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[Ph 34]

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Pyrazoles and Pyrazolo[3,4-*d*]pyrimidines as Biologically Active Agents

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Eingegangen am 12. August 1978

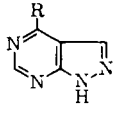
Syntheses of the pyrazole derivatives **2a–c** by different routes and cyclisation of the products to pyrazolo [3,4-d] pyrimidines are reported. The structures of the compounds were confirmed by spectroscopy. A few of them were screened as pesticides.

Pyrazole und Pyrazolo [3,4-*d*]pyrimidine als biologisch aktive Verbindungen

Es wird über die Synthese der Pyrazolderivate **2a–c** auf verschiedenen Wegen und ihre Zyklisierung zu Pyrazolo[3,4-*d*]pyrimidinen berichtet. Die Struktur der Verbindungen wird durch Spektroskopie abgesichert, einige werden auf pestizide Wirkung getestet.

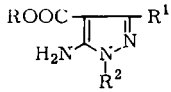
0365-6233/79/0707-0586 $ 02.50/0

The various bio-responses promoted by pyrazoles are illustrated by their insecticidal, analgesic, sedative, anticonvulsant, antipyretic, anti-inflammatory und diuretic activities[1]. Pyrazoles and pyrazolo[3,4-d]pyrimidines and their substituion products possess interesting enzyme-inhibiting properties. Many xanthine oxidase and choline-esterase inhibitors possess carcinostatic activity and are effective pesticides. Several 1-substituted pyrazoles elicited[2,3] significant activity against tumor CA-755. They are less effective on S-180. The anti-tumor activity of pyrazolo[3,4-d]pyrimidine and its substitution products was more extensive than any of the other bio-active pyrazoles. 4-Aminopyrazolo[3,4-d]pyrimidine **(1a)** was active against CA-755 and numerous other tumors. Substitution of certain alkyl group in the position 1 (corresponding to position 9 in purine) provided active compounds. Substitution of alkyl group in amino function at position 4 (position 6 in purine) produced less toxic products which had superior indexes, while simultaneous substitution at position 1 and 4 usually diminished the anti-tumor activity. Use of allopurinol **(1b)** in a dose of 0.2–1.0 g showed higher therapeutic index than any of the other neoplastic agents[4,5]. Based on these observations a few pyrazoles were prepared by the action of ethoxymethylene-ethylcyanoacetate and dimethylmercaptomethylene-methylcyanoacetate with substituted hydrazines.



1

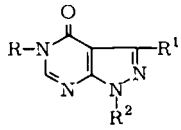
a: R = NH_2
b: R = OH



2

a: R = Et; R^1 = H; R^2 = Cyclopentyl
b: R = Me; R^1 = SMe; R^2 = Cyclopentyl
c: R = Me; R^1 = SMe; R^2 = p-Nitrophenyl

The resulting derivatives **2a,b,c** were cyclised into pyrazolo[3,4-d]pyrimidines **3a,d,e** by refluxing with formamide. N-Alkyl derivatives **3b,c,f,g** were prepared by stirring a mixture of **3a,d** with suitable alkyl halide in 1 % aqueous sodium hydroxide.



3

a: R = R^1 = H; R^2 = Cyclopentyl
b: R = Me; R^1 = H; R^2 = Cyclopentyl
c: R = Benzyl; R^1 = H; R^2 = Cyclopentyl
d: R = H; R^1 = SMe; R^2 = Cyclopentyl
e: R = H; R^1 = SMe; R^2 = p-Nitrophenyl
f: R = Me; R^1 = SMe; R^2 = Cyclopentyl
g: R = Benzyl; R^1 = SMe; R^2 = Cyclopentyl

Ir spectra of **2b,c** showed two peaks in the region 3310–3420 cm^{-1} due to NH stretching vibrations. The peak in the region 1670–1680 cm^{-1} is attributed to the carbonyl function. A small shift of the >C=O and NH bands (~20 cm^{-1}) to lower region is expected due to intramolecular hydrogen bonding. Two sharp and strong peaks at 1550 and 1340 cm^{-1} in compound **2c** are assigned to the NO_2 group. Ir spectra of pyrazolo [3,4-d]pyrimidines **3** showed a strong and sharp band in the region of 1680–1700 cm^{-1} due to /C=O stretching vibrations in a cyclic amide. Two weak peaks at 3100 and 3140 cm^{-1} are attributed to NH stretching vibrations **(3a,d,e).** The presence of two strong peaks at 1510 and 1345 cm^{-1} is assigned to the $-NO_2$ group.

