Table III. Per Cent Activity[a] of Compound 25 against African Trypanosomes

T. rhodesiense			*T. gambiense*			*T. congolense*		
Dose, mg/kg	Route	% act.	Dose, mg/kg	Route	% act.	Dose, mg/kg	Route	% act.
12.5 × 4	ip	100	10 × 4	po	100	50 × 4	ip	79
10 × 4	po	100	25 × 1	po	100	25 × 4	sc	100
50 × 1	po	89						
25 × 1	po	97						
2 × 4	sc	100						
5 × 1	sc	80						

[a] % activity calculated as on Table II.

Experimental Section

Melting points were taken on a Gallenkamp apparatus (Registered Design No. 889339) using capillaries and are uncorrected. All compounds were characterized by ir, uv, nmr, and elemental analyses (C, H, N) which were within ±0.4% of the theoretical values.

Acethydrazide, benzhydrazide, and Girard reagents "T" and "P" were purchased from BDH; Girard reagent "D" was obtained from Eastman-Kodak. All the other required hydrazides were prepared by literature methods.[10]

The hydrazines used for the preparation of compounds 13–18 were prepared by literature methods[11] and kindly supplied by Mr. J. P. Verge.

General Method for Thioacetals. The aldehyde **1b** (0.05 mol) was refluxed with the appropriate thiol (0.05 mol) in benzene and in the presence of *p*-toluenesulfonic acid (100 mg) for 2 hr, the water from the reaction being removed *via* a Dean-Stark separator. After cooling, the benzene solution was washed with $NaHCO_3$ solution, followed by H_2O, and then evaporated *in vacuo* to give a bright yellow solid which was crystallized from an appropriate solvent, *e.g.*, ethanol.

General Method for Hydrazones. The aldehyde **1b** (0.025 mol) and the hydrazine or hydrazide (0.025 mol) were refluxed in a solvent such as EtOH or $CHCl_3$, with or without the addition of a few drops of glacial acetic acid, for 2–4 hr. On cooling the resultant crystalline solid was filtered off and recrystallized from an appropriate solvent.

2-(4-*N,N*-Dimethylglycinamidoiminomethinestyryl)-5-nitro-1-vinylimidazole (25). Aldehyde **1b** (6.7 g, 0.025 mol), *N,N*-dimethylglycine hydrazide hydrochloride (3.8 g, 0.025 mol), and sodium acetate hydrate (4 g) were refluxed in EtOH (300 ml) for 2 hr. On cooling a small amount of inorganic material separated and was filtered off. The filtrate was evaporated *in vacuo* to give an oil which on treatment with $CHCl_3$ gave further inorganic solid which was filtered off. The bright yellow filtrate was carefully diluted with petroleum ether (bp 40–60°) and allowed to crystallize: yield 6.1 g (66%); mp 190–191°.

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Synthesis and Biological Evaluation of Xanthine Oxidase Inhibitors. Pyrazolo[3,4-*d*]pyrimidines and Pyrazolo[3,4-*b*]pyridines†

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1-, 3-, and 5-substituted pyrazolo[3,4-*d*]pyrimidines and pyrazolo[3,4-*b*]pyridines related to allopurinol were synthesized and evaluated as xanthine oxidase inhibitors. Among these compounds, 4-hydroxypyrazolo[3,4-*b*]pyridine-5-carboxylic acids **12** were found to possess potency in the same order of allopurinol. The influence of the substitutions on the enzyme inhibitory effect and the bulk tolerance of the enzyme–inhibitor complex are discussed.

4-Hydroxypyrazolo[3,4-*d*]pyrimidine (allopurinol, **1a**) has been shown to be an effective inhibitor of xanthine oxidase and thus a clinically useful agent in gout treatment; it is also an effective adjuvant in antitumor chemotherapy.[1] The objective of the work now reported is to develop more effective enzyme inhibitors using the parent skeleton (pyrazolo[3,4-*d*]pyrimidine) of allopurinol as the basis species, both by exploring the effects of substituent insertion on the activity of allopurinol and also by exploring various deaza species related to this molecule. Previ-

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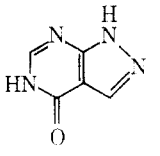


