DERIVATIVES OF PYRIMIDINE OBTAINED BY CONDENSATION OF *p*-NITROPHENYLBIGUANIDE AND *p*-NITROPHENYLAMIDINEUREA WITH ETHYL ACETOACETATE AND ACETYLACETONE, AND THEIR BIOLOGICAL ACTIVITY

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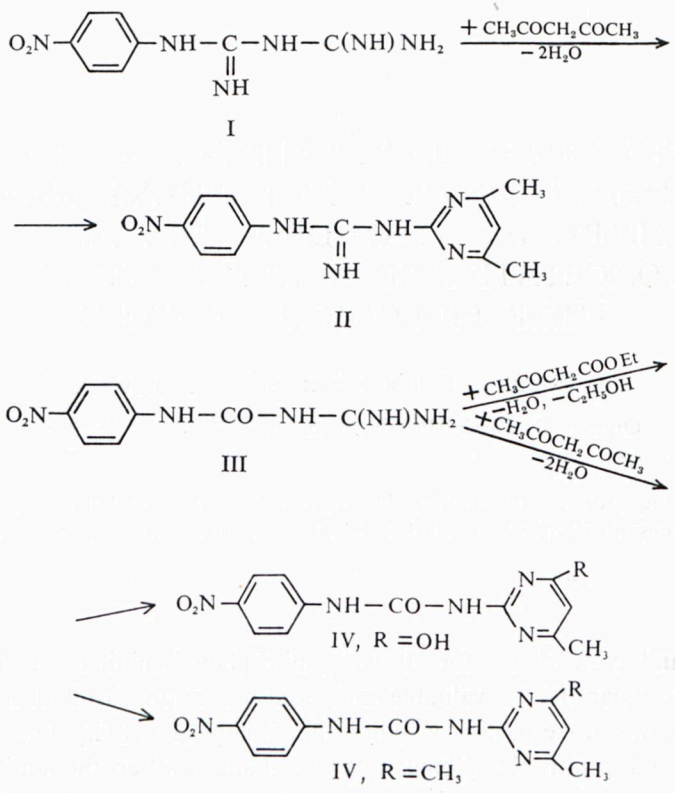
Abstract—Synthesis of new pyrimidine derivatives of *p*-nitrophenylguanidine and *p*-nitrophenylurea is described. The antimalarial activity *in vivo* and toxicity of the products was established.

URBAŃSKI and co-workers[1] found that *p*-nitrophenylamidineurea (III) hydrochloride (Nitroguanil) is a valuable and nontoxic antimalarial drug. The aim of this work was to establish whether the biological activity and toxicity of Nitroguanil and *p*-nitrophenylbiguanide (I) changed after the amidine group had been converted into the pyrimidine ring. 1-(*p*-Nitrophenyl)-3-[(4,6-dimethyl)-2-pyrimidyl]-guanidine (II) was obtained by condensation of I with acetylacetone. Condensation of III with ethyl acetoacetate and acetylacetone yielded 1-(*p*-nitrophenyl)-3-[(4-hydroxy-6-methyl)-2-pyrimidyl]-urea (IV, R=OH) and 1-(*p*-nitrophenyl)-3-[(4,6-dimethyl)-2-pyrimidyl]-urea (IV, R=CH_3), respectively.

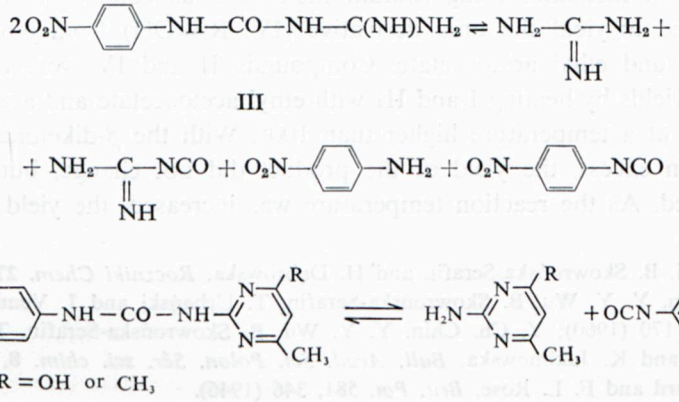
A compound of this group, 1-(*p*-nitrophenyl)-3-[(4-hydroxy-6-methyl)-2-pyrimidyl]-guanidine, has been prepared by Curd and Rose[2] from I and ethyl acetoacetate in methanol using sodium methoxide as condensing agent. This method failed to yield the urea derivative (IV, R=OH) from *p*-nitrophenylamidineurea and ethyl acetoacetate. Compounds II and IV were obtained in fairly good yields by heating I and III with ethyl acetoacetate and acetylacetone, respectively, at a temperature higher than 100°. With the β-diketone or β-keto ester used in excess, the yield of the product did not change, but its purity was enhanced. As the reaction temperature was increased, the yield as well as

