with 5.3 g of CH_2N_2 gave 8.9 g of a dark viscous oil which gradually solidified, mp 110–111° (MeOH).

A soln of guanidine (from 9.7 g of guanidine hydrochloride and 5.4 g of NaOMe) and 8.9 g of the above described methylated aldehydonitrile in 125 ml of MeOH was refluxed for 18 hr. The MeOH was evapd and the residue extd with hot THF. The residue from the THF soln was chromatogd through base-treated silica gel. The product, 1.2 g, was found in the second Me_2CO eluate.

2,4-Diamino-5-(3,4,5-trimethoxyphenylazo)pyrimidine Sulfate (2). To a soln of 20.6 g of 3,4,5-trimethoxyaniline in 250 ml of 1 *N* HCl at 0° was added 8 g of $NaNO_2$ in 40 ml of H_2O. This soln was added to 11 g of 2,4-diaminopyrimidine in 110 ml of H_2O at 0°. After 5 min sufficient $NaHCO_3$ (about 35 g) was added to raise the pH to 8. The red-brown solids, formed after standing overnight, were filtered and extd with 500 ml of boiling 1 *N* H_2SO_4. On cooling the H_2SO_4 soln pptd 3 g of **2** as a yellow solid.

2,4-Diamino-5-(3,4,5-trimethoxybenzamido)pyrimidine Monohydrate (3). To a cold soln of 2,4,5-triaminopyrimidine[7] (1 g) and 1.5 g of Et_3N in 20 ml of H_2O was added 1.95 g of 3,4,5-trimethoxybenzoyl chloride in 20 ml of THF. The mixt was stirred for 20 min and concd. The pptd solid was filtered and recrystd, yield 2 g.

2,4-Dichloro-5-pyrimidinecarbonyl Chloride. 2,4-Dihydroxy-5-pyrimidinecarboxylic acid was converted into the titled compound by the procedure Gershon[8] used to prep the analogous 6-pyrimidinecarbonyl chloride, bp 90–91° (0.3 mm), 50% yield. *Anal.* ($C_5HCl_3N_2O$) N, Cl.

2,4-Dichloro-5-pyrimidinecarbox-3,4,5-trimethoxyanilide (4a). Et_3N (1.62 g) and 2.84 g of 3,4,5-trimethoxyaniline in 100 ml of THF was added to a cold soln of 3.3 g of 2,4-dichloro-5-pyrimidinecarbonyl chloride in 50 ml of THF. After standing overnight the reaction was filtered and the THF evapd. The residue was washed with cold dil $NaHCO_3$ and recrystd from PhH–hexane, mp 196–198°. *Anal.* ($C_{14}H_{13}Cl_2N_3O_4$) N, Cl.

2,4-Diamino-5-pyrimidinecarbox-3,4,5-trimethoxyanilide Monohydrate (4). A mixt of 5 g of **4a**, 13 ml of NH_4OH, and 66 ml of liquid NH_3 was heated in an autoclave for 8 hr at 180°. The bomb was cooled, and the contents were filtered and recrystd, yield 3 g.

2,4-Diamino-5-(3,4,5-trimethoxybenzoyloxy)pyrimidine (5). To a cold soln of 1 g of 2,4-diamino-5-hydroxypyrimidine hydrochloride[9] and 3.1 g of Et_3N in 20 ml of THF–H_2O was added 1.36 g of 3,4,5-trimethoxybenzoyl chloride in 15 ml of THF. After 20 min the reaction mixt was concd, poured into a large vol of H_2O, filtered, H_2O washed, and recrystd, yield, 1.8 g.

3,4,5-Trimethoxybenzylideneisopropylamine. A soln of 40 g of 3,4,5-trimethoxybenzaldehyde and 35 ml of *i*-$PrNH_2$ in 200 ml of THF was refluxed for 5 hr. Evapn of solvent left 48 g of a white solid, mp 76.5–77.5° (MeOH–H_2O). *Anal.* ($C_{13}H_{19}NO_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzylideneamino)pyrimidine (6). An EtOH soln, 350 ml, contg 15.4 g of 3,4,5-trimethoxybenzylideneisopropylamine and 8.1 g of 2,4,5-triaminopyrimidine was refluxed for 20 hr and then allowed to stand at room temp for 2 days. The pptd yellow solid was filtered and recrystd. Addl quants of **6** could be obtd by concn of the filtrate.

