SYNTHESIS AND PROPERTIES OF PYRIMIDO[4,5-b][1,4]OXAZIN-7-ONE DERIVATIVES.

A NOVEL HETEROCYCLIC SYSTEM

Manuel Melguizo, Manuel Nogueras*, and Adolfo Sánchez
Departamento de Química Orgánica, Facultad de Ciencias Experimentales de Jaén, 23071 Jaén (Spain)

Abstract—The synthesis of two pyrimido[4,5-b][1,4]oxazin-7-one derivatives (5 and 10) from 4,5-diaminopyrimidine-6(1H)-one derivatives (2 and 8) and ethyl pyruvate is reported. By treatment of a 5-amino-4-(xylosylamino)pyrimidine (2) with ethyl pyruvate, a 8-xylosylpteridine derivative (4) or a 4-(xylosylamino)pyrimido[4,5-b][1,4]oxazin-7-one derivative (5) can be obtained depending on solvent employed. This solvent-dependent regioselectivity was rationalized on the basis of instability of compound 5 in hydroxlic media. This feature of instability is analyzed and conditions necessary for obtaining pyrimido[4,5-b][1,4]oxazin-7-one derivatives are reported.

The preparation of pteridine derivatives by reaction of 4,5-diaminopyrimidines with a-dicarbonyl compounds is known as "The Gabriel-Colman reaction". It constitutes the most widely employed procedure in the synthesis of pteridines from pyrimidine precursors. It has also been adapted to the synthesis of 8-glycosylpteridines by treating 5-amino-4-glycosylaminopyrimidines with a-carbonyl esters.

As a part of our work focused on the synthesis of nonnatural nucleosides, we planned the preparation of 8-glycosylpteridine derivatives by Gabriel-Colman reaction. Our original strategy involved the reduction of 5-nitroso-4-(glycosylamino)pyrimidines to 5-amino-4-(glycosylamino)pyrimidines which upon treatment with an a-carbonyl ester would afford 8-glycosylpteridine derivatives. However, in addition to the expected pteridine derivative, we obtained other unusual products. One of these unusual products was a pyrimido[4,5-b][1,4]oxazin-7-one derivative. In this paper the results thus obtained and the conditions necessary for obtaining pyrimido[4,5-b][1,4]oxazin-7-one derivatives are reported.

RESULTS AND DISCUSSION

We found that treatment of a diaminopyrimidine derivative (2) with ethyl pyruvate produced three different final products (3, 4 and 5) depending on the nature of reaction medium and on the molar ratio between 2 and ethyl pyruvate. There are two interesting features in these results:

1) In addition to the expected pteridin-7-one derivative (4), compounds 3 and 5 were isolated. The obtention of compounds similar to 3 or 5 from Gabriel-Colman reactions appears to be unknown.
2) The reaction product changes from pteridin-4,7-dione (4) to pyrimido[4,5-b][1,4]oxazin-7-one (5) simply by changing the reaction solvent from methanol to benzene.

A rationalization of these results is depicted in the following paragraphs. Firstly, formation of compounds 3, 4 and 5 can be explained from a common intermediate, that is the azomethine 6 formed by reaction of the 5-amino group in 2 with the 2-keto group in ethyl pyruvate. Two compounds (one yellow and another red, observed by tlc monitoring) were rapidly formed after mixing the reactants in all reaction conditions outlined in Scheme 1. Only the yellow compound was formed when the reaction was carried out in benzene at room temperature and in the presence of 4A molecular sieves. It was isolated and characterized as the expected azomethine (6) (see Scheme 1).

