Eur J Med Chem (1995) 30, 769–777

Competition between two metabolic pathways: oxidation and desulfuration in the thiobarbiturate series

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(Received 28 February 1995; accepted 31 May 1995)

Summary — In order to study the competition between hepatic hydroxylation and desulfuration in the thiobarbiturate series, two compounds bearing a branched side chain with a tertiary carbon atom in position ω–1 were administered to rats over about one week. Urine and faeces were collected and extracted. The metabolites isolated were identified. It was shown that desulfuration was not the major metabolic pathway, and that, when it took place, it remained a minor process and was accompanied by γ-hydroxylation into a tertiary alcohol.

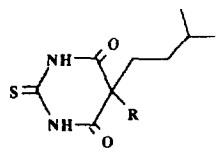
thiobarbiturate series / metabolism / oxidation / desulfuration

Introduction

The main metabolic pathway in the barbiturate series is a cytochrome P450 dependent hepatic oxidation. Desulfuration of thiobarbiturates has been described as a major biodegradation process [1–3], but the literature data are disparate and do not allow a rationalization of the competition between these two pathways.

In order to study this competition on a model with no side reactions, compounds with a tertiary carbon atom in the ω-1 position were selected, namely, 5-ethyl-5-(3-methylbutyl)-2-thiobarbituric acid **A1** and 5-(3-methylbutyl)-5-propyl-2-thiobarbituric acid **B1** (scheme 1). These conditions are in favour of an univocal oxidation.

The branched side chain is the same for the two compounds **A1** and **B1**, but the lengthening of the linear side chain induces an increase in lipophilicity ($\Delta \log P = 0.3$).



Scheme 1. **A1**: R = CH_2CH_3; **B1**: R = $CH_2CH_2CH_3$.

First models of the various potential metabolites of these xenobiotics were synthesized. The two thiobarbiturates were then administered to rats and urinary metabolites were extracted and identified.

Chemistry

Search for potential metabolites

According to literature data and previous work in our laboratory [4–7], the potential metabolites of the corresponding barbiturates are those listed in scheme 2. The first kind of potential metabolites result from a γ-oxidation of the branched side chain **A3**, R = CH_2CH_3, and **B3**, R = $CH_2CH_2CH_3$.

The oxidation of the linear side chain of **A1** leads to a single β-hydroxy compound **A5**. A similar oxidation on **B1** leads to either a β-hydroxy compound (secondary alcohol) **B5** or a γ-hydroxy compound (primary alcohol) **B4**.

Subsequent reactions could be observed from **A5**. Oxidation of the primary alcohol into an aldehyde function, **A6**, is followed by a further oxidation into a carboxylic acid, **A7**. Alternatively, intramolecular alcoholysis of **A5** into a thioallophanyl-γ-lactone, **A8**, is followed by a hydrolysis into a carboxylic lactone **A9**.

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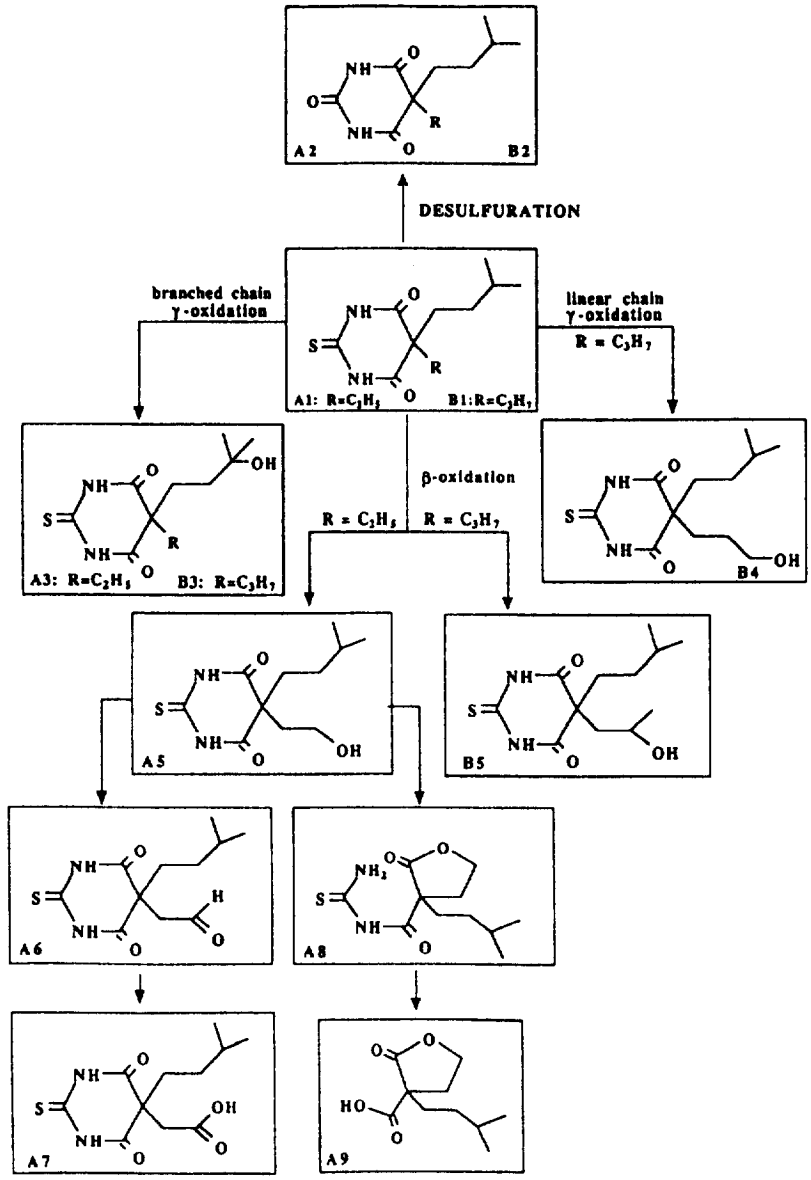


