TABLE II

ANTIMICROBIAL ACTIVITY[a] OF BROMO COMPOUNDS OF EUGENOL

		Antibacterial									Antituberculous		Antifungal			
Compd tested	pH	*Micrococcus pyogenes var aureus*	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhosa*	*Vibrio cholerae*	*Shigella dysenteriae*	*Diplococcus pneumoniae*	*Streptococcus pyogenes*	*Corynebacterium diphtheriae*	*Mycobacterium phlei*	*Mycobacterium 607*	*Microsporum gypseum*	*Trichophyton mentagrophytes*	*Candida albicans*	*Helminthosporium sativum*
I	4.9	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	7.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
IX	5.2	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	7.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
XI	5.5	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	7.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
II	5.5	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	7.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
VII	4.9	+	+	+	+	+	+	+	+	+	++	++	−	−	−	−
	7.0	+	+	+	+	+	+	+	+	+	++	++	−	−	−	−
VIII	4.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	7.0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
III	5.8	+	+	−	−	±	−	+	+	+	++	++	+	++	−	+
	7.0	±	+	−	−	−	−	−	+	+	++	++	+	++	−	+
Ia	5.5	−	−	−	−	−	−	−	−	−	++	++	−	±	−	−
	7.0	−	−	−	−	−	−	−	−	−	++	++	±	±	−	−
IIa	5.5	±	±	±	±	+	±	−	−	−	−	+	±	±	−	−
	7.0	±	±	±	±	+	±	−	−	−	+	+	±	±	−	−
IV	5.5	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
	7.0	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
X	5.5	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
	7.0	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
IIIa	5.8	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
	7.0	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−

[a] The concentration of the compound in the ditch was 10%. The tests for each compound were conducted at the normal pH of the compound and also at pH 7 by using a buffered solution. The activity is shown by: −, confluent growth across the ditch, *i.e.*, no inhibition; ±, sparse growth across the ditch, *i.e.*, slight inhibition; +, growth up to either side of the ditch, *i.e.*, moderate inhibition; ++, absence of growth across the ditch and ending some distance beyond the ditch on either side, *i.e.*, marked inhibition.

mann benzoylation of 2,3-dibromoeugenol (II) produced the corresponding benzoate (IIa) and bromination of the benzoate yielded 2,3-dibromoeugenol dibromide benzoate (IIIa).

Table I summarizes data of these compounds. The bromo compounds of eugenol were tested against a wide range of microorganisms by the ditch-plate technique[9] and have shown marked and, in some cases, specific activity. Results of these tests are summarized in Table II.

Experimental Section[10]

Bromination. General Method.—A solution of the requisite amount of bromine in 100 ml of acetic acid was added dropwise to a well-stirred, cooled solution of 0.1 mole of the compound to be brominated in 100 ml of acetic acid. The reaction mixture was allowed to stand for 1 hr after the addition of bromine was completed. If any solid product separated, it was filtered and recrystallized. The filtrate was diluted with water, filtered if necessary, and extracted with three 100-ml portions of ether. The ether extract was washed with water and dried (Na_2SO_4). The ether was evaporated and the residual oil was distilled *in vacuo*.

Side-Chain Debromination. General Method.—A solution of 5 g of the dibromide in 100 ml of ethanol was heated with 10 g of granulated zinc at 70–80° for 2 hr. The reaction mixture was then filtered, the filtrate was diluted with water, and the oil that separated was extracted with three 100-ml portions of ether. The ether extract was dried (Na_2SO_4). The ether was evaporated to give a residual oil which was distilled *in vacuo*.

Hydrolysis of Eugenol Dibromide Benzoates. General Method.—To a solution of 0.01 mole of the dibromide benzoate in acetic acid was added 3 ml of 65% H_2SO_4. Sufficient acetic acid was added to redissolve any solid that was precipitated on addition of the H_2SO_4. The mixture was refluxed gently on a steam bath until a test portion showed that the benzoate was completely hydrolyzed. After cooling and diluting with water, the reaction mixture was extracted with ether. The ether was evaporated and the residual liquid was digested repeatedly with hot water to remove all the benzoic acid. The oil was again taken up in ether. After drying the extract ($MgSO_4$), the ether was evaporated and the residual oil was distilled *in vacuo*.

Conversion of Bromoeugenols to Bromoveratric Acids. General Method.—The phenol (2.0 ml) from which the side-chain bromine atoms had been removed was dissolved in 20% NaOH solution. To this solution, sufficiently cooled, was added dropwise 5 ml of dimethyl sulfate. The reaction mixture was then refluxed for 0.5 hr. After cooling and diluting with water, it was extracted with ether. The ether extract was washed with dilute H_2SO_4 and then with water until the washings were neutral to litmus. The ether was evaporated and the residual oil was refluxed for 1 hr with alkaline $KMnO_4$. The reaction mixture was acidified (H_2SO_4) and excess $KMnO_4$ was reduced with sodium bisulfite. The solid obtained was filtered and recrystallized from aqueous ethanol or from ligroin (bp 100–120°).