1a

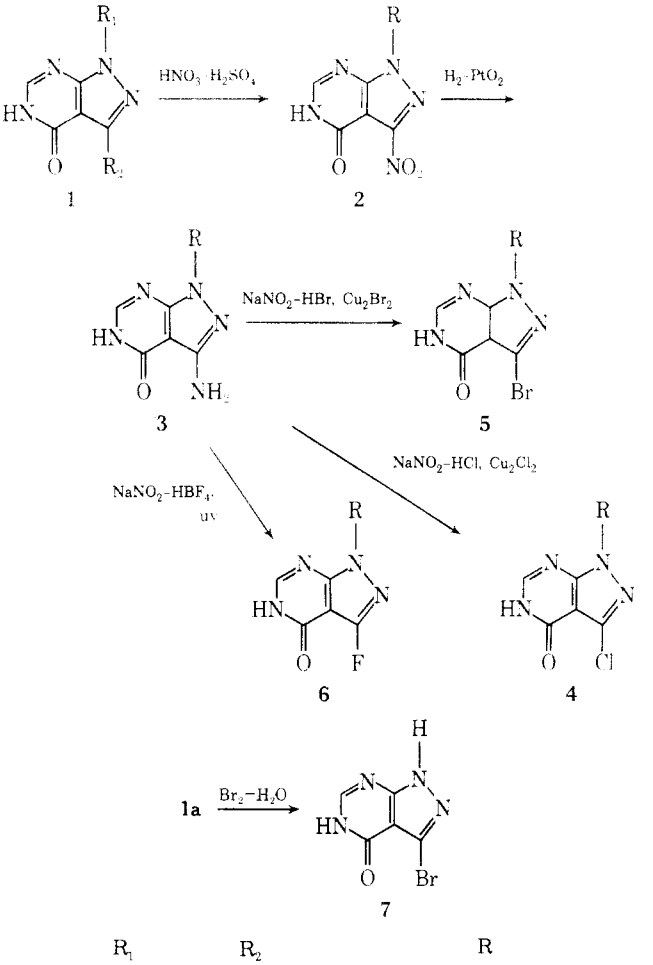
ous work by Baker and his coworkers[2] has established some of the effects of substituent variation in **1a** on enzyme-inhibitory effectiveness, but most structural variations were limited to the 1, 4, and 6 positions, and a key review account of the chemistry of pyrazolo[3,4-*d*]pyrimidines[3] makes scant mention of 3-substituted derivatives of **1a**. If the 3 position is not in contact with the enzyme surface, insertion of functional groups at this location should not greatly affect enzyme-to-inhibitor binding involving that surface, and it may be possible to place bond-forming groups at the 3 position, leading to "active-site directed irreversible inhibition by the exo-mechanism" (see Baker's monograph[4]). To this end, we have undertaken an exploratory study of syntheses and enzyme-inhibitory effects of pyrazolo[3,4-*d*]pyrimidines carrying 3- and/or 1-substituents and have also examined some of the 5-deaza analogs (pyrazolo[3,4-*b*]pyridines) with carboxyl-derived groups attached to ring carbon, instead of the 5-NH- function of allopurinol.

Chemistry. Pyrazolo[3,4-*d*]pyrimidines. The various 4-hydroxypyrazolo[3,4-*d*]pyrimidines **1** were prepared by Cheng and Robins' method.[5] Most recorded substitution reactions[3] of pyrazolo[3,4-*d*]pyrimidines involve nucleophilic attack in the pyrimidine ring, but molecular orbital calculations published by our group[6] indicate a relatively high π-electron density at the 3 position, suggesting that electrophiles could be introduced there (a similar expectation has been verified for nitrations and brominations of pyrazolo[3,4-*b*]pyridine derivatives[7] and for nitration of the neutral indazole molecule[7,8]). In agreement with this suggestion, prolonged treatment of 4-hydroxypyrazolo[3,4-*d*]pyrimidine with bromine water under reflux yielded the 3-bromo derivative **7** (Scheme I). Similar treatment of **1a** with chlorine water led to decomposition, and attempted nitration of **1a** in mixed nitrating acids gave no well-defined product. In view of the lability of **1a**, 1-alkyl or 1-aryl derivatives were employed and were less sensitive to decomposition; thus, the 1-methyl derivative **1b** was nitrated to the corresponding 3-nitro compound **2b** in mixed nitrating acids at 100°. The 1-phenyl derivative **1d** was more resistant to 3-nitration, with initial nitration in the phenyl ring leading to deactivation of the pyrazole moiety. 1-(2,4-Dinitrophenyl)-4-hydroxypyrazolo[3,4-*d*]pyrimidine is the sole product of mixed acids nitration of **1d**, but successful trinitration to **2d** was effected using fuming nitric acid and concentrated sulfuric acid at 100°. Using these conditions, the 1-aryl derivatives **1e** and **1f** underwent dinitration and trinitration to species **2e** and **2f**, where both the aryl moieties and the 3 position were nitrated. The 1-cyclohexyl derivative **1c** did not require forcing conditions, as the mild and selective acetyl nitrate effected 3-nitration.

