Synthesis of 3*H*-Thieno[3,2-*c*]-1,2-dithiole-3-thione and its Reaction with *n*-Butylamine

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A convenient synthesis of 3*H*-thieno[3,2-*c*]-1,2-dithiole-3-thione (**7**) is proposed. The reaction of **7** with *n*-butylamine afforded the *N*-butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione (**7a**) in dynamically equilibrium [1] with its 3*H*-thieno[3,2-*c*]-1,2-dithiole-*N*-butyl-3-imino isomer **7b**. Characterizations and antimicrobial activities of the synthesized products are reported.

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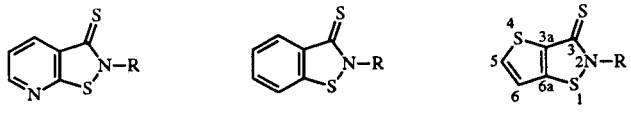
In connection with our program directed towards the preparation of new heterocyclic compounds which might exhibit antifungal and antibacterial activity, the synthesis of some *N*-substituted isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **A** [2], which are analogues of the much more studied 1,2-benzisothiazole-3(2*H*)-thiones **B** [3-8], has been recently described (Figure 1).



A **B** **C**

Figure 1

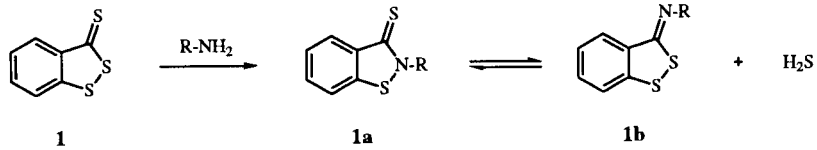
The *N*-alkyl derivatives having structures **A**, **B**, were prepared by reaction of the appropriate trithione with alkylamines. The 3*H*-1,2-benzodithiole-3-thione **1** reacts with 1.2 equivalents of various primary amines to give mixtures of *N*-substituted 1,2-benzisothiazole-3(2*H*)-thiones **1a** and 3-imino-3*H*-1,2-benzodithioles **1b** in "dynamic isomerism" [4,5] (Scheme 1, Equation 1). On the other hand the reactions of the 3*H*-1,2-dithiolo[3,4-*b*]-pyridine-3-thione **2** with alkylamines give the *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides in tautomeric forms **3a,b** and merely traces of *N*-alkylisothiazolo[5,4-*b*]-pyridine-3(2*H*)-thiones **2a** and *N*-alkyl-3-imino-3*H*-1,2-dithiolo[3,4-*b*]pyridines **2b** [2] (Scheme 1, Equation 2). These unexpected results in the pyridine series were explained with the decreased S-N bond stability which is more susceptible towards the reducing action of hydrogen sulphide. Compounds **A** were thus prepared by a successive iodine treatment of the *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamidic intermediates **3a,b**.

The desired trithiones **1,2** were themselves prepared by phosphorus pentasulfide treatment of 2,2'-dithiosalicylic acid **4** [9] and 2-mercaptonicotinic acid **5** [10] respectively (Scheme 2).

In continuation of the studies on annulated isothiazolethiones we have focused our attention on the synthesis of the *N*-alkylthieno[3,2-*c*]isothiazole-3(2*H*)-thiones **C** (Figure 1). In analogy with the synthesis of compounds **A**, **B**, the starting product of choice would be the 3*H*-thieno[3,2-*c*]-1,2-dithiole-3-thione **7**. This compound was prepared by other authors from 2-carbethoxy-3-hydroxy-

