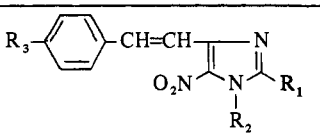
Table III



Compd	R_1	R_2	R_3	Yield,[a] %	Mp, °C	Formula[e]	MIC[f] (μg/ml), *T. vaginalis*
28	H	Me	Me	50	208	$C_{13}H_{13}N_3O_2$	2
29	H	Me	CO_2H	49	300–302 dec	$C_{13}H_{11}N_3O_4$	0.5
30	H	CH_2CH_2OH	Me	42	187	$C_{14}H_{15}N_3O_3$	2
31	H	CH_2CH_2OH	CO_2H	27	208 dec	$C_{14}H_{13}N_3O_5$	100–1000
32	H	CH_2CH_2OTs	Me	85[b]	190	$C_{21}H_{21}N_3O_5S$	
33	H	$CH{=}CH_2$	H	61[c]	146	$C_{13}H_{11}N_3O_2$	1
34	H	$CH{=}CH_2$	Me	58[c]	136	$C_{14}H_{13}N_3O_2$	2
35	H	$CH{=}CH_2$	CO_2H	38[c]	280 dec	$C_{14}H_{11}N_3O_4$	
36	Me	H	H	40	249[g]	$C_{12}H_{11}N_3O_2$	10–100
37	Me	H	Me	21	252[h]	$C_{13}H_{13}N_3O_2$	
38	Me	Me	H	76[d]	140[i]	$C_{13}H_{13}N_3O_2$	2
39	Me	Me	Me	78[d]	198[j]	$C_{14}H_{15}N_3O_2$	
40	Me	CH_2CH_2OH	H	78[b]	135	$C_{14}H_{15}N_3O_3$	
41	Me	$CH{=}CH_2$	H	32[c]	99	$C_{14}H_{13}N_3O_2$	

[a]Prepared by method A except where noted. [b]Preparation described in the Experimental Section. [c]Prepared by method C. [d]Prepared by method D. [e]All compounds analyzed for C, H, and N. [f]Determined by serial dilution *in vitro*. [g]Mp 245–246°, ref 4. [h]Mp 242°, ref 5. [i]Mp 136–138°, ref 5. [j]Mp 197–198°, ref 5.

stirred suspension of 18 (2.0 g, 0.0045 mol) in EtOH (5 ml) at 70°. The mixture was heated at 70° for 30 min and then evaporated and the residue was dissolved in water and extracted with $CHCl_3$. Evaporation of the extract gave an oil which crystallized from $CHCl_3$–petroleum ether to give the product 19, mp 178°.

4-Nitro-5-styryl-1-vinylimidazoles (7, $R_2 = CH{=}CH_2$). Method C. $SOBr_2$ (3.4 ml, 0.044 mol) was added over 30 min to a stirred solution of the diol 5 ($R_2 = CH_2CH_2OH$) (0.017 mol) in DMF (20 ml). The solution was stirred for 3 hr at 20–25° and poured onto ice. Neutralization with $NaHCO_3$ and extraction with EtOAc gave the crude dibromo compound 6 as an oil. This oil was dissolved in DMSO (50 ml) and 1,5-diazabicyclo[4.3.0]non-5-ene (13.5 ml) and the dark solution was heated at *ca.* 45° for 1 hr and then poured onto ice–H_2O, and the precipitate was recrystallized from ethanol.

4-Methyl-5-nitro-1-imidazoleethanol (8, $R_1 = H$; $R_2 = CH_2CH_2OH$). Ethylene oxide (25 ml, 0.5 mol) was added in small portions over 1 hr to a stirred solution of 4(5)-methyl-5(4)-nitroimidaozle (6.35 g, 0.05 mol) in 98% HCO_2H (150 ml) at 45° and the solution was stirred for a further 1 hr and then evaporated. The residue was diluted with H_2O (10 ml) and filtered to remove unreacted starting material. The filtrate was made alkaline with 5 *M* NaOH and extracted with EtOAc. Evaporation of the extract yielded an oil which crystallized from $CHCl_3$–petroleum ether to give 3.3 g (39%) of product, mp 100°.