Scheme 1

Compound 3 was obtained from 2 in refluxing benzene with an excess of ethyl pyruvate. Then, 3 could be formed
through nucleophilic attack of an enolate anion of a pyruvate molecule to the exocyclic imine bond of azomethine 6 followed by elimination of hydrogen and ethoxy carbonyl group. This rationalization was corroborated by an experiment in which 2 was treated with a four fold excess of ethyl pyruvate in the presence of 4A molecular sieves (the almost complete conversion of 2 into azomethine (6) under these conditions was confirmed by tlc monitoring), and subsequent addition of triethylamine in order to promote the formation of enolate ion from the excess pyruvate. By this method we obtained the best yield of 3 (80%), together with a minor amount (9%) of 5.

The lactam-lactin tautomerism of the N(1)H-C(6)=O moiety in the pyrimidine ring, provides a hydroxyl group 5 to the ethoxy carbonyl function in 6. Then, formation of 5 from 6 can be explained as lactonization of a 3-hydroxy ester. Similarly, formation of 4 from 6 arises from lactamization of a 5-amino ester. Under these structural circumstances, a competition between lactonization and lactamization should be expected. But, in fact, only one product of these two possible processes is obtained depending on the nature of solvent. The rationalization of this solvent-dependent regioselectivity is based on an interesting feature of 5, that is, its instability in hydroxylic solvent solutions. Thus, 5 decomposes rapidly in methanolic solutions at room temperature to produce a yellow intermediate for which we assume the structure 7 on the basis of the similarity between the uv absorption spectra of a solution of 5 in methanol (at 35 °C and 120 min after dissolution) and the corresponding spectra of 6 (see Figure 1). Analogously, rapid decomposition of 5 was observed in acetonitrile-water as well as in ethanolic solutions. We tried to obtain additional information about the instability of 5 in methanolic solutions by kinetic measurements on the methanolysis of 5 in neat methanol (solvolysis conditions) at 35 °C; we used the decrease in absorbance of this solution at a wavelength of 350 nm (see Figure 1) to monitor the decreasing concentrations of 5 as a function of time. The kinetic data indicated that the transformation of 5 into 7 under solvolysis conditions did not fit a pseudo first order rate equation (see Figure 2) as usually occurs for ester and lactone transesterification4 or enol ester hydrolysis;5 however, these data fitted quite well a pseudo second order rate equation (the pseudo second order rate constant value being 3.4 1-mol⁻¹·sec⁻¹) within a range in which the reaction had gone 90 % to completion (see Figure 2). These results indicate an unusual transesterification mechanism in which two molecules of 5 take part in the formation of the transition state pseudomolecule of the rate determining step. A model of self-assisted solvolysis might explain the rapid decomposition of 5 in hydroxyllic media and in the absence of any catalytic substance. Hence the synthesis of pyrimido[4,5-b][1,4]oxazin-7-one derivatives had never been previously reported probably due to the fact that reactions between compounds which fit the structural requirements to produce this type of heterocyclic derivatives (for example, Gabriel-Colman reactions between 4,5-diamino-6-hydroxypyrimidines and α-carbonyl esters) have usually been performed in hydroxyllic media.6

Additional information about the regioselectivity phenomenon under study can be deduced from the analogous
1722

Figure 1. UV absorption spectra of:

- - - - 5.5 \times 10^{-5} \text{ M solution of 5 in acetonitrile-methanol (v/v, 100:5).}

- - - - 5.1 \times 10^{-5} \text{ M solution of 6 in acetonitrile.}

- - - - quenched aliquot sample (4.8 \times 10^{-5} \text{ M}) obtained from a solution of 5 in methanol after 120 min of solvolysis at 35 °C (see kinetic procedure in Experimental Section).

Figure 2. Kinetic data plots for the solvolysis of 5 at 35 °C (see kinetic procedure in Experimental Section). These data fit a pseudo second order rate equation (upper plot) and not a pseudo first order rate equation (lower plot).