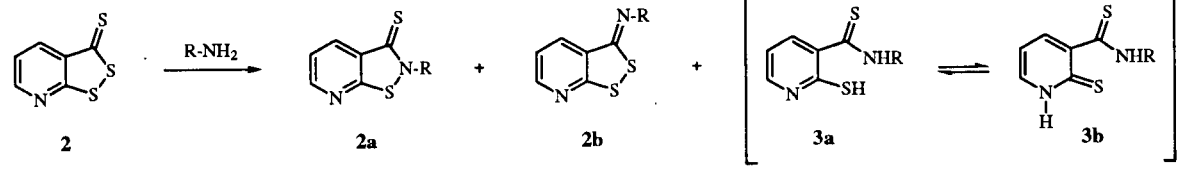
Scheme 1

Equation 1



1 **1a** **1b**

Equation 2

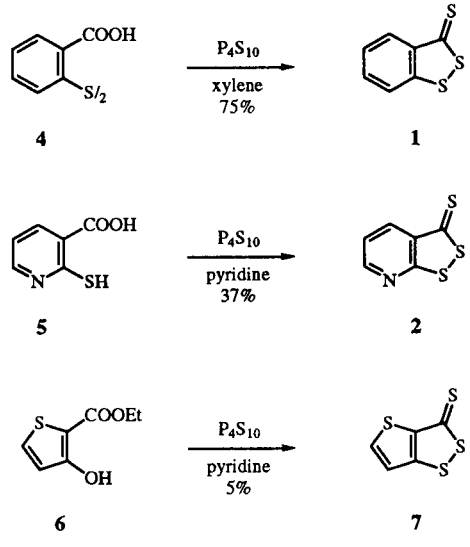


2 **2a** **2b** **3a** **3b**

R = *n*-C_4H_9

thiophene **6** and phosphorus pentasulfide albeit in a 5% yield [11] (Scheme 2).

Scheme 2



However as we needed substantial amount of compound **7** we planned a new synthesis of the above compound.

This paper deals with our results on the synthesis of the 3*H*-thieno[3,2-*c*]-1,2-dithiole-3-thione **7** and its reaction with *n*-butylamine, chosen as it is largely described as *N*-substituent for derivatives **A**, **B**. The reaction products were characterized and after an oxidative step the *N*-butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione **7a** in dynamically equilibrium with the 3*H*-thieno[3,2-*c*]-1,2-dithiole-*N*-butyl-3-imino **7b** were isolated and their antimicrobial activities evaluated.

Results and Discussion.

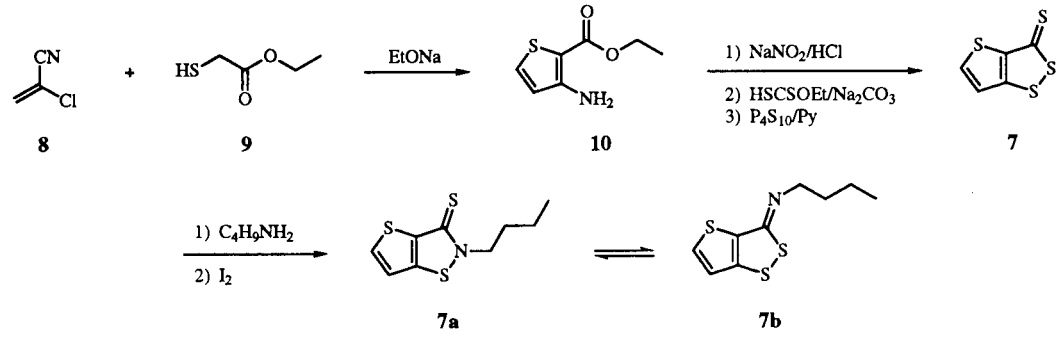
Chemistry.

A convenient three step synthesis of 3*H*-thieno[3,2-*c*]-1,2-dithiole-3-thione **7** was accomplished starting from 2-chloroacrylonitrile **8** and ethyl thioglycolate **9** [12] (Scheme 3).

The reaction involves a Michael addition of the thioglycolate anion to the acrylic derivative **8** followed by a Thorpe cyclization, and elimination of hydrogen chloride. Diazonium salt formation for the so obtained 3-amino-2-ethoxycarbonylthiophene **10** with hydrochloric acid and sodium nitrite, and subsequent treatment with potassium ethyl xantate [13] gave the 3-mercapto-2-ethoxycarbonylthiophene intermediate which was reacted without purification with phosphorus pentasulfide in pyridine solution to afford **7** in a 32% overall yield. The reactions of **7** with 1.2 equivalents of *n*-butylamine gave a mixture of **7a,b** and a polar product, probably the 3-mercaptothiophene-2-carbothioamide (^{1}H nmr signals of the compound in the mixture: δ 3.72 (m, 2H, N-CH_2), 7.20 (d, 1H, H-4, $J_{4,5}$ = 5.5 Hz), 7.39 (d, 1H, H-5, $J_{5,4}$ = 5.5 Hz), 8.50 (br s, 1H, SH)). This hypothesis was confirmed by oxidation of the crude reaction mixture with iodine which affected the complete conversion of the supposed 3-mercaptothiophene-2-carbothioamide into a mixture of the desired *N*-butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione **7a** and the 3*H*-thieno[3,2-*c*]-1,2-dithiole-*N*-butyl-3-imino isomer **7b**, without any other side products.

