Reaction of Tetraoxopyrrolo[2,3-*d*]pyrimidines, Isatin Analogues, with *o*-Phenylenediamine

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Received December 14, 1978

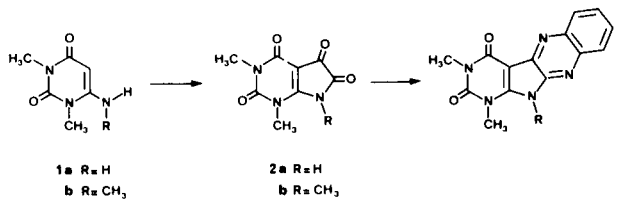
In the course of general studies on new antitumor tetracyclic intercalating agents, the condensation of *o*-phenylenediamine with two new tetraoxopyrrolo[2,3-*d*]pyrimidine derivatives has been carried out. The structure of the reaction products as arylketimines has been elucidated and mass spectral fragmentations are discussed.

J. Heterocyclic Chem., **16**, 717 (1979).

A wide variety of polycyclic molecules have been shown to interfere with the replication of DNA through intercalation between adjacent partially unwound base pairs as first described by Lerman (1). Actinomycin (2), ethidium bromide (3), acridines (4) and proflavine (5) are known to bind to DNA by this intercalative process. This type of binding to DNA is also observed for other planar heterocyclic ring systems such as ellipticine, a pyridocarbazole derivative with a high degree of activity against leukemia (6-8).

In connection with our studies on compounds related to this last-mentioned drug (9) and in order to enhance the affinity for DNA, we thought to introduce a uracil ring into the planar system. A good approach seemed to be the synthesis of a functionalized pyrrolopyrimidine **2**, an isosteric ring of isatin, followed by condensation with *o*-phenylenediamine (Scheme I). As starting materials,

Scheme I

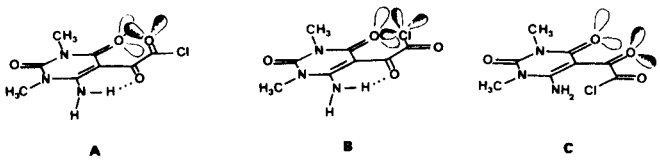


1a R=H
b R=CH_3

2a R=H
b R=CH_3

1,3-dimethyl-4-aminouracil (**1a**) and the methylamino derivative (**1b**) were used. It is known that such molecules easily react with acid chlorides at the 5-position, which can be considered as a nucleophilic site, the 4-amino group remaining unattacked even with a large excess of acid chloride (10). Nevertheless, we obtained in a one-step synthesis, the 7*H*-pyrrolo[2,3-*d*]pyrimidines **2** by the action of oxalyl chloride on **1** in the presence of pyridine. Such a cyclization can be accounted for due to an anomeric effect involving the free orbitals of the oxygen atom of the uracil group on one hand and the free orbitals of the oxygen for chlorine atom of the oxychloride moiety on the other hand. Thus, hydrogen bonding shown to stabilize the carboxyl group in other cases reported in the literature (10) does not occur and the intermediate form C (Scheme II) is much more probable, allowing for the further cyclizing amidification.

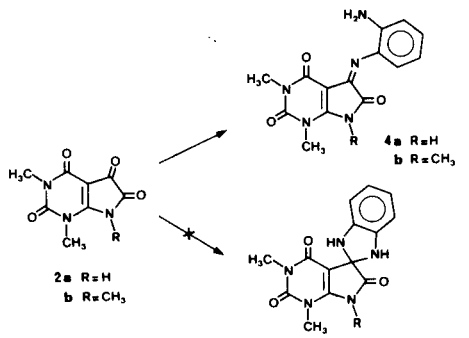
Scheme II



A **B** **C**

The tetraoxopyrrolopyrimidines **2a** and **2b** have been characterized by their mass spectra and by their thiosemicarbazones **3** (11,12). These interesting starting materials were allowed to react with *o*-phenylenediamine. Unfortunately, all attempts to obtain the tetracyclic compound failed. The same conditions applied to isatin were shown to lead to a spiro derivative (13,14). Such a reaction did not occur with **2**, which reacting only at the 3-position, affording *o*-aminophenylketimines **4** (Scheme III).

