compounds have exhibited significant CNS activity in the course of preliminary screening.

### Experimental[8]

**4-Dimethylamino-3-hydroxybutyric Acid (II).**—4-Amino-3-hydroxybutyric acid[9] (3 g.), water (150 ml.), 37% formaldehyde (4.08 g.) and 10% palladium on carbon catalyst (4 g.) were placed in a pressure bottle and hydrogenated on a Parr apparatus, using an initial pressure of 2.15 kg./cm.$^2$. Uptake of hydrogen was complete within 1 hr. and the shaking was continued for an additional 0.5 hr. with no additional change in pressure. The mixture was filtered free of catalyst (Celite) and the filter cake was washed with hot water. The combined filtrate and wash was evaporated on a steam bath *in vacuo* to give an oil which crystallized as white, flat needles when treated with ethyl acetate (3 g., 80%); m.p. 145–147°. For analysis a portion of this material was recrystallized 3 times by dissolving in a minimum of methanol, adding ethyl acetate and removing the methanol as an azeotrope. The melting point was unaffected by this treatment.

*Anal.* Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52; $NCH_3$, 20.43. Found: C, 48.67; H, 8.84; N, 9.43; $NCH_3$, 18.4.

**Ethyl 4-Dimethylamino-3-hydroxybutyrate (III).**—Anhydrous hydrogen chloride was bubbled for 2 hr. into a suspension of 4-dimethylamino-3-hydroxybutyric acid (558.5 g., 3.80 moles) in absolute ethanol (5.7 l.) containing ethyl orthoformate (283.8 g., 1.95 moles) while protecting the reaction mixture with a drying tube. The suspended material dissolved during this time yielding a clear yellow solution which was heated under reflux for 2 hr. and then concentrated to a small volume *in vacuo*. The syrupy residue was diluted with ice water and made basic with sodium carbonate. The basic solution was extracted 5 times with chloroform. The combined chloroform extracts were washed with a small volume of water and dried over anhydrous sodium sulfate. The dried chloroform solution was concentrated and the residue was distilled to yield the ethyl ester (532 g., 80%); b.p. 90–93° (4–5 mm.). The substance behaved as a pure compound when subjected to paper chromatography and electrophoresis.

*Anal.* Calcd. for $C_8H_{17}NO_3$: C, 54.83; H, 9.78; N, 7.99; eq. wt., 175.22. Found: C, 54.72; H, 9.88; N, 8.23; eq. wt., 179.7.

**4-Dimethylaminobutane-1,3-diol (IV).**—Ethyl 4-dimethylamino-3-hydroxybutyrate (12 g., 0.07 mole) was added slowly with stirring to lithium aluminum hydride (3.5 g.) in tetrahydrofuran (75 ml.). At the conclusion of the addition the mixture was heated to reflux and stirred for 45 min. It then was allowed to stand overnight at room temperature and decomposed by the cautious addition of water (6.5 ml.). The mixture was filtered free of alumina (Celite) and the filter cake was washed with several portions of tetrahydrofuran. The combined filtrate and wash was concentrated to remove solvent and the residue was distilled *in vacuo*. The alcohol was obtained as a main fraction, b.p. 103–104° (6 mm.) (85%), with very little forerun, ($n^{24}$D 1.4574). The infrared spectrum showed the absence of carbonyl absorption.

*Anal.* Calcd. for $C_6H_{15}NO_2$: C, 54.10; H, 11.35; N, 10.52. Found: C, 54.23; H, 11.34; N, 10.65.

**4-Dimethylamino-3-hydroxybutyramide (V).**—Ethyl 4-dimethylamino-3-hydroxybutyrate (48 g.) and liquid ammonia (450 ml.) were placed in a stainless steel bomb and autoclaved overnight at 150°. The bomb was cooled to room temperature and the excess ammonia was allowed to distil off leaving an oily residue which was taken up in chloroform and applied to a column of Merck acid washed alumina (15 × 4.35 cm.). The product was eluted with the solvent front and yielded crystals (25 g.) from a mixture of chloroform and Skellysolve C, m.p. 81–83°. A small portion of this material was recrystallized 3 times from the same solvent mixture to give crystals m.p. 85–86°.

*Anal.* Calcd. for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.17. Found: C, 49.21; H, 9.52; N, 18.99.

