TABLE I

1-SUBSTITUTED 5-NITROBENZIMIDAZOLES, 5-NITRO-2-METHYLBENZIMIDAZOLES, AND 5-NITRO-2-HYDROXYMETHYLBENZIMIDAZOLES

	5-Nitrobenzimidazoles						5-Nitro-2-methylbenzimidazoles						5-Nitro-2-hydroxymethylbenzimidazoles					
	M.p.[a]	Yield, %	Calcd.[b]		Found		M.p.	Yield, %	Calcd.		Found		M.p.	Yield, %	Calcd.		Found	
			C	H	C	H			C	H	C	H			C	H	C	H
CH_3	205–206[c]	44					231–2[d]	40					165	41	52.11	4.34	51.87	4.44
C_2H_5	131–132	81	56.54	4.71	56.84	4.87	176–7[e]	68					162	39	54.50	4.97	54.96	4.82
n-C_3H_7[f]	173–174	54	58.53	5.36	58.70	5.51	124–126	57	60.27	5.93	60.03	6.22	167–168	57	56.17	5.53	56.31	5.87
i-C_3H_7[g]	134–136	90	58.53	5.36	58.25	5.65	225–227	41	60.27	5.93	60.27	6.26	167–168	58	56.17	5.53	56.39	5.77
C_6H_5	162–163	76	65.27	3.76	64.99	4.01	166–168	26	66.27	4.34	66.01	4.01	186–187	53	62.45	4.08	62.90	4.37
$C_6H_5CH_2$[h]	158–159	73	66.40	4.34	66.86	4.52	148–150	30	67.41	4.86	67.23	5.07	205–207	24	63.60	4.59	63.98	4.75

[a] Fisher-Johns hot stage. [b] Analyses by C. F. Geiger, Ontario, Calif. [c] Davies, *J. Pharm. Pharmacol.*, **3**, 420 (1951) gives m.p. 209°. [d] Phillips, *J. Chem. Soc.*, 1931, 1143 gives m.p. 226°. [e] Foster, *J. Chem. Soc.*, 1957, 4687, gives m.p. 176°. [f] Prepared from 2-amino-4-nitro-*n*-propylaniline, m.p. 119–122 (80%); C, 55.41; H, 6.85: Calcd. C, 55.21; H, 6.65. [g] Prepared from 2-amino-4-nitro-*i*-propylaniline, m.p. 123–124 (61%); C, 55.32; H, 6.99: Calcd. C, 55.21; H, 6.65. [h] Prepared from 2-amino-4-nitrobenzylaniline, m.p., 153–154 (79%); C, 64.23; H, 5.56; Calcd. C, 64.30; H, 5.35.

nitrogen has been replaced by a substituent group has been carried out for the most part by direct substitution in the preformed benzimidazole. This leaves doubt concerning the identity of the isomers obtained. The structures of the benzimidazoles reported in this work result unequivocally from the method of synthesis.

EXPERIMENTAL

The compounds were prepared by treating 2,4-dinitrochlorobenzene with the appropriate secondary amine in 1:2 mole ratio in absolute ethanol.[4] The dinitro-substituted anilines were reduced by hydrogen sulfide in ethanolic–ammonium hydroxide solution.[5] The benzimidazoles and methylbenzimidazoles were obtained by treating the diamines with formic and acetic acids, respectively,[6] and the 2-hydroxy-methyl derivatives by the method of Phillips.[7]

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(4) Ref. *e*, Table I.
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Microbiological Transformations of Steroids. XVII.[1] Dehydrogenation of 5β-Pregnane-3,-11,20-trione 20-Ethylene Ketal by *Septomyxa affinis*

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The ability of the fungus *Septomyxa affinis* (ATCC 6737) to introduce ring A unsaturation into steroids has been described for a variety of substrates.[2,3] A particularly interesting aspect of the dehydrogenating ability of this microorganism is the fact that it dehydrogenates only the 1 and 2 positions of 3-keto steroids of the 5α- or 5β-pregnane series.[3] Other dehydrogenating organisms, such as *Fusarium solani*,[4] *F. caucasicum*,[4]

