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Short Communication

Chromatography of methyl derivatives of 5-ethyl-5-phenyl-2-thiobarbituric acid

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Abstract

Methylation of 5-ethyl-5-phenyl-2-thiobarbituric acid yields two pairs of monomethyl and dimethyl derivatives which are constitutional isomers differing in the N- *vs.* S-methyl substitution. These products were separated by column chromatography on silica gel and also by TLC and HPLC. The chiral methyl derivatives and closely related compounds were resolved using β-cyclodextrin in the mobile phase as a selector. The order of the eluted enantiomers was established by chemical transformation and correlation with enantiomers of known configuration.

1. Introduction

Several 5,5-disubstituted barbituric acids [2,4,6(1H,3H,5H)-pyrimidinetriones] and their 2-thio analogues are well known drugs with hypnotic and antiepileptic activity. Their N-methyl derivatives also show biological activity and are used in the pharmaceutical and toxicological gas chromatographic analysis of parent compounds [1]. Recently we investigated the methylation of 5-ethyl-5-phenyl-2-thiobarbituric acid (2-thiophenobarbital-5-ethyl-5-phenyl-2-thioxo-4,6-(1H,3H,5H)-pyrimidinedione, **1**) using methyl halides and a base (sodium hydride, tetra-*n*-butylammonium hydroxide or potassium hydroxide) [2]. All possible methylation products, *i.e.* N- (**2**), S- (**3**) monomethyl and N,N'- (**4**) and N,S- (**5**) dimethyl derivatives of 1 were obtained (Fig. 1). Compounds **2–4** were obtained previously by other methods [3,4]; **5** has not been described in the literature. We used various chromatographic methods to separate and identify these products. Compounds **2**, **3** and **5** are chiral, and we used chiral chromatography to resolve their enantiomers, as well as the enantiomers of closely related compounds (**6–9**).

2. Experimental

2.1. Reagents and materials

Compound **1**, **7** and **8** were obtained by condensation of diethyl ethylphenylmalonate with thiourea, N-ethylurea and N-ethylthiourea, respectively; compounds **2–5** were obtained by methylation of **1** [2]; compound **9** was obtained by oxidation of N-methyl-2-(N-methyl-thiocarbamyl)-imino-5-ethyl-5-phenylbarbituric acid [5]. Pure enantiomers of **6** with known configuration were kindly donated by Professor J. Knabe (Saarbrücken, Germany), β-cyclodextrin was obtained from Chinoin (Budapest, Hungary). Other reagents and solvents were of analytical

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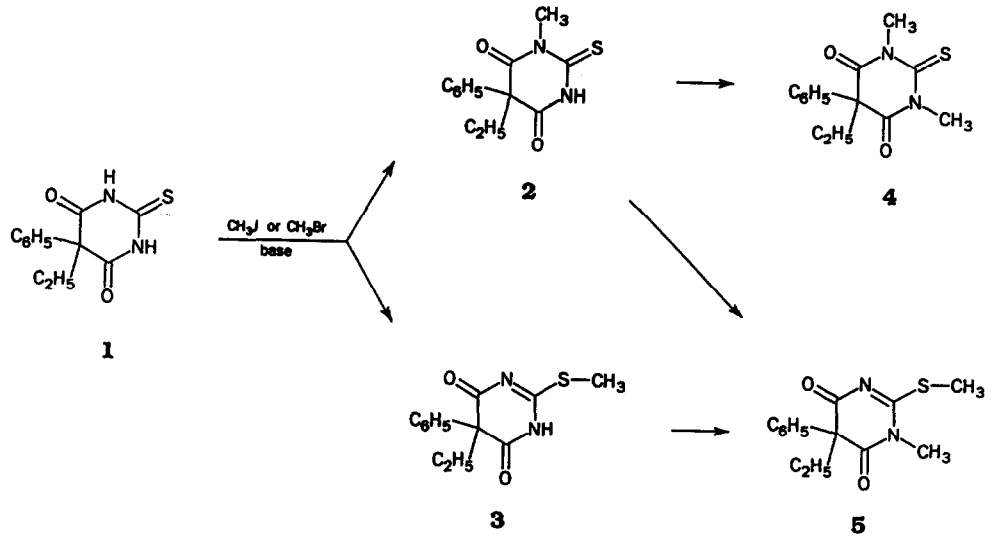


Fig. 1. Methylation of 5-ethyl-5-phenyl-2-thiobarbituric acid (1).

grade (POCh) and were used without purification.

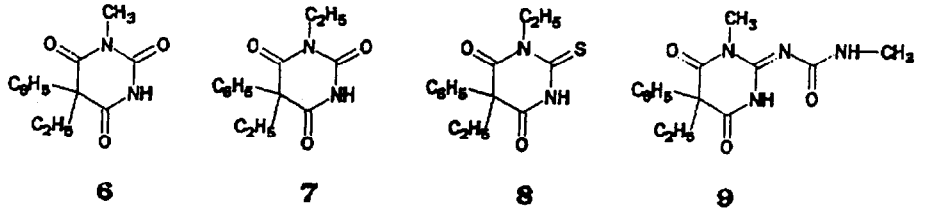
2.2. Chromatography

Column chromatography was carried out in a glass column of diameter 4 cm. Silica gel was used as the adsorbent for chromatography, with 70–325 mesh (Merck). The eluents used were cyclohexane–ethyl acetate [(A) 4:1, (B) 2:1, (C) 1:1]. The volume of collected samples was 5 ml and sample content was determined by TLC.