Scheme 2

reaction carried out in dry benzene with compound 8 (the aglycon of 2), which afforded the corresponding pyrimido[4,5-b][1,4]oxazin-7-one derivative (10) as minor product together with the pteridine-2,7-dione derivative (9) (see Scheme 2). The fact that pteridine 9 was obtained as major product in a non-hydroxylc
solvent (refluxing benzene), while the xylosylpteridine 4 was not obtained from 2 under similar conditions, reveals that the glycosidic moiety causes an important steric hindrance which makes the formation of the glycosylpteridine derivative difficult. Similar observations have been previously reported by other authors.\textsuperscript{2a,d,e}

Then, azomethine 6 formed by reaction of 2 with ethyl pyruvate, leads preferentially to the kinetically favoured pyrimido[4,5-b][1,4]oxazin-7-one derivative (5) in benzene as well as in methanol (trace amounts of 5 were observed by tlc near the beginning of the reaction when it was carried out in refluxing methanol). In the first medium, compound 5 remains stable and can be isolated; however, when methanol is the reaction medium, 5 should produce 7 through a solvolysis reaction. Molecules involved in equilibrium 4 $\rightleftharpoons$ 7 break away from this equilibrium when 7 cyclizes to the xylosylpteridine 4. Therefore, the most probable way which leads to 4 is the indirect pathway through 5 and 7, and not the direct way (see Scheme 3). In support of this hypothesis, 5 was converted into 4 by treating it with refluxing methanol for 6 h; the transformation was clearly observed by tlc and 41% of 4 was finally isolated.

From the above results we conclude that the isolation of pyrimido[4,5-b][1,4]oxazin-7-one derivatives requires a non-hydroxlyc reaction medium in addition to appropriate reactants (for example a 5-aminopyrimidin-6(1H)-one and an α-carbonyl ester).

Moreover, in reactions described in this paper, in which a competitive cyclization to pteridin-7-one derivative is possible, the formation of a pyrimido[4,5-b][1,4]oxazin-7-one seems to be favoured only when a bulky group sterically hinders the ring closure to pteridine derivative.

**EXPERIMENTAL SECTION**

**General Methods.**\textsuperscript{1}H-Nmr spectra were obtained by using a Hitachi-Perkin-Elmer R-600 spectrometer (60 MHz) with chemical shift (δ) reported in ppm downfield from tetramethylsilane.\textsuperscript{13}C-Nmr spectra were obtained in a Bruker AM-300 spectrometer from "Servicios Técnicos de la Universidad de Granada" (STUGRA), 17071 Granada (Spain), using TMS as internal reference. The following abbreviations were used to designate the multiplicity of individual signals: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were obtained in a Hewlett-Packard HP-5988-A from STUGRA. Elemental analyses were obtained in a Perkin-Elmer 240C from STUGRA. Uv spectra were recorded on a Bausch & Lamb Spectronic 2000 spectrophotometer. Ir spectra were recorded on a Beckman 4250 spectrophotometer. [α]$_D$ values were obtained by using a Perkin-Elmer
141 polarimeter. Melting points are uncorrected and were determined on a Gallenkamp Melting Point apparatus. Thin-layer chromatography (tlc) was performed with Merck 60 F$_{254}$ silica gel plates (0.2 mm). Preparative tlc was performed on Merck Kieselgel 60 F$_{254}$ (2 mm).

Methanol and acetonitrile used in the kinetic study were purified by fractional distillation through a 75 cm Hempel column packed with 6 mm Rasching rings. Acetonitrile was stored on 4A molecular sieves to keep dry.