(8) Microanalyses by H. V. Tashinian, Microchemical Specialties Co., Berkeley, California. Melting points are uncorrected.

(9) H. E. Carter and P. K. Bhattacharya, *J. Am. Chem. Soc.*, **75**, 2503 (1953); M. Bergmann, E. Brand and F. Weinmann, *Z. physiol. Chem.*, **131**, 1 (1923).

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## Synthesis of Isothiocyanates as Potential Antineoplastic Agents[1,2]

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The hypothesis that known alkylating agents react *in vivo* with the nucleophilic centers encountered in the biological system (amino groups, sulfhydryl groups, organic and inorganic anions)[4] has encouraged chemists to synthesize a large number of compounds which have a high reactivity toward the above mentioned nucleophilic centers as antineoplastic agents. Hendry and colleagues,[5] and later Ross[4] studied some acid chlorides, anhydrides, isocyanates and isothiocyanates (compounds reactive toward amino and alcoholic groups) as antineoplastic agents. Most of these agents were simple organic compounds, *e.g.*, 1,5-diisothiocyanatonaphthalene and 2,4,6-triisothiocyanatotoluene. Later, Bergel and Stock[6] attempted to replace the dichloroethylamino grouping in DL-phenylalanine nitrogen mustard by an isothiocyanate group, in the search of a more selective tumor inhibiting compound. These workers were unable to demonstrate any antitumor effects with such compounds. However, it will be noted from the structures that solubilizing groups are absent. The lack of biological activity might be attributed to a lack of their solubility in the system.

Interest in diisothiocyanates as possible cross-linking agents arose in this Laboratory during the development of fluorescent isothiocyanates as diagnostic agents.[7] It was proposed that solubilizing or conductophoric groupings, *i.e.*, basic side chains, be attached to such molecules. It was expected that basic side chains might transport these compounds throughout the blood stream and to desired sites of action, such as the liver, as has been noted with antimalarial agents. Two isothiocyanates and two diisothiocyanates were synthesized (II, V, VIII and X). It will be noted that X possesses two types of reactive groupings, the isothiocyanato and dichloroethylamino groups.

In this Laboratory, the synthesis of certain purine and pyridine nitrogen mustard compounds has been carried out already, and it was encouraging to note that a purine nitrogen mustard has shown promising results in leukemia.[8] It was proposed, therefore, to

(1) Abstracted from a portion of the Ph.D. Thesis of D. S. Bariana, The University of Kansas, 1960.

(2) Supported by Grant CY-3573, National Cancer Institute, U. S. Public Health Service.

(3) The University of Michigan, Ann Arbor, Mich.

(4) W. C. J. Ross, *Ann. N. Y. Acad. Sci.*, **68**, 669 (1958).

(5) J. A. Hendry, F. L. Ross, and A. L. Walpole, *Brit. J. Pharmacol.*, **6** 201 (1951).

(6) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954).

(7) J. L. Riggs, R. J. Seiwald, J. H. Burckhalter, C. M. Downs, and T. G. Metcalf, *Am. J. Pathol.*, **34**, 1081 (1958).

(8) J. H. Burckhalter and D. S. Bariana, *J. Med. Chem.*, in press.

synthesize analogous compounds by introducing diisothiocyanato groups into purine and benzimidazole (a purine antimetabolite) nuclei as in compounds XII and XIV.

2-Diethylaminoethyl *p*-isothiocyanatobenzoate (II) hydrochloride was prepared by the action of thiophosgene on procaine (I) hydrochloride in dilute acetone, according to the general procedure used in this Laboratory for the synthesis of fluorescein isothiocyanates.[7]

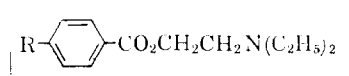
N,N-Diethyl-N'-(2,4-dinitrophenyl)-ethylenediamine (III) was obtained by the condensation of 2,4-dinitrochlorobenzene and N,N-diethylethylenediamine in alcoholic solution according to the general procedure of Hippchen.[9] Catalytic reduction of III gave a quantitative yield of diamine IV, which could not be isolated as the hydrochloride owing to its hygroscopic nature. Diamine IV on treatment with thiophosgene in chloroform solution, in accordance with the general procedure of Billeter and Steiner,[10] gave N-[2-(diethylamino)-ethyl]-2,4-diisothiocyanatoaniline (V) hydrochloride in 60% yield.

