Table I.

Effect	ED_{50}, mg/kg 8	9
Loss of muscle tone	100	24
Loss of righting reflex	132	200
Loss of corneal reflex	152	~214
Loss of spinal reflex	168	~238
Anticonvulsant activity (metrazol)	68	68
Anticonvulsant activity (MES)	100	147
LD_{50} (48 hr)	563	320

with assigned structures; combustion values for C, H, and N were within 0.4% of theory.

DL-1-(α-Methylbenzyl)imidazole-2-methanol (3). To a soln of 52 g (2.25 g-atoms) of Na in 600 ml of MeOH was added 170 g (2.50 moles) of imidazole. Most of the solvent was distilled off whereupon 450 ml of DMF was added and solvent removal was resumed till the internal temp reached 125°. The mixture was cooled to 30°. Addition of 327 g (2.32 moles) of α-methylbenzyl chloride resulted in an exothermic reaction, which required cooling. The reaction was then allowed to proceed overnight. Benzene (*ca.* 2 l.) was added and the soln was scrubbed 5 times with H_2O. The product was extracted from the organic phase with 3 *N* HCl and was regained from the acidic aqueous phase by basification. It was extracted into C_6H_6. Drying of the organic phase and removal of solvent left 160 g (42%) of an oil. To this residue was added 800 ml of CH_2O (37%) and the solution was refluxed for 72 hr. Excess reagent was removed by distillation until stopping up of the system became bothersome. PhMe and H_2O were then added whereupon more of the aqueous phase was removed azeotropically. The mixt was cooled, basified, and stirred for 1 hr, and the phases were separated. Extraction of the aqueous phase with fresh PhMe, drying of the combined organic layers, and stripping of solvent left a residue which was rendered crystalline by addition of Me_2CO; yield 93 g (50%), mp 88–92°. An analytical sample (Me_2CO) had mp 94–96°; nmr (CF_3COOH) τ 5.00–5.06 (d, 2, $ImCH_2OH$), 2.4–3.0 (m, 7, C_6H_5 and $C_3H_2N_2$). *Anal.* ($C_{12}H_{14}N_2O_2$) C, H, N.

DL-1-(α-Methylbenzyl)-2-chloromethylimidazole Hydrochloride (4). A soln of 150 g (0.74 mole) of **3** in 700 ml of $SOCl_2$ was refluxed for 1 hr. Addition of *i*-Pr_2O to the cloud point, cooling, and filtration afforded 177 g of **4**. A recrystallized sample (*i*-PrOH–*i*-Pr_2O) had a decomposition point of ~260°; nmr (CF_3COOH) τ 5.22 (s, 2, $ImCH_2Cl$). *Anal.* ($C_{12}H_{13}ClN_2 \cdot HCl$) C, H, N.

Reaction of 4 with NaCN in 80% Acetone. Compd **4**, 177 g (0.69 mole), was dissolved in 200 ml of H_2O at 60°; to this was added 1.1 l. of Me_2CO. The resulting saturated soln was added dropwise and with stirring to a slurry of 72 g (1.48 moles) of NaCN in 75 ml of H_2O (Caution: Good ventilation is necessary. HCN is liberated). Stirring was continued overnight whereupon 850 ml of Me_2CO was distilled out of the reaction flask. Benzene (700 ml) was then added. Inorganic material was removed from this mixture by repeated scrubbing with H_2O whereby the organic phase was kept in the flask, aqueous phases being withdrawn by aspirator suction. The C_6H_6 layer was dild with 1 l. of *i*-Pr_2O, and saturated HCl–*i*-PrOH was added cautiously until further addition failed to produce cloudiness. Cooling of this mixt gave semisolid HCl salts from which solvent was removed by decantation. Subsequent trituration (Me_2CO) rendered one component crystalline. This was filtered off, yielding 79 g (47%) of material characterized as DL-1-(α-methylbenzyl)-2-cyanomethylimidazole hydrochloride (**5**). An analytical sample was recrystallized from *i*-PrOH–*i*-Pr_2O; mp 180–185°; nmr (CF_3COOH) τ 5.60 (s, 2, $ImCH_2CN$), 2.31–2.91 (m, 7, arom H); ir (KBr) 2262 cm^{-1} (C≡N). *Anal.* ($C_{13}H_{13}N_3 \cdot HCl$) C, H, N.