5-Amino-2-methylthio-4-8-D-[(tri-O-acetyl)xylopyranosylamino]pyrimidin-6(1H)-one (2). A solution of sodium dithionite (2.00 g, 11.5 mmol) in water (20 ml) was added to a suspension of 2-methylthio-5-nitroso-4-8-D-[(tri-O-acetyl)xylopyranosylamino]pyrimidin-6(1H)-one (1) (0.89 g, 2.0 mmol) in MeOH (20 ml). The mixture was stirred at room temperature for 10 min (at this time the initial deep blue mixture became a colorless solution). Water was added and the resulting solution was extracted with CHCl$_3$ and the solvent was evaporated in vacuo (oil pump) to give compound 2 as a solid foam$^7$ (0.76 g, 88%). UV$\lambda_{max}$ (nm) (log e): (MeOH) 224 (4.20), 226 (3.94) shoulder, 305 (3.98). IR (KBr): 3380, 1750, 1630, 1450, 1365, 1240, 1070, 1060, 1035 cm$^{-1}$. $^1$H-Nmr (CDCl$_3$): $\delta$ 11.4 (br, s, 1H, D$_{2}$O exchangeable, N(1)H), 6.0 (d, J = 9.0 Hz, 1H, D$_{2}$O exchangeable, 4-NH), 5.8-4.8 (m, 6H, two D$_{2}$O exchangeable protons, 5-NH$_2$ and four sugar protons), 4.2 (m, 1H, sugar), 3.6 (m, 1H, sugar), 2.5 (s, 3H, S-CH$_3$), 2.0 (s, 9H, acetates). $^1$H-Nmr (DMSO-d$_6$): $\delta$ 12.3 (br, s, 1H, D$_{2}$O exchangeable, N(1)H), 6.5 (d, J = 9.6 Hz, 1H, D$_{2}$O exchangeable, 4-NH), 5.8-4.6 (m, 6H, two D$_{2}$O exchangeable protons, 5-NH$_2$ and four sugar protons), 4.2-3.0 (m, 2H, sugar), 2.5 (s, 3H, S-CH$_3$), 2.0 (s, 9H, acetates).

5-[(1-Ethoxycarbonyl)but-2-en-1-one-3-yl]amino-2-methylthio-4-8-D-[(tri-O-acetyl)xylopyranosylamino]pyrimidin-6(1H)-one (3). Procedure A: Ethyl pyruvate (2.03 ml, 18.6 mmol) was added to a suspension of 2 (2.00 g, 4.65 mmol) in benzene (30 ml) and the mixture was stirred at reflux temperature for 48 h. The solvent was evaporated to dryness in vacuo. The solid residue was dissolved in 5 ml of boiling MeOH and, after cooling, 100 ml of ethyl ether were slowly added to the solution under continuous stirring. The precipitate was collected by filtration, washed with ethyl ether and dried in vacuo (water pump) to give compound 3 (1.54 g, 58%). An analytical sample was prepared by recrystallisation from MeOH as colorless crystalline powder; mp: 178 °C. [a]$^0_D$ = -30.9° (c = 1, MeOH). UV$\lambda_{max}$ (nm) (log e): (MeOH) 221 (4.28), 230 (4.26), 276 (3.99). IR (KBr): 3380, 1750, 1665, 1635, 1590, 1550, 1420, 1370, 1240, 1022, 1050, 1030 cm$^{-1}$. $^1$H-Nmr (CDCl$_3$): $\delta$ 11.4 (br, s, 1H, D$_{2}$O exchangeable, N(1)H), 6.1 (d, J = 8.9 Hz, 1H, D$_{2}$O exchangeable, 4-NH), 5.4-4.7 (m, 6H, one D$_{2}$O exchangeable proton, 5-NH$_2$ and HC=C and four sugar protons), 4.4-3.8 (m, 3H, ethyl CH$_2$ and one sugar proton), 3.5 (m, 1H, sugar), 2.5 (s, 3H, S-CH$_3$), 2.0 (s, 6H, two acetates), 1.9 (s, 3H, acetate), 1.6 (s, 3H, C=C-CH$_3$), 1.2 (t, J = 7.4 Hz, 3H, ethyl CH$_2$). $^1$H-Nmr (DMSO-d$_6$): $\delta$ 12.2 (br, s, 1H, D$_{2}$O exchangeable, N(1)H), 7.0 (d, J = 8.9 Hz, 1H, D$_{2}$O exchangeable, 4-NH), 6.6 (s, 1H, D$_{2}$O exchangeable, 5-NH), 6.0-4.6 (m, 5H, HC=C and four sugar protons), 4.5-3.6 (m, 3H, ethyl CH$_2$ and one sugar proton), 3.3 (m, 1H, sugar), 2.6 (s, 3H, S-CH$_3$), 2.0 (s, 6H, two acetates), 1.9 (s, 3H, acetate), 1.6 (s, 3H, C=C-CH$_3$), 1.2 (t, J = 7.4 Hz, 3H, ethyl CH$_2$). $^1$C-Nmr (CDCl$_3$): $\delta$ 170.22, 169.76, 169.17 (acetates and carboxyethyl C=O), 161.61, 160.95, 156.34 (C-2, C-4 and C-6), 132.66 (C=O a to carboxyethyl), 99.01 (C-5), 84.21
(NH·Cl), 113.31 (CH₃), 80.98 (C-1'), 72.49, 70.63, 69.04 (C-2', C-3' ard C-4'), 64.47 (C-5'), 62.23 (ethyl CH₂), 23.39 (allyl CH₂), 20.78, 20.71 (acetate CH₂), 14.11 (ethyl CH₃), 13.44 (S-CH₃). Anal. Calcd for C₇H₇NO₃S: C, 48.42; H, 5.30; N, 9.82. Found: C, 48.10; H, 5.14; N, 10.15.

