Polycondensed Nitrogen Heterocycles. Part 22. Pyrrolo[3,4-*d*]-1,2,3-triazines: a New Ring System as Potential Antineoplastic Agent [1]

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Diazotization of the 3-aminopyrrole-4-carboxamides **6a,b** under different conditions directly led to the new ring system pyrrolo[3,4-*d*]-1,2,3-triazine in excellent yields through an intramolecular coupling reaction. In all the cases it was impossible to isolate the intermediate 3-diazopyrroles.

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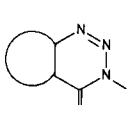
The 1,2,3-triazine nucleus, annelated at the 5,6- (or 4,5) position with the benzene or with an azine or azole moiety (structure **1**), is capable of versatile interactions with biologically significant macromolecules. In fact annelated triazines of type **2a-c** are structural analogues of naturally occurring purines and can be potential substrates or inhibitors of many enzymes controlling the metabolism of purine nucleotides [2]. These compounds can undergo fission at the 2,3, 3,4 or 1,6 bond depending on the conditions [3], and the ionic or radical reactive species thereby generated can take part in potential covalent reactions with biological substrates.



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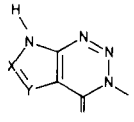
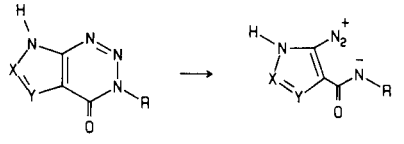


2 a X=N; Y=CH
b X=CH; Y=N
c X=Y=N

Although azolotriazine derivatives have not found yet commercial application as medicinal agents, compounds of type **3** exhibit broad spectrum of activity [4-7]. Of particular interest is their activity as inhibitors of neoplastic cells, that is related to the possibility of generating ring-opened azolotriazenes **4** or diazoazoles **5**.



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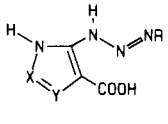

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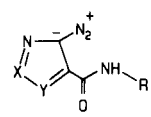

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In connection with our studies on 3-diazopyrroles [8-10], as key intermediates in the synthesis of potential antineoplastic agents, we thought to annelate a pyrrole nucleus to the 1,2,3-triazine ring.

Therefore we required 4-carboxamido-3-diazopyrrole derivatives that, through an intramolecular coupling reaction, can cyclize to the hitertho unknown pyrrolo[3,4-*d*]-1,2,3-triazine ring system.

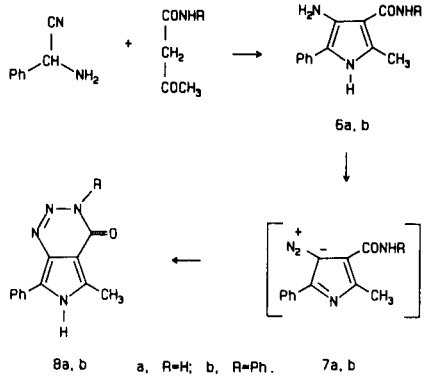
To this purpose 3-amino-4-carboxamidopyrroles **6a,b** were prepared by condensation of 2-amino-2-phenylacetonitrile with the opportune *N*-substituted acetoacetamides. Compounds **6a,b** were diazotized with sodium nitrite in hydrochloric acid and the reaction mixture was neutralized with aqueous ammonia. The pyrrolo[3,4-*d*]-1,2,3-triazin-4-ones **8a,b** were directly obtained in excellent yields. Evidently, the diazonium salts immediately coupled with the carboxamido substituent to give derivatives **8**, before it is possible to isolate the diazo compounds **7**.

Although the synthesis of the target compounds was achieved, the isolation of the intermediate 3-diazopyrroles was of interest since such compounds can show antineoplastic activity themselves and are useful synthons for the preparation of biologically interesting pyrrolyltriazenes, as it is well recognized in azole series [11,12]. Moreover in the case of 4-diazoimidazole-5-carboxamide and of the 2-azahypoxanthine, readily interconvertible into each other *in vitro* and *in vivo*, sometimes the interpretation of the biological results was not straightforward since both forms can be responsible for the activity [13].

Considering that in imidazole series, in acid conditions, the diazotization of the 4-amino-5-carboxamidoimidazole directly leads to imidazotriazines, and that only in opportune *p*H range (2.5-7.5) the 4-diazoimidazole-5-carboxamide was isolated [14], 3-aminopyrroles **6a,b** were diazotized in acetic acid and under buffered conditions

(*p*H 7). In both cases the pyrrolotriazines **8** were obtained in nearly quantitative yields and the 3-diazopyrrole-4-carboxamides were never isolated.

Scheme



These experimental results demonstrate that these diazopyrroles **7** are so reactive that the intramolecular coupling reaction takes place as soon as they are formed.

The screening tests for the evaluation of the antineoplastic activity of the pyrrolotriazine derivatives **8a,b** are in progress.

EXPERIMENTAL

All melting points were taken on a Buchi-Tottoli capillary apparatus; ir spectra were determined in bromoform with a Perkin-Elmer 299 spectrophotometer; the nmr spectra were obtained with a JEOL JMN-100FT spectrometer (TMS as internal reference); mass spectra were obtained with a JEOL JMS-01 SG-2 double focusing mass spectrometer operating with an electron beam energy of 75 eV and 10 Kv accelerating voltage.

