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.

Acknowledgments.—Carcinostatic assays were carried out by Mr. B. C. V. Mitchley and toxicity assays by Mr. M. Jones. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign and by Public Health Service Grant No. CA-03188-10 from the National Cancer Institute, U. S. Public Health Service.

(8) W. C. J. Ross, *J. Chem. Soc.*, 2257 (1950).

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

GLYNN P. WHEELER AND SUE CHUMLEY

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama

Received October 26, 1966

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-

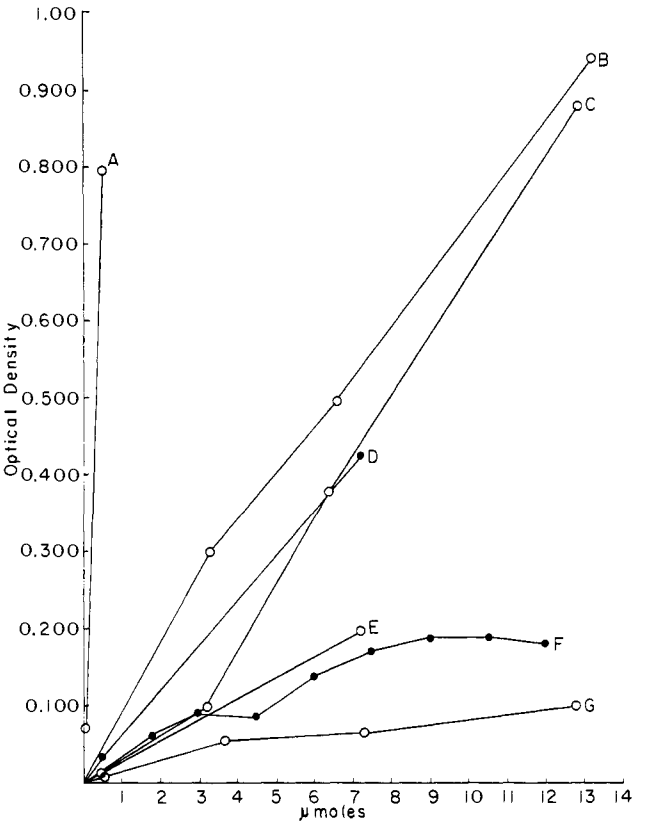


Figure 1. Measurement of the alkylating activities of several compounds. With the exception of the tests with diazomethane, the reaction mixtures were heated in a boiling-water bath for 20 min. The reaction with diazomethane occurred at room temperature in the course of approximately 3 min. The numbers 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.

(1) This work was supported by a grant from The John A. Hartford Foundation, Inc.

(2) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).

(3) R. W. Sidwell, G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr., *Appl. Microbiol.*, **13**, 579 (1965).

(4) (a) F. M. Schabel, Jr., T. P. Johnston, G. S. McCaleb, J. A. Montgomery, W. R. Laster, and H. E. Skipper, *Cancer Res.*, **23**, 725 (1963); (b) A. Goldin, J. M. Venditti, J. A. R. Mead, and J. P. Glynn, *Cancer Chemotherapy Rept.*, **40**, 57 (1964); (c) G. P. Wheeler and B. J. Bowdon, *Cancer Res.*, **25**, 1770 (1965).

(5) (a) D. P. Rall, M. Ben, and D. M. McCarthy, *Proc. Am. Assoc. Cancer Res.*, **4**, 55 (1963); (b) V. DeVita, P. P. Carbone, A. H. Owen, Jr., G. L. Gold, M. J. Krant, and J. Edmonson, *Cancer Res.*, **25**, 1876 (1965).

(6) R. F. Pittillo, A. J. Narkates, and J. Burns, *Cancer Res.*, **24**, 1222 (1964).

(7) B. A. Kihlman, *Exptl. Cell Res.*, **20**, 657 (1960).

(8) F. Arndt, *Org. Syn.*, **15**, 4 (1935).

(9) A. F. McKay, *Chem. Rev.*, **51**, 301 (1952).

(10) (a) W. A. Skinner, H. F. Gram, M. O. Greene, J. Greenberg, and B. R. Baker, *J. Med. Pharm. Chem.*, **2**, 299 (1960); (b) L. Pasternak, *Acta Biol. Med. Ger.*, **10**, 436 (1963); (c) H. Druckrey, S. Ivankovic, and R. Preussmann, *Z. Krebsforsch.*, **66**, 389 (1965).

