TABLE I

Compd	Code	Mp, °C	Formula
p-Dimethylaminoazobenzene	DAB	*a*	*a*
3′-Methyl-*p*-dimethylaminoazobenzene	3′-MeDAB	*a*	*a*
N,N-Dimethyl-*p*-(2-benzthiazolylazo)aniline	BT-2	130–132	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(4-benzthiazolylazo)aniline	BT-4	209	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(5-benzthiazolylazo)aniline	BT-5	171	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(6-benzthiazolylazo)aniline	BT-6	159	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(7-benzthiazolylazo)aniline	BT-7	150–151	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(4-benzimidazolylazo)aniline	BI-4	215–216[b]	*b*
N,N-Dimethyl-*p*-(5-benzimidazolylazo)aniline	BI-5	215[b]	*b*

[a] See ref 6. [b] See ref 4.

unable to prepare N,N-dimethyl-*p*-(2-benzimidazolylazo)aniline from 2-aminobenzimidazole either by the normal diazotization and coupling or the procedure of Brown and Faessinger.[6]

The 2-, 4-, 5-, 6-, and 7-benzthiazole analogs of DAB were all prepared from the corresponding amines and their melting points and formula are listed in Table I.

Experimental Section

All melting points were determined on a Fisher-Johns apparatus and are corrected. The C–H analyses were performed in this department on an F and M Model 185 analyzer by Mr. Daryl Sharp. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

4-Aminobenzimidazole was prepared according to van der Want.[7] Both this compound and 5-aminobenzimidazole were diazotized and coupled with N,N-dimethylaniline using the procedure reported by Montanari[5] to produce the benzimidazole dyes previously reported. This method and several minor modifications were then applied to 2-aminobenzimidazole but we did not obtain any dye. We then applied the sodium coupling with *p*-nitrosodimethylaniline as applied successfully to other 2-amino heterocycles by Brown and Faessinger[6] but again there was no evidence of azo dye formation.

4-, 6-, and 7-nitrobenzthiazoles were separated from the mixture formed on nitration of benzthiazole.[8,9] 7-Nitrobenzthiazole was also prepared by the method of Ward.[10] 5-Aminobenzthiazole was obtained according to Spieler.[11] All the nitrobenzthiazoles were reduced in the usual manner with $SnCl_2$–HCl. The azo compounds were prepared by diazotization and coupling of these amines. A typical procedure is given below and the various dyes prepared are listed with melting point and formula in Table I.

N,N-Dimethyl-*p*-(4-benzthiazolylazo)aniline.—4-Aminobenzthiazole (13.4 g) was diazotized in 14 ml of concentrated HCl and 150 ml of H_2O at 0–5° with 6.3 g of $NaNO_2$. Excess nitrite was destroyed after 1 hr by addition of urea, and coupling with 10.8 g of N,N-dimethylaniline and 12 g of anhydrous NaOAc in 100 ml of 50% EtOH–H_2O was allowed to proceed for 2 hr. At the end of this time the mixture was treated with excess NH_4OH. The dye was filtered, washed well (H_2O), and dried to give 19.8 g of crude azo compound. This was dissolved in 1500 ml of C_6H_6 and chromatographed on alumina. The red fraction eluted by C_6H_6 was concentrated and recrystallized from EtOH. See Table I.

Biological Properties.—Young male rats of the Sprague–Dawley strain, approximately 8 weeks of age and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of ten animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller, *et al.*,[12] to which had been added one of the azo compounds at a level of 0.03%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; cerelose, 770 g; Osborne and Mendel salt mixture, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate, 67,500 IU.

In each experiment, groups received DAB at the 0.06 as well as at the 0.03% level. The control group received only the basal diet. All of the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Results and Discussion

DAB (butter yellow) at the 0.06% level gave tumor incidences of 7/10 at 4 months and 9/10 at 6 months while at the 0.03% level it gave 5/10 in 6 months. On the other hand, 3′-MeDAB at 0.03% gave 5/10 in 4 months and 9/10 in 6 months. Our most active compound, BT-6, at 0.03% gave 5/10 in 1 month and 10/10 in 2 months. BI-4 gave 10/10 in 2 months at 0.03%, while BT-7 gave 10/10 in 3 months at this level. BT-2 at 0.06% and BT-4, BT-5, and BI-4 at 0.03% gave no tumors in 6 months at which time the experiment was terminated. The order of their carcinogenicity is BT-6 > BI-4 > BT-7 > 3′-MeDAB > DAB > BI-5, BT-4, BT-5, BT-2. The first two compounds mentioned, which produced multiple tumor nodules verified macroscopically and microscopically in 2 months or less, are certainly among the most powerful rat hepatocarcinogens ever reported.

