Notes

Synthesis and Antitrichomonal Activity of Certain Pyrazolo[1,5-*a*]pyrimidines

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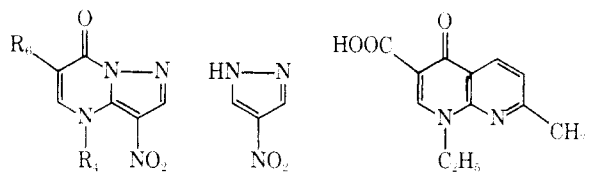
Several bridgehead nitrogen heterocycles were synthesized to be screened as antimicrobial agents, modeled after nalidixic acid. The activity of these new compounds, all derivatives of 3-nitro-4,6-disubstituted pyrazolo[1,5-*a*]pyrimidin-7-ones (**3, 7, 8,** and **9**), however, was found to be highly specific for *Trichomonas foetus* and completely lacking in activity against bacteria, fungi, and parasites other than *Trichomonas*. Of the nine compounds synthesized, including the intermediate 4,6-disubstituted pyrazolo[1,5-*a*]pyrimidin-7-ones (**2–6**) and the 6-substituted or unsubstituted pyrazolo[1,5-*a*]pyrimidin-7-ones (**1** and **4**), only 6-carbethoxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (**7**) was found to be a potent antitrichomonal agent, being comparable or perhaps better than metronidazole. From a tentative structure–activity relationship study, it was apparent that the combination of the 3-nitro, 4-ethyl, and 6-carbethoxy groups imparted specific activity, whereas other substitutions imparted little or no antitrichomonal activity.

Various nitrated heterocycles, particularly heterocycles with five-membered rings, have shown potent antimicrobial and antiparasitic properties.[1] For example, antitrichomonal activity has been found with nitropyrroles,[2] nitroimidazoles,[3] nitropyrazoles,[4] nitrofurans,[5] nitrothiophenes,[6] and nitrothiazoles.[7]

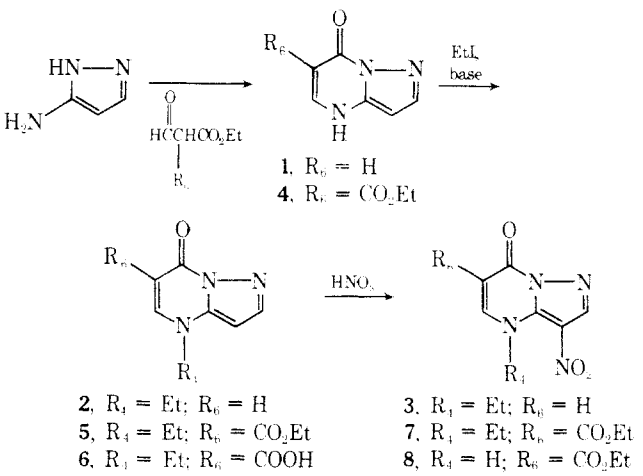
Current research of the syntheses and examination of the biological properties of pyrazolo[1,5-*a*]pyrimidines[8] prompted us to explore the possibility of developing antiparasitic compounds containing a fused nitropyrazole ring, *e.g.*, 3-nitropyrazolo[1,5-*a*]pyrimidine derivatives (I) in which R_4 is H or C_2H_5 and R_6 is H, $COOC_2H_5$, or COOH. Nitration of the pyrazolo[1,5-*a*]pyrimidine ring had previously been demonstrated to occur in the 3 position.[8c] The model compound chosen for this study was 6-carbethoxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (I, R_4 = C_2H_5; R_6 = $COOC_2H_5$) which was designed to incorporate the features of both 4-nitropyrazole (II) and the important antimicrobial agent nalidixic acid (III).[9]



I II III

Chemistry. Condensation of 3-aminopyrazole[10] with diethyl ethoxymethylenemalonate has been reported to yield 6-carbethoxy-7-hydroxypyrazolo[1,5-*a*]pyrimidine (**4**).[11] Alkylation of **4** with ethyl iodide afforded 6-carbethoxy-4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (**5**). Alkaline hydrolysis of **5** afforded 6-carboxy-4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (**6**). Nitration of 5 gave the desired product 6-carbethoxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (**7**). Similarly, alkylation of 7-hydroxypyrazolo[1,5-*a*]pyrimidine (**1**) afforded 4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (**2**) which was subsequently nitrated to give 4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (**3**). The reaction of 3-amino-4-nitropyrazole[12] with diethyl ethoxymethylenemalonate in acetic acid afforded 6-carbethoxy-7-hydroxy-3-nitropyrazolo[1,5-*a*]pyrimidine (**8**) which upon alkylation gave a product consisting of a mixture of 6-carboxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (**9**) and **7**. The product **9** apparently resulted from the hydrolysis of **7** (Scheme I).

