Table III. Pharmacological Activities of Pyridoxal Derivatives[a]

Compds	Behavioral observation	Barbiturate potentiating action: Relative activity	Judgement	Traction test: 30 min TT[b]	30 min FT[c]	90 min TT[b]	90 min FT[c]	Analgetic action: 45 min	90 min	Judgement
Aminophylline								1.63	1.25	++
VII	Pain response Flexor reflex	0.82	–	–	0	–	0	1.7	1.5	+++
VIII	No	0.90	–	–	0.07	–	0	1.0	0.9	–
IX	No	0.82	–	–	0	–	0	0.9	1.1	±
X	No	0.91	–	–	0	–	0	1.6	1.1	++
XI	No	1.46	±	–	0	–	0.07	1.6	1.4	++
XII	No	1.23	–	–	0	–	0	1.8	1.0	+++
XIII	No	1.13	–	–	0	–	0	1.0	0.8	–
XIV	No	1.36	±	–	0	–	0	0.9	0.7	–

[a] [Each number shows the mean value for 5 mice (ddy strain, ♂, 23-28 g)]. [b] TT = tranquilizing tendency. [c] FT = fallen tendency.

to recovery of the starting material (VII-XI); the lack of absorption due to C=N was observed in the ir spectrum; and, although the H_x proton of XV was resonant at τ 1,[8] there appeared no proton at a lower field than τ 2.1 in our product. Moreover, the uv spectrum showed the maximum characteristic of pyridoxal derivatives at 320 nm,[9] and of tetrahydroisoquinoline derivatives of 280 nm.[4-7] The compounds, VII, VIII, and X, were acetylated in the usual way to afford the Ac derivatives, which showed the absorption band attributable to NHC=O at around 1650 cm^{-1} in the ir (KBr).[8] Compounds VII and VIII were identical with the authentic sample prepared by Heyl and his coworkers.[10,11] These facts are consistent with the cyclic structures presented here.

Pharmacology. The compounds so obtained were tested for analgetic effect, traction, and hypnotic action using mice as described later in the Experimental Section. The results are listed in Table III. Compounds VII and XII, which are derivatives of histamine, were found to have slightly more analgetic activity than aminophylline; X and XI have the same analgetic effects as aminophylline, used as a control.

Experimental Section†

Cyclization of Pyridoxal (1) with Amines (Table I). A mixt of 150 mg (0.9 mmole) of pyridoxal, 92 mg (0.9 mmole) of histamine, and 5 ml of EtOH was heated on a water bath for 7 hr. Evap of the solvent gave a pale yellow powder, which was dissolved in 5 ml of 5% NaOH. After filtration, followed by neutralization with 5% HCl, the sepd crystals were collected by filtration. Since the compd was insol in all the solvents, purification was done by repptn.

Acetylation of Pyridoxal Derivatives (Table II). A mixt of 100 mg (0.5 mmole) of 4-pyridoxyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridine (VII), 2 ml of Ac_2O, and 1 ml of pyridine was heated on a water bath for 3 hr, and the excess reagents were distd off to leave the Ac derivative XII, recrystn of which from MeOH-Et_2O gave 120 mg (61%) of colorless prisms, mp 181-183°.

Pharmacological Tests (Table III). To male mice (ddy strain, 23-28 g), 100 mg/kg of each compd suspended in 1% arabic gum was administered per os. Barbiturate potentiating action, tractive action, and analgetic action were tested by the methods of Kuhn and coworkers,[12] Courvosier[13] and Burn,[14] respectively. After administration, general behavior was observed for 90 min and classified into 43 types.

Acknowledgments. The authors are indebted to the Psychopharmacology Division of Research Laboratories, Chugai Pharmaceutical Co. Ltd., for the pharmacological data presented.

†Melting points were determined on a Yanagimoto microapparatus (MP-S2) and uncorrected.

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Antimalarial Compounds.† 12.[1] Guanidine Derivatives of Diphenyl Sulfone and Related Compounds

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Continuing our research on potential antimalarial agents,[2] a series of biguanide and amidineurea derivatives of *p*-halodiphenyl sulfones, *p*-nitrodiphenyl ether, *p*-nitrodiphenylmethane, and *p*-nitrodiphenylamine was prepared. In the present work we tried to investigate to what extent the nitro and sulfo group were responsible for the antimalarial activity of *p*-nitrodiphenyl sulfone derivatives.[2]

Chemistry. The starting amines I-VI were prepared according to the literature[3-6] and subsequently caused to react

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Table I.