Acknowledgment.—The authors are indebted to Dr. Daniel L. Weiss, Department of Pathology, University of Kentucky College of Medicine, for the microscopic evaluation of the tumors.

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Phosphorus–Nitrogen Compounds. X.[1,2] Sulfur Analogs

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Since 4,4,′4″-phosphinylidynetrissemicarbazide[2] (I) has shown confirmed activity against one tumor test system[3] it was advisable to prepare a related sulfur-con-

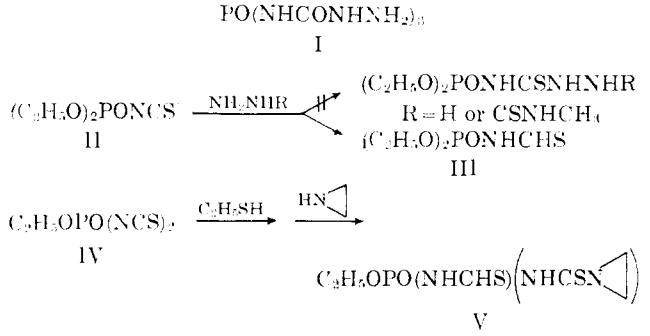
(1) This investigation was supported by Grant CA-08711 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see L. A. Cates, *J. Med. Chem.*, **11**, 382 (1968).

(3) Against Walker 256 carcinoma (im). Data from CCNSC. Confirmation defined according to *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

taining compound for screening.[4] Reactions between hydrazine or 4-methyl-3-thiosemicarbazide and diethyl phosphoroisothiocyanatidate (II) were expected to yield condensation products (Scheme I). All evidence

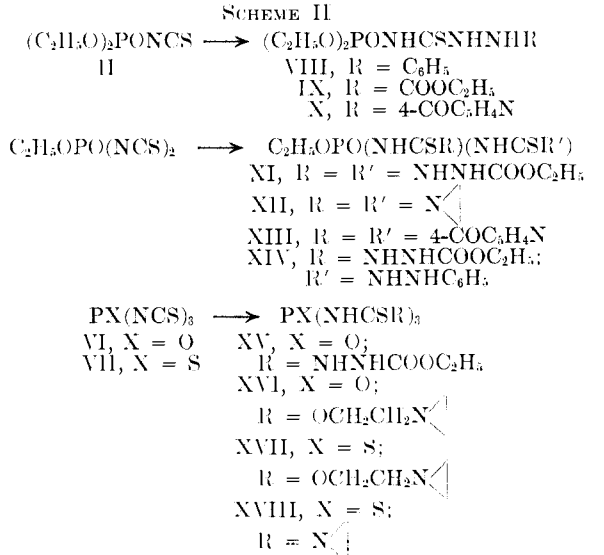
SCHEME I



indicates, however, that reduction of II to diethoxyphosphinylthioformamide (III) was the prevailing reaction. A similar reaction between ethyl phosphorodiisothiocyanatidate (IV) and ethanethiol, followed by addition of aziridine, gave a product (V) of both reduction and condensation. Elmore and Ogle[5] and Kulka[6] have shown that lowered yields of amine–phosphoroisothiocyanatidate condensation products possibly result from hydrolysis and isothiocyanatidate decomposition on storage. In the case of easily oxidized reactants the reduction of isothiocyanatidates may constitute a third source of interference.

The remaining products were synthesized *via* normal condensations between an amine, alcohol, hydrazine, or hydrazides and II, IV, phosphinylidyne trisisothiocyanate (VI), or thiophosphinylidyne trisisothiocyanate (VII) (Scheme II) and several of these are related to

SCHEME II



anticancer drugs and previous compounds of this series. XVIII, for example, is a thiocarbamoyl-containing thio-TEPA and sulfur analog of N,N',N''-phosphinylidynetris-1-aziridinecarboxamide.[7] The incorporation of the ethoxy, in lieu of urethan,[7,8] moiety in most of these derivatives is based on the recent findings of Chmielewicz, *et al.*,[9] that the former group confers greater antitumor activity.

