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STUDIES OF THE REACTION OF 1,2-DITHIOLE-3-THIONES WITH NUCLEOPHILES

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Abstract - Substituted 1,2-dithiole-3-thiones react with nucleophiles (alkoxides, thiolates) to give various reaction products depending on the nucleophiles and on the substituents on the 1,2-dithiole-3-thione ring. The mechanistic aspects of these reactions are discussed.

1,2-Dithiole-3-thiones substituted at the C-4 and C-5 positions are endowed with pharmacological properties. The derivative in which R^4= methyl, R^5= pyrazinyl (OLTIPRAZ, 35972 RP)[1] exhibits schistosomicidal activity[2] and is metabolized to pyrrolo [1,2-a] pyrazine derivatives[3] in vivo.

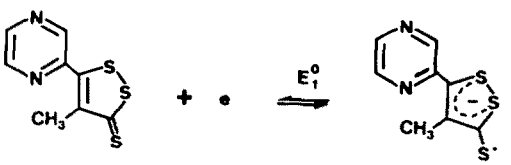
Compounds in which the thione sulphur is replaced by an oxygen atom are inactive. Complete loss of activity is also observed when the pyrazine is replaced by a phenyl or a thiophene ring.

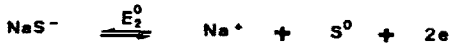
OLTIPRAZ is a slow-acting-drug and approximately two months are required until its full schistosomicidal effect becomes evident. One of the earliest effects of the drug is a reduction of the glutathione stores (GSH) of the worms (SCHISTOSOMA MANSONI). The close association between antischistosomal and GSH-lowering activities suggests the possibility of a causal relationship[4].

In a previous paper[5] it was shown that reduction of 5-(2-pyrazinyl)-1,2-dithiole-3-thione by sodium sulphide, followed by alkylation of the intermediate, yields pyrrolo [1,2-a] pyrazine. In addition, electrochemical studies in dimethylformamide[6,7] were carried out in our laboratory in order to establish a possible relationship between electrochemical and pharmacological properties. These studies led us to the following conclusions:

1) the life-time of the radical anion produced by the addition of one electron to a molecule of OLTIPRAZ is considerably longer than that of various other substituted 1,2-dithiole-3-thione radical anions. This result may be of biological importance if the antischistosomal activity occurs via a radical mechanism.

2) experimental oxidation-reduction potentials (v.s saturated calomel electrode), in dimethylformamide, for the following equilibria are: E_1°= -920 mV (measured) and E_2°= -700 mV (calculated).





This tends to show that the reduction of OLTIPRAZ by sodium sulphide as suggested[5] should not occur since $E_2^\circ > E_1^\circ$.

The present paper provides evidence for a novel mechanistic pathway involving attack by nucleophiles yielding a thione intermediate which can easily be reduced alternatively by sulphur species generated in the first step, when alkoxides (RO^-) are used as nucleophile, or by the excess of the thiolate reagent (RS^-).

RESULTS AND DISCUSSION

The processes involved in the transformation are discussed according to the nature of the nucleophiles (hard or soft character and steric hindrance) on one hand, and according to the nature of substituents at positions 4 and 5 on the other.

Reaction of 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione 1 and 5-(2-pyrazinyl)-1,2-dithiole-3-thione 2 with sodium ethoxide (EtONa).

After addition of an excess of sodium ethoxide to the ethanolic stock solution of compound 1, an increase of the two absorption bands at 278 nm and 400 nm is observed (see experimental section). Spectral changes show three isosbestic points at 286 nm, 340 nm and 424 nm indicating a shift in a simple equilibrium. Compound 2 behaves similarly, and for both derivatives the following kinetics can be calculated:

$v = dA_{278nm} / dt = dA_{400nm} / dt = k_{exp}(1)(EtO^-)$; $k_{exp} = 0.037\ mn^{-1}$ at 25°C.

$v = dA_{275nm} / dt = dA_{410nm} / dt = k_{exp}(2)(EtO^-)$; $k_{exp} = 0.115\ mn^{-1}$ at 25°C.

Reaction pathway:

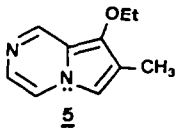
Previous experimental results suggest a nucleophilic attack at the C-5 position[8] {eqn. (1)} followed by an intramolecular ring-closure {eqn. (2)} according to scheme 1.

Compound 4a is isolated after acidification in 60% yield. Methylation of 3 and 4 gives 3b and 4b in 80% yield. ^1H, ^{13}C N.M.R, I.R, mass spectroscopy and U.V-visible absorption data of these compounds are consistent with a pyrrolo [1,2-a] pyrazine ring[3].

However, attack at the C-3 position[9] cannot be ruled out (scheme 2).

We were unable to distinguish between the two possibilities by Nuclear Overhauser Effect (N.O.E) techniques. However, the compound obtained on Raney Nickel desulphuration 5 allowed us to determine the position of the OEt group by N.M.R.[10-14] (see Experimental section).

Structure 5 is in agreement with attack at the C-5 position of the dithiole-thione ring.