No.	Toxicity, mg/kg (mice) LD_{50} po	$LD_{0/4}$[a] sc	$LD_{0/4}$[a] po	Antimalarial activity (parasitaemia relative to controls) mg/kg per day sc 1	3	10	30	100	300
VII	1166	100	200				93.7	89.8	41.4
VIII	2500	100	200				100	55.9	55.9
IX		100						73.1	52.8
X		< 100							Inactive
XI		> 300							Inactive
XII	450	100	17			100	71.6	18.7	0
XIII	660	100	17		100	85.1	77.8	58.7	9.0
XIV	830	100	75				83.3	83.5	51.5
XV		> 300							Inactive
XVI		> 300							Inactive
XVII		> 100						Inactive	

[a]The highest dose, administered on 4 consecutive days, that produced no deaths.

VII-IX and XII-XIV showed a similar level of subacute toxicity sc but the latter were more toxic orally, probably due to better oral absorption than VII-IX. XI and XV-XVII were significantly less toxic sc while X was the most toxic.

Antimalarial Activity.§ The antimalarial activity of VII-XVII was tested against *Plasmodium berghei* in mice sc (Table I). Compounds VII-IX and XII-XIV showed some antimalarial action, XII being the most active. In no case was complete activity obtained at the $LD_{0/4}$ or below. No activity was detected at the maximum doses tested in X, XI or XV-XVII.

Experimental Section

All analytical data of the new compounds were in agreement with the calcd ones for the expected structures which were also confirmed by ir spectra.

Table II.

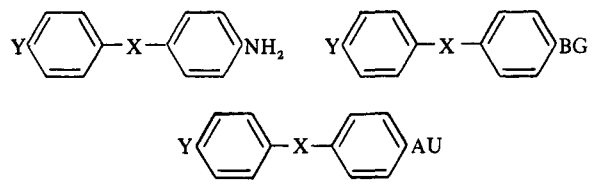
Amine No.	Amine Wt, g	Cyanoguanidine, g	35% alc HCl, ml	EtOH, ml	Reaction time, min	BG	Mp, °C	Yield g	Yield %	Recrystn solvent
II	7.5	5.0	10.0	30.0	60	VII	178-179	3.8	40	40% EtOH
III	6.0	6.0	7.0	25.0	60	VIII	175-176	3.5	35	40% EtOH
IV	7.5	5.0	5.0	50.0	60	IX	180-181	7.0	65	50% EtOH
V	7.5	5.0	7.5	20.0	120	X	174-175	6.1	65	MeOH + EtOH

Table III.

Biguanide No.	Biguanide Wt, g	10% HCl, ml	Reaction time, min	Amidineurea	Mp, °C	Yield g	Yield %	Recrystn solvent
VII	2.0	10	15	XIII	190-191	1.1	52	60% EtOH
VIII	2.0	10	15	XIV	187-188	1.2	60	60% EtOH
IX	1.0	20	120	XV	215-217	0.2	20	80% EtOH
X	1.0	20	90	XVI	206-208	0.2	15	80% EtOH
XI	1.5	20	150	XVII	>260	0.7	37	DMF

with cyanoguanidine in alcoholic HCl. *p*-Nitro-*p'*-biguanidodiphenylamine (XI) was prepared from VI and cyanoguanidine in the presence of pyridine hydrochloride. Amidineureas XII-XVII were prepared by hydrolysis of the corresponding biguanides. For details see Experimental Section.

Scheme I



BG = -NHCNHCNH$_2$ (NH, NH) AU = -NHCONHCNH$_2$ (NH)

Amine	BG	AU	X	Y
I		XII	SO_2	F
II	VII	XIII	SO_2	Cl
III	VIII	XIV	SO_2	Br
IV	IX	XV	O	NO_2
V	X	XVI	CH_2	NO_2
VI	XI	XVII	NH	NO_2

Toxicity.‡ Acute toxicity of VII, VIII, and XII-XIV was tested by oral administration and subacute toxicity of VII-XVII (4 daily doses) by oral and sc administration (Table I).