Since antibacterial effect is often used as preliminary indication of potential antitumor activity these compounds were screened against three gram-positive and gram-negative microorganisms using the agar diffusion filter paper disk method (Table I). These results show that X possesses the broadest spectrum of activity and XIII the highest complete and partial inhibition of *Mycobacterium smegmatis*. These compounds contain isonicotinoylhydrazide (INH) moieties but are more closely related to 1-isonicotinoyl-3-thiosemicarbazide which has also been investigated for antimicrobial properties.[10] The minimum inhibition concentrations (MIC) of VIII, X, and XIII against *M. smegmatis* were determined using serial dilution tests in broth. XIII gave an MIC of 4 μg/ml. whereas VIII and X were 62 μg/ml each. Under these test conditions INH and ethanol gave an MIC of 8 and 500 μg/ml. respectively. Similar testing using *Mycobacterium tuberculosis* yielded MIC of 62 (XIII) and 125 (INH) μg/ml. These results indicate that XIII is more potent than INH against two acid-fast microorganisms *in vitro* and this activity may be due to a property of the intact phosphorylated compounds since XIII contains only 57% INH. Regarding a possible relationship between bioactivity and physical properties it is noted that XIII is soluble 25% in water compared to 14% for INH (25°). Studies[11] have shown that carbonyl and arylsulfonyl acylation of INH diminishes or abolishes antituberele activity, whereas a similar investigation of the effect of phosphorylation apparently has yet to be reported.

Experimental Section

Chemistry.—All compounds shown in Schemes I and II were analyzed for C, H, and N[12] (Coleman C-H and N analyzers). Ir spectra of all starting materials and products were taken on a Beckman IR-8 spectrophotometer and gave the expected absorptions. All products either decomposed on melting (III and VIII–X) or decomposed over a wide range before melting (Fisher-Johns apparatus).

Nmr spectroscopic examination[13] of II and V using a Varian HA-100 spectrometer revealed single, intense peaks at 3.12 (D_2O) and 3.45 (DMSO-d_6) ppm, respectively, and, with all other absorptions referable, these were assigned to thioaldehydic hydrogens. Positive Tollens tests were given by III, VIII–XI, and XIII–XV at 25° and positive Schiff tests by III, VII–X, XIII, and XVIII.[14] The thials, III and V, possess considerably

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(12) Analytical results obtained for these elements were within ±0.4% of the theoretical values.

(13) Performed by the Department of Chemistry, University of Houston, Houston, Texas.

(14) Positive reactions by compounds other than thials may be due to reduction by the sulfurous acid required in the test.

TABLE I
ANTIBACTERIAL ACTIVITY

Compd	Solvent	Zone of inhib, mm: *M. smegmatis*	*S. aureus*	*γ Strep.*	*E. coli*	*P. vulgaris*	*Ps. aeruginosa*
III	EtOH	25[b] (35)[c]					
III	H_2O	17 (25)					
VIII	EtOH	25 (33)	20	24			
IX	H_2O						
X	EtOH	42 (54)	19	20	18 (22)	19	19
XI	H_2O	15 (21)	20				
XII	EtOH[a]						
XIII	H_2O	36 (69)					15
XIV	EtOH	18	17				15
XV	H_2O	19	18				
XVI–XVIII	EtOH[a]						

[a] Suspension. [b] Complete inhibition. [c] Complete and partial inhibition.

greater odor than the other derivatives and gave qualitative P and S tests.