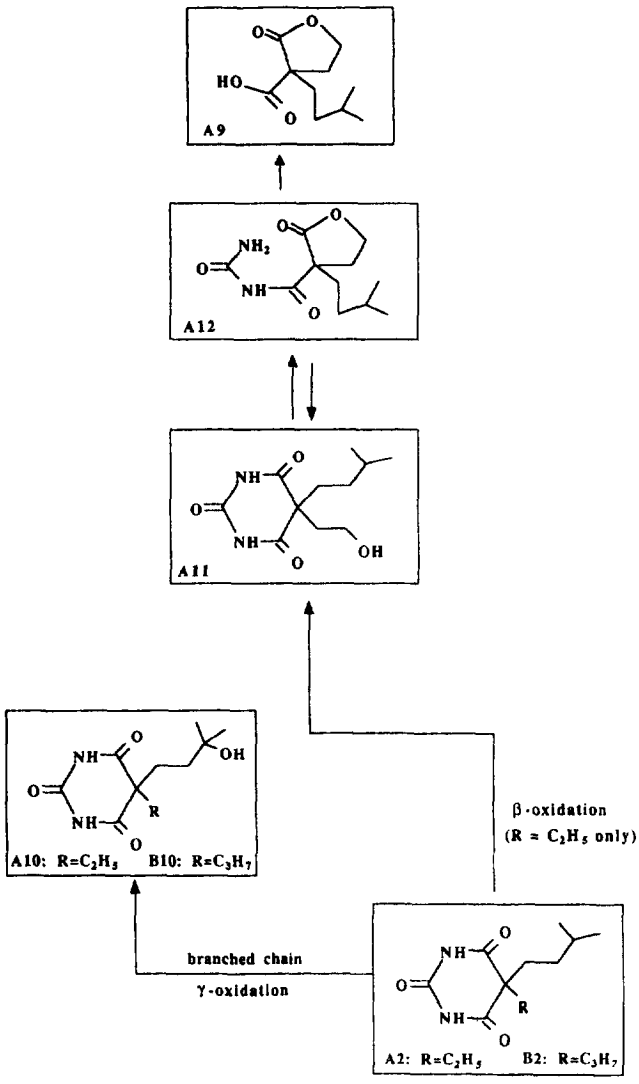
Scheme 2.

Another major potential metabolic pathway consists of a desulfuration leading to the corresponding barbiturates, **A2** or **B2**, and to all the metabolites previously isolated for these compounds [6–8] (scheme 3), *ie* γ–hydroxymetabolites, **A10** and **B10**, β-hydroxymetabolite, **A11**, and its degradation products, allophanyl-γ-lactone, **A12**, and carboxylic lactone, **A9**.

Syntheses of potential metabolites

Synthesis of hydroxybarbiturates

The method usually used for this purpose in the barbiturate series is a chromic oxidation [9]. However, for thiobarbiturates, a desulfuration is obtained without any oxidation of the side chain (scheme 4). Two methods were therefore used: (i) introduction of the



Scheme 3.

alcohol function before the condensation with thiourea and protection of this alcohol as the tetrahydropyranylether (scheme 5); and ii) introduction of a precursor of the alcohol function on the diethylmalonate. First, the diethyl 2-alkylmalonate was alkylated by a bromoalkene and then condensed with thiourea. The hydration of the double bond was obtained either directly using sulfuric acid in acetic acid medium, **B5**, or by a two-step process involving addition of hydrobromic acid followed by a substitution of the bromide atom by water, **A3** and **B3** (scheme 6).