Procedure B): Ethyl pyruvate (1.62 ml, 14.9 mmol) was added to a suspension of 2 (1.60 g, 3.73 mmol) in benzene (24 ml) containing 8 g of 4Å molecular sieves. The mixture was stirred at room temperature for 23 h (the almost complete conversion of 2 into azomethine 6 at this time was confirmed by tlc, eluent CH₃Cl/MeOH, v/v, 9/1). Triethylamine (0.26 ml, 1.9 mmol) was then added and the resulting mixture was stirred for 32 h at room temperature. Molecular sieves were filtered off and washed several times with benzene. The filtrate and the washing liquors were combined and evaporated in vacuo to afford a residue which was chromatographed on silica gel by eluting with CH₃Cl/MeOH mixtures (v/v, from 99/1 to 95/5). By tlc monitoring (CH₃Cl/MeOH, v/v, 9/1), appropriate fractions were combined and evaporated in vacuo (oil pump) to afford (in order of elution) compounds 5 (0.16 g, 9 %, see below) and 3 (1.69 g, 80 %).

6-Methyl-2-methylthio-8-B-D-(tri-O-acetyl)xylopyranosylpteridine-4,7(3H,8H)-dione (4). Ethyl pyruvate (0.90 ml, 8.05 mmol) was added to a solution of 2 (3.30 g, 7.67 mmol) in MeOH (20 ml). The mixture was stirred at reflux temperature for 32 h and the solvent was then evaporated in vacuo to afford a residue which was chromatographed on silica gel by eluting with CH₃Cl/MeOH mixtures (v/v, from 99/1 to 91/9). Fractions containing compound 4 (tlc, CH₃Cl/MeOH, 9/1) were combined and evaporated in vacuo (oil pump) to furnish a solid (1.03 g, 29 %). An analytical sample was obtained by recrystallisation from ethyl acetate as pale-yellow crystalline powder; mp: 265 °C decmp. [α]D² = -51.6' (c = 1, CHCl₃). Uvaλₘₚ (nm) (log ε): (MeOH) 299 (3.88), 346 (4.13), 400 (2.78) shoulder, IR (KBr): 1760, 1660, 1580, 1560, 1520, 1425, 1365, 1240, 1220, 1090, 1060, 1030 cm⁻¹. ¹H-Nmr (CDCl₃): 5 12.2 (br s, 1H, D₂O exchangeable, N(3)H), 5.4 (m, 2H, sugar), 5.2 (m, 3H, sugar), 4.2 (m, 1H, sugar), 3.5 (m, 1H, sugar), 2.8 (s, 3H, 6-CH₃), 2.4 (s, 3H, 5-CH₃), 2.0 (s, 9H, two acetates), 1.8 (s, 3H, acetate). Anal. Calcd for C₃H₇NO₃S: C, 47.30; H, 4.60; N, 11.61. Found: C, 47.11; H, 4.37; N, 11.80.