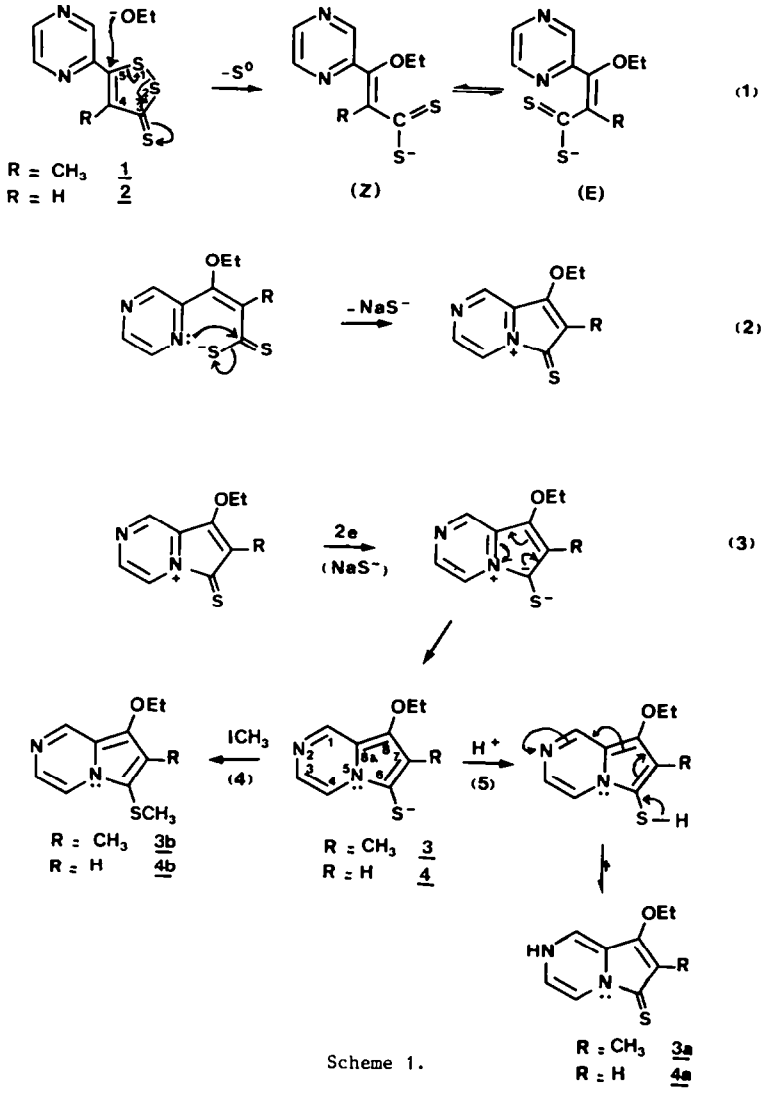
5

It is noteworthy that the chemical behaviour is not modified when an hydrogen atom is introduced at the C-4 position. Accordingly, the electron-donating effect exerted by the methyl group does not hinder the attack of EtONa at the C-5 position.

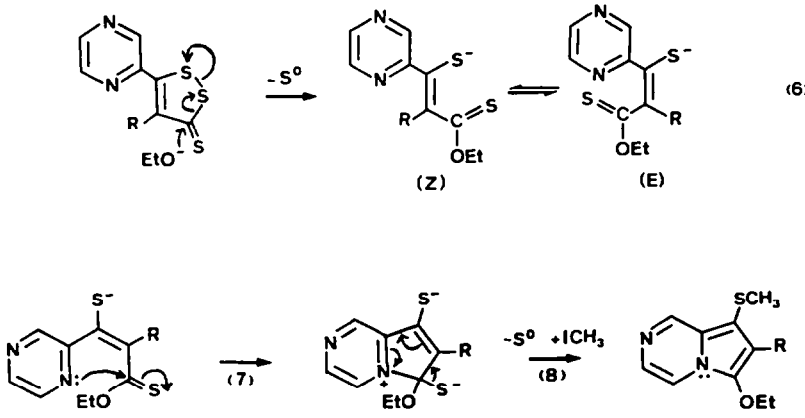
A similar behaviour is observed in alkaline aqueous-ethanol medium. 1 and 2 undergo attack by EtONa at the C-5 position, yielding 3b and 4b as their hydroiodide salts. Change in U.V-visible absorption enable us to determine the pKa values of the acid-base equilibrium: $H3b^+$ / 3b = 6.7 and $H4b^+$ / 4b = 6.9.

Reaction of 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione 1 with potassium t-butoxide.

As t-BuOK is a bulkier and a stronger base than EtONa, it may be expected that its use results in a change in the position of the nucleophilic attack.



Scheme 1.



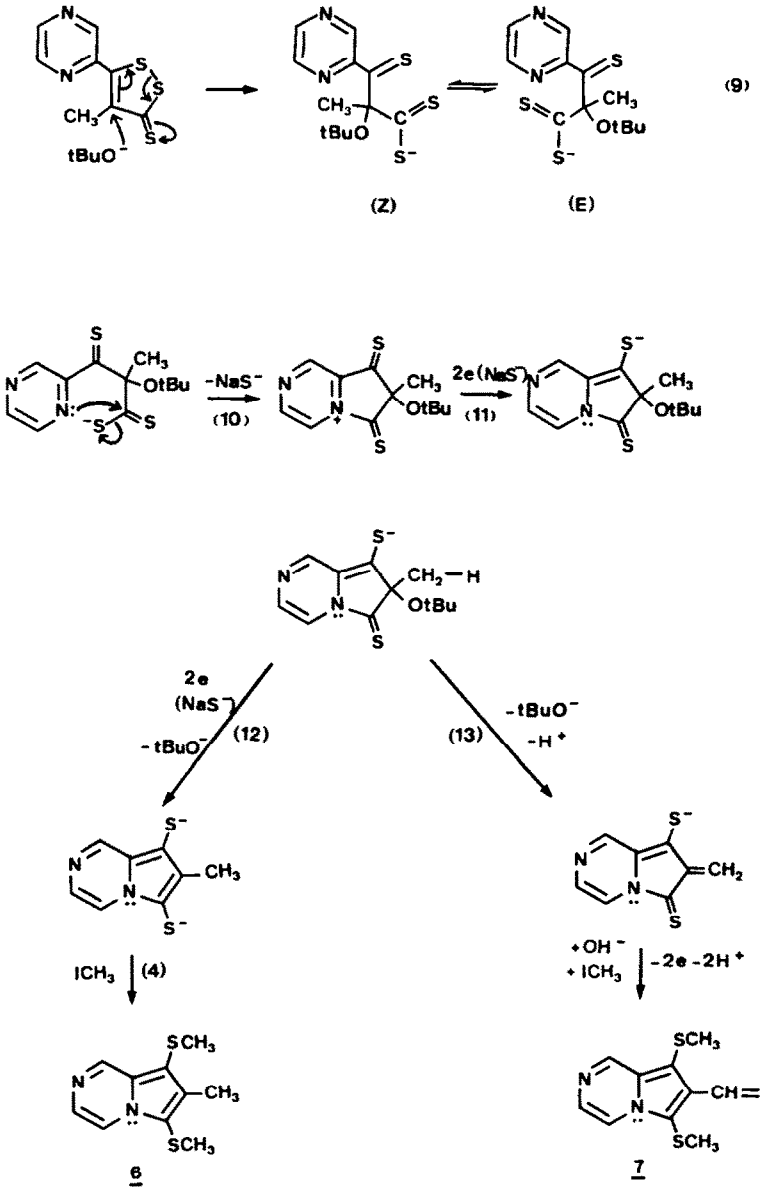
Scheme 2.