4-Methyl-5-nitro-1-(2-*p*-toluenesulfonyloxyethyl)imidazole (8, $R_1 = H$; $R_2 = CH_2CH_2OTs$), mp 130°, was prepared in 87% yield as described above for 4 ($R_1 = Me$; $R_2 = CH_2CH_2OTs$). Treatment of this tosylate with NaOEt in EtOH at 70° gave a dark brown solution from which only unreacted tosylate could be isolated.

5-Nitro-4-styrylimidazoles (Table III). Method A. 4-Methyl-5-nitroimidazoles were condensed with aromatic aldehydes using the procedure described above in method A for the preparation of 5. The products were recrystallized from EtOH (+DMF where $R_3 = CO_2H$). In the preparation of compounds 36 and 37, the reaction mixtures were heated under reflux for 1–2 hr and the products were isolated by extraction of the diluted and neutralized reaction mixture with EtOAc.

Method C. Compounds 9 ($R_2 = CH_2CH_2OH$) were converted to the corresponding *N*-vinyl compounds 33–35 and 41 by the procedure described above in method C for the preparation of compounds 7. The intermediate bromo compounds were solids but were not purified. Compound 35 was purified by acidification of an EtOH–H_2O solution of the Na salt.

Method D. Compounds 36 and 37 were methylated with a slight excess of Me_2SO_4 in refluxing dioxane. The solid products were crystallized from EtOH–dilute NH_3.

2-Methyl-5-nitro-4-styryl-1-imidazoleethanol (40). Ethylene oxide (50 ml) was added in small portions over 1 hr to a stirred solution of 36 (9.0 g, 0.039 mol) in 98% HCO_2H (150 ml) at 50–60°. The mixture was stirred for a further 1 hr and then evaporated, and the residue was heated to 70° with EtOH (100 ml) and 5 *M* NaOH (130 ml) to hydrolyze some esterified product. On cooling the solution yellow solid formed and was crystallized from $CHCl_3$–hexane and then from EtOH to give pure 40. Further material obtained from the crystallizations was a mixture of 40 with 36 and was again allowed to react with ethylene oxide in HCO_2H to give more product.

4-(4-Methylstyryl)-5-nitro-1-(2-*p*-toluenesulfonyloxyethyl)-imidazole (32). Compound 30 was tosylated as described above for compound 4 ($R_1 = Me$; $R_2 = CH_2CH_2OTs$). An attempted elimination reaction using NaOEt in EtOH at 70° gave a mixture of tarry material and starting tosylate.

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4-Vinyl Analog of Pyridoxal, a Potent Antagonist of Vitamin B_6†

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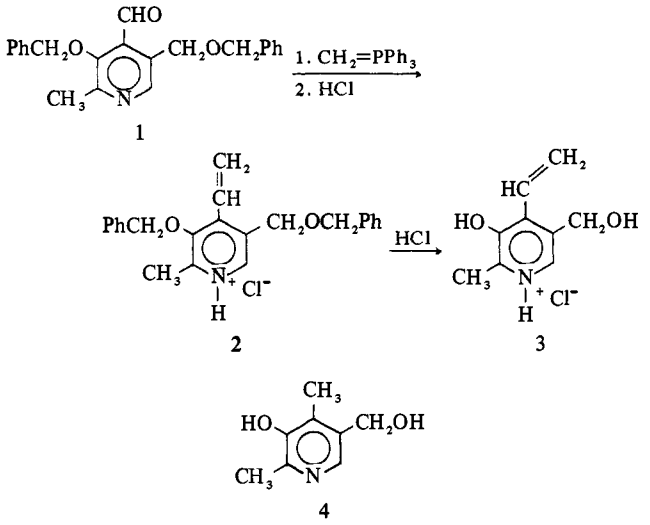
As part of our program for the development of new antagonists of vitamin B_6,[3] we have synthesized a close analog 3 of pyridoxal, in which the O atom of the aldehyde has been replaced with methylene. The synthesis of 4-deformyl-4-vinylpyridoxal ("4-VPAL," 3)‡ starts with 3,α^5-*O*-di-

†Chemistry and Biology of Vitamin B_6. 32. For the preceding paper in this series, see ref 1. Subseries: Selective Modification of the α^4 Position of Pyridoxol. 2. For the preceding paper in this subseries, see ref 2.

