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.[5] 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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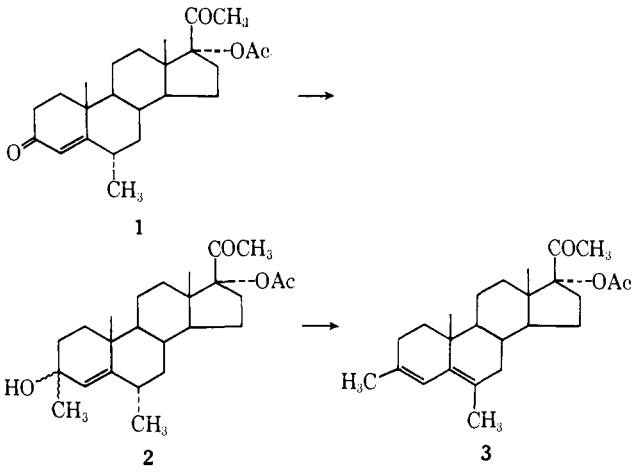
(2) Merck and Co., Inc., British Patent 877,087 (1961).

(3) Hiromu Mori, *Chem. Pharm. Bull.* (Tokyo), **12**, 1224 (1964).

(4) Bohumil Pelc., Czechoslovakian Patent 111,047 (1964).



pure **2** was treated with dilute methanolic HCl, **3** was obtained in quantitative yields.

Biological Data.—The progestational activity of **2** and **3** was determined by the Clauberg test[5] and the endometrial response was scored according to the index of McPhail.[6] Compound **2** at dose levels of 0.5 and 1.0 mg/kg exhibited a McPhail index of 1.9 and 2.8, respectively, whereas the corresponding diene **3** showed a McPhail index score of 1.0 at 0.5 mg/kg.

Experimental Section[7]

3ξ,17α-Dihydroxy-3ξ,6α-dimethylpregn-4-en-20-one 17-Acetate (2).—A solution containing 3.0 g of 17α-acetoxy-6α-methylprogesterone (1) in 50 ml of dry THF was added dropwise to a cooled solution of 15 ml of MeMgBr (Arapahoe Chemicals) in 15 ml of dry THF. The mixture was stirred at 20–25° for 0.5 hr and the excess Grignard reagent was decomposed with 100 ml of cold H_2O. The mixture was extracted with 100 ml of ether and the etheral solution was washed several times (H_2O), dried (Na_2SO_4), and evaporated to dryness. Repeated recrystallization of the residue from ether gave 900 mg (29%) of **2**: mp 195–197°; $[\alpha]D$ +48.2°; λ_{max}^{KBr} 2.84, 5.76, and 5.85 μ; no uv absorption between 200–300 mμ. *Anal.* ($C_{25}H_{38}O_4$) C, H.

17α-Acetoxy-3,6-dimethylpregn-3,5-dien-20-one (3).—Compound **2** (700 mg) was treated with 5.0 ml of dilute methanolic HCl at room temperature for 24–36 hr. The mixture was then poured into a large amount of ice and water and the solid material thus separated was filtered off. Recrystallization from MeOH gave **3** in quantitative yield, mp 153–154°, $[\alpha]D$ −44.5°, λ_{max}^{KBr} 5.77 and 5.84 μ, λ_{max}^{EtOH} 243 mμ. *Anal.* ($C_{25}H_{36}O_3$) C, H.

Acknowledgment.—I wish to thank Dr. R. P. Blye for biological evaluations.

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(6) M. K. McPhail, *J. Physiol.* (London), **83**, 145 (1935).

(7) All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. The uv and ir data were obtained on Cary Model 11 and Beckman IR-5 spectrophotometers, respectively. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Anabolic Cyclic Esters of 19-Nortestosterone

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Esterification of steroid hormones has frequently resulted in the intensification and prolongation of the hormonal response. Several reviews have been published on steroid esters and their pharmacology.[1] A recent study from these laboratories has indicated the utility of the highly symmetrical cagelike adamantane molecule in the form of adamantoic acid in the ester portion of anabolic steroid derivatives.[2]

The utility of cycloalkyl or bicycloalkyl as alkyl substituents has been studied in several pharmacological areas.[3] We wish to report the preparation and evaluation of several cycloalkyl esters of the anabolic steroid, 19-nortestosterone, in a duration myotrophic assay.

The nortestosterone 17β-esters were prepared *via* the acid chlorides as previously described.[2] The cycloheptane- and cycloundecanecarboxylic and cyclooctaneacetic acids are now available commercially, while the homoadamantoic and adamantaneacetic acids were prepared from adamantoic acid by the procedure described by Stetter and Rauscher.[4] The *exo*-2-norbornene-5-carboxylic acid was obtained by purification of a commercial sample utilizing the procedure of VerNooy and Rondestvedt.[5]

Treatment of the above acids with thionyl chloride produced the respective acid chlorides. The α-chloro-substituted acid chlorides were prepared by prolonged treatment with aged thionyl chloride.[6]

Evaluation of potency and duration of effect was established by the myotrophic–androgenic assay method of Hershberger, Shipley, and Meyer[7] in immature castrate male rats. The data are reported in Table I.

The cyclic esters I[8]–III show an early strong androgenic response as measured by the seminal vesicles while the cycloundecanecarboxylate ester V does not, and this response begins to diminish after the third week. Significantly increasing levator ani responses are shown also by the c-$C_{7,8}$-carboxylate esters. While ester I begins to diminish in myotrophic activity about the sixth week, it is noteworthy that the cyclooctanecarboxylate ester (III) continues to be active to the eighth week. This potency and duration of activity is also seen in the adamantoate ester.[2]

While ester VIII shows a good ratio of myotrophic activity *vs.* androgenic activity at the four- and six-week levels, the over-all potency is lower compared to the other esters. Similarly, the cyclooctaneacetate ester VI has good separation of activity but the anabolic potency is only half that of the cyclooctane ester III. Both of the α-chloro-substituted esters IV and VII were of weaker activity.

Two variations on the adamantane molecule were

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