After addition of an excess of potassium t-butoxide to a solution of 1 in t-butanol, a decrease of the absorption bands shown by the starting material is noted at 430 nm and T.L.C. analysis indicates the formation of several compounds.

The two major compounds possess a pyrrolo [1,2-a] pyrazine skeleton as confirmed by ^{1}H, ^{13}C N.M.R and U.V-visible absorption data: the major one 6 (40% yield) corresponds to the primary metabolite of OLTIPRAZ[3] and the secondary compound 7 (7% yield) has an aldehyde function in place of the methyl group.

Reaction pathway:

A nucleophilic attack at the C-4 position, according to equations (9)-(12) provides an interpretation of the results (scheme 3).



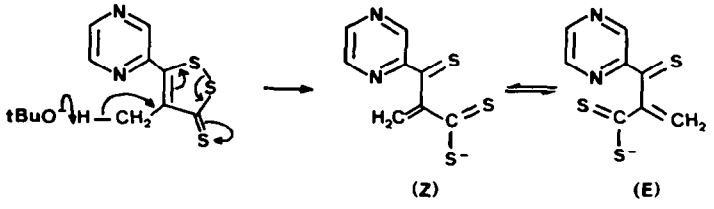
Scheme 3.

It is worth mentioning with regard to this pathway that:

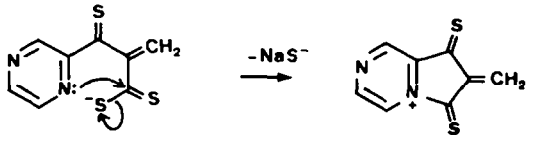
a) the reduction occurs via the hydrogenosulphur ion generated in equation (10), which, when oxidised to sulphur S°, liberates 2e. Since the pyrrolo[1,2-a] pyrazine metabolite is obtained by reduction of two thione groups, { 4e involved in steps (11) and (12) } , the yield of the metabolite cannot be greater than 50% (found: 40%).

b) beside the major compound 6, the reaction yields an aldehyde 7; the intermediate species yielded by step (11) reacts via two competitive routes according to equations (12) and (13).

Owing to the strong basicity of t-BuOK versus EtONa, an alternative route can be proposed for the formation of 6; this implies the cleavage of the C-H bond of the methyl group {eqn.(15) } followed by the intramolecular ring-closure {eqn.(16) } yielding the same kind of intermediate which can be reduced to give 6.

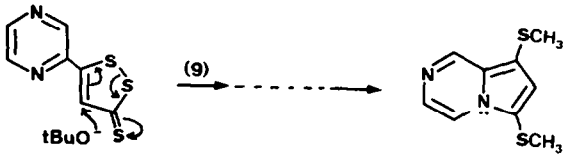


(15)



(16)

When the reaction is carried out with 2, it gives the pyrrolo [1,2-a] pyrazine 8. This result is obviously not consistent with the mechanistic pathway {eqn. (15)-(16)}.



2 8

Reaction of 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione 1 with thiolates.