Scheme I



1, R_6 = H
4, R_6 = CO_2Et

2, R_4 = Et; R_6 = H
5, R_4 = Et; R_6 = CO_2Et
6, R_4 = Et; R_6 = COOH

3, R_4 = Et; R_6 = H
7, R_4 = Et; R_6 = CO_2Et
8, R_4 = H; R_6 = CO_2Et

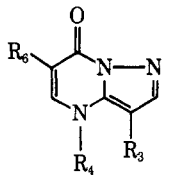
Results and Discussion

Of the compounds examined, only **7** exhibited significant antitrichomonal activity *in vitro* using *Tritrichomonas foetus* as the test organism, while **3** and **9** had minimal activity (Table I). All other compounds were inactive. The inhibitory and lethal effects for **7** were somewhat better than metronidazole. One striking and particularly interesting feature of **7** was its complete lack of activity in antibacterial, antifungal, *Trypanosoma cruzi*, and *Schistosoma mansoni* screens, indicating a high degree of specificity for trichomonas and possibly related organisms.

Admittedly, more compounds could be synthesized in this series to complete the information needed for a firm structure–activity relationship. However, a glance at Table I does reveal certain minimal requirements in this series, which account for the high degree of specificity for trichomonas.

It is clear that the 3-nitro, 4-ethyl, and 6-carbethoxy groups are all necessary to impart antitrichomonal activity, for example, by contrasting the *in vitro* data of **7** with that of **8**, which has no 4-ethyl group, or **9**, in which the 6-carbethoxy group has been replaced by a 6-carboxylic acid function, or **3**, which is unsubstituted in the 6 position.

Table I. *In Vitro* Antitrichomonal Activity



Compd	R_3	R_4	R_6	Concn, μg/ml MIC[b]	Concn, μg/ml MLC[c]
1	H	H	H	>100	NT[d]
2	H	C_2H_5	H	>100	NT
3	NO_2	C_2H_5	H	100	>100
4	H	H	$COOC_2H_5$	>100	NT
5	H	C_2H_5	$COOC_2H_5$	>100	NT
6	H	C_2H_5	COOH	>100	NT
7	NO_2	C_2H_5	$COOC_2H_5$	1.0	3.2
8	NO_2	H	$COOC_2H_5$	>100	NT
9	NO_2	C_2H_5	COOH	100	>100
Metronidazole[a]				3.2	10

[a] 1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole. [b] MIC, minimum inhibitory concentration. [c] MLC, minimum lethal concentration. [d] NT, not tested (since the MIC of these compounds was insufficient for further screening).

Clearly, the lack of activity of **1, 2, 4, 5,** and **6** substantiates the requirement for the 3-nitro group.

At this time, it has not been established what differentiates between the excellent activity of **7,** with the 4-ethyl group, and the lack of activity of **8,** which lacks the 4-ethyl group. Further synthetic work in this area may help to elucidate this unique specificity. However, in *in vivo* studies, no systemic antitrichomonal activity of **7** was observed in *T. foetus* infected mice at doses of 200 mg/kg/day.

Experimental Section

Melting points were recorded on a Hoover-Thomas capillary melting point apparatus and were uncorrected. All pmr spectra were recorded on a Hitachi Perkin-Elmer high-resolution Model R-20A (60 MHz) instrument. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal standard for all pmr spectra run in deuterated dimethyl sulfoxide (DMSO-d_6). The ir spectra were recorded in potassium bromide disks with a Perkin-Elmer Model 257 instrument. All uv spectra were recorded in methanol on a Cary 15 instrument. All analyses for C, H, and N were performed by Heterocyclic Chemical Corp. of Harrisonville, Mo. Pmr assignments were recorded as parts per million (δ).