‡Nomenclature and the abbreviations used were those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature; see, *e.g.*, ref 4.

benzylpyridoxal (1), which was described by us earlier and used for the introduction of various groups into the 4 position of vitamin B_6.[2] The aldehyde group has been converted to vinyl by means of a Wittig reaction in which the required ylide was generated from triphenylphosphonium bromide. In this reaction it was necessary to use both *n*-butyllithium and potassium *tert*-butoxide since, when either of the reagents was used alone, the yield of 2 was low.



Both benzyl groups in 2 were removed by refluxing with 4 *N* HCl giving the target compound in surprisingly good yield. The reactivity of the 4-vinyl group in 4-vinylpyridine[5] has apparently been reduced in 4-VPAL (3) due to the electron-donating power of the phenolic group ortho to the vinyl, resulting in a relatively stable compound.

In order to assess the potency and the relationship of the new analog 3 with vitamin B_6, we have determined its acute toxicity in mice maintained on a complete or B_6-deficient diet.[6] A comparison was made with 4-deoxypyridoxine (4-DOP, 4) under the same conditions§ (Table I). As evident from the data presented in Table I, 4-VPAL is a much more potent vitamin B_6 antagonist than 4-DOP, since its acute toxicity occurs at doses 100 times smaller than those of 4-DOP. Approximately 1 hr after the administration of toxic levels of 4-VPAL, mice exhibited clonic seizures followed by tonic convulsions indicating CNS toxicity. In comparison to their toxicity in animals on a complete diet, the toxicity of 4-DOP is increased tenfold in mice kept on a B_6-deficient diet for 6 weeks, whereas that of 4-VPAL is increased threefold (Table I). This slight increase in acute toxicity in animals depleted of vitamin B_6 is difficult to explain since the acute mortality of 4-VPAL is reversed competitively by pyridoxal (Figure 1). The effects of 4-VPAL are also competitively reversed by pyridoxal in mouse mammary adenocarcinoma (TA3) cells grown in culture.[8]

Forms of the vitamin other than pyridoxal, while less effective, also completely reversed the acute toxicity of 4-VPAL when administered by either the ip or ic route (Table II). In all cases administration by the ic route was much more effective than ip injection, again indicating the CNS toxicity of 4-VPAL. The effectiveness of the various vitamers in reversing the toxicity of 4-VPAL is in the following order: pyridoxal (PAL) > pyridoxine (PIN) > pyridoxamine (PAM). It is interesting to note that PAL is the least polar compound and PAM the most polar. This suggests that the lipid solubility of the vitamer may be a determining factor in its efficacy as a reversing agent. Subsequently, we have tested three lipid-soluble derivatives of PIN and PAL with the hope that they would permeate more readily into brain tissue and thus increase the selectivity of the protection. 5'-Adamantoyl-PIN[9] was found to

§ Rosen, *et al.*,[7] have reviewed the pharmacological aspects of 4-DOP and other B_6 antagonists.

Table I. Comparison of the Acute Toxicity of 4-DOP and 4-VPAL in Mice on Complete Synthetic or B_6-Deficient Diets[a]

Complete synthetic			B_6 deficient		
Drug	mg/kg	30-Day survivors total	Drug	mg/kg	30-Day survivors total
4-DOP	150	5/5	4-DOP	10.0	5/5
4-DOP	250	0/5	4-DOP	25.0	1/5
4-VPAL	1.0	5/5	4-VPAL	0.5	5/5
4-VPAL	3.0	0/5	4-VPAL	1.0	0/5

[a] Female DBA/2HaDD mice (19–22 g) were maintained on the respective diets for 6 weeks prior to a single injection of drug (ip).