TABLE I
REACTION OF VARIOUS COMPOUNDS WITH 4-(*p*-NITROBENZYL)PYRIDINE

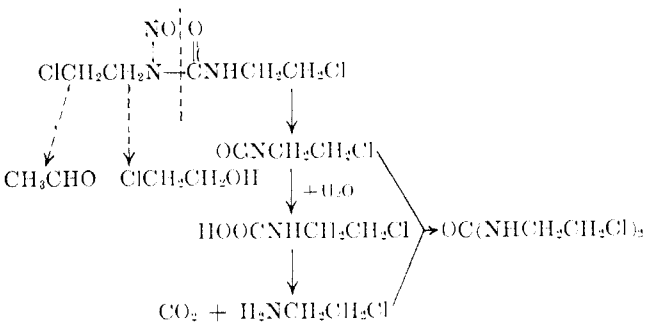
Compd	Optical density, Time of incubation, min				
	10	20	30	40	50
1,3-Bis(2-chloroethyl)-1-nitrosourea	0.517 (2)[a]	0.813 (2)	0.916 (3)	0.938 (3)	0.999 (2)
1-(2-Chloroethyl)-1-nitrosourea	0.006	0.009	0.005	0.015	0.014
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea	0	0.013	0.026	0.031	0.029
1-Methyl-1-nitrosourea	0	0	0	0	0
2-Chloroethyl isocyanate	0.376 (2)	0.820 (2)	0.879 (3)	0.887 (3)	0.834 (2)
2-Chloroethylamine hydrochloride	0.360 (3)	0.858 (3)	0.923 (4)	1.009 (4)	0.935 (2)
1,3-Bis(2-chloroethyl)urea	0.054	0.149	0.367	0.518	0.577
Ethyl isocyanate	0	0	0	0	0
3-Chloropropyl isocyanate	0	0	0.010	0.010	0.022
2-Chloroethanol	0.008	0.038		0.045	0.057

[a] The numbers in parentheses indicate the number of experiments included in the given mean value. Where parentheses are not used, the number of experiments was one. Triplicate tubes were used in each experiment.

cate that under the described conditions I yields compounds that have alkylating activity, but the major portion of this activity is probably due to the formation of 2-chloroethylamine rather than to the generation of diazoalkanes.

There is evidence that I cleaves, either directly or indirectly, between the nitrosated nitrogen atom and the carbonyl group, and that the amide portion of the molecule yields 2-chloroethyl isocyanate, which upon hydrolysis and subsequent decarboxylation yields 2-chloroethylamine[11] (Scheme 1). Reaction of 2-chloro-

SCHEME I



ethyl isocyanate with 2-chloroethylamine can then yield 1,3-bis(2-chloroethyl)urea. Under specified conditions the nitrosamine portion of I can yield acetaldehyde or 2-chloroethanol.[11a] It was therefore of interest to compare the alkylating activity of I with some of the compounds derived from it and also with several other 1-alkyl-1-nitrosoureas.

4-(*p*-Nitrobenzyl)pyridine has been used for the detection and assay of a number of alkylating agents[12] and for the comparison of the alkylating activities of various compounds.[13] The procedure of Friedman and Boger[12c] was the basis of the method used in the present study.

Some of the experimental results are given in Table I. The data in the upper section of the table show that the presence of the 2-chloroethyl group on N-3 of the nitrosourea is necessary for extensive color formation. Since 2-chloroethyl isocyanate yielded color, but ethyl isocyanate and 3-chloropropyl isocyanate did not, it seems evident that a chlorine atom on C-2 is required for color formation to occur. Color formation occurred in the test with 1,3-bis(2-chloroethyl)urea, but the density of the color was much less than that obtained with I. As the densities of the colors obtained with I, with 2-chloroethyl isocyanate, and with 2-chloroethylamine were essentially the same, it seems that most of the color obtained with I could be ascribed to reaction of 2-chloroethylamine with the 4-(*p*-nitrobenzyl)-pyridine. It is also implicit in these results that the 2-chloroethylamine that is generated combines more extensively with the 4-(*p*-nitrobenzyl)pyridine than with the 2-chloroethyl isocyanate.