2-(Diethylamino)-ethyl 3,5-diisothiocyanatobenzoate (VIII) hydrochloride was synthesized by the condensation of 3,5-dinitrobenzoyl chloride and 2-diethylaminoethanol in benzene solution according to the general procedure of Einhorn.[11,12] Catalytic reduction of VI gave a quantitative yield of VII which on treatment with thiophosgene in chloroform solution gave the desired product VIII in 60% yield.

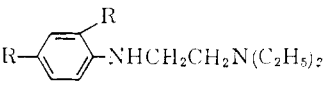
3-[Bis-(2-chloroethyl)-aminomethyl]-4-methoxyphenylisothiocyanate (X) hydrochloride was made in 60% yield by the action of thiophosgene on 2-[bis-(2-chloroethyl)-aminomethyl]-4-aminoanisole (IX) dihydrochloride.[8]

2,4-Dinitrobromobenzene was condensed with 6-purinethiol and 2-benzimidazolethiol in alcohol solution to give, respectively, thioethers XI and XIII, according to the general procedure of Bost and coworkers.[13] But owing to their lack of solubility in the commonly used solvents, such as the alcohols, dinitrophenyl thioethers XI and XIII could not be reduced satisfactorily to the corresponding diamines. Thus, XII and XIV were not synthesized.

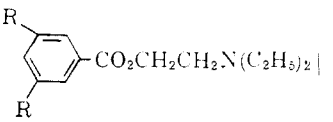
**Pharmacological Results.**—The summary of results (Table I) which are available to date was prepared from reports furnished by Dr. Joseph Leiter, Cancer Chemotherapy National Service Center, Bethesda, Maryland. Detailed information concerning test procedures may be found in publications from that office.[14]



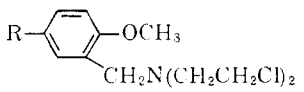
I, R = $NH_2$
II, R = SCN–



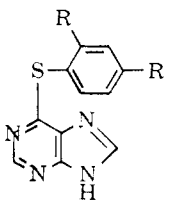
III, R = $NO_2$
IV, R = $NH_2$
V, R = SCN–



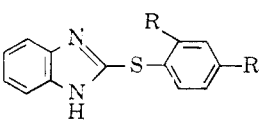
VI, R = $NO_2$
VII, R = $NH_2$
VIII, R = SCN–



IX, R = $NH_2$
X, R = SCN–



XI, R = $NO_2$
XII, R = SCN–



XIII, R = $NO_2$
XIV, R = SCN–

TABLE I

PHARMACOLOGICAL DATA

| Compound | NSC No. | Tumor[a] | Test status |
|---|---|---|---|
| II | 34051 | CA | Non-toxic, inactive[b] |
| | | SA | Non-toxic, inactive[b] |
| | | LE | Non-toxic, inactive[b] |
| V | 46241 | DL | Inactive[c] |
| VIII | 46239 | DL | Inactive[c] |
| X | 34050 | CA | Non-toxic, inactive[c] |
| | | SA | Non-toxic, inactive[b] |
| | | LE | Non-toxic, inactive[b] |

[a] CA, adenocarcinoma 755; DL, Dunning leukemia (solid); LE, lymphoid leukemia L-1210; SA, sarcoma 180. [b] Tested by Armour Research Foundation. [c] Tested by University of Miami (5DL).

## Experimental

**General Method of Preparation of Isothiocyanates and Diisothiocyanates.**—The appropriate amine or diamine (hydrochloride salts in the case of I and IX) was dissolved in a suitable solvent (dilute acetone, ethyl acetate or chloroform) and to it was added the calculated amount of thiophosgene[15] (1 mole for each amino group). The mixture was heated at reflux for 30–90 min. Excess thiophosgene and solvent were removed under reduced pressure. The residue was washed repeatedly with acetone and finally was recrystallized as the hydrochloride.

A summary of experimental details is given in Table II.

**6-(2,4-Dinitrophenyl)-thiopurine (XI).**—A solution of 2.47 g. (0.01 mole) of 2,4-dinitrobromobenzene in 10 ml. of alcohol was added to a solution which contained 1.52 g. (0.01 mole) of 6-purinethiol in 30 ml. of alcohol and 0.4 g. (0.01 mole) of sodium hydroxide in 4 ml. of water. The resulting solution was warmed gently on a water bath. It was filtered while hot, and the clear filtrate gave 2.5 g. (80% yield) of solid upon cooling. The product was recrystallized from a 3:1 mixture of alcohol and dimethylformamide as yellow needles, m.p. 244–246°.