Biological Activity

The pyrazolo[3,4-d]pyrimidines **3a–d,f,g** were screened for pesticidal activity. Only the compounds **(3b,f)** exhibited fungicidal activity against Rhizoctonia solani and Pythium at 64 ppm concentration with phytotoxicity rating of 7 and 8 respectively.

The fungicidal activity was evaluated by foliage spray and soil drench method. After 14 days phytotoxicity was rated on the scale of 0–11 where 0 corresponds to no injury and 11 implies death of the plants. Stunting was also rated on the scale of 1 (slight) to 9 (severe).

The screening results revealed that the N-methyl substituted pyrazolo[3,4-d]pyrimidines **3b,f** were active against fungi while the N-benzyl derivatives **3c,g** were completely inactive. The presence of the methyl-mercapto group also increases the fungicidal activity. The nature of the substitution at position 1 in **3** is not very significant for pesticidal activity.

The authors are thankful to Prof. *W. Pfleiderer,* University of Konstanz, West Germany, for spectral and elemental analyses and Prof. *R.P. Rastogi,* Head, Chemistry Department, University of Gorakhpur, India, for valuable suggestions and encouragements.

Experimental

All the m.ps. are uncorrected.

1-Cyclopentyl-3-methylmercapto-4-methoxycarbonyl-5-aminopyrazole **(2b)**

A solution of 10.1 g dimethylmercaptomethylenemethylcyanoacetate in ethanol was treated with cyclopentylhydrazine. The resulting mixture was refluxed for 1 h, cooled and diluted with water, which afforded a viscous liquid. It was again refrigerated overnight, the solid obtained was dried i. vac. d. From water-ethanol mixture: white crystalline solid, yield 10.0 g, m.p. 82 °C. UV: (methanol) λ max (log ε): 250, 216 nm (4.02, 4.36). MS: 50°, 70 ev, m/e (% abundance): 255 (100); 240 (6); 224 (8); 208 (6).

$C_{11}H_{17}N_3O_2S$ Calcd.: C 51.8 H 6.6 N 16.5 Found: C 51.7 H 6.8 N 16.5.

1-(p-)Nitrophenyl-3-methylmercapto-4-methoxycarbonyl-5-aminopyrazole **(2c)**

A mixture of dimethylmercaptomethylenemethylcyanoacetate and p-nitrophenylhydrazine in equimolar ratio was refluxed for 1 h in ethanol. During this time a yellow precipitate separated out which was crystallised with DMF, yield 61 %, m.p. 204 °C. MS: 130°, 70 ev, m/e (% abundance): 308 (100); 292 (10); 277 (45); 262 (10); 261 (11). $C_{12}H_{12}N_4O_4S$ Calcd.: C 46.8 H 3.89 N 18.2 Found: C 46.6 H 3.78 N 18.3.

1-Cyclopentyl-4-hydroxypyrazolo[3,4-d]pyrimidine **(3a)**

A solution of 3.4 g ethoxymethyleneethylcyanoacetate and 2.0 g cyclopentylhydrazine in ethanol was mixed and refluxed overnight. After complete refluxing the solvent and excess of hydrazine were removed under reduced pressure. A syrupy product was obtained which could not be solidified. It was directly cyclised to pyrazolo[3,4-d]pyrimidine by refluxing with 15 ml formamide. The solid was crystallised with ethanol, yield 50 %, m.p. 232 °C. UV: (methanol) λ max (log ε): 252.5, 211 nm (3.67,

4.24). MS: 125°, 70 ev, m/e (% abundance); 204 (25); 189 (4); 176 (6); 135 (8).
$C_{10}H_{12}N_4O$ Calcd.: C 58.8 H 5.88 N 27.5 Found: C 58.9 H 5.6 N 27.5.

1-Cyclopentyl-4-oxo-5-methylpyrazolo[3,4-d]pyrimidine **(3b)**

0.6 g **3a** were dissolved in a few ml of DMF and potassium carbonate (6 %). To this solution 2 ml methyl iodide were added and the reaction mixture was stirred overnight at room temp. The clear solution was diluted with water and crystallised with methanol, yield 0.51 g (73.4 %), m.p. 168 °C. UV: (methanol) λ max (log ε): 255, 211 nm (3.80, 4.46). MS: 175°, 70ev, m/e (% abundance), 218 (100); 203 (16); 190 (8); 189 (18).
$C_{11}H_{14}N_4O$ Calcd.: C 58.8 H 5.92 N 27.4 Found: C 59.0 H 6.06 N 27.5.