The mixture obtained appeared as a single spot on tlc and the two compounds were not separable by usual liquid-solid chromatography techniques. However the gc-ms of the reaction products gave two peaks (Rt = 17.15 and 19.45 min) with the same molecular ions (m/z: 229). In order to assign the correspondence of each peak and better elucidate the types of fragmentation of each isomeric form, the gc-ms behavior of the *N*-butylsubstituted benzene and pyridine analogues **1a,b** and **2a,b** respectively, were compared. The two isomeric forms **2a,b** could be isolated by suitable preparative chromatography [2] and the successive gc-ms analyses showed the retention times of 18.15 minutes and 16.52 minutes respectively. The presence of an ion peak at m/z 195 for compound **2a** was attributed to a loss of the ethyl group from the side chain, which produced a tricyclic ion containing a new five

Scheme 3



membered ring including nitrogen, sulphur and two carbon atoms deriving from the 2-side chain [2]. This kind of fragmentation was absent in the less retained imino form **2b**. Similar behavior was observed for the *N*-butyl-1,2-benzisothiazole-3(2*H*)-thiones **1a** (Rt = 19.90 minutes) which gave an ion peak at m/z 194 assigned to the tricyclic ion bearing the benzene ring instead of the pyridine one while the mass spectrum of compound **1b** (Rt = 17.70 minutes) does not exhibit the peak at m/z 194.

It is thus reasonable to assume that the order of retention of the isomers in the gc analysis is reproducible for the two classes of compounds **A**, **B** where the 3-imino forms are less retained and the 3-thione forms give higher retention times. Considering the mixture **7a,b**, the ms analyses for the gc peak at 19.45 minutes showed the presence of an ion peak with m/z 200 that was ascribed to the tricyclic ion bearing the thiophene ring instead of the pyridine or benzene ones. This behavior is diagnostic for the *N*-butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione **7a**, the gc peak at 17.15 minutes is therefore assigned to the imino isomer **7b**. The ^1H and ^{13}C nmr of the mixture confirmed the structure of the synthesized compounds. As previously reported [2] a good diagnostic feature for the isomers **2a,b** is the chemical shift of the methylene protons adjacent to the nitrogen atom, at δ 4.40 and 3.40 respectively. The same chemical shifts were observed for compounds **1a,b** [5]. Regarding the mixture of **7a,b**, the ^1H nmr spectra showed two triplets at δ 4.31 and 3.33 and the ^{13}C nmr two singlets at δ 178.0 (C=S) and 155.7 (C=N). These signals were thus assigned to compounds **7a** and **7b** respectively.

Table 1

In Vitro Antimicrobial Activity: MIC μg/ml [a]

	Compounds				
	7	**7a,b**	Gentamicin	Cefotaxime	Clotrimazole
Tm [b]	5	3	—	—	0.5
Ca [c]	10	10	—	—	1.25
Sau [d]	30	15	2.5	1.5	—
Sal [e]	30	10	1.5	0.5	—
Bs [f]	20	10	0.5	0.5	—
Kp [g]	>50	30	2.5	1.5	—
Eco [h]	50	30	2.5	1.5	—
Sty [i]	50	30	2.5	1.5	—
Pa [j]	>50	50	12.5	10	—
Cp [k]	20	10	—	—	—

[a] Microbiological assays were performed as previously reported [15]. The evaluation of minimum inhibitory concentrations (MICs) was carried out by the medium dilution technique for the fungal strains [16] and by the Bioscreen Analyzer for the bacterial strains [17]. [b] *Tricophyton mentagrophytes* (ATCC 9129). [c] *Candida albicans* (ATCC 2091). [d] *Staphylococcus aureus* (ATCC 6538). [e] *Staphylococcus albus* (ATCC 12228). [f] *Bacillus subtilis* (ISM 6513). [g] *Klebsiella pneumoniae* (ATCC 4352). [h] *Escherichia coli* (ISM 6585). [i] *Salmonella typhi* (ATCC 19430). [j] *Pseudomonas aeruginosa* (ATCC 15442). [k] *Clostridium perfrigens* (ATCC 12916).

Biological Results.

The mixture of compounds **7a,b** and the precursor **7** were subjected to biological assays for their antimicrobial activity. Table 1 summarizes their *in vitro* antimicotic and antibacterial activities against two fungal strains and three Gram-positive, four Gram-negative, and an anaerobic bacterial strains.