The trituration liquors *(vide supra)* were evaporated. The residue, taken up in H_2O was basified ($NaHCO_3$) and seeded to give solid DL-1-(α-methylbenzyl)-2-methylimidazole-5-carbonitrile (**6**). Upon filtration and trituration (Me_2CO), 14.8 g (10%) of material was obtained. Recrystallization (Me_2CO–*i*-Pr_2O) furnished analytical material; mp 106–107°; nmr τ 7.22 (s, 3, $ImCH_3$), τ 2.4–2.9 (m, 5, (C_6H_5), τ 2.01 (s, 1, ImH); ir (KBr) 2235 cm^{-1} (C≡N). *Anal.* ($C_{13}H_{13}N_3$) C, H, N.

DL-1-(α-Methylbenzyl)-2-methylimidazole-5-carboxylic Acid (7). A soln of 10 g (0.047 mole) of **6** in 100 ml of MeOH was alternately saturated with HCl gas and refluxed (2 hr). After 3 such cycles followed by a final 18-hr reflux period, the solvent was removed and was replaced with a soln of 20 g of NaOH in 50 ml of H_2O. The mixt was refluxed for another 3 hr; addition of a soln of 30 g of AcOH in 50 ml of H_2O, seeding, and cooling provided 9.4 g (87%) of **7**; mp 203–205°. Analytical material (H_2O) had mp 208–209°; nmr τ 7.64 (s, 3, $ImCH_3$). *Anal.* ($C_{13}H_{14}N_2O_2 \cdot H_2O$) C, H, N.

DL-1-(α-Methylbenzyl)-2-methylimidazole-5-carboxylic Acid Methyl Ester Nitrate (8). A mixt of 3.0 g of acid **7** and 20 ml of $SOCl_2$ was refluxed for 1 hr. Excess reagent was removed and was replaced with 30 ml of MeOH. Refluxing (1 hr) and solvent evaporation left an oil which was taken up in H_2O. The soln was treated with K_2CO_3 from which the product base was extracted into Et_2O. Addition of HNO_3 to the dried ethereal soln furnished 3.7 g of product; mp 92–94°. It was recrystallized from MeOH–Me_2CO–*i*-Pr_2O to melt at 93–94°; ir (KBr) 1745 cm^{-1} (C=O). *Anal.* ($C_{14}H_{16}N_2O_2 \cdot HNO_3$) C, H, N.

DL-1-(α-Methylbenzyl)-2-methylimidazole-5-carboxylic acid ethyl ester nitrate (9), prepared analogously to **8**, had mp 143–144° (EtOH–*i*-Pr_2O). *Anal.* ($C_{15}H_{18}N_2O_2 \cdot HNO_3$) C, H, N.

Acknowledgment. The authors wish to thank Professor Dr. C. Koningsberger for continued interest and encouragement. Thanks are also due to Mr. P. van den Bosch (microanalyses), Miss E. W. A. Kraak (nmr spectra), and Miss M. J. E. Elissen (ir spectra).

References

(1) (a) E. F. Godefroi, P. A. J. Janssen, C. A. M. Van der Eycken, and C. J. E. Niemegeers, *J. Med. Chem.*, **8**, 220 (1965); (b) E. F. Godefroi and C. A. M. Van der Eycken, U. S. Patent 3,354,173 (1967).

(2) (a) E. F. Godefroi, J. van Cutsem, C. A. M. Van der Eycken, and P. A. J. Janssen, *J. Med. Chem.*, **10**, 1160 (1967); (b) E. F. Godefroi and C. A. M. Van der Eycken, U. S. Patent 3,547,942 (1970).

(3) R. G. Jones, *J. Amer. Chem. Soc.*, **71**, 644 (1949).

(4) R. G. Jones, *ibid.*, **71**, 383 (1949).

(5) G. J. Durant, M. E. Foottit, C. R. Ganellin, J. M. Loynes, E. S. Pepper, and A. M. Roe, *Chem. Commun.*, 108 (1968).

(6) E. F. Godefroi, *J. Org. Chem.*, **33**, 860 (1968).

(7) G. B. Frank and K. Jhamandas, *Brit. J. Pharmacol.*, **39**, 696 (1970).

Cysteine Scavengers. 1. Bis(3-pyridylmethyl) Phosphate and Bis(3-pyridylmethyl) Pyrophosphate†

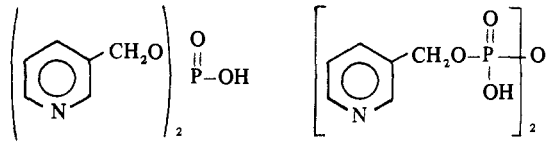
Mervyn Israel,* Marilyn A. Whitesell, Edward J. Modest, George E. Foley, Herbert Lazarus, and Franz Bergel‡

The Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, Massachusetts 02115. *Received July 21, 1971*