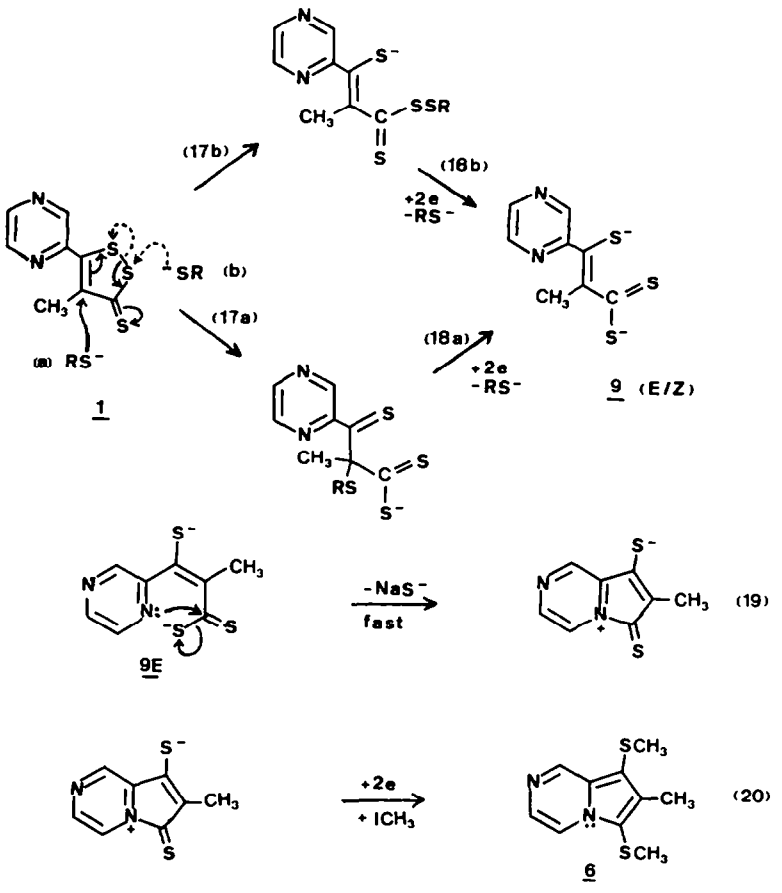
In addition to their nucleophilic properties, thiolates exert a reducing effect. Provided that the initial concentration of sodium ethanethiolate is 5xc, c being the initial concentration of OLTIPRAZ, the reduction reaction proceeds in high yield (≥ 80%).

The progress of the reaction is followed either by U.V-visible absorption spectrometry or by T.L.C. After addition of an excess of RS^- reagent (R = ethyl, cysteinyl, glutathionyl) to the ethanolic stock solution of 1, (see experimental section), an increase of the absorption band at 295 nm and a decrease of that at 440 nm are observed. When the reaction is driven to completion, the major compound is isolated as a yellow solid (80% yield), whose ^1H, ^{13}C N.M.R., U.V-visible spectra and mass spectroscopic data are in agreement with structure 6.

Mechanistic pathway:

From these experimental results, it can be deduced that 1 is transformed via the following route: attack by RS^- at the C-4 or S-2 position according to pathway (17a) or (17b) yielding the intermediate 9 which must exist as a mixture E/Z because of free rotation along the C-4 - C-5 bond in the precedent step (17a). Intramolecular ring closure of 9 (E) involving NaS^- as leaving group {eqn.(19)

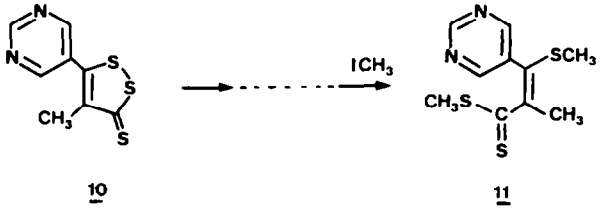
and reduction by an excess of the RS^- reagent (or alternatively by the NaS^- leaving group) of the transient cationic thione { eqn. (20) } affords after methylation the pyrrolo [1,2-a] pyrazine species 6 (scheme 4).



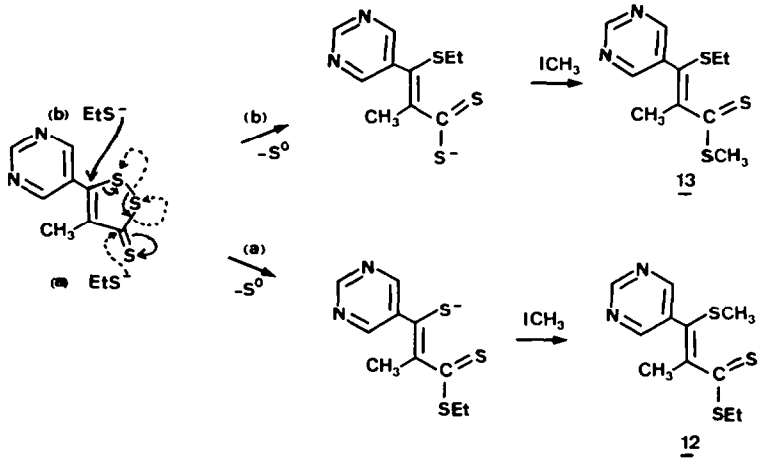
Scheme 4.

It is not possible to distinguish between the two possible routes, pathway (a) or (b), as they afford the intermediate 9 which is not isolated owing to its rapid intramolecular ring-closure.

In an attempt to isolate the same kind of intermediate, the corresponding reaction of 4-methyl-5-(5'-pyrimidinyl)-1,2-dithiole-3-thione 10 was studied. (The behaviour of this compound will be described in a subsequent paper.) The reaction progresses slowly leading to compound 11 after methylation.



Assuming that the replacement of pyrazine by pyrimidine does not strongly modify the reactivity of dithiole-thione ring, this result is important as it is in agreement with an attack at the C-4 or S-2 position; attack at the C-3 or C-5 position would afford after methylation compound 12 or 13 (scheme 5).



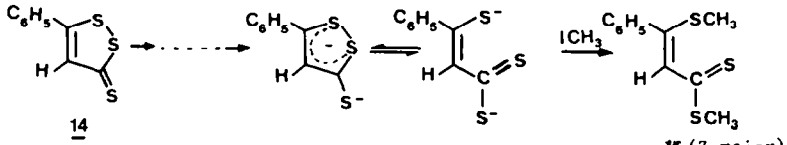
Scheme 5.

Reaction of 5-phenyl-1,2-dithiole-3-thione 14 with sodium ethanethiolate.