TLC was performed on precoated TLC aluminium sheets using silica gel 60 F_{254} (Merck). The solvent systems used were: (D) chloroform, (E) chloroform–toluene–acetone (2:2:1) and (F) cyclohexane–ethyl acetate (4:1). Visualization was with UV light at 254 nm.

A Liquochrom OE 312/1 chromatograph equipped with an OE 308 UV detector (Labor MIM, Budapest, Hungary) was used for HPLC. Compounds **2–5** were chromatographed in normal mode using a Sil 10-μm column (250 mm × 4.6 mm I.D.), 4% anhydrous ethanol in *n*-hexane as a mobile phase, a flow-rate of 3 ml/min and UV detection at 240 and 260 nm. Chiral chromatography was carried out in reversed-phase mode using a Chromsil C_{18} 10-μm column (250 mm × 4.6 mm I.D.). The mobile phase was composed of aqueous saturated solution of β-cyclodextrin, 96% ethanol, 0.1 *M* NaH_2PO_4 and 0.1 *M* Na_2HPO_4 (80:10:7.5:2.5) and the flow-rate was 1.5 ml/min. UV detection was at 240 nm and the temperature of chromatographic runs was 25°C.

For configuration assignment the collected eluates of the second-eluted enantiomer of compounds **2**, **5** and **7** were desulphurized to the enantiomers of the corresponding 2-oxo analogues, **6** and **8**, by intensive stirring with 100 mg



of red HgO for 3 h at room temperature. Then the solvent was evaporated, the residue was extracted with 1 ml of ethanol, and the extract was concentrated to 20–30 μl and co-chromatographed under conditions described above with the enantiomer of **6**. The eluate with the second-eluted enantiomer of compound **9** was transformed into the enantiomer of **6** by heating with concentrated HCl for 1 h, storing for 24 h at room temperature, evaporating the solvent, extracting with ethanol (1 ml) and concentrating of the sample and chromatography under conditions given above.

3. Results

Liquid chromatography of products **2–5** is exemplified by the results of elution after chromatography of the reaction mixture obtained after methylation of **1** (0.94 g) on 150 g of silica gel:

Eluent A: 165 ml + 145 ml (compound **4**, 0.1 g) + 60 ml, eluent change.

Eluent B: 80 ml + 100 ml (compound **2**, 0.23 g) + 110 ml + 65 ml (compounds **5**, 0.13 g) + 50 ml (compound **5** and **1**, 0.10 g) + 65 ml (compound **1**, 0.06 g) + 90 ml; eluent change.

Eluent C: 135 ml + 125 ml (compound **3**, 0.35 g).

The results of TLC and HPLC separations for compounds **2–5** are presented in Table 1, while

Table 1
Results of TLC and HPLC (normal-phase mode) separations of methyl derivatives of 2-thiophenobarbital

Compound	TLC (R_F values)			HPLC (t_R, min)
	Solvent system			
	D	E	F	
1	0.13	0.60	0.29	–
2	0.25	0.69	0.44	1.5
3	0.04	0.56	0.13	7.8
4	0.51	0.74	0.58	1.1
5	0.13	0.66	0.21	3.1

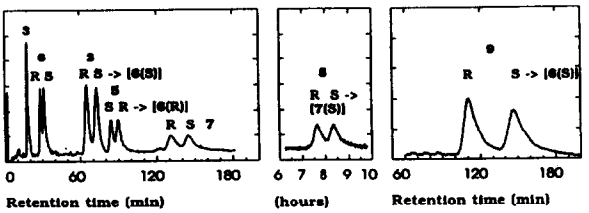


Fig. 2. Resolution of enantiomers of methyl derivatives of 5-ethyl-5-phenyl-2-thiobarbituric acid and closely related compounds. For conditions see the Experimental section. For compound designation and structures see Fig. 1 and the text.

those of chiral chromatography are given in Fig. 2 and Table 2.

4. Discussion

The results presented above show that the chromatographic methods used are well suited for separation and identification of all products of methylation of 2-thiophenobarbital, which are two pairs of constitutional isomers. In each pair the N-CH_3-substituted compound migrated faster than its S-CH_3-substituted counterpart in all chromatographic systems used with the exception of chiral chromatography. The fastest migration was observed for the N,N′-dimethyl derivative.

Of the three chiral methyl derivatives of 2-thiophenobarbital (compounds **2**, **3** and **5**) and four other closely related compounds (**6–9**), only one (**3**) was not resolved into enantiomers with

Table 2
Retention times and enantioseparation factors for chiral products of methylation of 2-thiophenobarbital and related compounds

Compound	t_R (min)		α
	R	*S*	
2	65.5	74.0	1.13
3	18.0	18.0	1.0
5	90.1	84.8	1.06
6	28.8	31.5	1.10
7	132.5	146.5	1.12
8	457.0	501.0	1.10
9	112.0	148.0	1.32

β-cyclodextrin in the mobile phase used as the chiral selector. For all but one resolved compounds the first eluted was the enantiomer with the *R* configuration. For the N,S-dimethyl derivative of **1** (compound **5**) the first eluted was the (*S*)-enantiomer, probably because of the C_2–N_3 double bond in the ring —the structural feature unique among the resolved compounds.

The elution order and configuration were confirmed by transformation of the second-resolved enantiomer of compounds **2**, **5** and **8** to the corresponding 2-oxo analogue (compound **6**), of which the elution order and configuration under similar conditions were determined previously [6] based on synthetic specimens of individual enantiomers of **6** [7].

The elution order of the enantiomers of compound **7** was assumed to be the same as that of compound **6**. The elution order of the enantiomers of compound **9** was correlated with that of **6** by hydrolytic splitting of the exocyclic N-methylurea moiety.

5. References

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