A recent report[1] on the absolute nutritional requirement *in vitro* of lymphoblastic leukemic cells of human origin for L-cysteine (or L-cystine) prompted us to consider the synthesis of modified transport forms of pyridoxal for use as an enzymomimetic system for cysteine desulfhydrase.[2] In connection with the synthesis of bis(pyridoxalyl) phosphate and bis(pyridoxalyl) pyrophosphate, 2 key target compounds in this investigation, we chose as a model compound for study 3-pyridylmethanol, which, like pyridoxal, possesses a carbinol function at the β position on the pyridine nucleus. We should like now to report the preparation of the hitherto unknown bis(3-pyridylmethyl) phosphate (**1**)

†This work was supported in part by a research grant (C6516), research career award (K6-CA-22,150) (to G.E.F.), and research career development award (K3-CA-22,151) (to E.J.M.) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

‡Professor of Chemistry, Emeritus, University of London and Scientific Consultant, The Children's Cancer Research Foundation.



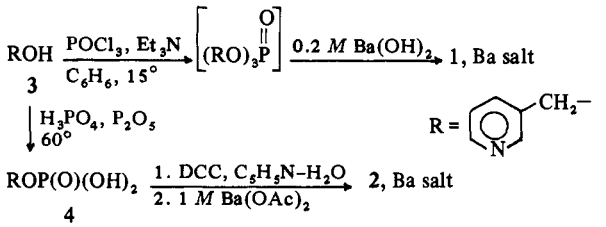
1 2

and bis(3-pyridylmethyl) pyrophosphate (2), each of which was isolated and characterized as its Ba salt.

Some time ago, Noller and Dutton reported the synthesis of some trialkyl phosphates by reaction of $POCl_3$ with the appropriate alcohol in the presence of pyridine.[3] Essentially by this method, except that Et_3N was substituted for pyridine, 3-pyridylmethanol (3) was treated with freshly distd $POCl_3$; however, the resulting triester was not isolated, but was hydrolyzed *in situ* to 1 by the action of dil $Ba(OH)_2$.

The preparation of the pyrophosphate required, as starting material, 3-pyridylmethyl phosphate (4). Compound 4 was obtained by treatment of 3 with P_2O_5 and orthophosphoric acid.[4,5] The Dowex 50 purification method for 4 described by Murakami, *et al.*,[5] was superior to the methods reported by Long and Morrison.[4]

Formation of the pyrophosphate resulted when 4 was treated with dicyclohexylcarbodiimide in pyridine containing a few drops of H_2O.[6,7] H_2O served 2 purposes in this reaction: first, it kept 4 in solution (4 is essentially insol in



anhyd pyridine), and, secondly, it prevented the formation of a more complex product mixture consisting of pyrophosphate (2), unchanged 4, and phosphodiester (1). According to Khorana,[7] carbodiimide-induced pyrophosphate bond formation under anhyd conditions is accompanied by further reactions leading to phosphodiester products.

The reaction mixture, after hydrolysis of excess carbodiimide and separation of dicyclohexylurea, was treated with $Ba(OAc)_2$ solution. Addition of acetone resulted in the pptn of the Ba salt of 2, while the more sol Ba salt of 4 remained in solution.

Compounds 1 and 2, as their Ba salts, were evaluated for growth-inhibitory activity against cysteine-dependent leukemic (CCRF-SB) cells in culture according to procedures previously described[8] and were found to be inactive at concentrations up to 8.9×10^{-4} and $5.8 \times 10^{-4} M$, respectively, near the limits of their solubility. These results were not unexpected, since the model compounds lack the phenolic aldehyde system of pyridoxal. The methods developed for the synthesis of 1 and 2 are now being applied to the preparation of the corresponding pyridoxal derivatives.

Experimental Section§

Melting points were taken by the capillary method on a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are uncorrected. Ir spectra were determined with a Perkin-Elmer Model 137B spectrophotometer, nmr spectra by means of a Varian Associates A-60 spectrometer in D_2O (sodium 3-(trimethylsilyl)-propanesulfonate). Tlc was done on Eastman Chromagram sheets without fluorescent indicator; the developing system was "formix" (*tert*-BuOH–H_2O–HCO_2H, 70:15:15 v/v).[9] Visualization was accomplished by means of the ammonium molybdate–$HClO_4$–H_2S technique.[10]