The nitro compounds **2** had very limited solubilities in the common hydrogenation solvents; only **2b** (1-methyl) and **2c** (1-cyclohexyl) were sufficiently soluble in boiling ethanol for catalytic hydrogenation. The resulting amines **3b** and **3c** were converted to the chloro compounds **4b** and **4c** and to the bromo compounds **5b** and **5c** using the standard Sandmeyer reactions. 3-Fluoro-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (**6b**) was also synthesized photochemically from the precursor **3b**.[9]

Pyrazolo[3,4-*b*]pyridines. The general synthetic route

Scheme I



	R_1	R_2	R	
1a	H	H	**2b**	CH_3
1b	CH_3	H	**2c**	cyclohexyl
1c	cyclohexyl	H	**2d**	2′,4′-dinitrophenyl
1d	phenyl	H	**2e**	4′-chloro-3′-nitrophenyl
1e	*p*-chlorophenyl	H	**2f**	3′,5′-dinitro-4′-methyl phenyl
1f	*p*-tolyl	H		
1g	CH_3	CH_3	**3b-5b**	CH_3
			3c-5c	cyclohexyl
			6b	CH_3

for the pyrazolo[3,4-*b*]pyridines is outlined in Scheme II. The required aminopyrazoles **9** were prepared by decarboxylation of the precursors **8** with 85% phosphoric acid. Replacement of the ethoxy group of diethyl ethoxymethylenemalonate by the 5-amino function of the appropriate pyrazoles **9** afforded the intermediates, diethyl 5-pyrazolylaminomethylenemalonates, which were cyclized in excellent yields giving the ethyl 1-alkyl- (and aryl-) 4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **10**. Unsuccessful attempts were made to decarboxylate **10** by various techniques, including heating in quinoline–copper powder, or in concentrated sulfuric acid, and simple pyrolysis. The compounds **10** usually decomposed before decarboxylated products were produced. Under dilute acidic or alkaline conditions, only hydrolysis occurred. Finally, we found that decarboxylation took place smoothly in 85% phosphoric acid, and the pyrazolo[3,4-*b*]pyridines **11** were obtained in good yield. The structure assignments of **11** were substantiated by their nmr spectra, which revealed doublets (δ 8.20–8.50, $J_{5,6}$ = 7.0 Hz) arising from the C-5 proton coupled to the C-6 proton. A simple and general route is thus provided to hitherto difficultly ac-

cessible 4-hydroxypyrazolo[3,4-*b*]pyridines (there is a literature precedent for the preparation of the parent 4-hydroxypyrazolo[3,4-*b*]pyridine,[10] but low yields are recorded).