(1) Paper XVI of this series: *J. Org. Chem.*, **25**, 1968 (1960).
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(3) R. C. Meeks, P. D. Meister, S. H. Eppstein, J. P. Rosselet, A. Weintraub, H. C. Murray, O. K. Sebek, L. M. Reineke, and D. H. Peterson, *Chem. & Ind.* (London), **1958**, 391.
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and *Nocardia blackwellii*,[5] bring about dehydrogenation resulting in $\Delta^{1,4}$-dien-3-ones. In the case of *N. blackwellii* the Δ^4-3-one was reported to be the first intermediate. It thus appeared that the use of *Septomyxa affinis* represented the method of choice for the preparation of 5β-pregn-1-en-3-ones. Compounds of this type were desired to permit the preparation of modified cortical hormones. Unfortunately incubation of 5β-pregnane-3,11,20-trione with *S. affinis* results in degradation of the side-chain as well as dehydrogenation of the A-ring, resulting in 5β-androst-1-ene-3,11,17-trione.[3] Our studies[6] of the mechanism of microbial side-chain degradation seemed to indicate that a 20-keto function was required for side-chain degradation to occur, perhaps as an enzyme-substrate contact site. We felt that suitable chemical modification of this function, for example as the cyclic ketal, might result in blocking of the enzymatic side-chain degradation reaction without interfering with the 1,2-dehydrogenation. The requisite 5β-pregnane-3,11,20-trione 20-ethylene ketal to test this hypothesis was prepared by *N*-bromoacetamide oxidation[7] of 3α-hydroxy-5β-pregnane-11,20-dione 20 ethylene ketal[8] and subjected to fermentation by *S. affinis*. There was thus readily obtained, in about 43% yield, a crystalline substance exhibiting infrared absorption bands at 1700 cm.$^{-1}$, 1672 cm.$^{-1}$, and 1608 cm.$^{-1}$, as well as an ultraviolet absorption maximum at 225 mμ with ε 8800. These spectral properties, together with the elemental analysis, support formulation of the product as 5β-pregn-1-ene-3,11,20-trione 20-ethylene ketal. On mild acid hydrolysis the ketal group was removed to give material having infrared absorption bands at 1707 cm.$^{-1}$, 1688 cm.$^{-1}$, 1670 cm.$^{-1}$, and 1610 cm.$^{-1}$, and an ultraviolet absorption maximum at 225 mμ with ε 7250. The substance can be formulated as 5β-pregn-1-ene-3,11,20-trione.

It would appear from these results that conversion of a steroid keto group to a ketal successfully blocks at least one of the enzymatic reactions normally occurring in the environment of the keto group. The application of this finding to other enzymatic reactions of steroids is being studied further.

EXPERIMENTAL[9]

5β-Pregnane-3,11,20-trione 20-ethylene ketal. To a solution of 75.5 g. of 3α-hydroxy-5β-pregnane-11,20-dione 20-ethylene ketal in 2000 ml. of *t*-butyl alcohol containing 100 ml. of pyridine was added 41.3 g. of *N*-bromoacetamide. After the mixture had been stirred overnight at room temperature, a solution of 25 g. of sodium bisulfite in 350 ml. of water was added and the solution evaporated at reduced pressure until most of the *t*-butyl alcohol had been removed. The resulting precipitate was filtered and washed with aqueous sodium bicarbonate, with water, and then dried to give 57.7 g. of crude product. Recrystallization of the crude material from acetone-petroleum ether (b.p. 60–70°) afforded 26.7 g. of 5β-pregnane-3,11,20-trione 20-ethylene ketal, m.p. 150–153°. The analytical sample, recrystallized again from acetone–petroleum ether, had m.p. 150–151°, $[\alpha]_D$ + 67° (*c* 0.8 in dioxane).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.18; H, 9.55.

5β-Pregn-1-ene-3,11,20-trione 20-ethylene ketal. A fermentor containing 10 l. of a medium at *p*H 4.9 made from commercial dextrose (10 g./l.) and corn steep (12 g. solids/l.) to which 1 ml. of Dow-Corning XC-120 antifoaming agent had been added was steam sterilized at 20 lb./in.^{2} (gauge) for 90 min. Upon cooling, the sterile medium was inoculated with 500 ml. of a 72-hour vegetative growth of *Septomyxa affinis* (A.T.C.C. 6737). After 48 hr. of agitation (300 r.p.m.) and aeration (0.5 l. of air/min.), a solution of 2.0 g. of 5β-pregnane-3,11,20-trione 20-ethylene ketal and 0.05 g. of 3-ketobisnor-4-cholen-22-al[2b] in 30 ml. of *N*,*N*-dimethylformamide was added, using 10 ml. of acetone to rinse the substrate addition flask. The fermentation was continued for 48 hr. under the same conditions. Extraction of the conversion product with methylene chloride and work-up as described previously[10] afforded a crude oily residue that was shown by paperchromatography, using the CM System,[11] to contain material of the same mobility as 5β-pregnane-3,11,20-trione 20-ethylene ketal, but which, unlike that substance, had ultraviolet absorbing properties.

The oily residue was dissolved in a minimum of methylene chloride and chromatographed over a 2.8 × 36 cm. column of Florisil[12] prewashed with petroleum ether. Elution with 5% acetone–petroleum ether afforded crystalline material that was recrystallized from acetone–petroleum ether to give 0.87 g. of 5β-pregn-1-ene-3,11,20-trione 20-ethylene ketal, m.p. 204–206°, λ_{max}^{EtOH} 225 mμ, ε 8800; $\nu_{cm^{-1}}^{Nujol}$ 1700, 1672, 1608.