Scheme III



2a R=H
b R=CH_3

4a R=H
b R=CH_3

Microanalysis and ir data are in good agreement with either the spiro or the ketimine form. Moreover, investigation of the mass spectral fragmentations of **4a** and **4b** brings to light a base peak at (M-H_2O) and a weak molecular ion, corresponding to the ketimine structure, according to the previous study of Ballantine and coworkers (15) who unambiguously established the mass

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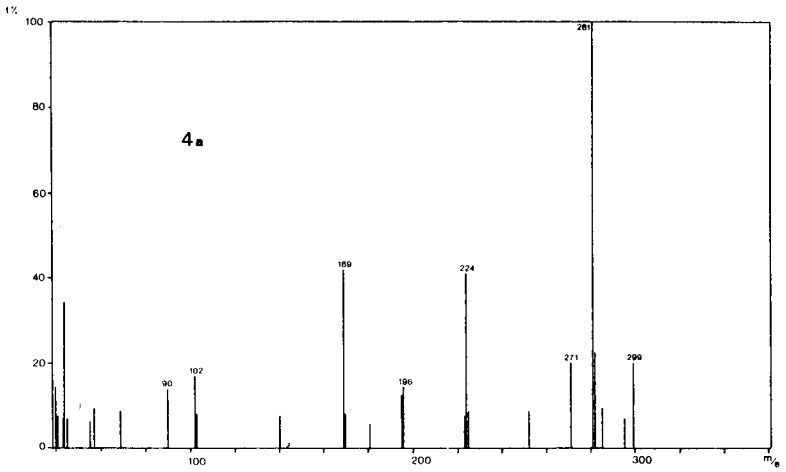


Figure 1: Mass spectrum of **4a**. Direct insertion probe at a temperature of 485°.

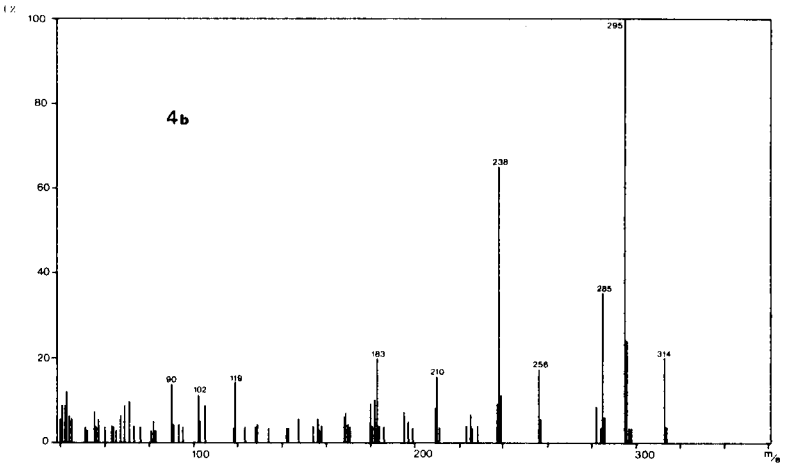
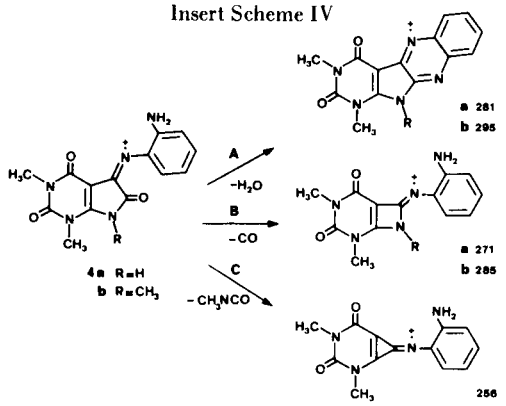


Figure 2: Mass spectrum of **4b**. Direct insertion probe at a temperature of 350°.

Insert Scheme IV

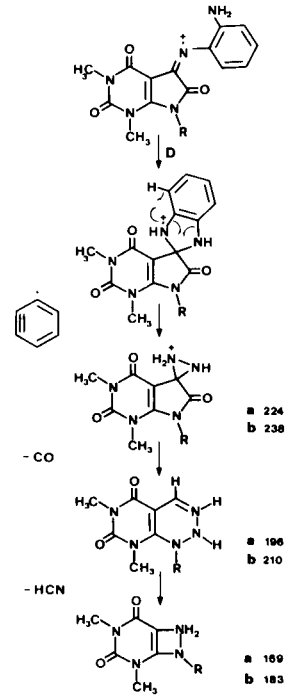


spectral differences between both forms (16).