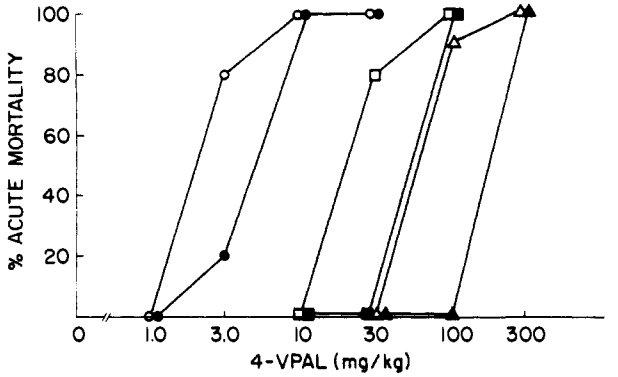


Figure 1. Reversal of the acute toxicity of 4-VPAL by PAL. Female DBA/2HaDD mice (19–22 g) were injected ip with 4-VPAL followed immediately by an ip injection of PAL: 0 or 0.3 mg/kg, ○–○; 1.0 mg/kg, ●–●; 3.0 mg/kg, □–□; 10 mg/kg, ■–■; 30 mg/kg, △–△; 100 mg/kg, ▲–▲. Ten mice were in each group. Mortality occurred within 48 hr after injection of the drugs.

Table II. Reversal of the Acute Toxicity of 4-VPAL (10 mg/kg) by B_6 Vitamers and Derivatives[a]

Vitamer or derivative	mg/kg ip	30-Day survivors total	mg/kg ic	30-Day survivors total
None		0/6		0/6
Pyridoxal (PAL)	0.3	0/6	0.1	2/9
Pyridoxal (PAL)	1.0	0/6	0.3	5/12
Pyridoxal (PAL)	3.0	6/6	1.0	6/6
Pyridoxamine (PAM)	3.0	0/6		
Pyridoxamine (PAM)	10.0	1/6	1.0	1/6
Pyridoxamine (PAM)	30.0	1/6	3.0	3/6
Pyridoxamine (PAM)	100.0	5/6	10.0	6/6
Pyridoxine (PIN)	3.0	0/9	1.0	0/3
Pyridoxine (PIN)	10.0	5/6	3.0	3/3
5'-Adamantoyl-PIN	3.0	0/3		
5'-Adamantoyl-PIN	10.0	5/6	1.0	2/3
Isopropylidene-5'-adamantoyl-PIN	10.0	0/6	3.0	0/3
Isopropylidene-5'-adamantoyl-PIN	30.0	3/3	10.0	1/3
3-*p*-Nitrobenzoyl-4',5'-isopropylidene-PIN	10.0	0/3	1.0	0/3
Dipalmitoyl-PAL	10.0	0/3	1.0	0/3

[a] Female DBA/2HaDD mice (19–22 g) were injected ip with 4-VPAL (10 mg/kg) followed immediately by injections of the various vitamers (either ip or ic). Mortality occurred within 48 hr after injection of the drugs. Limited availability of drugs precluded the use of larger numbers of animals per group.

be a slightly better reversing agent than PIN on a molar basis (Table II). Presumably, the adamantoyl group is removed by hydrolysis before the compound penetrates the cell membrane.# The other two derivatives, which have the less readily hydrolyzable ester and isopropylidene groups, were either less effective (isopropylidene-5′-adamantoyl-PIN[9]) or completely ineffective (3-*p*-nitrobenzoyl-4′,5′-isopropylidene-PIN[10] and dipalmitoyl-PAL[11]).

Thus, by a minor modification of the 4 position, we have obtained an analog of pyridoxal which is a more potent vitamin B_6 antagonist than 4-DOP and also has a different type of action. The biochemical basis for the potency of 4-VPAL is currently under investigation.**

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Tlc (Silica gel) was used routinely as described earlier.[13] Ir spectra were determined with a Perkin-Elmer 457 spectrophotometer, and nmr spectra with a Varian A-60A instrument. Peaks were assigned on the basis of previous work.[14]