6-Methyl-2-methylthio-4-B-D-[(tri-O-acetyl)xylopyranosylamino]pyrimido[4,5-b][1,4]oxazin-7-one (5). Ethyl pyruvate (0.29 ml, 2.56 mmol) was added to a suspension of 2 (1.10 g, 2.56 mmol) in benzene (12 ml). The mixture was stirred at reflux temperature for 6 h, and the solvent was then evaporated in vacuo to afford a residue which was chromatographed on silica gel by eluting with a mixture of ethyl ether and CH₂Cl₂ (v/v, 1/9). Fractions were collected, and those containing compound 5 (tlc, CH₃Cl/MeOH, v/v, 9/1) were combined and evaporated in vacuo (oil pump) to a solid foam (0.382 g, 31 %). A crystalline analytical sample was prepared by dissolving the foam in MeOH and immediate cooling to -10 °C; mp 194 °C. [α]D² = -12.0' (c = 1, CHCl₃). Uvaλₘₚ (nm) (log ε): (acetonitrile) 214 (4.12), 266 (4.14), 295 (3.74), 350 (4.17). IR (KBr): 3380, 1750, 1600, 1565, 1420, 1370, 1245, 1225, 1066, 1030 cm⁻¹. ¹H-Nmr (CDCl₃): 6 7.0 (d, J = 10.0 Hz, 1H, D₂O exchangeable, 4-NH), 5.8-4.7 (m, 4H, sugar), 4.15 (m, 1H, sugar), 3.5 (m, 1H, sugar), 2.6 (s, 3H, 6-CH₃), 2.5 (s, 3H, 5-CH₃), 2.1-2.0 (three s, 9H, acetates). ¹H-Nmr (DMSO-d₆): 6 8.5 (d, J = 8.2 Hz, 1H, D₂O exchangeable, 4-NH), 5.8 (m, 1H, sugar), 5.4 (m, 3H,
sugar), 4.9 (m, 1H, sugar), 4.2-3.4 (m, 2H, sugar), 3.6 (s, 3H, 6-CH_3), 3.4 (s, 3H, 5-CH_2), 2.0 (s, 6H, two acetates), 1.9 (s, 3H, acetate). \^1H-Nmr (CDCl_3): δ 173.36 (C-7), 170.64, 169.90 (acetate C=O), 157.74, 157.17, 153.20, 151.45 (C-2, C-4, C-6 and C-8a), 107.22 (C-4a), 79.73 (C-1'), 72.18, 70.63, 69.00 (C-2', C-3' and C-4'), 54.33 (C-5?), 20.96 (6-CH_2), 20.71 (acetate CH_3). Anal. Calcd for C_9H_13N_7S_2: C, 47.30; H, 4.60; N, 11.61. Found: C, 47.15; H, 4.59; N, 11.71.

Transformation of 5 into 4. Compound 5 (0.139 g, 0.29 mmol) was suspended in MeOH (5 ml) and the mixture was refluxed for 6 h (during this time the transformation of 5 into 4 was monitored by tlc; eluent, CH_2Cl_2-MeOH, v/v, 9:1). The resulting orange solution was evaporated and the residue was separated by preparative tlc (CH_2Cl_2-MeOH, v/v, 9:1; two developments) to afford compound 4 (0.057 g, 41%), whose spectroscoical properties (uv and h-nmr) coincided with those of a sample obtained as described above.