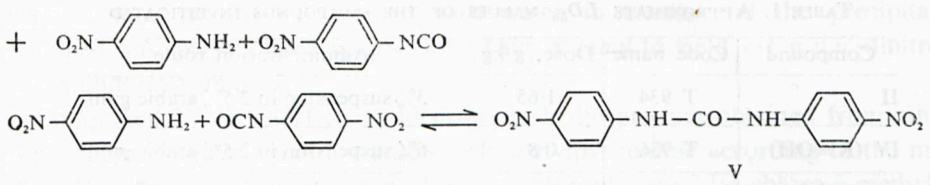
[1] T. Urbański, B. Skowrońska-Serafin and H. Dąbrowska, *Roczniki Chem.* **27**, 65 (1953); Y. Ch. Chin, Y. Y. Wu, B. Skowrońska-Serafin, T. Urbański and J. Venulet, *Nature, Lond.* **186**, 170 (1960); Y. Ch. Chin, Y. Y. Wu, B. Skowrońska-Serafin, T. Urbański, J. Venulet and K. Jakimowska, *Bull. Acad. Sci. Polon. Sér. sci. chim.* **8**, 109 (1960).

[2] F. H. S. Curd and F. L. Rose, *Brit. Pat.* 581, 346 (1946).



the recovery of the starting materials (I and III) decreased. When carried out in more severe conditions (increased temperature and prolonged heating), the condensation of III with ethyl acetoacetate and acetylacetone yielded, besides the pyrimidine derivative, a considerable amount (up to 20%) of N,N′-di-(*p*-nitrophenyl)-urea (V). This compound is formed probably by decomposition of the product or the starting material according to one of the equations:



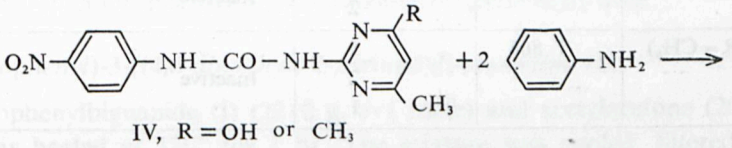


V

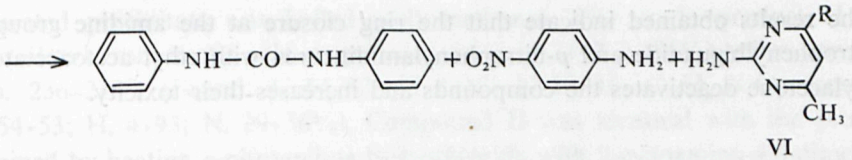
The decomposition products, *p*-nitrophenyl isocyanate and *p*-nitroaniline, can react together to give *p,p'*-dinitrocarbanilide (V). This course of the reaction is supported by the fact that small amounts of ethyl *p*-nitrophenylurethane were isolated from the condensation product of III with ethyl acetoacetate. The urethane is formed during the condensation as the product of interaction of *p*-nitrophenyl isocyanate and ethanol. The formation of these compounds as intermediates is in agreement with the results obtained by Mukaiyama and Matsunaga[3] for urea, which on thermal decomposition forms ammonia and isocyanic acid:

$$NH_2CONH_2 \rightleftharpoons NH_3 + HNCO$$

The highest yields of compounds II and IV were obtained when a large 2–4-fold excess of acetylacetone or ethyl acetoacetate was applied and the reaction was carried out at 120–140° for about 1 hr. Compounds II and IV were also prepared by a known method from respective *p*-nitrophenyl and pyrimidine derivatives for identification. Their physical properties and I.R. spectra were identical with those of the compounds synthesized from I or III.



IV, R = OH or CH_3



VI

When heated with aniline, the two urea derivatives IV yielded carbanilide, *p*-nitroaniline and 2-amino-4-hydroxy-6-methylpyrimidine (VI, R=OH) or 2-amino-4,6-dimethylpyrimidine (VI, $R=CH_3$). Carbanilide was isolated from the reaction product in a nearly quantitative yield.

Toxicity of these compounds was investigated on white mice. The compounds were administered intraperitoneally. The approximate LD_{50} values are collected in the Table 1.

[3] T. Makaiyama and T. Matsunaga, *J. Amer. Chem. Soc.* **75**, 6209 (1953).

TABLE 1. APPROXIMATE LD_{50} VALUES OF THE COMPOUNDS INVESTIGATED

Compound	Code name	Dose, g/kg	Administration route
II	T 934	1·65	3% suspension in 2·5% arabic gum
IV (R=OH)	T 936	0·8	6% suspension in 2·5% arabic gum
IV ($R=CH_3$)	T 866	0·6	4% suspension in 2·5% arabic gum

Antimalarial activity of these compounds was established by testing them upon *Plasmodium berghei* in mice. The compounds were administered intraperitoneally. Results are collected in Table 2.