Pyrazolo[1,5-*a*]pyrimidin-7-one (1). A mixture of 37 g (0.5 mol) of ethyl formate and 44 g (0.5 mol) of ethyl acetate was added dropwise to a stirred suspension of 27 g (0.5 mol) of NaOMe in 1 l. of anhydrous ether, maintaining the temperature below 5°. After the addition was completed, the mixture was stirred at 5–10° for 1.5 hr and then gradually allowed to come to room temperature (3 hr). The mixture was filtered and the solid sodium salt thus prepared was added to 24.9 g (0.3 mol) of 3-aminopyrazole in 1 l. of absolute EtOH. This mixture was heated, with stirring, for 4 hr (reflux). The mixture was then cooled and the precipitated sodium salt was filtered. The solid was dissolved in 250 ml of H_2O and acidified to pH 5 with 6 *N* HCl. The product precipitated upon cooling the solution overnight at 4°. The title product was filtered the next day and recrystallized from EtOH–H_2O to yield 10.6 g (31%) of a white powder, mp 331° dec. *Anal.* ($C_6H_5N_3O$) C, H, N.

4-Ethylpyrazolo[1,5-*a*]pyrimidin-7-one (2). A mixture of 9.45 g (0.07 mol) of **1,** 21.84 g (0.14 mol) of EtI, 9.66 g (0.07 mol) of anhydrous K_2CO_3, and 50 ml of dry DMF was stirred at room temperature for 20 hr. The mixture was evaporated (*in vacuo*) and the residue was dissolved in 150 ml of H_2O and acidified with dilute HCl (to pH 1). The solution was extracted with $CHCl_3$ and the organic solvent was dried (Na_2SO_4) and evaporated to yield an oil. The oil was triturated with ether to give a solid, which upon recrystallization from EtOAc–acetone yielded 5.3 g (46%) of the title compound, mp 86–88°. *Anal.* ($C_8H_9N_3O \cdot H_2O$) C, H, N.

4-Ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (3). To a suspension of 1.63 g (0.01 mol) of 4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (**2**)[12] in 10 ml of concentrated H_2SO_4, 5 ml of 90% HNO_3 (*d* 1.5) was added dropwise over a period of 30 min at −10 to −5° with stirring. After stirring 30 min at the same temperature, the reaction mixture was allowed to stand at room temperature for 1 hr. The solution was poured onto 300 g of ice and kept at room temperature overnight. The precipitate was filtered, washed with H_2O, and dried to give 0.8 g (38%) of analytically pure product: mp 177–179°; nmr (DMSO-d_6) δ 1.49 (t, 3, CH_3), 4.38 (q, 2, OCH_2), 6.82 (d, 1, $J_{5,6}$ = 2 Hz), 8.16 (d, 1, $J_{5,6}$ = 2 Hz), 9.53 (s, 1, 2 H). *Anal.* ($C_8H_8N_4O_3$) C, H, N.

6-Carbethoxy-4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (5). A mixture of 2.07 g (0.01 mol) of 4-carbethoxy-7-hydroxypyrazolo[1,5-*a*]pyrimidine (**4**),[11] 1.38 g (0.01 mol) of anhydrous K_2CO_3, and 2.34 g (0.015 mol) of EtI in DMF (50 ml) was heated at reflux for 5 hr. The reaction mixture was evaporated *in vacuo* to give an oil. The residue was dissolved in H_2O (50 ml) and the pH was adjusted to 1 with dilute HCl. The solution was extracted with $CHCl_3$ (50 ml × 3) and the $CHCl_3$ solution was dried over Na_2SO_4. The $CHCl_3$ extracts were evaporated *in vacuo* and the residue was recrystallized from a mixture of *n*-hexane and acetone to give 1.55 g (66%) of an analytically pure product: mp 164–166°; nmr (DMSO-d_6) δ 1.33 (t, 3, CH_3), 1.45 (t, 3, CH_3), 4.28 (q, 4, $2OCH_2$), 6.63 (d, 1, $J_{2,3}$ = 2 Hz), 8.03 (d, 1, $J_{2,3}$ = 2 Hz), 8.75 (s, 1, 5 H). *Anal.* ($C_{11}H_{13}N_3O_3$) C, H, N.