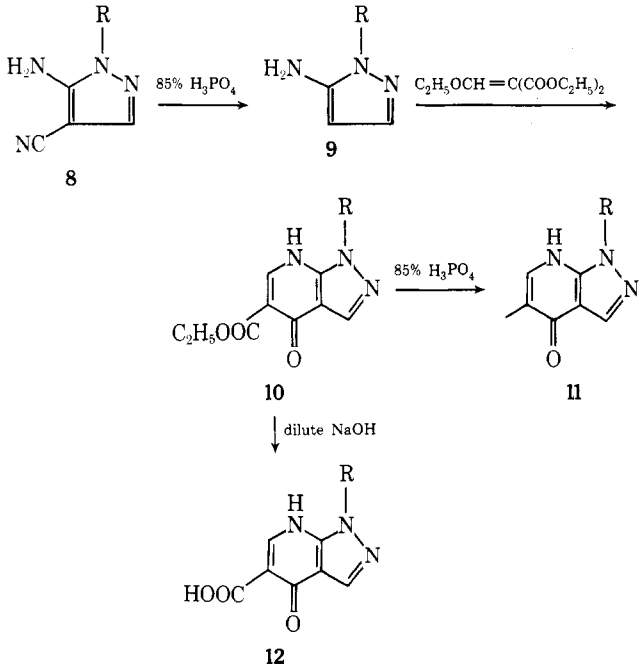
On nitration, the 4-hydroxy-1-methyl-5-carboxylic acid species **10b** behaved similarly to the parent pyrazolo[3,4-*b*]pyridine,[7] with substitution at the 3 position yielding **13b**.

Biological Results. Details of xanthine oxidase inhibition for the fully assayed compounds are given in Table I, where potencies are expressed in terms of inhibitor concentration (μM) required to produce 50% enzyme inhibition (I_{50}) and by the ratio of the concentration of inhibitor to substrate (I/S) at 50% inhibition. Allopurinol was used as reference standard. Many of the synthesized compounds were insufficiently soluble in the aqueous medium to obtain inhibitor concentrations causing 50% enzyme inhibition, and these were subjected to preliminary assay using an inhibitor concentration of 66 μM and a hypoxanthine concentration of 8 μM. Using these conditions, the following compounds showed less than 5% inhibition of xanthine oxidase: 4-hydroxy-1-methyl-3-nitropyrazolo[3,4-*d*]pyrimidine (**2b**) and the derived 3-amino compound **3b**; 1-cyclohexyl-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidine (**2c**) and the derived 3-amino (**3c**), 3-bromo (**5c**), and 3-chloro (**4c**) species; and the 1-aryl-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidines **2e** and **2f**.

Thus, virtually all the new pyrazolo[3,4-*d*]pyrimidines are but feebly active. The bromo compound **7** is 1/18 (I_{50} = 23 *vs.* 1.3), exhibiting some bulk tolerance to 3-substitution, but all the 1,3-disubstituted compounds show very low inhibitory effectiveness. The weak activities shown by these species suggest that the pyrazole moiety is in contact with the active-site surface and that effective inhibitors among 3-substituted pyrazolo[3,4-*d*]pyrimidines must be of the "endo-mechanism" type.[4] Further work is in progress seeking to synthesize 3-substituted pyrazolo[3,4-*d*]pyrimidines lacking 1-substituents.

In contrast to the disappointing results with the pyrazolo[3,4-*d*]pyrimidine series, the 4-hydroxypyrazolo[3,4-*b*]pyridine species of general structures 10, 11, and 12 show significant activity (see Table I) and are at least an order of magnitude more active than the pyrazolo[3,4-*d*]pyrimidines with corresponding 1- and/or 3-substituents. The 5-unsubstituted species 11 and the 5-ethoxycarbonyl species 10 possess activities of the same order, indicating that the presence of groups at the 5 position is compatible with strong binding to the enzyme surface (it may be noted here that the 5-propyl derivative of allopurinol binds tightly to xanthine oxidase[11]). When the 5-substituent is capable of reversible binding, the activity is further enhanced as the class of pyrazolo[3,4-*b*]pyridine-5-carboxylic acids 12 show potent inhibiting properties, approaching that of allopurinol. It appears likely that the carboxylate anion affords additional binding to the cationic site(s) of the enzyme. Since some 6-substituted pyrazolo[3,4-*d*]pyrimidines have been reported as having xanthine oxidase inhibitory activity close to that of allopurinol,[2] it would appear that there is bulk tolerance for sub-

Scheme II



8b-11b, R = CH_3
8c-11c, R = cyclohexyl
8d-11d, R = phenyl
8e-11e, R = *p*-chlorophenyl

Table I. Effects of Pyrazolo[3,4-*d*]pyrimidines and Pyrazolo[3,4-*b*]pyridines as Inhibitors of Xanthine Oxidase