TABLE 2. ANTIMALARIAL ACTIVITY OF THE COMPOUNDS EXAMINED

Compound	Code name	Dose, mg/20g mouse/day	Result
II	T 934	20 10 5	Toxic, mice died in 2–4 days
		2 1 5 2	Inactive
IV (R=OH)	T 936	10	Toxic, mice died in 4 days
		5 2	Inactive
IV ($R=CH_3$)	T 866	5 2 1	Inactive

The results obtained indicate that the ring closure at the amidine group of *p*-nitrophenylbiguanide and *p*-nitrophenylamidineurea with ethyl acetoacetate or acetylacetone deactivates the compounds and increases their toxicity.

EXPERIMENTAL

1-(p-*Nitrophenyl*)-3-[(4,6-*dimethyl*)-2-*pyrimidyl*]-*urea* (IV, $R=CH_3$)

p-Nitrophenylamidineurea (III) (22·3 g, 0·1 mole) and acetylacetone (20 ml, 0·2 mole) were heated at 120° for 1 hr. The reaction mixture was cooled, the excess of acetylacetone was filtered off and the precipitate was boiled with methanol in order to remove the starting materials. The crude product was crystallized from acetic acid or pyridine to yield 12·1 g (42%) yellow crystals, m.p. 270–271°. (Found: N, 24·64. Calcd. for $C_{13}H_{13}N_5O_3$: N, 24·37%). The

acetylacetone filtrate was treated with water in excess and the precipitate obtained was recrystallized several times from ethanol to yield 1·6 g *p,p'*-dinitrocarbanilide (V), m.p. 312°.

Compound IV (R=CH_3) was identical with the product obtained from ethyl *p*-nitrophenylurethane and 2-amino-4,6-dimethylpyrimidine according to the method described by Ashworth *et al.*[4] for 1-(*p*-chlorophenyl)-3-[(4-chloro-6-methyl)-2-pyrimidyl]-urea. Compound IV (R=CH_3), m.p. 269–270°, was also obtained by O'Neill and Basso.[5]

1-(p-*Nitrophenyl*)-3-[(4-*hydroxy*-6-*methyl*)-2-*pyrimidyl*]-*urea* (IV, R=OH)

p-Nitrophenylamidineurea (III) (22·3 g, 0·1 mole) was heated with ethyl acetoacetate (20 ml, 0·2 mole) at 140° for 1·5 hr. The reaction mixture was cooled, the resulting precipitate was filtered, boiled with methanol and refiltered. The crude product was dissolved in alcohol-water 2% sodium hydroxide solution. The precipitated crystalline sodium salt was separated and acidified with diluted acetic or mineral acid. The operation was repeated 2–3 times to afford 10·2 g (35%) yellow product, m.p. 306–310° with decomposition. (Found: C, 49·45; H, 3·95; N, 24·32. $C_{12}H_{11}N_5O_4$ requires: C, 49·83; H, 3·83; N, 24·21%). The ethyl acetoacetate filtrate was evaporated *in vacuo* to dryness. *p,p'*-Dinitrocarbanilide (V) m.p. 312° (2·2 g) and ethyl *p*-nitrophenylurethane, m.p. 127–128° (0·3 g), were separated by fractional crystallization from ethanol. Compound IV (R=OH) is identical with the product obtained from *p*-nitroaniline and 2-ureido-4-hydroxy-6-methylpirimidine according to the method described by Ashworth *et al.*[4] for 1-(*p*-chlorophenyl)-3-[(4,6-dimethyl)-2-pyrimidyl]-urea.

1-(p-*Nitrophenyl*)-3-[(4,6-*dimethyl*)-2-*pyrimidyl*]-*guanidine* (II)

p-Nitrophenylbiguanide (I) (22·2 g, 0·1 mole) and acetylacetone (20 ml, 0·2 mole) was heated at 120° for 1 hr. The mixture was cooled, filtered and the separated precipitate was boiled with methanol. The crude product II was crystallized from acetic acid or pyridine to yield 24·2 g (85%) yellow crystals, m.p. 236–238°. (Found: C, 54·82; H, 5·12; N, 29·36. $C_{13}H_{14}N_6O_2$ requires: C, 54·53; H, 4·93; N, 29·36%). Compound II was identical with the product obtained by heating *p*-nitroaniline hydrochloride with 2-cyanamino-4,6-dimethylpyrimidine in ethanol according to the method described by Birtwell[6] for 1-(*p*-chlorophenyl)-3-[(4,6-dimethyl)-2-pyrimidyl]-guanidine.

[4] R. de B. Ashworth, A. W. Crowther, F. H. S. Curd and F. L. Rose, *J. Chem. Soc.* 581 (1948).

[5] R. C. O'Neill and A. J. Basso, *U. S. Pat.* 2,762,742 (1956).

[6] S. Birtwell, *J. Chem. Soc.* 1725 (1953).