Since 1-(2-chloroethyl)-1-nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and 1-methyl-1-nitrosourea are active against L1210 leukemia,[2,13,14] it is evident that the generation of 2-chloroethylamine is not a requisite for this biological activity. The small but significant quantities of color obtained with 1-(2-chloroethyl)-1-nitrosourea and with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea are evidently due to compounds that have alkylating activity and are derived from the nitrosated ends of the molecules. The curves of Figure 1 make possible a rough comparison between the alkylating activities of these compounds and the alkylating activity of diazomethane. The comparison is "rough," because the diazomethane was allowed to react with the color reagent at room temperature during a period of a few minutes, while the other compounds were incubated with the reagent in a boiling-water bath for 20 min. If it is assumed that the elevated temperature promotes the generation of the reactive compound but has little effect upon the observed rate of reaction of this compound with the 4-(*p*-nitrobenzyl)pyridine, it appears that these nitrosoureas yield compounds having alkylating activities of the same order of magnitude as the alkylating activity of diazomethane. It is of interest that much of the alkylating activity of 1,3-bis(2-fluoroethyl)-1-nitro-

(11) (a) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, Abstracts of the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 6–10, 1964, p 27M; (b) T. L. Loo, R. L. Dion, R. L. Dixon, and D. P. Rall, *J. Pharm. Sci.*, **55**, 492 (1966).

(12) (a) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955); (b) O. Klatt, A. C. Griffin, and J. S. Stahlin, Jr., *Proc. Soc. Exptl. Biol. Med.*, **104**, 629 (1960); (c) O. M. Friedman and E. Boger, *Anal. Chem.*, **33**, 906 (1961); (d) R. K. Ansman, G. E. Crevar, H. Hogedora, T. J. Bardos, and J. L. Ambrus, *J. Am. Med. Assoc.*, **178**, 735 (1961); (e) H. G. Petering and G. J. Van Giessen, *J. Pharm. Sci.*, **52**, 1159 (1963).

(13) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).

(14) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *ibid.*, **9**, 892 (1966).

sourea must be associated with the nitrosated end of the molecule, since considerably less color was obtained with equimolar quantities of 2-fluoroethylamine. Therefore, it remains possible that under physiological conditions 1-alkyl-1-nitrosoureas might yield small quantities of compounds having alkylating activity, but it is still an open question whether this activity is sufficient to cause the observed biological effects.

Experimental Section

A mixture of 2 ml of water, 1 ml of acetate buffer (0.025 *M*, pH 6.0), 1 ml of an acetone solution containing 0.504 μmole/ml (unless otherwise indicated) of the test compound, and 0.4 ml of a 5% (w/w) solution of 4-(*p*-nitrobenzyl)pyridine in acetone was heated in a boiling-water bath for designated periods of time. The mixture was then cooled in an ice bath and to it were added 2 ml of acetone, 5 ml of ethyl acetate, and 1.5 ml of 0.25 *N* NaOH. The tube was shaken 20 times, and the two phases were separated by centrifugation in a clinical centrifuge for 2 min. A portion (2–3 ml) of the upper layer was transferred by pipet to a cuvette, and the optical density at 540 mμ was determined with a Beckman Model DU spectrophotometer. The operation from the introduction of the NaOH through the determination of optical density was performed within a period of 5 min in subdued light.

Diazomethane[15] from N-methyl-N-nitrosourethan was assayed by reaction with benzoic acid and titration of the excess benzoic acid. Measured volumes of this solution were used for the color tests, and equal volumes of ether were added to the control tubes.

(15) W. E. Bachmann, W. Cole, and A. L. Wilds, *J. Am. Chem. Soc.*, **62**, 824 (1940).

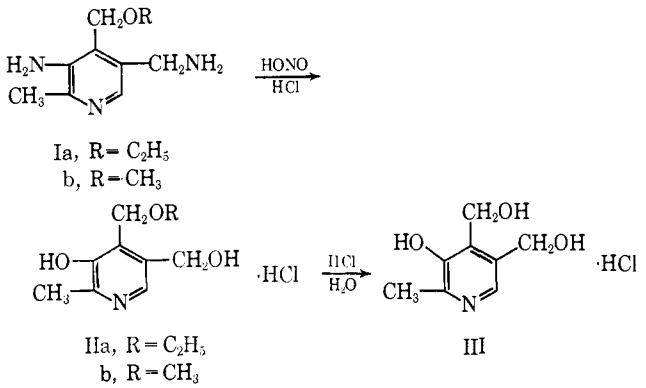
Synthesis of Vitamin B_6. III. 3-Deoxypyridoxine

STANTON A. HARRIS, ELBERT E. HARRIS, ELWOOD R. PETERSON, AND EDWARD F. ROGERS

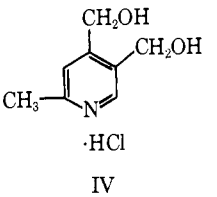
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