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(10) Melting points were observed in capillary tubes and are corrected.

Arylazo Derivatives of Pyridoxine

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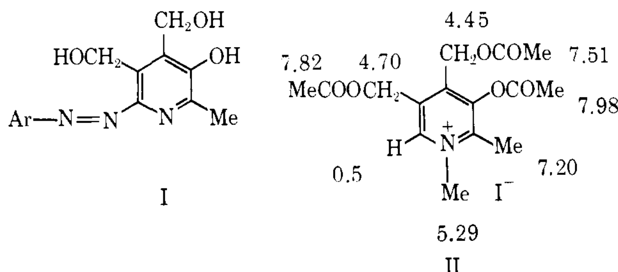
Pyridoxine couples readily at pH 8 with aryldiazonium chlorides to give good yields of 6-substituted derivatives (I, Table I). The coupling of pyridoxine with diazonium salts has been recorded as a color

TABLE I

ARYLAZOPYRIDOXINE HYDROCHLORIDES

6 substituent	Mp, °C dec	Formula	Calcd, % C	H	N	Found, % C	H	N
Phenylazo	190	$C_{14}H_{16}ClN_3O_3$	61.5	5.8	15.4	61.7	5.5	15.3
p-Chlorophenylazo	180	$C_{14}H_{15}Cl_2N_3O_3$	48.9	4.7	12.2	49.0	5.1	11.9
p-Tolylazo	180	$C_{15}H_{18}ClN_3O_3$	55.6	5.6	13.0	56.0	6.4	12.7
p-Nitrophenylazo	175	$C_{15}H_{15}ClN_4O_5$	47.3	4.2	15.8	47.6	4.4	15.4
p-Sulfonylazo[a]	180	$C_{14}H_{15}N_3O_6S$	47.6	4.3	11.9	47.3	4.7	11.6

[a] Not hydrochloride.



test,[1,2] but crystalline derivatives have not previously been reported. Hydrogenation or dithionite reduction of the phenylazo compounds gave 6-aminopyridoxine which was characterized as the tetrabenzoyl derivative. The structure of these compounds was confirmed by the nmr spectra in trifluoroacetic acid which showed no peak due to a ring proton in the 6 position.

Triacetoxypyridoxine gave the methiodide (II; nmr spectrum on τ scale); attempts to condense this with aromatic aldehydes were unsuccessful. Pyridoxine was condensed with ethyl bromoacetate, and also the N-oxide of the tribenzoyl derivative of pyridoxine was prepared. These and some further compounds[3] were tested for *in vivo* inhibition of Sarcoma 180 tumors in mice (see Table II). Antimicrobial tests were also carried out, but no significant biological activity was disclosed.

TABLE II

COMPOUNDS TESTED FOR INHIBITION OF SARCOMA 180 TUMORS

Compd	Activity[a]
2,4,5-Trihydroxymethyl-3-hydroxypyridine	−
3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-aldoxime hydrochloride	−
N-Hydroxypyridoxinium chloride	−
5-Acetoxy-3,4-dihydroxymethylpyridine-2-aldehyde N-oxide p-dimethylaminoanil	−
3-Ethoxymethyl-4-acetoxymethyl-5-hydroxy-6-methylpyridine N-oxide	−
3,4-Dihydroxymethyl-5-hydroxy-6-aminomethylpyridine dihydrochloride	−
Pyridoxinium-1-acetic acid	−
2-(N-Pyridoxinium)acetophenone	−
6-Phenylazopyridoxine hydrochloride	−
6-(p-Chlorophenylazo)pyridoxine hydrochloride	−
6-Tolylazopyridoxine hydrochloride	±[b]
p-[2-(3,4-Dihydroxymethyl-5-hydroxy-6-methyl)-pyridylazo]phenylsulfonic acid hydrochloride	−
2-Chloro-3,4,5-triacetylpyridoxine	−[c]
2-Aminopyridoxine hydrochloride	−
Pyridoxine N-oxide hydrate	−

[a] Compounds were tested at 500 mg/kg. [b] One test only. [c] Negative at 250 mg/kg (insufficient material for test at higher dosage).

Experimental Section

6-Phenylazopyridoxines.—The diazonium solution (from 9.3 g of aniline and 100 ml of 6 *N* HCl) was added at 5–10° with stirring to pyridoxine hydrochloride (21.2 g) in water (500 ml). Sodium hydroxide (2.5 *N*) was added simultaneously to keep pH 8. After stirring for a further 1 hr at 20°, 12 *N* HCl was added to pH 4, and the precipitate was filtered off and dried in a vacuum desiccator. Substituted compounds were prepared analogously. The products (Table I) crystallized from dilute HCl.