‡Tests were carried out partly at the Institute of Drugs, Warsaw (acute and subacute oral tests) and the Liverpool School of Tropical Medicine (subacute sc tests).

Biguanides VII-X were prepared as follows. The amine was dissolved in alc HCl, cyanoguanidine was added, and the mixt was refluxed. The resulting hydrochloride was made alk with 5% NaOH, dried, and boiled with PhMe to remove the unreacted starting amine (Table II).

The biguanide XI was prepared by refluxing 2.3 g of VI, 1.2 g of pyridine hydrochloride, and 1.0 g of cyanoguanidine in 10 ml of pyridine for 4 hr. The soln was poured into H_2O and made alk with 5% NaOH. The crude product upon washing with acetone crystd from 50% EtOH: mp 201-202°; yield, 1.1 g (48%).

Amidineureas XIII-XVII were obtained from biguanides VII-XI, respectively, on heating in dil HCl (Table III).

Toxicity.‡ Acute toxicity on oral administration was tested in white mice (Porton breed) in groups of 20. The compds were administered by gavage in a 5% suspension of aq gum arabic in a vol of 0.7-0.8 ml/20 g of body wt. The LD_{50} was calcd graphically by Litchfield and Wilcoxon's method as modified by Roth. The animals were observed for 7 days. Subacute toxicity was assessed by detg the highest dose administered on 4 consecutive days orally (as above) or sc (in a 4% soln of Tween 80), that produced no deaths in groups of 10 or 15 mice. Doses (sc) were contained in a vol of 0.2 ml/20 g of body wt (Table I).

Antimalarial Activity.§ All tests were carried out in white mice (CFl line) infected ip on day 0 with donor blood contg approximately 10^7 parasitised red blood cells. Animals received 4 consecutive daily doses of drug in 0.2 ml of soln or suspension sc from day 0 through day +3. The percentage of parasitised red blood cells was counted in treated groups of animals on day +4 and compared with the percentage in saline-treated controls.

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§Tests were carried out by the method of Peters,[7] at the Liverpool School of Tropical Medicine.

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Synthesis and Enzymological Activity of 3-Hydroxy-2-*n*-propyl-4,5-pyridinedimethanol

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The conversion of 3-hydroxy-2-ethyl-4,5-pyridinedimethanol and 3-hydroxy-2-isopropyl-4,5-pyridinedimethanol to their corresponding aldehydes by yeast pyridoxine dehydrogenase was described by Melius and Marshall.[1] Also 3-hydroxy-2-methyl-6-chloro-4,5-pyridinedimethanol was found to be oxidized by the enzyme with an activity of the order of that for the *i*-Pr analog. The rate of reaction for the Me and Et analogs was about 4 times that for the *i*-Pr analog and the hydroxychloro compound. In the present report the synthesis and yeast pyridoxine dehydrogenase action on 3-hydroxy-2-*n*-propyl-4,5-pyridinedimethanol (VIII) is described.

The synthesis of VIII involved an initial condensation of ethyl-*n*-butyropyruvate and cyanoacetamide to form 4-carbethoxy-3-cyano-6-*n*-propyl-2-pyridone (I).[2] I was then carried through a sequence of reactions involving nitration,[3] chlorination, reduction with $SnCl_2$,[4] reduction with Pd-H_2,[3] hydrolysis with HCl,[3] diazotization, and reduction with $NaBH_4$,[6] to give finally VIII. Thus a modification of the reduction of the NO_2 group was utilized here, in which $SnCl_2$ was used in place of Fe which had been utilized in the preparation of the *i*-Pr analog.

The pyridoxine dehydrogenase enzyme used here was a preparation described by Morino and Sakamoto.[2] The assay of enzymatic activity toward VIII is given in Table I and compared with the activities of the compds prepared and studied by Melius and Marshall.[1]

Experimental Section†

4-Carbethoxy-3-cyano-6-*n*-propyl-2-pyridone (I) was prepd refluxing a soln of the Na salt of ethyl *n*-butyropyruvate[5] (208 g; 1.0 mole) and cyanoacetamide (92 g; 1.1 moles) in abs EtOH (1400 ml) for 3 hr. After standing at room temp overnight, the reaction mixt was chilled and treated with an ice cold soln made up by dilg concd HCl (200 ml) to 1200 ml with ice and H_2O. The crude product thus pptd was washed thoroughly with H_2O before being crystd from aq EtOH (3000 ml; (60:40) EtOH-H_2O) to give 152 g (65%) of I, mp 146–148°.