The target compounds **7a,b** showed a good antimicotic activity in particular towards *T. mentagrophytes*. Relevant activities have also been exhibited towards Gram-positive strains and *C. perfrigens*, while lower activities were obtained with respect to Gram-negative strains. Surprisingly the trithione **7** showed a certain activity, especially towards fungal strains.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and were not corrected. Elemental analyses were performed on a Carlo Erba model 1106 Elemental Analyzer. Infrared ir spectra were recorded in nujol on a Perkin Elmer model 1310 spectrophotometer. The 300 MHz ^1H and 75 MHz ^{13}C nmr spectra were recorded on a Bruker ACE-300 spectrometer, in deuteriochloroform using tetramethylsilane as internal standard; chemical shifts are in δ (ppm) and coupling constants (J) in Hz; only representative proton signals were assigned. Mass spectra (ms) (EI, 70 eV) were taken on a gc-ms Finnigan ITD instrument. The chromatographic column was a 30 m long DB-5 (J&W Scientific), with a 0.25 mm ID and a 0.25 μm film thickness. The chromatographic conditions were: injector temperature 240°; the oven temperature was maintained at 100° for 1 minute and then heated to 300° by a temperature increment of 10°/minute. The tlc analyses was run on silica gel 60 F_{254} Merck and visualized by uv (λ = 264 nm); flash column chromatography on silica gel 60 (60-200 μm, Merck) was performed as described in the original paper [14]. Conditions for microbiological assays are reported in Table 1.

3-Aminothiophene-2-carboxylic Acid Ethyl Ester (**10**).

Ethyl thioglicolate (21 ml, 0.19 mole) was added to a solution of sodium ethoxide (from 9.75 g of sodium) in 190 ml of absolute ethanol. A solution of 2-chloroacrylonitrile (15 ml, 0.187 mole), in absolute ethanol (20 ml), was added dropwise with stirring at room temperature. The mixture was stirred for further one hour and then concentrated *in vacuo* to one-third of its original volume. Water (150 ml) was added and organic products were extracted with ether (4 x 100 ml). The combined organic phases were dried (anhydrous sodium sulphate) and evaporated under reduced pressure. The residue was purified through flash chromatography (*n*-hexane/ethyl acetate (8:2)) and fractions containing only product **10** (by tlc analysis) were combined and concentrated. The crude product was then recrystallized from light petroleum to give 26 g of light yellow crystals (80% yield), mp 42-43°; R_f (*n*-hexane/ethyl acetate (8:2)) = 0.40; ^1H nmr: δ 1.25 (t, 3H, CH_3), 4.20 (q, 2H, CH_2), 6.55 (bs, 2H, NH_2), 6.63 (d, 1H, H-4, $J_{4,5}$ = 5.45 Hz), 7.55 (d, 1H, H-5, $J_{5,4}$ = 5.45 Hz).

Anal. Calcd. for $C_7H_9NO_2S$: C, 49.11; H, 5.30; N, 8.18. Found: C, 49.05; H, 5.23; N, 8.12.

3*H*-Thieno[3,2-*c*]-1,2-dithiole-3-thione (**7**).

To a 6 *N* hydrochloric acid suspension (100 ml) of 3-aminothiophene-2-carboxylic acid ethyl ester (**10**) (24 g, 0.14 mole) at 0°, an aqueous solution (70 ml) of sodium nitrite (9.7 g, 0.14 mole) was added. The cold reaction mixture was stirred for one hour and the resulting red solution of diazonium salt was slowly added to a stirred solution of sodium carbonate (17.8 g, 0.17 mole) and potassium ethyl xanthate (22.43 g, 0.14 mole) in water (300 ml). The temperature was then maintained at about 70° under vigorous stirring, until the nitrogen has completed evolving. After cooling, the water solution was extracted with ether (4 x 100 ml) and the collected organic layers were dried over anhydrous sodium sulphate. Evaporation of organic solvent gave 20 g of a crude product that was dissolved in 200 ml of anhydrous pyridine. Phosphorous pentasulfide (7.64 g, 0.0174 mole) was added and the reaction mixture was refluxed for two hours. Then the reaction mixture was refluxed for further four hours with a phosphorous pentasulfide (7.64 g, 0.0174 mole) addition at any hour. After the last addition, refluxing was continued for further four hours and then quenched with dilute hydrochloric acid and brine. The organic product was extracted with ethyl acetate (3 x 80 ml) and the collected organic phases were dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure and the residue was flash chromatographed (*n*-hexane/ethyl acetate (7:3)) to give 8.64 g (32% yield) of pure product **7**, mp 127°; R_f (*n*-hexane/ethyl ether (9:1)) = 0.35; ir: ν C=S 1310; ^{1}H nmr: δ 7.21 (d, 1H, H-6, $J_{5,6}$ = 6 Hz), 8.02 (d, 1H, H-5, $J_{6,5}$ = 6 Hz); ^{13}C nmr: δ 202.6 (s, C-3), 156.3 (s, C-3a), 149.3 (s, C-6a), 142.5 (d, C-5), 122.3 (d, C-6); ms: m/z (% ra) 190 (100), 174 (47), 146 (26), 114 (33), 100 (23), 69 (28).