Syntheses of metabolites at higher oxidation rates

Synthesis of aldehyde A6. The standard method for the synthesis of an aldehyde function on a plurifunctional compound, *ie* oxidation of a primary alcohol by pyridinium or pyrazinium chlorochromate [10–12], could not be used because the various reactants were not soluble in the same solvents. The aldehyde function was therefore introduced on the diethyl alkylmalonate after protection as a dioxolane. After condensation with thiourea, the protective group was removed in acidic medium (scheme 7).

Synthesis of carboxylic acid A7. The carboxylic function was introduced on the monoalkylheterocycle by action of bromoacetic acid (scheme 8).

Synthesis of a thioallophanyl lactonic potential metabolite A8

This compound was obtained *via* an intramolecular alcoholysis of the corresponding β-hydroxy compound, **A5**, at pH 7.5 in the presence of chloroform and reflux for 2 h (equation [1]).

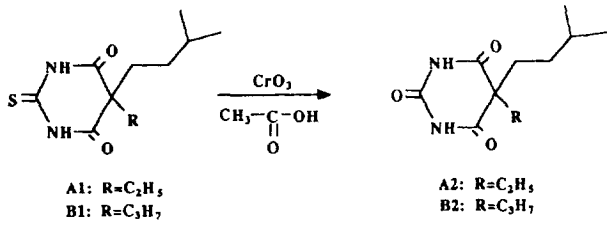
$$\mathbf{A5} \xrightarrow[\text{pH 7.5 (reflux)}]{H_2O/CHCl_3} \mathbf{A8} \qquad [1]$$

Synthesis of the biodegradation products of A2 and B2

The barbiturates **A2** and **B2** are potential metabolites of **A1** and **B1**. It was therefore necessary to synthesize their metabolites (scheme 3). The γ-hydroxymetabolites **A10** and **B10**, β-hydroxymetabolite **A11** and corresponding allophanyl-γ-lactone **A12** and carboxylic lactone **A9** were thus prepared according to methods described elsewhere [7].

Biology

Compounds **A1** and **B1** were administered to rats at a dose of 20 mg/kg/d for 6–9 d. Urine and faeces were extracted and the isolated compounds were identified. The results of these experiments are summarized in table I. Most of the previously synthesized potential metabolites were not recovered in urine and faeces under the extraction conditions. This would have allowed their isolation, were they present at a level of less than 0.1% of the dose. Untransformed compound **A1** was recovered in both urine (2.7 ± 0.3% of the dose) and faeces (1.6 ± 0.4% of the dose), while compound **B1** was present in neither urine nor faeces.



Scheme 4.

For **A1** and **B1**, the major metabolites were the tertiary γ-hydroxy metabolites **A3** (52.8 ± 1.8% in urine) and **B3** (54.03 ± 3.23% in urine; 0.54 ± 0.03% in faeces), respectively. No primary hydroxy compounds (**A5** and **B4**) were present in the excreta. In the case of **B1**, compound **B10** was isolated from urine (8.93 ± 1.24% of the dose). This resulted from desulfuration and γ-hydroxylation.

Discussion and conclusion

The first significant observation is that while compound **A1** could be recovered untransformed in both urine and faeces, even if it was present at a low level, the more lipophilic, homologous, compound **B1** was not isolated at all. This could be considered as a consequence of the increase in lipophilicity making the biodegradation of **B1** easier. Moreover, the regioselectivity for hydroxylation of a tertiary carbon in position ω-1 was significant for both compounds (in the two cases it represented more than 50% of administered drug). In contrast to what was observed with an ethyl group in the barbiturates series [6, 13], no oxidation of the linear side chain was observed, and therefore no β-hydroxymetabolite was formed. As regards desulfuration, only the more lipophilic compound **B1** underwent this kind of biodegradation, which proceeded with a γ-hydroxylation in the tertiary position ω-1.

The results show that desulfuration was in no way the major metabolic pathway for these thiobarbiturates bearing a branched side chain. This observation refutes the hypothesis that desulfuration is the main biodegradation for all thiobarbiturates, and demonstrates that when a favourable position, *ie* a tertiary carbon atom in position ω-1, was present on a side chain, hepatic hydroxylation constituted the major biodegradation process. Even when some desulfuration took place, this transformation remained a minor pathway and, moreover, was accompanied by hydroxylation into a tertiary alcohol.