3,α^5-*O*-Dibenzyl-4-deformyl-4-vinylpyridoxal (2). Triphenylmethylphosphonium bromide (1.62 mg as a 21.5 wt % hexane solution) and potassium *tert*-butoxide (900 mg)–*tert*-butyl alcohol (1:1) in dry ether (8 ml). After stirring for 2 hr, 3,α^5-*O*-dibenzylpyridoxal (1, 1.05 g, 3 mmol) in THF (4 ml) was slowly added to the ylide solution, and the reaction mixture was stirred for about 15 hr, when the reaction was complete, as indicated by tlc (EtOAc was used for development). After filtration the solution was washed with concentrated $NaHSO_3$ solution, 20% NH_4Cl solution, and H_2O. After drying ($MgSO_4$), the filtrate was evaporated and the oil was taken up in a small amount of EtOAc and chromatographed on a silica gel column (BIO-SIL "A," 100–200 mesh). Elution with EtOAc was followed by tlc; the combined fractions were evaporated yielding 740 mg (71%) of a pale yellow oil (740 mg, 71%) which was converted to the hydrochloride in ethereal HCl: mp 114° (from acetone); nmr [$(CF_3)_2CO \cdot D_2O$, from Tier's salt] δ 2.45 (2-CH_3), 4.70 (2CH_2), 5.00 (CH_2), 6.55–7.03 (m,†† 4-C*H*=CH_2), 5.90–6.22 (m,†† 4-CH=C*H*$_2$), 7.40 (phenyl), 8.27 (C_6-H); ir λ_{max} (KBr) 1630 cm^{-1} (CH=CH_2). *Anal.* ($C_{23}H_{24}ClNO_2$) C, H, Cl, N.

4-Deformyl-4-vinylpyridoxal (3). Hydrolysis of benzyl groups in **2** (200 mg, 0.5 mmol) was accomplished by refluxing with 4 *N* HCl (5 ml) for 22 hr. The solution was evaporated to dryness and coevaporated with H_2O to remove the benzyl alcohol. The solid residue was recrystallized from acetone, yielding 75 mg (72%) of a yellowish powder: mp 204° dec (from MeOH–acetone); nmr (D_2O, from Tier's salt) δ 2.70 (2-CH_3), 4.83 (5-CH_2), 6.80–7.13 (m,†† 4C*H*=CH_2), 5.85–6.18 (m,†† 4-CH=C*H*$_2$), 8.28 (C_6-H); ir λ_{max} (KBr) 1625 cm^{-1}.

Acknowledgment. This work was supported by U. S. Public Health Service Grants CA-13038 and CA-08793. The technical assistance of Mrs. Jo Ellen Budnick is gratefully acknowledged.

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#5′-Adamantoyl-PIN was found to be inactive as an inhibitor of mouse mammary adenocarcinoma cells but was found to be a weak inhibitor of *S. carlsbergensis*; see ref 9.

**The 5 isomer of 4-VPAL, 3-hydroxy-4-(hydroxymethyl)-2-methyl-5-vinylpyridine, has recently been prepared and was found to be inactive as an inhibitor of the growth of mammary adenocarcinoma cells in tissue culture.[12]

††These complex multiplets are typical of an ABC spectrum as found in styrene; see, *e.g.*, ref 15.

Book Reviews

Progress in Drug Research. Vol. 16. Edited by E. Jucker. Birkhäuser Verlag, Basel and Stuttgart. 1972. 472 pp. 23.6 × 18 cm.

While research on drug design and drug action plods ahead in a few new and many older fields, most medicinal scientists in the pharmaceutical industry anxiously contemplate whether even their best efforts will *ever* lead to a new clinically useful drug. In an article on the art and science of contemporary drug development, A. J. Gordon and S. G. Gilgore (Pfizer Pharmaceuticals) review all stages of development to final FDA approval and product marketing for therapeutic chemicals. Although one cannot generalize a typical chronology of a new program of conception, development, and preclinical and clinical testing, one can estimate average time ranges for these activities. They vary with the type of drug (life-saving antibiotics take the least time), the number of investigators and patients involved, the sociopolitical climate at the time of the study, and FDA attitudes reflecting such a climate. Overall time periods from initiation of chemical research to approval of marketing average 5.5 to 17 years, with 11.25 years appearing as a desirable goal for a functional drug. These sobering figures and the hurdles along the way should be considered carefully by any company embarking on such a program. They suggest that only a few of the strongest organizations can undertake the development of a *novel* drug. The days when a small newcomer could enter the competition are over, probably once and for all; those were the days, my friend. . . .

One of the fields in which novel drugs are generally expected is immunosuppression. Effective immunosuppressive agents are available and the present wide search is aimed at *better* drugs than those we now have. G. W. Camiener and W. J. Wechter (Upjohn Co.) present all stages of chemical immunosuppression, their complex mechanisms of action, accessory procedures such as enhancement, thymectomy, and radiation assistance, and pitfalls and applications in diseases with autoimmune components. Since the latter reach into such divergent symptomatology as arthritis and cancer, the well-