6-Carboxy-4-ethylpyrazolo[1,5-*a*]pyrimidin-7-one (6). A mixture of 1.18 g (0.005 mol) of **5** and 10 ml of 5% NaOH was stirred at room temperature for 16 hr. The solution was acidified with dilute HCl and the precipitate was filtered. Recrystallization from EtOH gave 0.5 g (48%) of pure crystals: mp 187–189°; nmr (DMSO-d_6) δ 1.46 (t, 3, CH_3), 4.35 (q, 2, NCH_2), 6.75 (d, 1, $J_{2,3}$ = 2.5 Hz), 8.15 (d, 1, $J_{2,3}$ = 2.5 Hz), 8.98 (s, 1, 5 H), 12.85 (b, 1, COOH). *Anal.* ($C_9H_9N_3O_3 \cdot 0.5H_2O$) C, H, N.

6-Carbethoxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (7). To a suspension of 1.18 g (0.005 mol) of **5** in concentrated H_2SO_4 (10 ml), 90% HNO_3 (*d* 1.5, 5 ml) was added over a period of 10 min at the range of −10 ~ 0° with stirring. After stirring at this temperature for 10 min the solution was stirred at room temperature for 1 hr. The resultant clear solution was poured onto ice (300 g), and the H_2O solution was extracted with $CHCl_3$ (50 ml × 3). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was recrystallized from benzene to give 1.0 g (71%) of an analytically pure product: mp 146–148°; nmr (DMSO-d_6) δ 1.32 (t, 3, CH_3), 1.41 (t, 3, CH_3), 4.32 (q, 4), 4.63 (q, 4), 8.75 (s, 1), 8.83 (s, 1). *Anal.* ($C_{11}H_{12}N_4O_5$) C, H, N.

6-Carbethoxy-7-hydroxy-3-nitropyrazolo[1,5-*a*]pyrimidine (8). A mixture of 5.12 g (0.04 mol) of 3-amino-4-nitropyrazole[13] and 8.64 g (0.04 mol) of diethyl ethoxymethylenemalonate in acetic acid (50 ml) was heated at reflux for 7 hr. After cooling the reaction mixture, the precipitate was filtered, washed with EtOAc, and dried. Recrystallization from DMF–EtOH gave 5.5 g (61%) of an analytically pure product: mp 277° dec; nmr (DMSO-d_6) δ 1.35 (t, 3, CH_3), 4.32 (q, 2, OCH_2), 8.47 (s, 1), 8.73 (s, 1), 9.4 (b, 1). *Anal.* ($C_9H_8N_4O_5$) C, H, N.

6-Carboxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (9). A mixture of 2.52 g (0.01 mol) of **8,** 7.7 g (0.05 mol) of diethyl sulfate, and 2.76 g (0.02 mol) of anhydrous K_2CO_3 in DMF (50 ml) was heated on the steam bath for 1.5 hr. The reaction mixture was evaporated *in vacuo*. The residue was dissolved in H_2O (50 ml) and the pH was adjusted to 1 with dilute HCl. The precipitate was filtered, washed with H_2O, and dried to give 1.3 g of crystals. Recrystallization from benzene afforded 0.5 g (188%) of **7** which was identical in all respects with the product prepared by the nitration of **5.**

After the separation of **7,** the benzene-insoluble material was recrystallized from DMF–EtOH to give 0.4 g (16%) of analytically pure 6-carboxy-4-ethyl-3-nitropyrazolo[1,5-*a*]pyrimidin-7-one (**9**): mp 290° dec; nmr (CF_3COOD) δ 1.48 (t, 3, CH_3), 4.54 (q, 2, NCH_2), 8.70 (s, 1), 9.07 (s, 1). *Anal.* ($C_9H_8N_4O_5$) C, H, N.

Antitrichomonal Evaluation. *Tritrichomonas foetus* A 20075[13] was used in both *in vitro* and *in vivo* studies.

In *in vitro* studies, compounds were initially dissolved in DMSO (reagent grade) and serially diluted in TYM medium,[14] pH 7.0, containing 3% inactivated horse serum. End points were determined by microscopic examination of cultures and obtained as a 99% reduction of organisms for the minimum inhibitory concentra-

tion (MIC) at 48 hr of incubation, or an absence of organisms in a subculture after 24 hr of incubation for the minimum lethal concentration (MLC). Cultures were incubated at 35°.