With this derivative, the intramolecular ring-closure {eqn. (19)} can no longer occur. After addition of an excess of sodium ethanethiolate to the ethanolic stock solution of 14, an increase of the absorption band at 435 nm and a decrease of that at 310 nm are observed immediately. The absorption at 435 nm, ε = 18000 $M^{-1}cm^{-1}$, is compatible with a dianion cyclic species[6,15]. ^1H, ^{13}C N.M.R., mass spectroscopic data are in agreement with structure 15 for the methylated product.

Mechanistic pathway:

From these experimental results, it can be deduced that EtS^- attacks at the C-4 or S-2 position according to the sequence proposed in scheme 4. Starting from 14 a similar intermediate compound is obtained yielding 15 after methylation (scheme 6).



Scheme 6.

It is worth mentioning that with 5-phenyl-4-methyl-1,2-dithiole-3-thione 16, the reaction does not proceed to a measurable extent. This result may be attributed either to the hindrance effect or to the electron-donating effect of the methyl group. As the reaction occurs rapidly in the case of OLTIPRAZ (1) or 10, it can be deduced that the withdrawing effect of a pyrazine or a pyrimidine ring overcomes the electron-donating effect of a methyl substituent. Furthermore, this is in favour of a nucleophilic attack of RS^- reagents at the C-4 position in OLTIPRAZ.

In the reaction of 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (OLTIPRAZ) with nucleophiles (alkoxides or thiolates), in alcoholic medium, it is possible to distinguish three steps:

1) attack of nucleophile (Nu^-) at the C-5, C-4 or S-2 position;

when $Nu^- = EtO^-$, attack occurs at the C-5 position of the dithiole-thione ring[9] {eqn. (1)}. This reaction provides a convenient route to compounds 3b and 4b.

when $Nu^- = t\text{-}BuO^-$, attack occurs at the C-4 position according to equation (9). To our knowledge,

nucleophilic additions at the C-4 position have not been previously reported.

when $Nu^- = RS^-$, attack occurs at the C-4 or S-2 position according to equation (17a) or (17b).

2) Intramolecular ring-closure involving NaS^- as leaving group affording the pyrrolo [1,2-a]-pyrazine skeleton {eqn. (2), (10) or (19)} ;

3) redox equilibrium: eqn. (3), {(11) and (12)} or (20);

when $Nu^- = t\text{-}BuO^-$, as only one mole of NaS^- (2e) is produced in equation (10) whilst 4e are needed for the overall reaction, the yield of the pyrrolo [1,2-a] pyrazine derivative cannot be greater than 50%. In the presence of methyl iodide, the pyrrolo [1,2-a] pyrazine species is transformed into the major metabolite of OLTIPRAZ[3], 6. In contrast, thiolates exerting a reducing effect, the reduction reaction proceeds in high yield (⩾ 80%).

Finally, several points should be underlined:

a) as shown in equation (12), NaS^- acts as a reducing agent versus a dithione cationic transient species, not versus the starting material (OLTIPRAZ). This result is not in agreement with a previously reported hypothesis[5], but can be deduced from values found for E_1° and E_2°.

b) the major metabolite 6 of OLTIPRAZ can be obtained either by electrochemical reduction[5] or by the use of nucleophiles.

c) the initial step of the chemical transformation of OLTIPRAZ is attack at the C-4 or S-2 position by RS^- reagents. Results obtained with 16 ($R^5 = C_6H_5$, $R^4 = CH_3$) favour attack at the C-4 position.

d) the ability of glutathionate (GS^-) to produce quantitatively the metabolite 6 may explain the simultaneous depletion of the GSH stores of the worms[6], during administration of OLTIPRAZ to infected mice.

Acknowledgements: The authors thank Dr. M. MESSER for his helpful guidance and discussion.

EXPERIMENTAL

General: 1,2-dithiole-3-thiones 1, 2, 10, 14, 16, substituted at the C-4 and C-5 positions were supplied by RHONE-POULENC-SANTE.

The solvents used for extractions and chromatography were obtained from S.D.S (puran purity grade). T-butanol, potassium t-butoxide and methyl iodide were MERCK products. Raney Nickel was obtained from ALDRICH.

A stock solution of 0.1 M tetrabutylammonium hydroxide (T.B.A.H.) in isopropanol was purchased from FLUKA. Cysteine and glutathione were obtained from FLUKA.

Sodium ethanethiolate (10g) was prepared by adding under nitrogen, at 5°C, to a suspension of sodium hydride (2.88g, 80% pure) in anhydrous tetrahydrofurane (T.H.F.), a solution (60 ml) of ethanethiol (7.4 ml) in T.H.F. (60 ml). The resulting mixture was used after diluting with an equal volume of ethanol.

The conjugated bases of RSH compounds (5 mmol) were prepared in ethanol solution by adding an excess of sodium ethoxide (10 mmol when R = cysteinyl) or of T.B.A.H. (15 mmol when R = glutathionyl).

U.V-visible absorption spectral data of substituted 1,2-dithiole-3-thiones in ethanol at 20°C.

U.V-visible spectra were recorded using a VARIAN DMS 90 spectrophotometer.

1: λ_{nm} (ε_M-1 $_{cm}$-1): 220 (11500), 278 (12500, sh), 295 (14000), 442 (7800).

2: 225 (5000), 275 (12000, sh), 308 (18000), 455 (8500).

10: 225 (11500), 235 (10500,sh), 275 (10500), 320 (5000), 425 (8500).

14: 225 (11000), 240 (8500), 270 (9500), 310 (18000), 430 (9300).

^1H N.M.R. spectra were recorded on a BRUKER WM 250 (250 MHz) spectrometer. Chemical shifts are reported in ppm relative to internal T.M.S. The multiplicity of the signal, the number of protons and the coupling constant are listed in this order.