5-[(Ethyloxycarbonyl)ethylidene]amino-2-methylthio-4-4-D-[(tri-O-acetyl)lxyopyranosilamino]pyrindin-6-[(1H-one (6). Ethyl pyruvate (1.37 ml, 12.24 mmol) was added to a suspension of 2 (1.32 g, 2.06 mmol) in benzene (20 ml) containing 6.5 g of 4A molecular sieves. The mixture was stirred at room temperature for 6 h and molecular sieves were then filtered off. Hexane was added to the filtrate until a yellow solid precipitated. The solid was collected by filtration, washed with hexane and dried in vacuo (oil pump) to give compound 6 (0.29 g, 18%); mp: 205 °C. \^1H-nmr (nm) (log c): (acetoneitrile) 225 (4.09), 244 (3.97) shoulder, 291 (3.83), 366 (3.53). Ir (KBr): 3320, 1750, 1655, 1580, 1540, 1425, 1365, 1240, 1220, 1055, 1030 cm\(^{-1}\). \^1H-Nmr (CDCl_3): δ 12.0 (br s, 1H, D_2O exchangeable, N(1)H), 6.8 (d, J = 6.2 Hz, 1H, D_2O exchangeable, 4-NH), 5.6-4.6 (m, 3H, sugar), 4.5-3.9 (m, 4H, ethyl CH_2 and two sugar protons), 3.4 (m, 1H, sugar), 2.5 (s, 6H, S-CH_3 and N=O-CH_2), 2.0 (s, 9H, acetates), 1.3 (t, J = 7.2 Hz, 3H, ethyl CH_3). Anal. Calcd for C_9H_13N_7S_2: C, 47.72; H, 5.34; N, 10.60. Found: C, 47.40; H, 5.17; N, 10.25.

5-Amino-3-methyl-7-methylthiopyridin-4,5-D-oxidin-2-one (10) and 6-Methyl-2-methylthiopseudidine-4,7(3H,8H)-dione (9). Ethyl pyruvate (0.67 ml, 6.0 mmol) was added to a suspension of 6 (1.03 g, 6.0 mmol) in benzene (40 ml) with of 4A molecular sieves (10 g). The mixture was refluxed for 25 h, then the precipitate together with molecular sieves were collected by filtration and washed several times with boiling benzene. The filtrate and the washing liquors were combined, the solvent volume was reduced in vacuo to ca. 20 ml and the resulting solution was kept at room temperature to give compound 10 as pale yellow crystals which were collected by filtration, washed with benzene and dried in vacuo (0.04 g, 2.8%); mp: 221 °C. Ei-ms (m/z): 224 (M\(^{+}\)). \^1H-nmr (nm) (log c): 265 (4.08), 296 (3.66) shoulder, 353 (4.13). Ir (KBr): 3340, 3290, 1735, 1650, 1630, 1695, 1545, 1425, 1340 cm\(^{-1}\). \^1H-Nmr (CDCl_3): δ 5.9 (br s, 2H, D_2O exchangeable, 4-NH), 2.6 (s, 3H, 6-CH_3), 2.55 (s, 3H, S-CH_3). \^1C-Nmr (CDCl_3): δ 172.71 (C-7), 159.50, 157.28, 152.65, 150.93 (C-2, C-4, C-6 and C-8a), 107.04 (C-4a), 20.70 (6-CH_2), 14.57 (5-CH_3). Anal. Calcd for C_9H_13N_7S_2: C, 42.85; H, 3.60; N, 24.98. Found: C, 42.91; H, 3.56; N, 24.78.