2,4-Diamino-5-(3,4,5-trimethoxybenzylamino)pyrimidine Acetate Salt (7). Low pressure hydrogenation of 2.5 g of **6** in 140 ml of HOAc over PtO_2 occurred in 15 min. The reaction mixt was filtered, poured into a large vol of Et_2O, filtered, and repeatedly washed with Et_2O, yield 2 g.

2,4-Diamino-5-(3,4,5-trimethoxybenzylamino)pyrimidine (8). An H_2O soln of **7** was made basic with NH_4OH, cooled, filtered, and recrystd.

3,4,5-Trimethoxyphenyl Isocyanate. To a slurry of NaN_3, 19.5 g in 125 ml of PhMe was added 23.1 g of 3,4,5-trimethoxybenzoyl chloride in 250 ml of PhMe. Stirring was contd until the characteristic acyl halide ir absorption was absent. The mixt was then heated on a steam bath until N_2 evoln ceased. It was cooled, filtered, and distd (14.4 g), bp 120–140° (0.1 mm). This product solidified on standing, mp 43–43.5°. *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

3,4,5-Trimethoxyphenyl Isothiocyanate. To $CSCl_2$ (25 g) suspended in 180 ml of ice H_2O was added 30.7 g of 3,4,5-trimethoxyaniline in 300 ml of $CHCl_3$, maintg the temp below 5°. After 15 min the $CHCl_3$ layer was sepd and dried over $CaCl_2$. Distn gave 24.8 g of product, bp 145° (0.5 mm). The distillate solidified on standing, mp 63° (hexane). *Anal.* ($C_{10}H_{11}NO_3S$) C, H, S.

1-[5-(2,4-Diaminopyrimidinyl)]-3-(3,4,5-trimethoxyphenyl)urea Monohydrate (9). To a soln of 2 g of 2,4,5-triaminopyrimidine in 150 ml of 50% EtOH was added 2.8 g of 3,4,5-trimethoxyphenyl isocyanate. After several hours stirring at room temp the ppt was filtered and washed with H_2O and EtOH, yield 4 g.

1-[5-(2,4-Diaminopyrimidinyl)]-3-(3,4,5-trimethoxyphenyl)-thiourea (10). Powdered 3,4,5-trimethoxyphenyl isothiocyanate (2 g) was added to a soln of 2,4,5-triaminopyrimidine in 40 ml of 75% EtOH. After 1 hr stirring the ppt was filtered and washed thoroughly with H_2O and EtOH.

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Preparation and Antitumor Activity of Derivatives of 1-Phenyl-3,3-dimethyltriazene†

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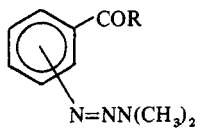
Received May 20, 1971

A number of 5-triazenoimidazoles are active against experimental tumors.[1] An excellent and comprehensive review of this subject has recently appeared.[2] Of the 5-triazenoimidazoles, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) in particular, is clinically useful for the induction of temporary remission in malignant melanoma.[3] The mechanism of the antitumor action of DIC remains obscure. However, on the basis of studies of the biotransformation of DIC[4,5] and the carcinogenesis of phenyltriazenes, it has been proposed that the triazenes may act as alkylating agents through the *in vivo* generation of carbonium ions.[6] Nevertheless, it is entirely possible that as a derivative of 5-aminoimidazole-4-carboxamide (AIC), the precursor of the purine base, 5-triazenoimidazole may somehow interfere with imidazole and purine metabolism.[7]

Antitumor activity has been observed in several derivatives of phenyltriazene.[8] To extend these observations and, above all, to elucidate the structural requirements for antitumor activity in the triazenes and specifically to ascertain whether an imidazole ring with a carboxamide moiety ortho to the triazeno side chain is indispensable in DIC, we have synthesized 6 derivatives of 1-phenyl-3,3-dimethyltriazene (**1a–1f**). Except for **1d**,[9] these compounds have not been

†Supported in part by Contracts PH 43-66-1156 and PH 43-68-1283 with Chemotherapy, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.