In vivo activity was determined by a mouse-mortality model. Male CF-1 mice, 18–20 g, were inoculated with 2×10^6 *T. foetus* intraperitoneally. Compounds were suspended in a carboxymethylcellulose vehicle[15] and administered orally twice daily for 2 days beginning 24 hr after infection. The median survival time and number of survivors were used as criteria to assess activity. The dose of metronidazole affording 50% survival was approximately 22 mg/kg.

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3-Substituted 2′,3′-Dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ols as Potential Estrogens

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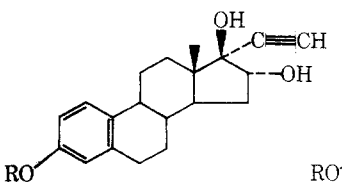
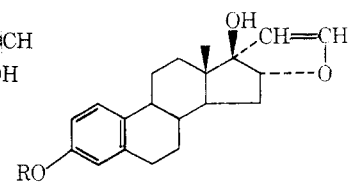
The preparation, characterization, and estrogenic activity of the two new steroids 3-(cyclopentyloxy)-2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ol and 2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-3,17β-diol are described. The compounds were found to be 0.1 and 0.002, respectively, as potent as estrone in a test designed to measure the uterine weight gain of treated immature mice relative to controls.

A potent, orally active estriol-like steroid, diol **1a**, useful in the treatment of menopausal syndrome and other conditions of estrogen deficiency has been synthesized recently in our laboratories.[1,2] In the course of studying the chemical reactivity of this diol, it was noted from thin-layer chromatographic observations that the reaction of **1a** with mild base resulted in transformation to a less polar compound.

High-resolution mass spectrometry indicated that this new compound had an identical molecular formula to the starting diol, while the mass spectrum of the silylated derivative (TMSI) of the compound showed the presence of only one trimethylsilyl group. The infrared spectrum revealed that the –C≡CH moiety was absent and a double resonance nmr experiment indicated the presence of two 1-proton doublets in the olefinic region. These data are consistent with alcohol **2a**, 3-(cyclopentyloxy)-2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ol, as the structure of the less polar transformation product from **1a**.



1a, R = cyclopentyl
b, R = H



2a, R = cyclopentyl
b, R = H

An alternative structure containing a 16α,17α-methyleneoxetane moiety is mechanistically possible;[3-5] however, it does not fit the analytical data. The ir spectrum of the product assigned structure **2a** lacks the strong absorption indicative of a methylene moiety exocyclic to a cyclobutane or oxetane ring[6] and, furthermore, the chemical shifts (H_2 = 6.2, H_3 = 4.8) and J value (J = 2.6) of the olefinic protons in the nmr spectrum of the model compound 2,3-dihydrofuran[7] are in excellent agreement with the observed data.

Although there are many examples of γ-lactones fused onto the D ring of a steroid nucleus[8-10] and there are a few sapogenin derivatives that contain a furan or reduced furan ring in a similar position,[11,12] compound **2a** is the first example of a steroid containing a [16α,17α-*b*]furan moiety with an alcohol function at C_{17}.

The relative stereochemistry of **2a** was ascertained by noting the pyridine-induced solvent shift of the C_{18}-methyl group in its nmr spectrum. The observed shift (δ $CDCl_3$–C_5D_5N) indicates the C_{18} methyl moiety is syn to the C_{17} OH group.[13] Diol **2b** was made from **1b** in a manner similar to the preparation of **2a** from **1a**. The spectroscopic and analytical data for **2b** are consistent with the proposed structure.

The estrogenic activity of **2a,b** was measured by treating groups of ten immature female mice each, subcutaneously, with the compound under test for 3 days at different dose levels (see Figure 1). A control group is maintained which receives only the injection vehicle. The average increase in the uterine weights of the treated mice as compared to those of the untreated mice determines estrogenic activity.[14] Using groups of ten mice each yields statistically significant results. Relative to estrone, the potency of **2a** was found to be 0.1 while that of **2b** was approximately 0.002. This demonstrated biological activity of the two new compounds is not surprising. Diverse studies continue to reveal that the structural criteria for estrogenic activity are much