An examination of the whole spectral fragmentation agrees well with the proposed structures (Figures 1 and 2). The major fragmentation pathway (route A; Scheme IV) would require the loss of water, involving the amino group of the anil moiety, with the formation of a diazine ring. Other predominant pathways result in the elimination of neutral molecules of carbon monoxide (route B) or methyl isocyanate (route C). These paths are in accordance with previous investigations concerning oxoindolinylidene anil parent compounds (15). However, further explanations could be advanced to account for the peaks occuring at m/e = 224 (a) or M/e = 238 (b) (M^+ - 75), corresponding to the elimination of a molecule of benzyne or for the peaks at m/e = 196 or m/e = 210 (M^+ - 103), corresponding to the further loss of a molecule of carbon monoxide (route D; Scheme V).

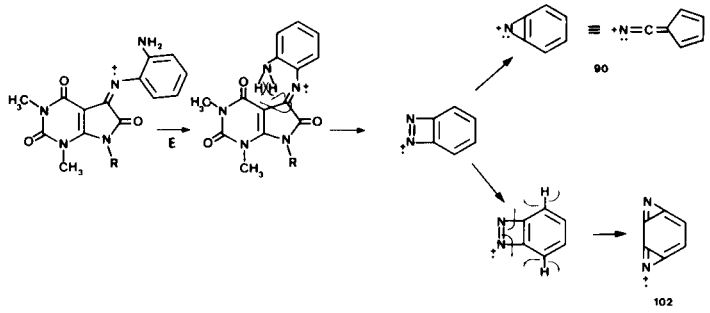
The peaks of moderate intensity occuring at m/e = 90 and m/e = 102 are common to both spectra. It could be postulated that they correspond to a fragmentation pattern involving the common portion of the two molecules, the anil moiety. These peaks were also present in the indolinylidene derivatives spectra having the same

Scheme V



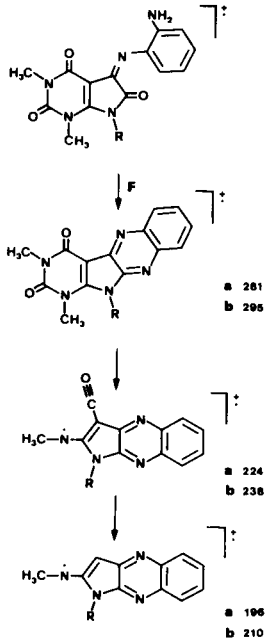
common fragment (15), curiously not considered by Ballantine in his proposed fragmentation. The cleavage of the uracile-nitrogen atom and the rearrangements reported here (route E; Scheme VI) involve a well-known *ortho*-effect (17,18).

Scheme VI



The uracil ring is relatively stable under electron impact as previously described (19). Nevertheless, its major fragmentation corresponds to ring cleavage with the expulsion of a molecule of CH_3NCO and subsequent loss of CO giving rise to the formation of ions at m/e = 223 (a), m/e = 238 (b) and m/e = 196 (a), m/e = 210 (b), respectively. Uracil mass spectra were discussed by Rice (20) who found that the first step could be explained by a "retro Diels-Alder" mechanism. His results, confirmed by other authors (21-24), are in perfect agreement with the fragmentation of the pyrimidinyl moiety reported here (route F; Scheme VII).

Scheme VII



EXPERIMENTAL

6-Amino-1,3-dimethyluracils (**1a**, R = H; **1b**, R = CH_3).

6-Amino-1,3-dimethyluracil (**1a**) was prepared as reported by Blicke (26) and 6-methylamino-1,3-dimethyluracil (**1b**) as described by Pfleiderer (27).

1,3-Dimethyl-2,4,5,6-tetraoxo-1,2,3,4,5,6-hexahydro-7*H*-pyrrolo-[2,3-*d*]pyrimidine (**2a**).