1

1a, R = NH_2, *o*-
b, R = NH_2, *m*-
c, R = NH_2, *p*-
d, R = OCH_3, *o*-
e, R = OCH_3, *m*-
f, R = OCH_3, *p*-

described previously. They were readily prepared by treating the corresponding diazotized methyl aminobenzoates and *m*- and *p*-aminobenzamide, respectively, with Me_2NH in Na_2CO_3 soln.[9] However, because the diazotization of *o*-benzamide directly afforded 1,2,3-benzotriazin-4(3*H*)-one instead of the required diazo intermediate, **1a** was synthesized by a modified procedure. An ethyl benzoyl carbonate was prepared from *o*-(3,3-dimethyl-1-triazeno)-benzoic acid and ethyl chloroformate. Ammonolysis of the ethyl benzoyl carbonate which was not isolated gave **1a**.

Antitumor Activities. In Table I we compare the antileukemic activity of the 1-phenyl-3,3-dimethyltriazene derivatives with that of DIC. From these results, it is clear that although the tolerated dose range is lower for the Ph analogs, they are nevertheless as effective as DIC in increasing the survival time of the leukemic mice. In fact, at least one of these, **1f**, may have a more favorable therapeutic index than DIC. The determination of the activity of each compound against a variety of transplantable tumors is currently in progress.

The fact that 1-phenyl-3,3-dimethyltriazene with no annular substituent does not increase the survival of mice bearing L1210 leukemia (sensitive to methotrexate)[8] would suggest that the carboxamide moiety is necessary for antileukemic activity. However, our present work indicates that the $CONH_2$ group in the phenyltriazenes may be replaced with CO_2CH_3 with no adverse effect. Further, the orientation of either the $CONH_2$ or the CO_2CH_3 substituent with respect to the dimethyltriazeno side chain is not critical, as all 3 derivatives, (ortho, meta, and para) show comparable antileukemic activity. Finally, because these phenyltriazenes and DIC are equally active and because the pyrazole analog of DIC likewise displays antileukemic property,[10,11] it appears that the imidazole ring, the Ph ring, and the pyrazole ring are all equivalent therapeutically in the triazenes.

Experimental Section‡

Derivatives of 1-Phenyl-3,3-dimethyltriazene. The arom amine, (0.13 mole) was added to a mixt of 100 g of crushed ice and 33 ml of concd HCl with vigorous stirring at 0–5°. Diazotization was achieved by the slow addn, accompanied by thorough agitation, of $NaNO_2$ (9 g, 0.13 mole) dissolved in 25 ml of H_2O. After standing at 0–5° for 20 min, the above cold soln contg the diazo compound was carefully added to Me_2NH (40% in H_2O, 15.8 g, 0.14 mole) and ice (75 g) with stirring, the temp being kept between 5 and 15°. At the end of the mixing, the stirring was contd at room temp for an addl 2–3 hr. The light brown crude phenyltriazene derivative was collected by filtration and dried over P_2O_5 *in vacuo*; yields of the crude product were 94–100%.

The crude compound was dissolved in abs MeOH and treated with activated charcoal. The clarified MeOH soln was dild with H_2O to ppt the purified product. However, **1e** was recrystd from anhyd Et_2O instead.

***o*-(3,3-Dimethyl-1-triazeno)benzamide, 1a.** To a soln of *o*-(3,3-dimethyl-1-triazeno)benzoic acid (2 g, 10.35 mmoles in 30 ml of an equivol mixt of anhyd Et_2O and *p*-dioxane) was added Et_3N (1.39 ml, 10 mmoles) and ethyl chloroformate (1 ml, 10.3 mmoles, dild with 20 ml of anhyd Et_2O). After stirring at room temp for 30 min, the mixt was made ammoniacal with concd NH_4OH (2.1 ml, 15 mmoles) while stirring was contd for another 3.5 hr. The solid formed was removed by filtration. A second crop of the crude product was obtd by extg the filtrate with PhH followed by concn. The combined crude product was recrystd from PhH. An analytical sample was further recrystd from PhH–Et_2O (4:1 by vol, with the addn of a few drops of EtOH).