Compound	I_{50}[a]	$(I/S)_{0.5}$[b]
Allopurinol (**1a**)	1.3[c]	0.05
1-Cyclohexyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**1c**)	2380	95
1,3-Dimethyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**1g**)	3360	174
3-Chloro-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (**4b**)	1400	56
3-Bromo-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (**5b**)	1630	65
3-Fluoro-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (**6b**)	2170	87
3-Bromo-4-hydroxypyrazolo-[3,4-*d*]pyrimidine (**7**)	23	0.92
Ethyl 4-hydroxy-1-methylpyrazolo[3,4-*b*]pyridine-5-carboxylate (**10b**)	190	7.7
Ethyl 1-cyclohexyl-4-hydroxypyrazolo[3,4-*b*]pyridine-5-carboxylate (**10c**)	190	7.7
Ethyl 4-hydroxy-1-phenylpyrazolo[3,4-*b*]pyridine-5-carboxylate (**10d**)	206	8.2
4-Hydroxy-1-methylpyrazolo-[3,4-*b*]pyridine (**11b**)	405	16
1-Cyclohexyl-4-hydroxypyrazolo[3,4-*b*]pyridine (**11c**)	190	8.0
4-Hydroxy-1-phenylpyrazolo-[3,4-*b*]pyridine (**11d**)	280	11
1-*p*-Chlorophenyl-4-hydroxypyrazolo[3,4-*b*]pyridine (**11e**)	108	4.3
4-Hydroxy-1-methylpyrazolo-[3,4-*b*]pyridine-5-carboxylic acid (**12b**)	47	1.86
4-Hydroxy-1-phenylpyrazolo-[3,4-*b*]pyridine-5-carboxylic acid (**12d**)	10	0.40
1-*p*-Chlorophenyl-4-hydroxypyrazolo[3,4-*b*]pyridine-5-carboxylic acid (**12e**)	7.3	0.29

[a] Inhibitor concentration (μM) required to produce 50% inhibition. [b] Ratio of the concentrations of the inhibitor to substrate (hypoxanthine) for 50% inhibition. [c] Literature value[2] is 0.87.

stitution within the six-membered rings of 4-hydroxypyrazolo[3,4-*d*]pyrimidines and 4-hydroxypyrazolo[3,4-*d*]pyridines and that the 5 and 6 positions are not in contact with the active-site surface, so that there are good prospects that bond-forming groups may be introduced at these positions, giving more effective irreversible enzyme inhibitors. Further work is in progress to test these prospects.

Experimental Section

When analyses are indicated, analytical results for these elements were within ±0.4% of the theoretical values. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Nmr spectra were recorded on a Varian A-60A spectrophotometer and ir spectra recorded on a Beckman infrared spectrophotometer, Model Acculab 4. Enzyme inhibitory results were determined using a Beckman ACTA III spectrophotometer.

Nomenclature. Heterocyclic lactam or amide species are named as *hydroxy* compounds throughout, following Albert's[12] precedent. The structural representations in the figure and Schemes I and II depict the compounds in the predominant tautomeric form.

4-Hydroxy-1-methyl-3-nitropyrazolo[3,4-*d*]pyrimidine (2b). A mixture of 4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (**1b**)[5] (1 g, 0.005 mol), HNO_3 (*d* 1.42, 6 ml), and concentrated H_2SO_4 (12 ml) was heated on a boiling water bath for 2 hr. The cooled mixture was poured onto crushed ice and the precipitate was filtered giving **2b** (0.59 g, 46%), mp 300° (EtOH). *Anal.* ($C_6H_5N_5O_3$) C, H, N. Similarly prepared was 4-hydroxy-1-methyl-3-nitro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (**13b**) from **10b:** yield 91%; mp 241–243°. *Anal.* ($C_8H_6N_4O_5$) C, H, N.

1-(2,4-Dinitrophenyl)-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidine (2d). **2d** was synthesized from 4-hydroxy-1-phenylpyrazolo[3,4-*d*]pyrimidine (**1d**)[5] in a similar manner to **2b** except that fuming HNO_3 (90%, *d* 1.50) was used instead of concentrated HNO_3: yield 60%; mp >300°. *Anal.* ($C_{11}H_5N_7O_7$) C, H, N.

