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 ($C_{16}H_{21}N_6O_6PS_2$, >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 ($C_{15}H_{21}N_6O_4PS_2$, >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

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TABLE I

PHYSICAL CONSTANTS, YIELDS, AND ANALYTICAL DATA OF AMINO ACID ANALOGS

Compd	Yield,[a] %	Mp,[a] °C	R_f[b]	Formula[c]
5-Azanorleucine·2HCl	72	200–201	0.085 (A), 0.34 (B), 0.33 (C)	$C_5H_{12}N_2O_2 \cdot 2HCl$
4-Azanorleucine·2HCl	75	175–177	0.14 (A), 0.64 (B), 0.48 (C)	$C_5H_{12}N_2O_2 \cdot 2HCl$
4-Azanorvaline·2HCl	63	181–183	0.10 (A), 0.52 (B), 0.48 (C)	$C_4H_{10}N_2O_2 \cdot 2HCl$

[a] Melting points and yields are for analytical samples. [b] The R_f values given for the appropriate solvents are indicated by letter: A, *n*-BuOH–AcOH–H_2O (4:1:1); B, 65% pyridine; C, 95% MeOH. [c] All compounds were analyzed for C, H, N.

corresponding 2-acetamido-3-alkylaminopropionic acid, followed by mild acid hydrolysis.

Biological Results.—Using *Escherichia coli* 9723 as the test organism, 5-azanorleucine, 4-azanorleucine, and 4-azanorvaline did not exert any observable inhibitory activity even at a concentration level of 2 mg/ml.

Experimental Section[6]

Ethyl 2-Acetamido-2-(N-benzyl-N-methyl-2-ethylamino)malonate.—To a solution of 21.7 g of ethyl acetamidomalonate in 100 ml of Mg-dried EtOH containing 2.3 g of Na was added 18.3 g of freshly distilled N-benzyl-N-methyl-2-chloroethylamine[7] in small increments, and the reaction mixture was heated at reflux for 20 hr. The salt was removed by filtration, and the filtrate was taken to dryness *in vacuo*. Recrystallization of the residue (EtOH–H_2O) gave 5.0 g (14%) of product, mp 34–36°. *Anal.* ($C_{19}H_{28}N_2O_5$) C, H.

5-Azanorvaline (2-Amino-4-methylaminobutyric Acid) Dihydrochloride.—A sample of 4.0 g of ethyl 2-acetamido-2-(N-benzyl-N-methyl-2-ethylamino)malonate was hydrolyzed in the presence of 40 ml of 6 *N* HCl for 2 hr. The hydrolysis reaction mixture was taken to dryness under reduced pressure with warming. The resulting oil was freed of residual HCl by the repeated addition and evaporation of EtOH and the remaining residue was recrystallized (EtOH–Et_2O) to yield 2.1 g (65%) of product. The extreme hygroscopic nature of the sample was such that a melting point could not readily be determined and an acceptable analysis was not obtained. The material gave a positive test with ninhydrin, and the R_f values of this crude reaction product in *n*-BuOH–AcOH–H_2O (4:1:1), 65% pyridine, and 95% MeOH were 0.37, 0.80, and 0.63, respectively.

A sample of 0.40 g of the hygroscopic 2-amino-4-(N-benzylmethylamino)butyric acid in 50% MeOH–H_2O was agitated in the presence of 50 mg of Pd black under about 3.52 kg/cm² pressure of H_2 for 3 hr. The catalyst was removed by filtration and the filtrate was acidified to a pH 1 by addition of concentrated HCl. After the resulting solution was reduced to dryness *in vacuo*, the residue was recrystallized from absolute EtOH–Et_2O to yield 0.20 g of product (see Table I).

2-Acetamido-3-ethylaminopropionic Acid.—The synthesis of this compound was patterned after a previously described procedure for the general preparation of 3-amino-substituted derivatives of acetamidopropionic acid.[8] A mixture of 3.2 g of acetamidoacrylic acid and 50 ml of 33% aqueous $EtNH_2$ was allowed to stand at 40° for 72 hr. The reaction mixture was taken to dryness *in vacuo*, and the solid residue was recrystallized from absolute EtOH to yield 2.7 g (57%) of the product, mp 160–161°. *Anal.* ($C_7H_{14}N_2O_3 \cdot H_2O$) C, H.

4-Azanorleucine (2-Amino-3-ethylaminopropionic Acid) Dihydrochloride.—A solution of 1.0 g of 2-acetamido-3-ethylaminopropionic acid in 20 ml of 6 *N* HCl was heated under reflux for 4 hr. The reaction mixture was concentrated by removal of the acid under reduced pressure and absolute EtOH was then added. After chilling in the refrigerator, 0.80 g of product was obtained (see Table I).

2-Acetamido-3-methylaminopropionic Acid.—Using the general method[8] from 3.6 g of 2-acetamidoacrylic acid there was recovered 2.7 g (60%) of product, mp 164–165°, lit.[8] mp 164°.

4-Azanorvaline (2-Amino-3-methylaminopropionic Acid) Dihydrochloride.—2-Acetamido-3-methylaminopropionic acid (1 g) in 20 ml of 6 *N* HCl was heated at reflux for 4 hr. There was recovered 0.75 g of product from the hydrolysis reaction mixture after facilitating crystallization by the addition of absolute EtOH (see Table I).

Microbiological Assays.—A previously described inorganic salts–glucose medium[9] was used for *E. coli* 9723, and experimental detail has been reported elsewhere.[10]

The amino acid analogs were dissolved in sterile H_2O and added aseptically to the previously autoclaved assay tubes. In all assays the amount of growth was determined photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer, in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at zero absorbance.

(6) All melting points are corrected. The microanalyses were performed by International Chemical and Nuclear Corp., City of Industry, Calif. All R_f data were determined using the ascending technique of paper chromatography in the solvents indicated, and ninhydrin reagent was used for the development of the spots. Where analyses are indicated only by symbols of the elements, analytical results obtained by those elements were within ±0.4% of the theoretical values.

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Grignard Addition to 17α-Acetoxy-6α-methylprogesterone

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Grignard addition to a steroid α,β-unsaturated ketone has been reported by Musgrave[1] in 1951. He carried out 1,2 addition on cholestenone with methylmagnesium bromide to give 3-methylcholest-4-en-3-ol. A number of other investigators[2–4] have used cuprous chloride and cuprous acetate along with the Grignard reagent to obtain 1,4 addition. We wish to report in this communication a one-step 1,2 addition of methylmagnesium bromide to the α,β-unsaturated ketone, 17α-acetoxy-6α-methylprogesterone (1).

An attempt to add a stoichiometric amount of methylmagnesium bromide to **1** at room temperature gave only precipitates owing to complex formation. Varying the proportions of reactants and the temperature of the reaction gave mixtures of products. However, the use of a large excess of Grignard reagent at room temperature gave 3ξ,17α-dihydroxy-3ξ,6α-dimethylpregn-4-en-20-one 17-acetate (**2**) if acid was avoided in isolating the desired product. If acetic acid or dilute HCl was added in the work-up of the reaction, then dehydration occurred and 17α-acetoxy-3,6-dimethylpregn-3,5-dien-20-one (**3**) was isolated. When

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