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.38; H, 8.78.

5β-Pregn-1-ene-3,11,20-trione. A solution of 0.50 g. of 5β-pregn-1-ene-3,11,20-trione 20-ethylene ketal in 25 ml. of methanol was hydrolyzed by the action of 5 ml. of *N* hydrochloric acid at room temperature for several hours. Concentration of the solution to a small volume and cooling afforded a crystalline product, recovered by filtration and recrystallized[13] from acetone–petroleum ether to give 5β-pregn-1-ene-3,11,20-trione, m.p. 150.5–151.5°, λ_{max}^{EtOH} 225 mμ, ε 7250; $\nu_{cm^{-1}}^{Nujol}$ 1707 (shoulder), 1688, 1670 (shoulder), 1610.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 77.06; H, 8.96.

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(13) We are indebted to Dr. W. J. Wechter for purification of this material.

associates for infrared and ultraviolet absorption measurements, and to G. Staffen and Miss I. N. Pratt for technical assistance.

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Pyrimidines. VII. 2-Amino-4-(substituted anilino)pyrimidines[1]

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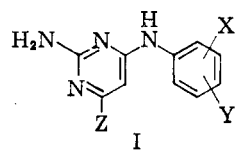
Arylaminopyrimidines of the general formula (I) have demonstrated a broad spectrum of pharmacological applications, including the antimalarials,[2] analgesic agents,[3] agents for the *Trypanosoma rhodesiense* infections,[4] antagonists to the folic-folinic acid system,[5] and bacteriostatic agents.[6-8] Rose and co-workers have suggested that this type of compound might act as a riboflavin antagonist.[2] Hence the synthesis of certain arylaminopyrimidines is logically incorporated in our general study of pyrimidines in the effort to obtain more information in the search for antitumor agents.



I

An extensive literature search indicated that very few of these 4-arylaminopyrimidines (I) where Z = CH_3, NH_2, OH, SH and Cl have been synthesized. In all cases the aniline-substitution is either at the *para* position or at the aniline nitrogen atom.

Existing methods for the preparation of these compounds are quite similar. Banks[3] has treated 2-amino-4-chloro-6-methylpyrimidine with aniline in aqueous ethanol, catalyzed by a trace of acid, to obtain 2-amino-4-anilino-6-methylpyrimidine. This general method of synthesis has been extended to include other 2-amino-4-(substituted anilino)-6-methylpyrimidines,[2,9,10] 2-amino-4-chloro-6-(substituted anilino)pyrimidines,[2,3] 2,4-diamino-6-(substituted anilino)pyrimidines,[4,9] and 2-amino-6-(substituted anilino)-4-pyrimidinols.[9]

When an *ortho* substituted aniline was used, according to the known procedures, the reaction failed to proceed. This prompted the investigation of a general method that could apply to all anilines. Peters and co-workers[11] have reported a fusion method for the preparation of 2-(methylthio)-4-(2′,6′-dichloroanilino)-5-carbethoxypyrimidine. A modification of this procedure has been utilized for the preparation of arylaminopyrimidines (I), where Z = CH_3, NH_2, and Cl.

Since 2-amino-4-chloro-6-(substituted anilino)-pyrimidines can be prepared quite readily by fusion, we have used these chloro compounds for the preparation of the corresponding hydroxy and thio derivatives (I, Z = OH, SH). Various attempts to convert these chloro groups under mild conditions have failed, due to the fact that chloro compounds of this type are extremely unreactive towards nucleophilic substitution. In order to convert these chloro groups, it was necessary to use sodium hydroxide or sodium hydrosulfide heated in ethylene glycol at 150°. These reactions proceed readily in 65–85% yield. By this procedure all the arylaminopyrimidines (I, Z = SH, OH) have been synthesized.

The compounds have been submitted for general screening at the Cancer Chemotherapy National Service Center.

EXPERIMENTAL[12]

Preparation of arylaminopyrimidines (I, Z = NH_2, CH_3, Cl, SH, OH). *Method A.* (See Tables I, II, and III.) One milliliter of concentrated hydrochloric acid was added to a mixture of chloropyrimidine (0.1 mole) and aniline (0.1 mole) in a round-bottom flask. The flask was immersed in an oil bath preheated to 175°. When the internal temperature reached 120° a complete melt resulted. At 155° an exothermic reaction took place and the temperature rose spontaneously to 185°. This temperature was maintained for 20 min. and the melt cooled. The glass-like substance was dissolved in 200 ml. of dilute hydrochloric acid, treated with charcoal, and filtered. The clear filtrate was made alkaline with ammonia solution. The white crystalline arylaminopyrimidine deposited on cooling. The product was filtered, dried at 80°, and recrystallized from a mixture of water and ethanol.

Method B. (See Table IV). To 150 ml. of ethylene glycol,

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