Similarly prepared were 1-(4-chloro-3-nitrophenyl)-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidine (**2e**) from 1-*p*-chlorophenyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**1e**)[5] [yield 40%; mp >300°. *Anal.* ($C_{11}H_5ClN_6O_5$) C, H] and 1-(3,5-dinitro-4-methylphenyl)-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidine (**2f**) from 4-hydroxy-1-*p*-tolylpyrazolo[3,4-*d*]pyrimidine (**1f**)[5] [yield 41%; mp 273–274° (HOAc). *Anal.* ($C_{12}H_7N_7O_7$) C, H, N].

1-Cyclohexyl-4-hydroxy-3-nitropyrazolo[3,4-*d*]pyrimidine (2c). 1-Cyclohexyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**1c**) was prepared using the general route for species 1 (Cheng and Robins[5]): yield 75%; mp 251–252°. *Anal.* ($C_{11}H_{14}N_4O$) C, H, N. This compound (6.0 g, 0.023 mol) was added to a mixture of HNO_3 (*d* 1.42, 19 ml) and Ac_2O (210 ml). The temperature was kept below 40° by external cooling and the mixture was stirred for 24 hr. The mixture was poured onto crushed ice, giving **2c** (6.1 g, 84%): mp >300° (EtOH). *Anal.* ($C_{11}H_{13}N_5O_3$) C, H, N.

3-Amino-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (3b). The nitro compound **2b** (1.0 g, 0.005 mol) in hot ethanol (300 ml) was hydrogenated over PtO_2 (0.1 g) overnight. The catalyst was filtered off, and the solvent was evaporated yielding 0.56 g (66%) of crude **3b:** mp 239–241° (EtOH). *Anal.* ($C_6H_7N_5O$) C, H, N.

3-Amino-1-cyclohexyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**3c**) was prepared similarly from the nitro compound **2c:** yield 70%; mp 219–221° (EtOH). *Anal.* ($C_{11}H_{15}N_5O$) C, H, N.

3-Chloro-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (4b). The amine **3b** (1.0 g, 0.005 mol) was diazotized at 0–5° in concentrated HCl (10 ml) by addition of $NaNO_2$ (0.4 g) in H_2O (2 ml) over 30 min. To the diazotization mixture was added Cu_2Cl_2 (1 g) in concentrated HCl (5 ml) and the mixture was heated at 60° for 0.5 hr. The solid separating after neutralization was filtered off and gave **4b** (0.6 g, 54%): mp 287–288° (EtOH). *Anal.* ($C_6H_5ClN_4O$) C, H, N.

Similarly prepared was 3-chloro-1-cyclohexyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**4c**) from **3c:** yield 28%; mp 194–196° (EtOH). *Anal.* ($C_{11}H_{13}ClN_4O$) N.

3-Bromo-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (5b). **3b** (1 g, 0.005 mol) was diazotized in 48% HBr (10 ml) with $NaNO_2$ (0.4 g) and was treated with Cu_2Br_2 (1 g) to yield **5b** (70%): mp >300° (EtOH). *Anal.* ($C_6H_5BrN_4O$) C, H, N.

3-Bromo-1-cyclohexyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**5c**) was prepared similarly from **3c:** yield 61%; mp 253–254° (EtOH). *Anal.* ($C_{11}H_{13}BrN_4O$) C, H, N.

3-Fluoro-4-hydroxy-1-methylpyrazolo[3,4-*d*]pyrimidine (6b). A solution of **3b** (1 g, 0.006 mol) in 49% HBF_4 (20 ml) was treated with $NaNO_2$ (0.49 g) in H_2O (2 ml) at 0°. The diazotized solution was then flushed with N_2 and stirred in a uv reactor (3500 Å) for 18 hr. The solution was adjusted to pH 5–6 with dilute NaOH and extracted repeatedly with $CHCl_3$. Evaporation of the extract afforded **6b** (0.25 g, 25%), mp 235–240° (EtOH). *Anal.* ($C_6H_5FN_4O$) C, H.