^{13}C N.M.R. spectra were recorded on a BRUKER WP 200 SY spectrometer. The multiplicity of the signals was determined by off-resonance or by spin echo J. modulation sequence[16].

I.R. spectra were recorded on a PERKIN-ELMER 700 spectrometer.

Mass spectra were recorded on a FINNIGAN 3000 spectrometer.

Chromatographies were conducted on MERCK silica gel H type 60 N° 7736 with a positive pressure. T.L.C. was performed on MERCK silica gel 60 F 254 N° 5765.

8-Ethoxy-7-methyl-6-methylthiopyrrolo [1,2-a] pyrazine 3b.

method A :

Sodium ethoxide (50 mmol) was added to a solution of 1 (0.5 mmol) in ethanol (500 ml), under nitrogen at 35°C. After allowing the mixture to react (see kinetic data), the resulting species was methylated with an excess of methyl iodide (20 mmol). The solution was neutralised with dry-ice and evaporated to dryness in vacuo at 35°C. The residue was poured into water (50 ml) and then extrac-

ted with ethyl acetate (40 ml). The organic phase was dried over anhydrous sodium sulphate and evaporated to dryness.

Chromatography of the residue (toluene-acetone 95:5), provided 3b (90 mg; 80% yield); Rf = 0.30 (toluene-acetone 80:20).

^{1}H N.M.R, 250 MHz, $CDCl_3$, δ: 1.40 (t,3H,CH_3,ethyl), 2.20 (s,3H,CH_3 or SCH_3), 2.30 (s,3H,CH_3 or SCH_3) 4.15 (q,2H,OCH_2,ethyl), 7.45 (d,1H,H-3,J_{4-3}= 5Hz), 8.10 (dd,1H,H-4,J_{4-3}= 5Hz, J_{4-1}= 1Hz), 8.75 (bd,1H,H-1,J_{4-1}= 1Hz).

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ:9.2 (CH_3), 15.9 (SCH_3), 18 (CH_3,ethyl), 71 (OCH_2,ethyl), 113.4 (C-8a) 115.7 (C-4), 121.3 (C-6), 122.4 (C-7), 127.2 (C-3), 138.2 (C-8), 142.2 (C-1).

mass spectrum (E.I):m/z = 222 ($M^{+\cdot}$); m/z = 207 (M-CH_3); m/z = 193 (100%).

U.V. (100% EtOH) : λ_{nm} (ε_M-1 $_{cm}$-1): 260 (16000), 290 (2800,sh), 305 (3400), 315 (3200), 350 (2200).

Elemental analysis corresponded to empirical formula, $C_{11}H_{14}N_2OS$.

method B:

1 (0.25 mmol) was dissolved under nitrogen at 35°C in an aqueous ethanol (1:1) solution of 0.1M sodium hydroxide (500 ml). After 3hr, the alkaline solution was acidified by sulphuric acid to pH = 4.0 and evaporated in vacuo at 35°C. The resulting solution (50 ml) was extracted with ethyl acetate (50 ml). The organic phase was dried and methylated with an excess of methyl iodide (2.5 mmol) and its volume reduced to 10 ml. A brown precipitate was filtered off, washed with ethyl ether and then dried over P_2O_5 at reduced pressure for 24hr. A brown solid (40 mg; 75% yield) was isolated (m.p. 186°C); its structural data were consistent with the hydroiodide salt of 3b.

U.V-visible (100% EtOH): λ_{nm} (ε_M-1 $_{cm}$-1): 250 (15000), 275 (4600), 325 (4400), 380 (2800).

8-Ethoxy-6-methylthiopyrrolo [1,2-a] pyrazine 4b.

Using the above mentioned method A (EtO^- = 25 mmol), 2 gave 4b in 80% yield.

^{1}H N.M.R., 250 MHz, $CDCl_3$, δ: 1.45 (t,3H,CH_3,ethyl), 2.30 (s,3H,SCH_3), 4.15 (q,2H,OCH_2,ethyl), 6.60 (s,1H,H-7), 7.45 (d,1H,H-3,J_{4-3}=5Hz), 7.90 (dd, 1H,H-4,J_{4-3}=5Hz, J_{4-1}=1Hz), 8.75 (bs,1H,H_1).

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ: 15.3 (SCH_3), 19 (CH_3, ethyl), 67.5 (OCH_2, ethyl), 106.4 (C-7), 114.1 (C-8a), 115.1 (C-4), 119.2 (C-6), 128.2 (C-3), 141.4 (C-8), 143.4 (C-1).

mass spectrum (E.I.): m/z = 208 ($M^{+\cdot}$); m/z = 193 (M-CH_3); m/z = 179 (100%).

U.V-visible (100% EtOH), λ_{nm} (ε_M-1 $_{cm}$-1): 242 (18000), 292 (3200,sh), 307 (4800), 318 (5000), 370 (2400).

Elemental analysis corresponded to empirical formula, $C_{10}H_{12}N_2OS$.

Using the same conditions of method A (EtO = 25 mmol), but excluding methylation, 2 yielded a mixture of products. Chromatography (chloroform-methanol 98:2) enabled the separation of compound 4a (60 mg; 60% yield); Rf = 0,15 (chloroform-methanol 95:5).

^{1}H N.M.R., 250 MHz, DMSO d_6, δ: 1.35 (t,3H,CH_3,ethyl), 4.10 (q,2H,OCH_2,ethyl), 6.35 (s,1H,H-7), 6.65 (d,1H,H-3 or H-4,J_{4-3} = 6Hz), 7.50 (d,1H,H-3 or H-4,J_{4-3} = 6Hz), 7.80 (s,1H,H-1), 10.60 (s,1H, NH).

mass spectrum (E.I.): m/z = 194 ($M^{+\cdot}$).