The solid filtered from the crude was dissolved in hot pyridine, molecular sieves were filtered off and the
solvent was evaporated in vacuo to give a solid residue which was treated with CHCl₃ at room temperature for 3 h. The solid was collected by filtration, washed with CHCl₃ and dried in vacuo (oil pump) to afford compound 9 (0.85 g, 64 %); mp: >300 °C. EI-ms (m/z): 224 (M⁺). UV λmax (nm) (log ε): (MeOH) 233 (4.08), 282 (4.21) shoulder, 289 (4.22), 356 (3.68) shoulder, 368 (3.72), 380 (3.53) shoulder. IR (KBr): 3130, 3010, 2920, 1675, 1650, 1560, 1420, 1365 cm⁻¹. ¹H-Nmr (DMSO-d₆): δ 12.8 (br s, 2H, O-O exchangeable, N(3)H and N(8)H), 2.6 (s, 3H, 6-CH₃), 2.5 (s, 3H, S-CH₃). Anal. Calcd for C₆H₇N₂O₅: C, 42.85; H, 3.60; N, 24.98. Found: C, 43.19; H, 3.51; N, 25.25. Kinetic procedure. Compound 5 (9.80 mg) was dissolved in MeOH (20 ml) at 35 °C. This solution was let be at 35 °C in a thermostatic bath. Aliquot samples of 0.5 ml were taken periodically, quenched by adding 10 ml of dry acetonitrile and the absorbance of the resulting solution at a wavelength of 350 nm was measured. Table 1 contains a kinetic run which data are plotted in Figure 2. The rate constant value was determined by the method of least squares, thus being 3.4 l·mole⁻¹·sec⁻¹ (correlation coefficient = 0.9992).

### Table 1. Methanalysis of 5, kinetic data.

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Measured absorbance</th>
<th>-ln [S]</th>
<th>1/[S]</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>0.514</td>
<td>7.324</td>
<td>1516</td>
</tr>
<tr>
<td>455</td>
<td>0.421</td>
<td>7.624</td>
<td>2046</td>
</tr>
<tr>
<td>670</td>
<td>0.363</td>
<td>7.870</td>
<td>2817</td>
</tr>
<tr>
<td>896</td>
<td>0.314</td>
<td>8.136</td>
<td>3423</td>
</tr>
<tr>
<td>1133</td>
<td>0.264</td>
<td>8.347</td>
<td>4220</td>
</tr>
<tr>
<td>1362</td>
<td>0.262</td>
<td>8.534</td>
<td>5087</td>
</tr>
<tr>
<td>1520</td>
<td>0.247</td>
<td>8.585</td>
<td>5916</td>
</tr>
<tr>
<td>1630</td>
<td>0.237</td>
<td>8.800</td>
<td>6638</td>
</tr>
<tr>
<td>2037</td>
<td>0.227</td>
<td>8.931</td>
<td>7560</td>
</tr>
<tr>
<td>2270</td>
<td>0.222</td>
<td>9.002</td>
<td>8124</td>
</tr>
<tr>
<td>2516</td>
<td>0.214</td>
<td>9.130</td>
<td>9225</td>
</tr>
<tr>
<td>2755</td>
<td>0.210</td>
<td>9.200</td>
<td>9866</td>
</tr>
<tr>
<td>2986</td>
<td>0.206</td>
<td>9.275</td>
<td>10672</td>
</tr>
<tr>
<td>3229</td>
<td>0.203</td>
<td>9.334</td>
<td>11339</td>
</tr>
</tbody>
</table>

1 Absorbance was measured at λ=350 nm. ¹ These values are referred to the [S] in the original methanolic solution, and not to the [S] in the quenched aliquot samples used for absorbance measurements.

**ACKNOWLEDGEMENTS**

The authors thank the "Consejería de Educación y Ciencia de la Junta de Andalucia" for the award of a fellowship to M. Helguizo. This investigation was supported by the spanish CICYT (FAR 89-0414).

**REFERENCES AND NOTES**


2. (a) W. Pfleiderer and E. Bühler, Chem. Ber., 1966, 99, 3022. (b) W. Pfleiderer and D. Söll, J. Heterocycl.


7. This foam was homogeneous by tlc. Analytical data of compound (2) were obtained directly from the foam, without further purification.


Received, 13th May, 1991