4-Carbethoxy-3-cyano-6-*n*-propyl-5-nitro-2-pyridone (II). Compd I (23.5 g; 0.1 mole) was nitrated with HNO_3-Ac_2O, essentially as described by Wuest,[3] to give, after recrystn from 50% aq EtOH, 18.5 g (66.3%) of II, mp 163–164°.

4-Carbethoxy-2-chloro-5-nitro-6-*n*-propylnicotinonitrile (III). Compd II (27.9 g; 0.1 mole) and PCl_5 (22.9 g; 0.11 mole) were mixed and heated together at 125 ±5° for 2 hr. $POCl_3$ was removed *in vacuo* before the residue was triturated with crushed ice until solidification was complete. Recrystn of the crude product from abs EtOH gave 21.2 g (71%) of III, mp 48–50°.

5-Amino-2-chloro-4-carbethoxy-6-*n*-propylnicotinonitrile (IV). Compd III (29.8 g; 0.1 mole), suspended in Et_2O was treated with a freshly filtered soln of $SnCl_2$ (78 g) in concd HCl (165 ml) in a manner analagous to that described by Greene and Montgomery.[4] The crude product was recrystd from abs EtOH (625 ml) to give 23 g (86%) of IV, mp 168–169°.

5-Amino-4-carbethoxy-6-*n*-propylnicotinonitrile (V). Hydrogenation of IV (26.8 g; 0.1 mole) over 5% Pd-$BaCO_3$[3] and work-up of the reaction mixt gave 10.5 g (45%) of V after recrystn from abs EtOH, mp 132–133°.

3-Amino-2-*n*-propylpyridine-4,5-dicarboxylic Acid (VI). Hydrolysis of V (15.6 g; 0.067 mole) with concd HCl[3] gave 9.0 g (60%) of the dicarboxylic acid VI, mp 215–216° dec.

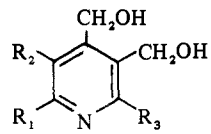
3-Hydroxy-2-*n*-propylpyridine-4,5-dicarboxylic Acid (VII). Compd VI (8.3 g; 0.037 mole) was diazotized at 80° in aq soln to give 4.1 g (49.4%) of VIII, mp 230–232°.

3-Hydroxy-2-*n*-propyl-4,5-pyridinedimethanol Hydrochloride (VIII). Reduction of the 2 CO_2H groups of VII (2.25 g; 0.01 mole) with $NaBH_4$-$AlCl_3$ as described by Blackwood[6] gave the *n*-Pr analog of pyridoxine hydrochloride (0.94 g; 41%); mp 210–212° dec. *Anal.* ($C_{10}H_{15}NO_3 \cdot HCl$) C, H, N.

Enzymatic assays were carried out as described by Melius and Marshall.[1] The yeast pyridoxine dehydrogenase (PDH) activity was measured by the reaction of phenylhydrazine with the aldehyde produced from the pyridoxine and its analogs.[7]

†Melting points are corrected and were determined in a Mel-Temp apparatus (Laboratory Devices, Cambridge, Mass.) Microanalyses were by Galbraith Laboratories, Knoxville, Tenn.

Table I. Enzyme Activity of Analogs

Compound	R_1	R_2	R_3	PDH activity, %
				
1 Pyridoxine	Me	OH	H	100
2 ω-Methylpyridoxine[a]	Et	OH	H	95
3 *i*-Pr analog[a]	*i*-Pr	OH	H	25
4 Cl analog[a]	Me	OH	Cl	22
5 *n*-Pr analog VIII	*n*-Pr	OH	H	5

[a] Data for these PDH activity estimates were obtd from Melius and Marshall.[1]

Acknowledgment. We wish to thank Miss Barbara K. Newton for her technical assistance.

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