Table I. Comparison of the Antitumor Activity of Phenyl Analogs of DIC with DIC Itself in Mouse Leukemia L1210[a]

Dose,[b] mg/kg	DIC MST[c]	DIC ILS[d]	1a MST	1a ILS	1b MST	1b ILS	1c MST	1c ILS	1d MST	1d ILS	1e MST	1e ILS	1f MST	1f ILS
Treatment Day 1 only														
833	12 (3)	20												
500	11 (2)	10			2	0	2	0	2	0	10 (2)	0	7 (6)	0
300	11 (2)	10	12.5 (3)	25	6 (6)	0	10.5 (5)	5	10.5 (5)	5	11 (0)	10	14 (3)	40
180	10 (2)	0	11 (2)	10	13 (4)	30	12.5 (3)	25	12 (3)	20	11 (0)	10	11 (0)	10
108	11 (1)	10	11 (2)	10	12 (0)	20	10 (0)	0	10.5 (1)	5	10 (0)	0	11 (0)	10
65	10 (0)	0			10.5 (0)	5	10 (0)	0	11 (0)	10	10 (0)	0	10 (0)	0
Treatment Days 1, 5, and 9														
180	12 (0)	20			10.5 (4)	5	10.5 (4)	5			12.5 (0)	25	15 (2)	50
108	11 (0)	10	10.5 (2)	5	13.5 (4)	35	14 (2)	40	13 (2)	30	11 (0)	10	12.5 (0)	25
65	11 (0)	10	10 (2)	0	12.5 (3)	25	13 (1)	30	11.5 (0)	15	10.5 (0)	5	13 (0)	30
Treatment Days 1–9														
108	11.5 (3)	15			6 (3)	0	6 (3)	0			8 (3)	0	10 (2)	0
65	14 (2)	40	11 (2)	10	9.5 (2)	0	10.5 (2)	5			13 (3)	30	14 (1)	40
39	12 (0)	20	10 (1)	0	15 (2)	50	14 (1)	40			13 (3)	30	14 (1)	40
23	12.5 (0)	25	10 (1)	0	13 (1)	30	13.5 (1)	35			11 (0)	10	13.5 (0)	35

[a] BDF, mice inoculated ip with 10^5 L1210 leukemic ascites cells; 8 mice per group and 46 untreated controls. Median survival time of untreated controls: 10 days. [b] Treatment ip [c] Median survival time, days. Number in parenthesis represents the average body loss in grams on the 6th day. [d] Increase in life span, %. ILS of 20% or greater is suggestive of reproducible activity.

Table II.

Compound	Yield, %	Mp, °C	Molecular formula[a]
1a	56	127–131	$C_9H_{12}N_4O$
1b	73	145–146	$C_9H_{12}N_4O$
1c	78	176–178	$C_9H_{12}N_4O$
1e	70	43–45	$C_{10}H_{13}N_3O_2$
1f	80	103–104	$C_{10}H_{13}N_3O_2$

[a] All compounds analyzed within ±0.3% for C, H, N.

‡ Mp's were detd with a Fisher-Jones apparatus. Microanalyses were performed by Dr. William C. Alford and associates of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, to whom we wish to express our gratitude.

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Biochemical Studies on Drugs and the Central Nervous System. 1. Synthesis and Activity of Pyridoxal Derivatives (Studies on the Syntheses of Heterocyclic Compounds. 438[1])

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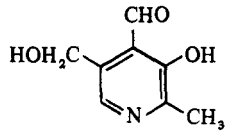
Received May 12, 1971

It is well known that vitamin B_6 has significant action on the CNS, and that it is of importance in the metabolism of brain cells. A number of studies on the pharmacological action of vitamin B_6 and its derivatives have been reported.[2,3]

We had found a method of synthesizing the various 1,2,3,4-tetrahydroisoquinoline derivatives by cyclization of the corresponding carbonyl compounds with 3-hydroxyphenethylamine derivatives III–VI without acid[4–7] as a catalyst. We wish to report the synthesis of 1,2,3,4-tetrahydro-1-pyridoxylisoquinoline derivatives (VIII–XI), 4-pyridoxyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridine (VII), and their Ac derivatives, by the application of this method using pyridoxal with several 3-hydroxyphenethylamines and histamine, respectively. Their activity on the CNS has also been examined.