6-Amino-4,5-bishydroxymethyl-3-hydroxy-2-methylpyridine. A.—6-Phenylazopyridoxine (1 g) in ethanol (150 ml) was shaken under 3 atm of hydrogen over 10% Pd-C (0.2 g) at 20°. Hydrogenation was complete in 3 hr; steam distillation followed by acidification with 12 *N* HCl gave the **amino derivative** (0.2 g, 32%) which crystallized from ethanol as microcrystals, mp 180–185° dec.

Anal. Calcd for $C_8H_{13}ClN_2O_3$: C, 43.4; H, 5.9; N, 12.7. Found: C, 43.6; H, 5.9; N, 12.7.

B.—Sodium dithionite (20 g) was added to the alkaline azo solution (prepared from 10.3 g of pyridoxine hydrochloride) at 75° with stirring. After cooling, aniline was extracted with ether. The aqueous residue was acidified with 12 *N* HCl, kept 2 hr at 50°, filtered, and evaporated to dryness to give **6-aminopyridoxine hydrochloride** (7.3 g, 66%).

Anal. Calcd for $C_8H_{13}ClN_2O_3$: C, 43.4; H, 5.9; N, 12.7. Found: C, 43.5; H, 6.0; N, 12.7.

Benzoyl chloride (3.5 g) and 2.5 *N* NaOH (30 ml) was treated with 6-aminopyridoxine hydrochloride (1 g) to give the **tetrabenzoyl derivative** which separated from methanol as pale yellow crystals, mp 86–88°.

Anal. Calcd for $C_{36}H_{28}N_2O_7$: C, 72.0; H, 4.7; N, 4.7. Found: C, 71.6; H, 4.4; N, 4.5.

3-Acetoxy-4,5-bisacetoxymethyl-1,2-dimethylpyridinium Iodide.—Pyridoxine triacetate (5 g) and methyl iodide (15 ml) were refluxed for 12 hr in methyl cyanide (100 ml). Evaporation of the solvent and recrystallization of the residue from ethyl acetate gave the **methiodide** (7.4 g) as yellow plates, mp 114–114.5°.

Anal. Calcd for $C_{15}H_{20}INO_6$: C, 41.2; H, 4.6; N, 3.0. Found: C, 40.9; H, 4.9; N, 2.9.

1-(Ethoxycarbonylmethyl)-4,5-bishydroxymethyl-3-hydroxy-2-methylpyridinium Bromide.—Pyridoxine (5 g) and ethyl bromoacetate (6.0 g) were refluxed 2 hr in ethanol (20 ml). Cooling gave the **bromide** (6.2 g, 62%) which separated from ethanol in needles, mp 128.5–129°.

Anal. Calcd for $C_{12}H_{18}BrNO_5$: C, 42.9; H, 5.4; N, 4.2. Found: C, 43.0; H, 5.6; N, 4.1.

Nmr spectrum (in D_2O with $(Me_4N^+)_2SO_4^{2-}$ as internal standard at 6.81): 1.71 (6-H), 4.30 (4-CH_4), 4.89 (5-CH_2), 5.15 (N-CH_2), 5.75 (quartet)(CH_2 of Et), 7.35 (2-CH_3), 8.70 (triplet) (CH_3 of Et).

3-Benzoxy-4,5-bisbenzoxymethyl-2-methylpyridine 1-Oxide.—Pyridoxine tribenzoate[4] (1 g) was treated with monoperphthalic acid (2 g) in chloroform (10 ml) and ethanol (10 ml) for 4 days at 20°. The mixture was shaken with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated to give the **oxide** (0.8 g, 76%), which crystallized from benzene as needles, mp 133–134°.

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(4) A. Itiba and K. Miti, *Sci. Papers Inst. Phys. Chem. Res.* (Tokyo), **35**, 73 (1938); *Chem. Abstr.*, **33**, 3430 (1939).

Anal. Calcd for $C_{29}H_{23}NO_7$: C, 70.0; H, 4.7; N, 2.8. Found: C, 70.2; H, 4.7; N, 2.9.