Anal. Calcd. for $C_5H_2S_4$: C, 31.56; H, 1.06. Found: C, 31.48; H, 1.11.

N-Butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione (**7a**) and 3*H*-Thieno[3,2-*c*]-1,2-dithiole-*N*-butyl-3-imine (**7b**).

To a stirred solution of 1.5 g (7.8 mmoles) of 3*H*-thieno-[3,2-*c*]-1,2-dithiole-3-thione (**7**) in 150 ml of absolute ethanol, at room temperature, 1.9 ml (19.5 mmoles) of butylamine was added. The reaction mixture was refluxed for 4 hours and then solvent and excess reagent were removed *in vacuo*. The residue was taken up in ethanol (100 ml) and an ethanolic solution of iodine (6%) was added dropwise until a persistent brown color is noted. The solvent was evaporated under reduced pressure and the residue was purified through flash chromatography (*n*-hexane/ethyl acetate (97:3)). Analysis of the reaction mixture by 300 MHz nmr indicated a 1:3 mixture of **7a/7b** (1.02 g, 57%). This mixture was inseparable by both flash chromatography and hplc although its elemental analysis was in accordance with an expected value for both isomers.

N-Butylthieno[3,2-*c*]isothiazole-3(2*H*)-thione (**7a**).

This compound was isolated by gc-ms: Rt = 19.45 minutes, m/z (% ra) 229 (80), 200 (58), 186 (77), 173 (100), 159 (67), 140 (20), 109 (42), 69 (72), 64 (43); ir: ν C=S 1280; ^{1}H nmr: δ 0.93 (t, 3H, CH_3), 1.38 (m, 2H, CH_2-CH_3), 1.79 (m, 2H, N-CH_2-CH_2), 4.31 (t, 2H, N-CH_2), 7.22 (d, 1H, H-5, $J_{5,6}$ = 5 Hz), 7.78 (d, 1H, H-6, $J_{6,5}$ = 5 Hz); ^{13}C nmr: δ 178.0 (s, C-3), 140.8 (s, C-3a), 136.0 (d, C-5), 134.2 (s, C-6a), 117.8 (d, C-6), 49.8 (t, N-CH_2), 31.2 (t, N-CH_2-CH_2), 18.6 (t, CH_2-CH_3), 13.5 (q, CH_3).

Anal. Calcd. for $C_9H_{11}NS_3$ [18]: C, 47.13; H, 4.83; N, 6.11. Found: C, 47.15; H, 4.71; N, 5.83.

3*H*-Thieno[3,2-*c*]-1,2-dithiole-*N*-butyl-3-imine (**7b**).

This compound was isolated by gc-ms: Rt = 17.15 minutes, m/z (% ra) 229 (15), 196 (100), 186 (30), 173 (83), 159 (10), 140 (18), 109 (62), 69 (39), 64 (62); ir: ν C=N 1610; ^{1}H nmr: δ 0.90 (t, 3H, CH_3), 1.38 (m, 2H, CH_2-CH_3), 1.66 (m, 2H, N-CH_2-CH_2), 3.33 (t, 2H, N-CH_2), 6.98 (d, 1H, H-5, $J_{5,6}$ = 5 Hz), 7.62 (d, 1H, H-6, $J_{6,5}$ = 5 Hz); ^{13}C nmr: δ 155.7 (s, C-3), 144.9 (s, C-3a), 141.5 (s, C-6a), 133.9 (d, C-5), 120.7 (d, C-6), 57.6 (t, N-CH_2), 32.3 (t, N-CH_2-CH_2), 20.5 (t, CH_2-CH_3), 13.8 (q, CH_3).

Anal. Calcd. for $C_9H_{11}NS_3$ [18]: C, 47.13; H, 4.83; N, 6.11. Found: C, 47.15; H, 4.71; N, 5.83.

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REFERENCES AND NOTES

* To whom correspondence should be addressed.

[1] The dynamic equilibrium occurs in solution making these compounds unseparable by hplc. However this equilibrium is evidently not active in the gas phase allowing the separation of the two isomers by gc.

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[18] Elemental analyses for compounds **7a** and **7b** were determined on a mixture.