(9) H. Hippchen, *Chem. Ber.*, **80**, 263 (1947).
(10) O. Billeter and A. Steiner, *Ber.*, **20**, 228 (1887).
(11) A. Einhorn and E. Uhlfelder, *Ann.*, **371**, 131 (1909).
(12) T. Canbäck, *Svensk Kem. Tidskr.*, **58**, 101 (1946) [*Chem. Abstr.*, **40**, 6060 (1946)].
(13) R. W. Bost, J. O. Turner, and R. D. Norton, *J. Am. Chem. Soc.*, **54**, 1985 (1932).
(14) *E.g.*, J. Leiter, A. R. Bourke, D. B. Fitzgerald, S. A. Schepartz, and I. Wodinsky, *Cancer Research* (Supplement), **22**, 363 (1962).
(15) Rapter Laboratories, Chicago, Ill.

TABLE II

| Compound | M.p., °C. | Yield, % | Formula | Calcd. C | Calcd. H | Found C | Found H |
|---|---|---|---|---|---|---|---|
| II[a] | 150[b] | 70 | $C_{14}H_{18}N_2O_2S\cdot HCl$ | 53.40 | 6.08 | 53.65 | 6.15 |
| V[c,d,e] | 241[f] | 60 | $C_{14}H_{18}N_4S_2\cdot 2HCl$ | 49.02 | 5.58 | 48.81 | 5.49 |
| VIII[d,g,h] | 113[b] | 60 | $C_{15}H_7N_3O_2S_2\cdot 2HCl$ | 48.44 | 4.87 | 48.50 | 5.04 |
| X[a,i] | 154[b] | 60 | $C_{13}H_{16}Cl_2N_2OS\cdot HCl$ | 43.88 | 4.81 | 43.97 | 4.84 |

[a] Reaction solvent, dilute acetone. [b] From dry acetone. [c] For III, see ref. 9. [d] Dinitro free amine reduced in low pressure hydrogenator in presence of catalytic amount of platinum oxide; diamine not converted to salt owing to hygroscopic nature. [e] Reaction solvent, ethyl acetate. [f] From acetone–dimethylformamide. [g] For VI, see ref. 11. [h] Reaction solvent, chloroform. [i] For IX dihydrochloride, see ref. 8.

*Anal.* Calcd. for $C_{11}H_6N_6O_4S$: C, 41.51; H, 1.90. Found: C, 41.72; H, 2.16.

**2-(2,4-Dinitrophenyl)-thiobenzimidazole (XIII).**—The procedure for XI, using 2-benzimidazolethiol, 2,4-dinitrobromobenzene and sodium hydroxide, gave a 70% yield of yellow product, m.p. 231–232°. It was recrystallized from alcohol.

*Anal.* Calcd. for $C_{13}H_8N_4O_4S$: C, 49.42; H, 2.73. Found: C, 49.41; H, 3.00.

## Nuclear Substituted 2-Amino-1-(2-pyridyl)propanes[1]

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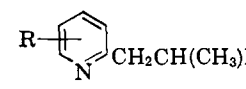
The postulate that histaminic activity may be expected if in a system, $-CH=N-C(=CH)-CH_2CH_2-NH_2$,[2] both the aliphatic and heterocyclic nitrogen atoms are members of a chelated ring,[3] has been made the basis of a working hypothesis for several attempts to produce hypotensive amines,[4,5] and to delineate the essential molecular fragment for histaminic properties.[2,3] Branching of the carbon chain $\alpha$ to the aliphatic amino group should render the resulting compounds more refractory to enzymatic deamination, and this prediction has indeed been realized in some cases.[6] However, the histaminic depressor properties of 2-amino-1-(2-pyridyl)-propane are lost when an ethyl group is introduced into position 5, or methyl into position 6 of the pyridine nucleus; these alkylpyridine derivatives exhibit, instead, marked analgetic activity.[6,7] In thiazolyl-2-aminopropane, two nuclear methyl groups intensified the analgetic effect,[8] while in methylpyrazolylethylamines the low depressor activity of the nuclear unsubstituted derivative was abolished by introduction of a C-methyl group.[5,6]

An explanation of these qualitative changes in activity is still lacking. The nuclear alkyl groups could perhaps change the distribution ratios of the resulting compounds, or somehow interfere with the stability of six-membered chelate rings. In the present study, the synthesis of two additional nuclear substituted 2-amino-1-(2-pyridyl)propanes is described. The 6-dimethylamino group in I should facilitate hydrogen bridging from the nuclear to the aliphatic nitrogen atoms.