3-Bromo-4-hydroxypyrazolo[3,4-*d*]pyrimidine (7). A mixture of 4-hydroxypyrazolo[3,4-*d*]pyrimidine (**1a**, 2 g, 0.015 mol), Br_2 (15 g), and H_2O (600 ml) was heated under reflux for 48 hr. The product separated from the solution after standing at room temperature overnight (1.86 g, 62%): mp >300° (H_2O). *Anal.* ($C_5H_3BrN_4O$) C, H, N.

5-Amino-1-methylpyrazole (9b). 5-Amino-1-methyl-4-cyanopyrazole (**8b**,[5] 5 g, 0.04 mol) was heated in 85% H_3PO_4 (20 ml) at 170–180° for 20 hr. The mixture was poured onto crushed ice, basified with 60% NaOH, and extracted with $CHCl_3$. Evaporation of the extracts afforded **9b** (3.5 g, 87%), mp 67–68° [lit.[13] bp 72–73° (0.02 mm)].

Similarly prepared were 5-amino-1-cyclohexylpyrazole (**9c**) from 5-amino-4-cyano-1-cyclohexylpyrazole (**8c**), mp 73–74° [lit.[14] bp 115–120° (0.5 mm)], and 5-amino-1-*p*-chlorophenylpyrazole (**9e**) from 5-amino-1-*p*-chlorophenyl-4-cyanopyrazole (**8e**),[5] mp 38–40°. *Anal.* ($C_9H_8ClN_3$) C, H, N.

Ethyl 4-Hydroxy-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (10b). **10b** was prepared as described previously:[15] mp 138–140° (lit. mp 141–143°).

Similarly prepared were ethyl 1-cyclohexyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (**10c**) from **9c** [yield 50%; mp 170–172°. *Anal.* ($C_{15}H_{19}N_3O_3$) C, H, N], ethyl 4-hydroxy-1-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxylate (**10d**) from **9d** [mp 148–150° (lit.[15] 152–154°)], and ethyl 1-*p*-chlorophenyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (**10e**) from **9e** [yield 85%; mp 187–189° (EtOH). *Anal.* ($C_{15}H_{12}ClN_3O_3$) C, H, N].

4-Hydroxy-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine (11b). **10b** (1 g, 0.004 mol) was dissolved in 85% H_3PO_4 (15 ml) and heated at 170–180° for 20 hr. After being poured onto crushed ice the pH was adjusted to pH 5–6 and the reaction mixture was repeatedly extracted with $CHCl_3$. Concentration of the combined extracts gave the decarboxylated product (0.55 g, 81%): mp 165–168°; nmr (TFA-*d*) δ 7.19 and 8.48 (two doublets, H_6 and H_5, J = 7.0 Hz). *Anal.* ($C_7H_7N_3O$) C, H, N.

The following compounds were prepared similarly: 1-cyclohexyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine (**11c**) from **10c** [yield 44%; mp 150–153°; nmr ($CDCl_3$) δ 6.55 and 8.32 (two doublets, H_6 and H_5, J = 7.0 Hz)], 4-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine (**11d**) from **10d** [yield 49%; mp 175–177°; nmr (TFA-*d*) δ 7.20 and 8.42 (two doublets, H_6 and H_5, J = 7.0 Hz). *Anal.* ($C_{12}H_9N_3O$) C, H, N], 1-*p*-chlorophenyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine (**11e**) from **10e** [yield 74%; mp 160–162°; nmr (TFA-*d*) δ 7.29 and 8.39 (two doublets, H_6 and H_5, J = 7.0 Hz). *Anal.* ($C_{12}H_8ClN_3O$) C, H, N], and 4-hydroxy-1-methyl-3-nitro-1*H*-pyrazolo[3,4-*b*]pyridine (**14b**) from **13b** [yield 62%; mp 181–183°; nmr (TFA-*d*-DMSO) δ 6.85 and 8.35 (two doublets, H_6 and H_5, J = 6.0 Hz). *Anal.* ($C_7H_6N_4O_3$) C, H, N].

4-Hydroxy-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (12b) and 4-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (12d) were prepared following a literature method and the physical constants were comparable to the literature[13] values.

Similarly prepared was 1-*p*-chlorophenyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (**12e**) from **10e:** yield 78%; mp 223–224°. *Anal.* ($C_{13}H_8ClN_3O_3$) C, H, N.

Biological Results. The xanthine oxidase inhibitory activities were evaluated *in vitro*, using a previously described method.[16] The results are given in Table I.