A solution of oxalyl chloride (17 ml.) in acetone (100 ml.) was added dropwise to a suspension of **1a** (15.5 g.) in acetone. The mixture was heated under reflux for 3 hours. The cooled yellow precipitated material, **2a**, was collected, washed with acetone and ethanol and dried to afford 9 g. of **2a** (43%). Recrystallization from water gave the analytically pure product, m.p. > 260°; ir (potassium bromide): ν 1740, 1790 (C=O), 3100 (N-H) cm^{-1}.

Anal. Calcd. for $C_8H_7N_3O_4$: C, 45.94; H, 3.37; N, 20.09; O, 30.60. Found: C, 45.92; H, 3.52; N, 19.91; O, 30.56.

1,3,7-Trimethyl-2,4,5,6-tetraoxo-1,2,3,4,5,6-hexahydro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**2b**).

Under the same conditions as described for the preparation of **2a**, **1b** (16.9 g.) was allowed to react with oxalyl chloride (17 ml.) to give **2b** in a yield of 55% as yellow crystals from water, m.p. > 260°; ir (potassium bromide): ν 1750, 1780 (C=O) cm^{-1}.

Anal. Calcd. for $C_9H_9N_3O_4$: C, 48.43; H, 4.06; N, 18.83; O, 28.67. Found: C, 48.39; H, 4.09; N, 18.76; O, 28.72.

1,3-Dimethyl-2,4,5,6-tetraoxo-1,2,3,4,5,6-hexahydro-7*H*-pyrrolo-[2,3-*d*]pyrimidine 5-Thiosemicarbazone (**3a**).

To a boiling solution of **2a** (2.1 g.) in water (200 ml.), acetic acid (1 ml.) and thiosemicarbazide (0.9 g.) were added. After stirring under reflux for 2 hours, the yellow crystalline precipitate was collected by filtration, washed with boiling water and dried to give 1.8 g. of **3a** (63%), m.p. > 260°; ir (potassium bromide): ν 1700, 1760 (C=O), 3170, 3300 (N-H) cm^{-1}.

Anal. Calcd. for $C_9H_{10}N_6O_3S$: C, 38.29; H, 3.57; N, 29.77; S, 11.36. Found: C, 38.29; H, 3.73; N, 29.93; S, 11.22.

1,3,7-Trimethyl-2,4,5,6-tetraoxo-1,2,3,4,5,6-hexahydro-7*H*-pyrrolo[2,3-*d*]pyrimidine 5-Thiosemicarbazone (**3b**).

Compound **2b** (2.2 g.) was converted to its thiosemicarbazone by the same process as described above yielding 2.1 g. of **3b** (71%), m.p. $> 260^\circ$; ir (potassium bromide): ν 1720, 1750 (C=O), 3400 (N-H) cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}N_6O_3S$: C, 40.53; H, 4.08; N, 28.36; S, 10.82. Found: C, 40.35; H, 4.12; N, 28.16; S, 10.75.

5-(2-Aminoanil)ketimine of **2a** (**4a**).

A mixture of **2a** (2.1 g.) and *o*-phenylenediamine (1.2 g.) in isobutyl alcohol containing 1 ml. of acetic acid was heated under reflux for 4 hours. After cooling, a yellowish precipitate was collected and dried to afford 2 g. of **4a** (66%), m.p. $> 260^\circ$. A crystallization in pyridine followed by a recrystallization from ethanol yielded analytically pure samples; ir: ν 1700 (C=O), 3200, 3400 (N-H) cm^{-1}; ^1H nmr (pyridine-d_5): 4.90 ppm (s, i = 2).

Anal. Calcd. $C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.38; N, 23.40; O, 16.04. Found: C, 56.08; H, 4.40; N, 23.34; O, 16.10.

5-(2-Aminoanil)ketimine of **2b** (**4b**).

The same conditions applied to **2b** (2.2 g.) yielded 2.2 g. of **4b** (70%), m.p. $> 260^\circ$ (pyridine and ethanol); ir: ν 1700 (C=O), 3400 (N-H) cm^{-1}; ^1H nmr (pyridine-d_5): 4.85 ppm (s, i = 2).

Anal. Calcd. $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35; O, 15.32. Found: C, 57.50; H, 4.86; N, 22.35; O, 15.26.

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