I, R = 6-N(CH$_3$)$_2$
II, R = 3-CH$_3$

The synthesis of I and II started with the metallation of 6-dimethylamino-2-picoline, and 2,3-lutidine, respectively. Phenyllithium had been fairly satisfactory for the metallation of 2-picoline, but had given lower yields with 2,6-lutidine, and had failed with 4-picoline and 2,4-lutidine.[6] The much more stable methyllithium was therefore employed in the present cases. Treatment of the lithium derivatives with acetonitrile gave the corresponding lithium picolyl ketimines, and attempts were made to reduce these adducts *in situ* to the amines I and II. This direct route was unsuccessful, and the adducts were therefore hydrolyzed to 6-dimethylamino- and 3-methyl-2-pyridylacetone, respectively. Reduction of the oximes of these ketones furnished the amines, I and II, in good yields. The infrared spectrum of 2-amino-1-(3-methyl-2-pyridyl)-propane (II) contained an N–H stretching band at 3250 cm.$^{-1}$, slightly lower than that expected for free $NH_2$, and perhaps indicative of intramolecular hydrogen bonding.

In early experiments designed to synthesize (3-methyl-4-pyridyl)acetone, 3,4-lutidine was treated with phenyllithium, and after the color of the mixture had lightened, acetonitrile was added as above. However, the oily reaction product contained no carbonyl group. Fractionation furnished two isomers, $C_{13}H_{13}N$, which boiled at 113–115° (2.2 mm.) (III) and at 124–129° (2.2 mm.) (IV), respectively, and gave different picrates. This suggested that 3,4-lutidine had been phenylated, and that III and IV represent two of the three structures, 4-benzyl-3-methyl-, 3,4-dimethyl-2-phenyl-, or 3,4-dimethyl-6-phenylpyridine. Since the melting point of the picrate of 6-phenyl-3,4-lutidine[9] differs from those of the picrates of III and IV, the first two structures must be assigned to our compounds.

A C-methyl determination for III indicated the presence of only one methyl group while the values obtained for IV were inconclusive. This discrepancy should be weighed against the experience that the analysis for aromatically bound methyl groups often yields only a fraction of the expected values.[10] Oxidation of both III and IV produced only benzoic acid, and no pyridinecarboxylic acids could be found. If III is 4-benzyl-3-methylpyridine, oxidation to benzoic acid

(1) This research has been supported by a Grant, B-1445, from the Institute of Neurological Diseases and Blindness, National Institutes of Health, U. S. Public Health Service.

(2) L. A. Walter, W. H. Hunt and R. J. Fosbinder, *J. Am. Chem. Soc.*, **63**, 2771 (1941).

(3) C. Niemann and J. T. Hays, *ibid.*, **64**, 2288 (1942).

(4) R. G. Jones, M. J. Mann, and K. C. McLaughlin, *J. Org. Chem.*, **19**, 1428 (1954).

(5) H. M. Lee and R. G. Jones, *J. Pharmacol. Exptl. Therap.*, **95**, 71 (1949).

(6) A. Burger and G. E. Ullyot, *J. Org. Chem.*, **12**, 342 (1947).

(7) E. J. Fellows and G. E. Ullyot in "Medicinal Chemistry," C. M. Suter, ed., Vol. 1, John Wiley & Sons, New York, N. Y., 1951, p. 390.

(8) M. Erne, F. Ramirez, and A. Burger, *Helv. Chim. Acta*, **34**, 143 (1951).

(9) G. J. Janz and W. J. H. McCulloch, *J. Am. Chem. Soc.*, **77**, 3143 (1955).

(10) F. Pregl and J. Grant, "Quantitative Organic Microanalyses," The Blakiston Co., Philadelphia, Pa., 1946, p. 169.