1-Cyclopentyl-4-oxo-5-benzylpyrazolo[3,4-d]pyrimidine

A mixture containing 0.6 g **3a** and 0.4 g benzyl chloride in 10 ml DMF was stirred for 3 h in presence of 0.45 g potassium carbonate. Water was added and a crystalline product separated out which was washed with petroleum ether (60–80°). It was crystallised with methanol, yield 0.5 g (60 %), m.p. 105 °C. UV: (methanol) λ max (log ε): 260, 213 nm (3.79, 4.54). MS: 175°, 70ev, m/e = 294.
$C_{17}H_{18}N_4O$ Calcd.: C 69.4 H 6.12 N 19.1 Found: C 69.4 H 6.2 N 19.3.

1-Cyclopentyl-3-methylmercapto-4-hydroxypyrazolo[3,4-d]pyrimidine **(3d)**

10.0 g **2b** were refluxed with 50 ml formamide for 3 h. The reaction mixture was cooled and the dark-brown residue after treating with animal charcoal was crystallised with ethanol as white crystalline solid, yield 5.0 g (51 %), m.p. 182 °C. UV: (methanol) λ max (log ε): 250, 231 nm (3.97, 4.19).
$C_{11}H_{14}N_4OS$ Calcd.: C 52.8 H 5.6 N 22.4 Found: C 52.6 H 5.8 N 22.6.

1-(p-)Nitrophenyl-3-methylmercapto-4-hydroxypyrazolo[3,4-d]pyrimidine **(3e)**

7.5 g **2c** were refluxed with formamide for 3 h. During this time a yellow precipitate separated which was washed with methanol and crystallised with DMF, yield was quantitative, m.p. 310 °C. UV: (methanol) λ max (log ε): 340, 255, 227.5 nm (4.22, 4.08, 4.37). MS: 175°, 70ev, m/e (% abundance), 302 (100); 287 (8); 270 (75); 256 (8).
$C_{12}H_9N_5O_3S$ Calcd.: C 47.5 H 2.97 N 23.0 Found: C 47.6 H 3.11 N 23.2.

1-Cyclopentyl-3-methylmercapto-4-oxo-5-methylpyrazolo[3,4-d]pyrimidine **(3f)**

0.8 g **3d** were dissolved in sodium hydroxide (5 %) and methyliodide (a little more than equimolar quantity) was added. To clear the solution ethanol was added and the whole mixture was stirred at room temp. for 1 h. Now water was added to the clear solution whereupon a white precipitate separated which was washed with water. Finally it was crystallised with ethanol as white needles, yield 0.5 g (62.7 %), m.p. 133 °C. UV: (methanol) λ max (log ε): 250, 230 nm (3.95, 4.11). MS: 175°, 70ev, m/e (% abundance), 264 (100); 217 (12); 189 (6); 163 (77).
$C_{12}H_{16}N_4OS$ Calcd.: C 54.5 H 6.06 N 21.2 Found: C 54.6 H 6.32 N 21.4.

1-Cyclopentyl-4-chloropyrazolo[3,4-d]pyrimidine

A mixture of 20 g **3a**, 100 ml $POCl_3$ and 25 ml dimethylaniline was refluxed for 5 h. At the end, excess of $POCl_3$ and dimethylaniline was removed under reduced pressure and the viscous liquid was poured

on ice cautiously with vigorous stirring. The suspension was neutralized with ammonia and the desired compound was extracted with chloroform and dried over fused calcium chloride. The filtrate was distilled under reduced pressure and the fraction between 107–111 °C (13 mm) was collected, yield 16.6 g (73.3 %), m/e = 222.
$C_{10}H_{11}ClN_4$ Calcd.: C 53.9 H 4.94 N 25.2 Found: C 54.0 H 5.1 N 25.3.

1-Cyclopentyl-3-methylmercapto-4-oxo-5-benzylpyrazolo[3,4-d]pyrimidine **(3g)**

3g was prepared by the reaction of **3d** and benzyl chloride, following the procedure described earlier. Attempts were made to solidify and crystallise the compound but always a gummy mass was obtained which could not be properly characterised, m/e = 340.

1-Cyclopentyl-4-hydrazinopyrazolo[3,4-d]pyrimidine

To a solution of 6.0g 1-cyclopentyl-4-chloropyrazolo[3,4-d]pyrimidine in little ethanol, 6.0g hydrazine hydrate 90 % were added and the resulting mixture was refluxed for 12h. After complete refluxing the solvent and excess of hydrazine were removed under reduced pressure. The white solid which separated was crystallised with ethanol, yield 40 %, m.p. 191 °C, m/e = 218. $C_{10}H_{14}N_6$ Calcd.: C 55.1 H 6.42 N 38.5 Found: C 55.2 H 6.55 N 38.6.

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