U.V-visible (chloroform-methanol 95:5): λ_{nm} (ε_M-1 $_{cm}$-1): 297 (12500), 330 (6000), 460 (3500), 530 (1000,sh).

Elemental analysis corresponded to empirical formula, $C_9H_{10}N_2OS$.

8-Ethoxy-7-methylpyrrolo [1,2-a] pyrazine 5.

1 mmol of Raney Nickel (50%, in neutral aqueous suspension) was added to a solution of 3b (0.5mmol) in dioxane (2.5 ml) under nitrogen at 80°C. After allowing the mixture to react for 5hr, a black precipitate was filtered off and the filtrate extracted with dichloromethane. The organic phase was dried and evaporated to dryness. Preparative T.L.C. (toluene-acetone 80:20) enabled the separation of compound 5 as an orange oil (50 mg; 50% yield).

^{1}H N.M.R., 250 MHz, $CDCl_3$, δ: 1.35 (t,3H,CH_3,ethyl), 2.20 (d,3H,CH_3,$J_{CH_3-(H-6)}$= 0.5 Hz), 4.10 (q,2H OCH_2,ethyl), 7.05 (quintuplet,1H,H-6,$J_{(H-6)-CH_3}$ = 0.5 Hz, $J_{(H-6)-(H-1)}$= 0.5 Hz), 7.25 (bd,1H,H-3, $J_{(H-3)-(H-4)}$= 5 Hz), 7.50 (dd,1H,H-4,$J_{(H-4)-(H-3)}$= 5 Hz, $J_{(H-4)-(H-1)}$= 1,5 Hz), 8.70 (bd,1H,H-1, $J_{(H-1)-(H-6)}$=0.5 Hz, $J_{(H-1)-(H-4)}$= 1.5 Hz), was in agreement with previous data[11].

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ: 9.3 (CH_3), 15.9 (CH_3,ethyl), 71.0 (OCH_2,ethyl), 111.6 (C-6), 117.1 (C-8a), 117.5 (C-4), 119.5 (C-7), 126.6 (C-3), 139.0 (C-8), 142.9 (C-1); these results were in agreement with these reported in literature[12,13,14].

mass spectrum (E.I.): m/z = 176 ($M^{+\cdot}$); m/z = 147 (100%); m/z = 107 (40%).

U.V-visible (100% EtOH), λ_{nm} (ε_M-1 $_{cm}$-1): 240 (26100), 297 (2890), 307 (2490), 357 (2250).

Elemental analysis corresponded to empirical formula, $C_{10}H_{12}N_2O$.

6,8-Dimethylthio-7-methylpyrrolo [1,2-a] pyrazine 6 and 6,8-dimethylthio-7-formylpyrrolo [1,2-a] pyrazine 7.

method I:

t-BuOK (10 mmol) was added to a solution of 1 (0.5 mmol) in t-butanol (500 ml) under nitrogen at 45°C. After 90 mn, the solution was methylated with an excess of methyl iodide (50 mmol). The solution was neutralised with dry-ice and evaporated to dryness in vacuo at 60°C. The residue was poured into water (50 ml) and extracted with ethyl acetate (50 ml). The organic phase was dried over anhydrous sodium sulphate and evaporated to dryness giving a brown oil. Chromatography (toluene-acetone 98:2) provided a major compound 6 (45 mg, 40% yield); Rf = 0.70 (ethyl ether) and a minor compound 7 (8 mg, 7% yield); Rf = 0.64 (ethyl ether).

6 was a pale yellow solid with m.p. 66°C.

^{1}H N.M.R., 250 MHz, $CDCl_3$, δ: 2.20 (s,3H,SCH_3 or CH_3), 2.30 (s,3H,SCH_3 or CH_3), 2.50 (s,3H,SCH_3 or CH_3), 7.70 (d,1H,H-3, J_{3-4}=5Hz), 8.20 (dd,1H,H-4,J_{3-4}= 5Hz,J_{4-1}= 1Hz), 8.95 (d,1H,H-1, J_{4-1}= 1Hz).

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ: 10.6 ($\underline{C}H_3$), 17.8 {$S\underline{C}H_3$(8)}, 20.5 {$S\underline{C}H_3$(6)}, 108.3 (C-8), 116 (C-6), 116.3 (C-4), 128.5 (C-3), 131.3 (C-8a), 136 (C-7), 143.1 (C-1).
mass spectrum (E.I.): m/z = 224 ($M^{+\cdot}$); m/z = 209 (M-CH_3); m/z = 191 (M-SH).
U.V. (100% EtOH) λ_{nm} (ε_M $l\,cm^{-1}$) : 240 (17500), 285 (2200,sh), 298 (2900), 308 (3000), 345 (3000).

7, an orange oil, had:

^1H N.M.R., 250 MHz, $CDCl_3$, δ: 2.40 {s,3H,SCH_3(8) or SCH_3(6)}, 2.50 {s,3H,SCH_3(8) or SCH_3(6)}, 7.80 (bs,1H,H-3), 8.30 (bd,1H,H-4), 9.25 (bs,1H,H-1), 10.60 (s,1H,CHO).