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Synthesis and Biological Activity of Some Ethers of Testosterone. Implications Concerning the Biological Activity of Esters of Testosterone

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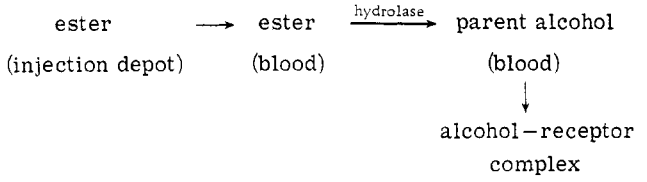
The benzyl (**2**), allyl (**4**), propyl (**10**), 3-hydroxypropyl (**12**), 2,3-dihydroxypropyl (**11**), 4-pentenyl (**7**), and pentyl (**8**) ethers of testosterone were synthesized. Compounds **2**, **4**, **7**, **8**, **10**, and **12** were found to be almost devoid of anabolic or androgenic activity in a modified Hershberger Assay, but **2**, **4**, **10**, and **12** were found to be effective inhibitors of testosterone 5α-reductase from human skin. These findings suggest that esters of testosterone and of 19-nortestosterone must hydrolyze before interacting with the hormonal receptors, but that the esters may competitively compete with the parent alcohols for interaction with enzymes. The latter effect may shift the distribution of metabolites of the esters relative to the alcohols and thus influence the pharmacological effect of these compounds.

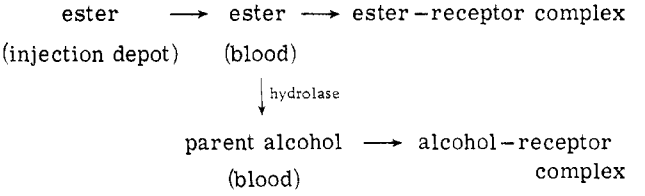
Esterification of testosterone with short-chain fatty acids gives compounds which, on intramuscular injection, show prolonged and enhanced biological activity.[1,2] Intramuscular injection of low doses of such agents also was found to produce an enhanced ratio of anabolic to androgenic activity when compared to the effect of brief intravenous infusion of higher doses of testosterone.[3]

A further enhancement of the anabolic–androgenic ratio has been reported for 19-nortestosterone[4] and its esters.[5,6] Van der Vies[7] claims to have demonstrated that, in the rat, both the duration of action and the degree of enhancement of the anabolic–androgenic ratio of various 19-nortestosterone esters can be closely approximated by subcutaneous injection of 19-nortestosterone essentially according to the schedule at which the particular ester was found to be released from the oily intramuscular injection depot. Van der Vies[7] also found that rat plasma contains an enzyme which rapidly hydrolyzes esters of 19-nortestosterone. Therefore, he hypothesized that the sole function of the acyl portion of the 19-nortestosterone esters is to control the rate of release of the ester from its injection depot and that the ester was immediately hydrolyzed in blood to afford 19-nortestosterone which interacted with the various biological receptors (Scheme I). Van der Vies acknowledged that his results did not rule out direct interaction between the ester and the hormonal receptor. His results also seem compatible with both the ester and the alcohol interacting (probably with different affinities) with the receptor, as shown in Scheme II.

Scheme I



Scheme II



While van der Vies' preferred hypothesis (Scheme I) has been widely accepted, considerable evidence supports the alternative hypothesis (Scheme II). Thus, van der Vies reported that 19-nortestosterone esters showed much less tendency to hydrolyze in the plasma of the dog or of man than in the rat, in spite of showing comparable biological effects in all of these species.[7] Moreover, he found that administration of esterase inhibitors to the rat failed to reduce the effect of 19-nortestosterone esters.[7] A number of very hindered esters of 19-nortesterone have been reported[8,9] to show more prolonged activity and more favorable anabolic–androgenic ratios than the compounds studied by van der Vies. Because the bulk of such esters makes their facile hydrolysis seem improbable, the need for such a hydrolysis has been questioned.[8]

In a recent series of papers, James has demonstrated that the prolongation of the biological response to testosterone and to 19-nortestosterone esters can be correlated with their lipid–water partition coefficients.[10-12] The ana-