Amide-*N*-Substituted 3-Amino-5-methyl-2-phenylpyrrole-4-carboxamides **6a,b**.

Using a procedure analogous to that described in the literature [15], 2-amino-2-phenylacetonitrile (20 mmoles) and acetoacetamide or acetoacetanilide (20 mmoles) were heated under azeotropic conditions. After treatment of the intermediate enamino carboxamide with sodium ethoxyde (25 mmoles) in ethanol (100 ml), the mixture was worked up by extraction with dichloromethane (3 x 50 ml) and the residue was recrystallized from benzene.

3-Amino-4-carboxamido-5-methyl-2-phenylpyrrole (**6a**).

This compound was obtained in a yield of 45%, mp 175° dec [16]; ir: 3390, 3310 and 3190 (br, NH_2, CO*NH*$_2$ and NH), 1630 (CO) cm^{-1}; nmr (DMSO-d_6): δ 2.50 (3H, s, CH_3), 4.90 (2H, br s, exchangeable NH_2), 6.80 (2H, br s, exchangeable $CONH_2$), 7.15-7.85 (5H, m, C_6H_5), 10.70 (1H, s, exchangeable NH); ms: M^+ = 215.

Anal. Calcd. for $C_{12}H_{13}N_3O$: C, 66.95; H, 6.09; N, 19.52. Found: C, 66.88; H, 6.15; N, 19.50.

3-Amino-4-(*N*-phenyl)carboxamido-5-methyl-2-phenylpyrrole (**6b**).

This compound was obtained in a yield of 45%, mp 189° dec; ir: 3440, 3360, and 3220 (br, NH_2, CO*NH*R and NH), 1610 (CO) cm^{-1}; nmr (DMSO-d_6): δ 2.50 (3H, s, CH_3), 4.40 (2H, s, exchangeable NH_2), 7.00-7.80 (10H, m, 2 x C_6H_5), 10.30 (1H, s, exchangeable CONHR), 10.90 (1H, s, exchangeable NH); ms: M^+ = 291.

Anal. Calcd. for $C_{18}H_{17}N_3O$: C, 74.20; H, 5.88; N, 14.42. Found: C, 74.11; H, 5.79; N, 14.39.

3-Substituted 5-Methyl-7-phenyl-6*H*-pyrrolo[3,4-*d*]-1,2,3-triazin-4(3*H*)-ones (**8a,b**).

Method a.

To a solution of the amines **6a,b** (10 mmoles) in the minimum volume of acetone, dilute hydrochloric acid (20 mmoles, 0.5 *N*) was added and then the mixture was cooled at 0° with stirring. An aqueous solution of sodium nitrite (10 mmoles, 18%) was added dropwise and the reactants were allowed to stir at room temperature for 3 hours. The mixture was neutralized with aqueous ammonia (2 *N*). The solid precipitated was collected, air dried and recrystallized from ethanol.

5-Methyl-7-phenyl-6*H*-pyrrolo[3,4-*d*]-1,2,3-triazin-4(3*H*)-one (**8a**).

This compound was obtained in a yield of 90%, mp 231° dec; ir: 3290 and 3190 (br, 2 x NH), 1680 (CO) cm^{-1}; nmr (DMSO-d_6): δ 2.70 (3H, s, CH_3), 7.40-8.40 (5H, m, C_6H_5), 13.40 (2H, br s, 2 x exchangeable NH); ms: M^+ = 226.

Anal. Calcd. for $C_{12}H_{10}N_4O$: C, 63.70; H, 4.46; N, 24.77. Found: C, 63.59; H, 4.44; N, 24.71.

3,7-Diphenyl-5-methyl-6*H*-pyrrolo[3,4-*d*]-1,2,3-triazin-4(3*H*)-one (**8b**).

This compound was obtained in a yield of 96%, mp 222° dec; ir: 3300 (NH), 1660 (CO) cm^{-1}; nmr (DMSO-d_6): δ 2.60 (3H, s, CH_3), 7.20-8.40 (10H, m, 2 x C_6H_5), 13.10 (1H, s, exchangeable NH); ms: M^+ = 302.

Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53. Found: C, 71.40; H, 4.59; N, 18.41.

Method b.

To a stirred solution of the amines **6a,b** (10 mmoles) in acetic acid (40 ml), a solution of sodium nitrite (10 mmoles) in water (10 ml) was added dropwise at 0-5°. After 1 hour the mixture was allowed to room temperature and poured onto crushed ice. The precipitate was filtered off, air dried and recrystallized to give compound **8a** in a yield of 93% and **8b** in a yield of 96%.

Method c.

To a solution of sodium nitrite (10 mmoles) in acetone (200 ml) and water (100 ml) amines **6a,b** (10 mmoles) were added. To the resulting solution, cooled at 0-5° and stirred, dilute hydrochloric acid (10 mmoles, 0.5 *N*) was added dropwise. After standing overnight at room temperature, the acetone was evaporated under reduced pressure. The solid was collected, air dried and recrystallized to give compounds **8a,b** in quantitative yield.

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[16] This compound was isolated as hydrochloride, mp 307-309°, ref [15].