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Studies on Latent Derivatives of Aminoethanethiols as Potentially Selective Cytoprotectants. V. Syntheses of S-(2-Aminoethyl) Methyl Hydrogen Phosphorothioate[1]

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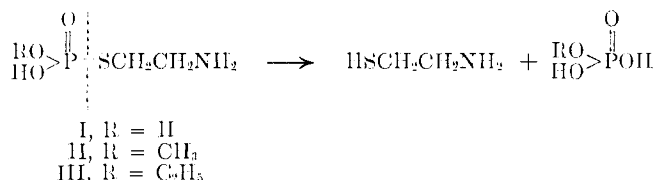
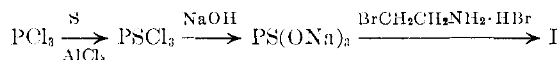
As prototypes of a class of latent cytoprotective agents, we became interested in the synthesis of derivatives of phosphorothioic acid which might release 2-aminoethanethiol *in vivo* as indicated. Should this release occur selectively in normal tissues sensitive to the damaging effects of either administered alkylating

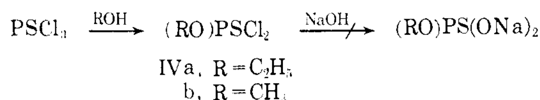


agents or radiation and not in a tumor, it would be possible to increase the safely tolerated dose of these therapeutic agents with consequent increase in therapeutic effect in cancer.

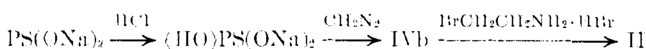
The sodium salt of the parent compound cysteamine S-phosphate (I) was prepared by the method of Åkerfeldt,[2] involving the reaction of 2-bromoethylamine hydrobromide with trisodium phosphorothioate, the latter being obtained by the reaction of sulfur with PCl_3 followed by treatment of the product with sodium hydroxide.[3]



We originally tried to synthesize III by an analogous route. Thiophosphoryl chloride was treated with ethanol to yield O-ethyl phosphorodichloridothioate. Hydrolysis of the latter with sodium hydroxide, however, failed to yield the desired disodium salt (IVa). The O-methyl analog (IVb) had been prepared[4] in 1911 by the partial hydrolysis of trimethyl phosphorothioate with sodium hydrogen sulfide. However, the procedure was cumbersome and the yields poor. Consequently, it was decided to seek a new route to IVb and hence to the desired II.



We have now succeeded in preparing II by the partial neutralization of trisodium phosphorothioate with hydrochloric acid, followed by methylation with diazomethane. This gave the monomethyl ester (IVb) in good yield. The latter reacted smoothly with 2-bromoethylamine hydrobromide to give S-(2-aminoethyl) methyl hydrogen phosphorothioate (II) as a yellow oil.



The diazomethane reaction is based on an earlier observation[5] that ionizable phosphoric acid groups in the acid form esterify readily and essentially quantitatively with diazomethane, whereas those in the salt form do not react at all. Since one would anticipate that the dianion of phosphorothioic acid would exist mainly in the (HO)POS⁻O⁻ form, we presumed that reaction with diazomethane would lead primarily to the O-methyl ester (IVb) that was indeed obtained.

Biological Studies.—When injected into Sprague–Dawley rats, cysteamine S-phosphate (I) produced significant concentrations of cysteamine in 13 of the 15 tissues studied, including spleen, brain, pancreas, intestine, thymus, lung, heart, blood, liver, kidney, colon, bone marrow, and tumor and produced insignificant levels in muscle and stomach. The compound was given intravenously at a dose of 300 mg/kg. The levels of cysteamine found in tissues were in the range of 0.5–2.5 μmoles/g of tissue. In contrast, the methyl ester II administered at the same dose level appeared to be essentially uncleaved *in vivo* since it gave undetectable amounts of cysteamine release in all of these tissues except bone marrow which has not been assayed.

Cysteamine was measured in tissue homogenates by a specific spectrophotometric method that was developed[6] for the purpose utilizing the known reaction[7] of aminoethanethiols with 3-fluoropyruvic acid. The reaction product absorbs maximally in the ultraviolet at 300 mμ (ε 5800).

Experimental Section

Disodium O-Methyl Phosphorothioate (IVb).—To 4.5 g (25 mmoles) of trisodium phosphorothioate, prepared according to the procedure of Yasuda and Lambert,[3] was added 25 ml of 1 N HCl and to this solution was added absolute methanol until the mixture became cloudy. An ethereal solution of CH_2N_2 was added to the cooled mixture and immediate effervescence occurred. The ether and water were removed under reduced pressure at 30°. The yellow oil which remained was taken up in hot methanol and filtered, and the filtrate was treated with charcoal and then evaporated to dryness. This procedure was repeated. Final removal of methanol and trituration with ether gave a white powder, mp 55.5–57.5°, yield 3.9 g (80%), lit.[4] mp 49°.

S-(2-Aminoethyl) Methyl Hydrogen Phosphorothioate (II).—To 2.39 g (14 mmoles) of IVb in 12 ml of water was added a solution of 3.3 g (16 mmoles) of 2-bromoethylamine hydrobro-

(1) Supported by Research contract PH43-62-170, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Bethesda, Md.

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