Synthesis.—The products were prepared using previously described methods[2,7] whereby II,[6] VI,[15] VI,[16] or VII[16] and the appropriate amine, alcohol, hydrazine, or hydrazide were treated to yield VIII ($C_{11}H_{18}N_3O_3PS$, 125°, 66%), IX ($C_8H_{18}N_3O_3PS$, 81°, 49%), X ($C_{11}H_{17}N_4O_4PS$, 147°, 38%), XI ($C_{10}H_{21}N_6O_6PS_2$, >80°, 53%, deliquescent), XII ($C_8H_{15}N_4O_2PS_2$, >115°, 76%), XIII ($C_{16}H_{19}N_8O_4PS_2$, >105°, 94%), XV ($C_{12}H_{24}N_9O_7PS_3$, >53°, 14%, deliquescent), XVI ($C_{15}H_{27}N_6O_4PS_3$, >95°, 91%), XVII ($C_{15}H_{27}N_6O_3PS_4$, >130°, 84%), and XVIII ($C_9H_{15}N_6PS_4$, >175°, 28%). Ether was the solvent, except in the case of XIII (CH_3CN), XVI and XVII (CH_3CN–ether, 1:1), and X (no solvent). Addition of II to hydrazine or 4-methyl-3-thiosemicarbazide (CH_3CN) gave III ($C_5H_{12}NO_3PS$, 129°, 61%). Consecutive additions of ethanethiol and aziridine to IV (ether) produced V ($C_6H_{12}N_3O_2PS_2$, >147°, 57%). Similar addition of phenylhydrazine and ethyl carbazate to IV (ether) yielded XIV ($C_{13}H_{21}N_6O_4PS_2$, >65°, 25%) which separated as an oil before solidifying. One mixture (XI) was heated (35°, 1 hr) to ensure complete reaction.

Antibacterial Screening.—The antibacterial spectra were determined by saturating filter paper disks (12.7 mm) with 2 drops of an aqueous or alcoholic solution or suspension of the compound (20 mg/ml) and placing these on agar (Bacto Nutrient Agar, Difco Lab.) seeded with 48-hr culture broths (Nutrient Broth, Baltimore Biological Lab.) of the test organisms (0.5 ml). The microbial spectrum consisted of *Mycobacterium smegmatis*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, a *γ Streptococcus*, and *Pseudomonas aeruginosa* from the collections maintained at the Biology Department, University of Houston. V did not dissolve or suspend well in either solvent and was not tested. Alcohol controls were also run. The zones of inhibition around the disks were measured after 4 days of incubation (37°).

The MIC of VIII, X, XIII, INH, and alcohol against *M. smegmatis* and XIII and INH against *M. tuberculosis*[17] were determined by a serial broth dilution method similar to that employed by Glasser and Doughty.[18] The tubes, containing concentrations of 1000 to 1 μg/ml, were examined for bacterial growth after incubation periods (37°) of 7 (*M. smegmatis*) and 10 days (*M. tuberculosis*).

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Some Aza Analogs of Amino Acids[1]

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In previous work, we described the preparation of 4-azaleucine (2-amino-3-dimethylaminopropionic acid) and its potent microbiological activities as a specific and competitive antagonist of leucine.[2] More recently, a compound identified as 2-amino-3-dimethylaminopropionic acid by structural studies was reported to have been isolated from culture media of a certain *Streptomyces* strain.[3] In view of the uniqueness of 4-azaleucine as a natural product and our particular interest in aza analogs of amino acids,[4,5] we undertook the synthesis of 4-azanorleucine, 5-azanorleucine, and 4-azanorvaline in order to determine their microbiological properties.

5-Azanorleucine was prepared in relatively poor over-all yield *via* an acetamidomalonic ester synthesis. Ethyl acetamidomalonate was condensed with N-benzyl-N-methyl-2-chloroethylamine in the presence of sodium ethoxide to form ethyl 2-acetamido-2-(N-benzyl-N-methyl-2-ethylamino)malonate. Acid hydrolysis of the condensation product gave the corresponding intermediate amino acid, 2-amino-4-(N-benzylmethylamino)butyric acid. Subsequent hydrogenolysis of the latter compound resulted in the formation of 5-azanorleucine.

The synthesis of 4-azanorleucine and 4-azanorvaline was accomplished by using the same general procedure in which the appropriately substituted amine underwent addition with 2-acetamidoacrylic acid to yield the

(1) The support of this work in part by Grant R-085 from the Robert A. Welch Foundation of Houston, Texas, and by Public Health Service Research Grant No. AM-4060-08 from the National Institute of Arthritis and Metabolic Diseases is gratefully acknowledged.

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