^{13}C N.M.R., 50.3 MHz, $CDCl_3$ + CD_3OD (1:1)
The aldehyde / aldehyde hydrate equilibrium was observed. Due to this mixture and the quantities available, it was not possible to observe quaternary carbons.
aldehyde form had δ: 18.9 {$S\underline{C}H_3$(8)}, 21.5 {$S\underline{C}H_3$(6)}, 117.3 (C-4), 130.1 (C-3), 143.5 (C-1), 188 ($\underline{C}$HO).
aldehyde hydrate form had δ: 18.6 {$S\underline{C}H_3$(8)}, 20.9 {$S\underline{C}H_3$(6)}, 101.6 {$\underline{C}H(OH)_2$}, 116.9 (C-4), 127.6 (C-3), 146.4 (C-1).
mass spectrum (E.I.): m/z = 238 ($M^{+\cdot}$); m/z = 223 (M-CH_3); m/z = 205 (M-SH).
(aldehyde)
U.V.(100% EtOH), λ_{nm} (ε_M $l\,cm^{-1}$): 240 (17000), 270 (7500), 295 (3000,sh), 322 (2900), 380 (2700).
Elemental analysis corresponded to empirical formula, $C_{10}H_{10}N_2OS_2$.

method II:
The nucleophile RS^- (R = ethyl, 1 mmol; R = cysteinyl, 2.5 mmol; R = glutathionyl, 5 mmol) was added to a solution of 1 (0.5 mmol) in ethanol (500 ml), under nitrogen at 35°C. The reaction was allowed to go to completion and the resulting mixture methylated with an excess of methyl iodide(50 mmol) . The solution was neutralised with dry-ice and evaporated to dryness in vacuo at 35°C. The residue was poured into water (50 ml) and then extracted with ethyl acetate (50 ml). The organic phase was dried over anhydrous sodium sulphate and evaporated to dryness. Chromatography of the residue (toluene-acetone 95:5) provided the metabolite 6 (90 mg, 80% yield).

6,8-Dimethylthiopyrrolo [1,2-a] pyrazine 8
Using the above mentioned method I, 2 gave 8 in 40% yield; Rf = 0.65 (ethyl ether).

^1H N.M.R., 250 MHz, $CDCl_3$, δ: 2.35 {s,3H,SCH_3(8) or SCH_3(6)}, 2.45 {s,3H,SCH_3(8) or SCH_3(6)}, 7.10 (s,1H,H-7), 7.70 (d,1H,H-3,J_{3-4} =5Hz), 8.10 (dd,1H,H-4,J_{3-4} = 5Hz, J_{4-1} = 1Hz), 8.95 (d,1H,H-1, J_{4-1} = 1Hz).
^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ:14.3 {$S\underline{C}H_3$(8)}, 21.3 {$S\underline{C}H_3$(6)}, 110.8 (C-8), 116.7 (C-4), 118.4 (C-6) 125.7 (C-7), 129 (C-3), 137 (C-8a), 144.4 (C-1).
mass spectrum (E.I.): m/z = 210 ($M^{+\cdot}$); m/z = 195 (M-CH_3); m/z = 147 (M-CH_3-CH_3SH, 100%).
U.V. (100% EtOH), λ_{nm} (ε_M $l\,cm^{-1}$): 235 (14000), 255 (8800,sh), 292 (3000), 302 (3000), 350 (3000).
Elemental analysis corresponded to empirical formula, $C_9H_{10}N_2S_2$.

3-Methylthio-3-(5'-pyrimidinyl)-2-propene-methyldithioate 11.
Using the above mentioned method II (EtS^- = 4 mmol and ICH_3 = 50 mmol) but working at 20°C and stopping the reaction after 10 mn, 10 gave an orange oil. Chromatography (hexane-acetone 5:1) enabled the separation of a major compound 11 as E isomer[17]; (70 mg, 60% yield, m.p. 49°C).

^1H N.M.R., 250 MHz, $CDCl_3$, δ: 2.00 (s,3H,CH_3), 2.40 (s,6H,2xSCH_3), 8.60 (bs,2H,H-4' and H-6'), 9.05 (bs,1H,H-2').

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ: 16.4 ($\underline{C}H_3$) , 20.4 ($S\underline{C}H_3$), 23.9 ($CS_2\underline{C}H_3$) , 128.9 (C-2), 132.5 (C-5'), 147.2 (C-3), 157.5 (C-6' and C-4'), 157.8 (C-2'), 234.1 (C-1).
mass spectrum (E.I.): m/z = 256 ($M^{+\cdot}$); m/z = 241 (M-CH_3); m/z = 226 (M-CH_3)-CH_3.
U.V-visible (100% EtOH), λ_{nm} (ε_M $l\,cm^{-1}$) : 235 (10500), 315 (10500), 345 (3500,sh), 495 (200).

3-Methylthio-3-phenyl-2-propene-methyldithioate 15.
With 14 as starting material, the previous method II (EtS^- = 2 mmol and ICH_3 = 50 mmol), provided after chromatography (hexane) compound 15 as Z isomer[17]: red solid m.p. 67°C, (70 mg, 70% yield); Rf = 0.8 (hexane-acetone 6:1).

^1H N.M.R., 250 MHz, $CDCl_3$, δ: 1.95 (s,3H,SCH_3), 2.65 (s,3H,CS_2CH_3), 7.15 (s,1H,vinyl H), 7.20 to 7.50 (m,5H,aromatic).

^{13}C N.M.R., 50.3 MHz, $CDCl_3$, δ: 17.7 (SCH_3), 19.4 ($CS_2\underline{C}H_3$), 128 to 132 (phenyl, $\underline{C}H$), 129.1 (C-2) 139.8 (phenyl, quaternary $\underline{C}$), 159.7 (C-3), 216 (C-1).
mass spectrum (E.I.): m/z =239 (M-1), m/z = 225 (M-CH_3).
U.V-visible (100% EtOH) : λ_{nm} (ε_M $l\,cm^{-1}$): 265 (3700), 365 (17600), 500 (200).
Elemental analysis corresponded to empirical formula, $C_{11}H_{12}S_3$.

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