Attempted Preparation of N-Nicotinoylethylenimine.**—Ethylenimine (5.1 ml) was added to a stirred solution of nicotinoyl chloride (14 g) and triethylamine (27.6 ml) in dry ether (100 ml) keeping the temperature below 10°. The filtered solution was evaporated and a benzene solution of the residue was passed through a column of activated alumina. Elution of the column ($CHCl_3$) gave a solid as plates, mp 66–68°, from petroleum ether (bp 30–40°). It formed a picrate, mp 140–142°.

*Anal.* Calcd for $C_8H_8N_2O$: C, 64.9; H, 5.4; N, 18.9. Found: C, 64.7; H, 5.3; N, 18.9.

The product, which failed to react with thiosulfate, was identical (infrared spectrum, melting point and mixture melting point of base and picrate) with the 2-(3-pyridyl)-2-oxazoline obtained

(7) Melting points were determined with a Townson and Mercer heated metal block apparatus and are corrected. The activated alumina used was Spence Type H.

by Braz and Skorodumov[4] by the action of alkali on chloroethylnicotinamide (IV).

**N-Nicotinoylethylenimine.**—Dicyclohexylcarbodiimide (20.6 g) in THF (100 ml) was added to a solution of nicotinic acid (12.3 g) and ethylenimine (5.1 ml) in THF (200 ml). After keeping for 1.5 hr at 25° the solution was filtered and evaporated under reduced pressure at 25°. The residue was extracted with petroleum ether (bp 30–40°, 1 l.). On chilling the extract in a Dry Ice–acetone bath, plates of mp 36° separated; yield 10 g.

*Anal.* Calcd for $C_8H_8N_2O$: C, 64.9; H, 5.4; N, 18.9. Found: C, 64.9; H, 5.5; N, 18.8.

The compound could be stored for several days at 0° without decomposition but at room temperature in an evacuated vessel it liquefied within a few hours; this behavior probably accounts for the previously reported "hygroscopic" nature of the product. A solution of the compound (156.4 mg) in 50% aqueous acetone (50 ml) containing sodium thiosulfate (1 g) rapidly developed alkalinity (phenolphthalein indicator) which was continuously titrated with 0.1 *N* HCl.[8] The final titer was 10.2 ml, indicating that the material contained 97% of nicotinoylethylenimine.

**N-4-(2-Chloroethylthio)nicotinoylethylenimine.**—4-(2-Chloroethylthio)nicotinic acid was similarly converted into its ethylenimide which formed plates, mp 66–68°, from benzene–petroleum ether (bp 40–60°); yield 40%. The product was unstable at room temperature and was stored at −30°.

*Anal.* Calcd for $C_{10}H_{11}ClN_2OS$: C, 49.5; H, 4.6; Cl, 14.6; N, 11.5; aziridine N, 5.77. Found: C, 49.2; H, 4.3; Cl, 14.6; N, 11.3; aziridine N (thiosulfate titration), 5.6.

**N,N′-Dinicotinoylpiperazine.**—Isobutyl chloroformate (2.6 g) in THF (10 ml) was added to a stirred, cooled (0°) solution of nicotinic acid (2.46 g) and triethylamine (2.8 g) in THF (40 ml). After allowing to stand for 30 min at 0°, a solution of piperazine (0.86 g) in THF (20 ml) was added and the mixture was allowed to warm up to room temperature during 1.5 hr. The filtered solution was washed successively with aqueous 2 *N* $Na_2CO_3$, aqueous 2 *N* HCl and water, and then dried ($Na_2SO_4$). The product obtained on evaporation formed prisms, mp 201°, from ethanol–ether (1:2).

*Anal.* Calcd for $C_{16}H_{16}N_4O_2$: C, 64.9; H, 5.4; N, 18.9. Found: C, 64.4; H, 5.6; N, 18.9.

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## Alkylating Activity of 1,3-Bis(2-chloroethyl)-1-nitrosourea and Related Compounds[1]

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1,3-Bis(2-chloroethyl)-1-nitrosourea[2] (I) is an active compound against lymphocytic choriomeningitis,[3] L-1210 leukemia and other experimental neoplasms,[2,4] and a number of neoplasms in humans.[5] Because a hamster plasmacytoma that was resistant to cyclophosphamide was also resistant to this compound, it was suggested that it might function as an alkylating agent.[4a] Cross resistance with alkylating agents was also observed with microorganisms,[6] and some of the biochemical effects of the agent are similar to those of several alkylating agents.[4c] It is known that treatment of N-nitroso-N-methylurethan,[7] 1-methyl-1-nitrosourea,[8] and 1-alkyl-1-nitroso-3-nitroguanidines[9] with alkali yields diazoalkanes, and it has been suggested that the biological effects of these agents might be due to such diazoalkanes,[7,10] which might function as alkylating agents. The results presented below indi-

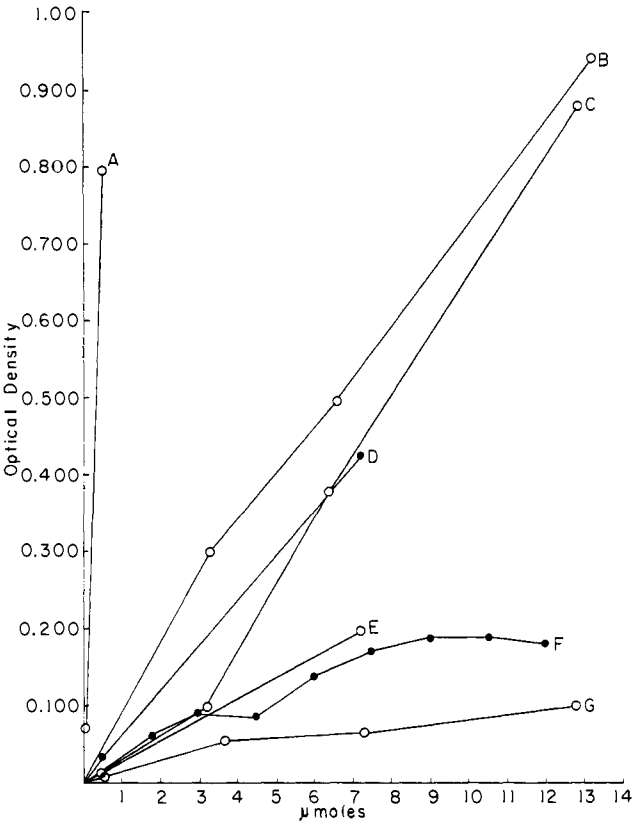


Figure 1. Measurement of the alkylating activities of several compounds. With the exception of the tests with diazomethane, the reaction mixtures were heated in a boiling-water bath for 20 min. The reaction with diazomethane occurred at room temperature in the course of approximately 3 min. The numbers on the abscissa indicate the total micromoles of compound present in the reaction mixture. A, 1,3-bis(2-chloroethyl)-1-nitrosourea; B, 1-(2-chloroethyl)-1-nitrosourea; C, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; D, 1,3-bis(2-fluoroethyl)-1-nitrosourea; E, 2-fluoroethylamine hydrochloride; F, diazomethane; G, 1-methyl-1-nitrosourea.

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