Received November 18, 1966

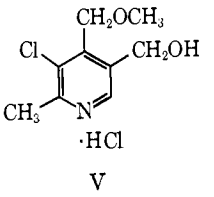
Preparations of 3-deoxypyridoxine (6-methylpyridine-3,4-dimethanol) have been previously reported.[1,2] We have now isolated it as an unusual by-product in the synthesis of pyridoxine. Harris and Folkers[3] described the following sequence of reactions: 1A → IIA → III. Compounds IV and V have now been



found in the mother liquors from pyridoxine prepared by this route without isolation of IIb, employing the



IV

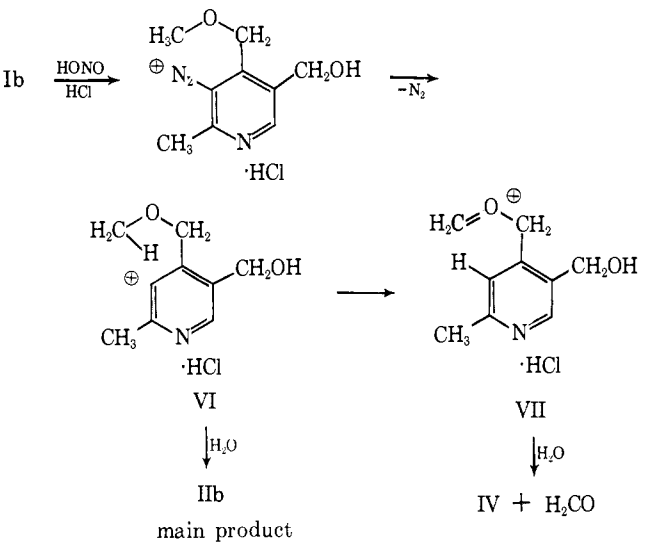


V

methyl ethers instead of the ethyl ether series described in the original publication.

An attempt was made to isolate the 4-methyl ether of IV from the mother liquors of 4-methoxypyridoxine (IIb), but unexpectedly only the demethylated compound IV was found at this stage also. The formation of 3-deoxypyridoxine (IV) during the diazotization can be explained by an intramolecular hydride transfer from the carbon of the neighboring methyl ether to the arylcarbonium ion VI formed from the diazonium salt. The resulting intermediate VII, comparable to that from the hydrolysis of an acetal, would immediately break down under the reaction conditions to form compound IV and formaldehyde (Scheme I).

SCHEME I



Analogous 1,5-hydride transfers have been proposed to explain abnormal products first observed[4] in the syntheses of certain phenanthridones by Pschorr cyclization and later extended to simpler anthranilamides by Cohen[5] and co-workers. For example, these authors reported a 9.9% yield of N-methylbenzamide and of formaldehyde from the diazotization of *o*-amino-N,N-dimethylbenzamide, along with the expected N,N-dimethylsalicylamide as the major product.

The formation of V by reaction of VI with chloride ion is a not-unexpected side path in the diazotization. It is of interest that the methyl ether linkage of V is resistant to the final hydrolytic conditions (II → III), illustrating the labilizing influence of the 3-hydroxyl group on the 4-methylene position.

(1) A. Ichiba, K. Michi, and S. Emoto, *Sci. Papers Inst. Phys. Chem. Res.* (Tokyo), **39**, 131 (1941).

(2) R. G. Jones and E. C. Kornfeld, *J. Am. Chem. Soc.*, **73**, 107 (1951).

(3) S. A. Harris and K. Folkers, *ibid.*, **61**, 3307 (1939).

(4) D. H. Hey and D. G. Turpin, *J. Chem. Soc.*, 2471 (1954); T. R. Govindachari and N. Arumugam, *J. Sci. Ind. Res.* (India), **14B**, 250 (1955); L. G. Humber, H. Kondo, K. Kotera, S. Takagi, K. Takeda, W. I. Taylor, B. R. Thomas, Y. Tsuda, K. Tsukamoto, S. Uyeo, H. Yajima, and N. Yanaihara, *J. Chem. Soc.*, 4622 (1954).

(5) T. Cohen, R. M. Moran, Jr., and G. Sorvinski, *J. Org. Chem.*, **26**, 1 (1961); T. Cohen, A. H. Dinwoodie, and L. D. McKeever, *ibid.*, **27**, 3385 (1962).