Chart I



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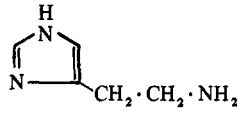

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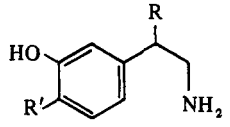

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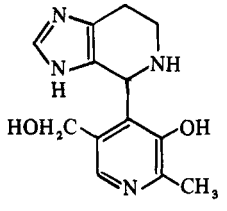
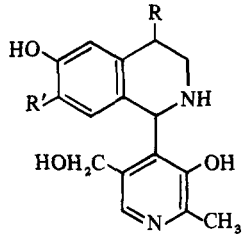
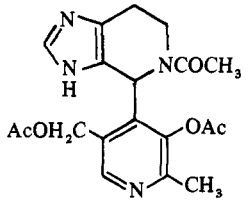
IV, R = OH; R′ = OH
V, R = OH; R′ = H
VI, R = H; R′ = OMe



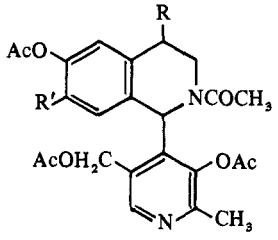
VII



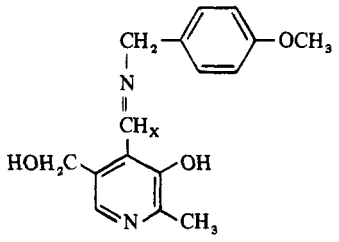
VIII, R = H; R′ = OH
IX, R = OH; R′ = OH
X, R = OH; R′ = OH
XI, R = H; R′ = OMe



XII



XIII, R = H; R′ = OAc
XIV, R = OAc ; R′ = H



XV

Table I. Products from Pyridoxal and 3-Hydroxyphenethylamines and with Histamine

	Starting materials			Product			
Compd	Pyridoxal, mg	Amine, mg		Yield, mg (%)	Mp, dec, °C	Appearance	Formula[e]
VII	92	II	150	160 (82)	252–254[b]	Colorless plates	$C_{13}H_{15}N_4O_2$[f]
VIII	420	III[a]-HCl	470	400 (53)	243–245[c]	Colorless needles	$C_{16}H_{18}N_2O_4$[f]
IX	836	IV	845	810 (51)	270–273	Colorless needles	$C_{16}H_{18}N_2O_5$
X	1050	V[a]-HCl	1020	630 (33)	243–245	Pale brown needles	$C_{16}H_{18}N_2O_4 \cdot 0.5H_2O$[d]
XI	410	VI[a]-HCl	500	120 (16)	198–200	Pale yellow needles	$C_{17}H_{20}N_2O_4 \cdot 0.5H_2O$[d]

[a]The free base was prepd as usual. [b]Lit.[10] mp 252–253° dec. [c]Lit.[11] mp 242–244° dec. [d]Dried over P_2O_5 at 120° (1 mm) for 24 hr. [e]C, H, N anal. [f]Not analyzed.

Table II. The Properties of Acetyl Derivatives of 1,2,3,4-Tetrahydro-6-hydroxy-4-pyridoxylisoquinolines and 4-Pyridoxyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridine

No.	Mp, °C	Recrystn solvent	Formula[a]
XII	181–183	MeOH-Et_2O[b]	$C_{21}H_{24}N_4O_6$
XIII	204–206	MeOH-Et_2O[c]	$C_{26}H_{28}N_2O_9$
XIV	241–243	MeOH[b]	$C_{26}H_{28}N_2O_9$

[a]C, H, N anal. [b]Colorless prisms. [c]Colorless needles.

Chemistry. 3-Hydroxyphenethylamine derivatives (III–VI), prepared by the usual method, were cyclized with pyridoxal to give the cyclized compounds listed in Table I.

Although the structure of the above products could be thought to be that of Schiff bases, this was ruled out by the following evidence. Treatment of VII–XI with dil HCl led