Bis(3-pyridylmethyl) Phosphate (1), Ba Salt. To a soln of 10.9 g (0.10 mole) of 3-pyridylmethanol (Aldrich Company) and 10.1 g (0.10 mole) of Et_3N in 150 ml of C_6H_6 cooled in an ice bath was added dropwise with stirring a soln of 5.10 g (0.035 mole) of freshly distd $POCl_3$ in 50 ml of C_6H_6. The reaction temp was not permitted to rise above 15° during the course of the addn. After complete addn, the ice bath was removed and the pink- to red-colored reaction mixt was stirred overnight at room temp. $Et_3N \cdot HCl$, which had begun to ppt early in the reaction, was sepd and washed with a small vol of C_6H_6; the C_6H_6 wash was added to the mother liquor. The C_6H_6 soln was heated at reflux for 1 hr, during which time it lost its pink color and became beige. The soln was then evapd under reduced pressure and the remaining nearly colorless oil was stirred with Et_2O to dissolve as much product as possible. The Et_2O soln was decanted from a small amount of insol oil and evapd to dryness. The residual oil (2.6 g, equiv to 7 mmoles of triester) was treated with 35 ml of 0.2 *M* $Ba(OH)_2$ soln (2 equiv); as the oil dissolved, the soln became light orange. After overnight refrigeration, the soln was mixed with 150 ml of EtOH and refrigerated for 1 hr. White solid (a mixt of $Ba(OH)_2$ and the Ba salt of 4) was sepd and discarded. To the aq EtOH filtrate was added 150 ml of Me_2CO and the resulting ppt was collected. Repptn of the solid from aq EtOH with Me_2CO gave 1.2 g (10%) of product: nmr δ 4.90 (doublet, J = 14 Hz, CH_2 split by P) and 7.30–8.60 (arom) ppm; ir λ_{max}^{KCl} 3.28, 6.32, 7.00, 8.10, 8.17, 9.18, 9.58, 9.77, 11.50, 11.67, and 11.82 μ. *Anal.* ($C_{24}H_{24}BaN_4O_8P_2 \cdot H_2O$) C, H, N, P.

Bis(3-pyridylmethyl) Pyrophosphate (2), Ba Salt. A sample of 100 mg (0.5 mmole) of 4,[4,5] was dissolved in 5 ml of C_5H_5N to which 0.75 ml of H_2O had been added. Dicyclohexylcarbodiimide (1.0 g, 5 mmoles) was added and the reaction mixt was stirred overnight at room temp; dicyclohexylurea began to ppt after 10 min. On the following day an additional 100 mg of carbodiimide was added and the reaction mixt was stirred for another 2 hr. C_5H_5N was removed under reduced pressure and the residue was treated with 5 ml of H_2O. Insol dicyclohexylurea was sepd and the aqueous filtrate was concd to 1 ml and mixed with 0.125 ml of 1 *M* $Ba(OAc)_2$ soln. Addn of Me_2CO resulted in pptn of product, the more sol Ba salt of 4 remaining in the aq Me_2CO medium. The product weighed 40 mg (30% yield) and was homogeneous by tlc: ir λ_{max}^{KCl} 6.04, 6.12, 6.34, 6.76, 7.02, 8.13, 8.82, 9.28, 9.74, 10.47, 11.29, and 11.86 μ. *Anal.* ($C_{12}H_{12}BaN_2O_7P_2 \cdot 4H_2O$) C, Ba, N, P. H was found 0.8% lower than theory.

Acknowledgment. We thank Dr. L. M. Jampolsky of Hoffman-La Roche Inc., Nutley, N. J., for a generous sample of 3-pyridylmethanol tartrate which was used for the initial studies.

References

(1) G. E. Foley, E. F. Barell, R. A. Adams, and H. Lazarus, *Exp. Cell Res.*, **57**, 129 (1969).
(2) F. Bergel, "Chemistry of Enzymes in Cancer," C. C. Thomas, Springfield, Ill., 1961, p 72; see also, F. Bergel, K. R. Harrup, and A. M. Scott, *J. Chem. Soc.*, 1101 (1962).
(3) C. R. Noller and G. R. Dutton, *J. Amer. Chem. Soc.*, **55**, 424 (1933).
(4) R. F. Long and A. L. Morrison, U. S. Patent 2,777,851 (1957).
(5) Y. Murakami, M. Takagi, and H. Nishi, *Bull. Chem. Soc., Jap.*, **39**, 1197 (1966).
(6) H. G. Khorana, *J. Amer. Chem. Soc.*, **76**, 3517 (1954).
(7) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," Wiley, New York, N. Y., 1961, p 134.
(8) G. E. Foley and H. Lazarus, *Biochem. Pharmacol.*, **16**, 659 (1967).
(9) E. A. Peterson and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 169 (1953).
(10) S. Burrows, F. S. M. Grylls, and J. S. Harrison, *Nature (London)*, **170**, 800 (1952).

§Analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn., and are within ±0.5% of theory, except where otherwise noted.