Experimental protocols

^{1}H-NMR spectra were recorded on a Varian T60 spectrometer using $(CH_3)_4Si$ as a reference. Melting points were recorded on a Kofler apparatus and are uncorrected. Elemental analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. Mass spectra were obtained on a GC-MS system. The mass spectrometer (Hewlett Packard 5970 MSD) was operated in electron impact mode and directly interfaced with a gas chromatograph (Hewlett-Packard 5890 GC).

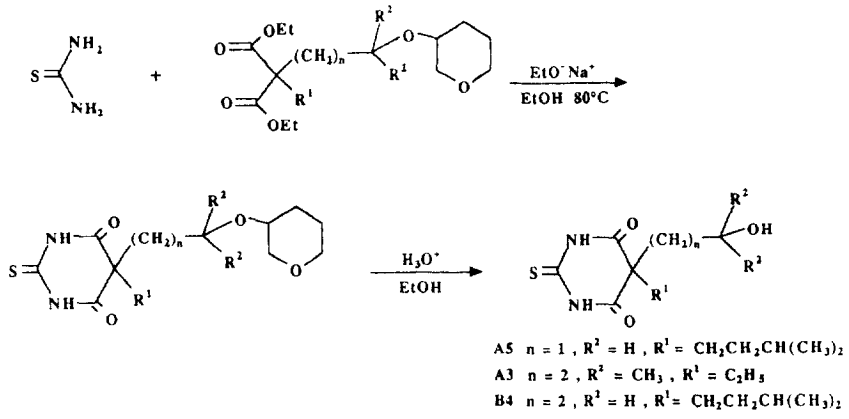
Chemistry

General

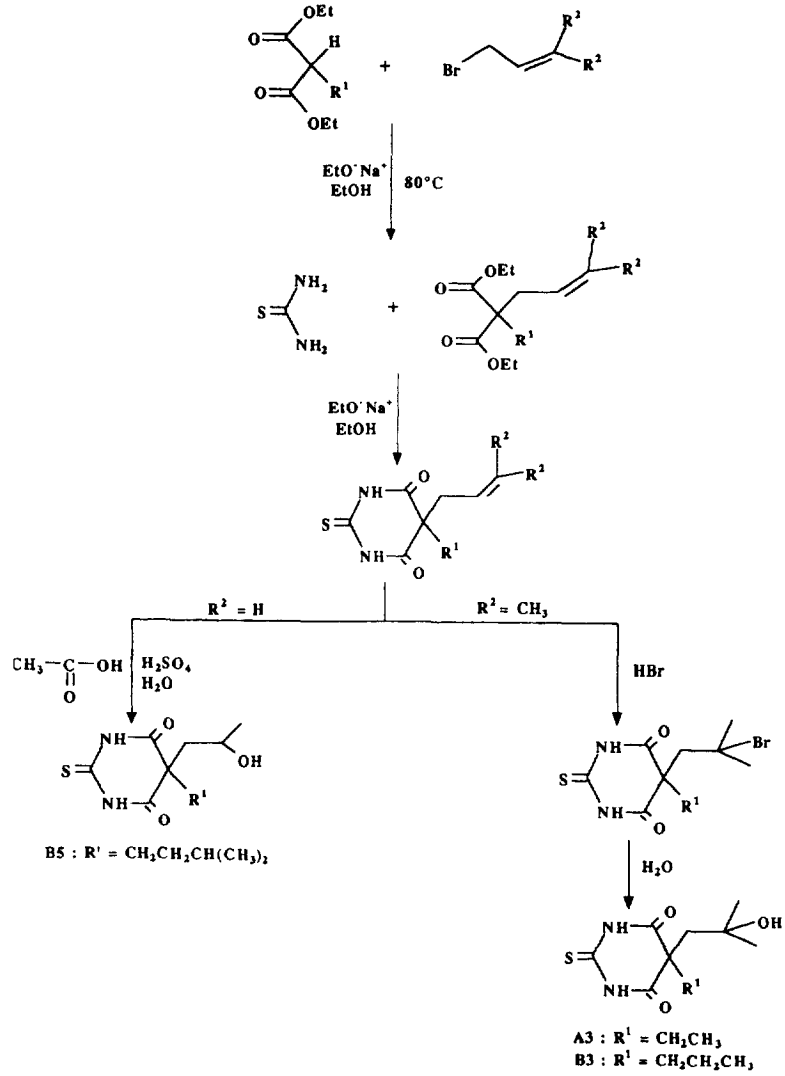
Diethyl-2-ethylmalonate, thiourea, transcutol (diethyleneglycolmonoethylether), ethyl acetate, petroleum ether, diethylether and chloroform were commercial products.

Thiobarbiturates A1 and B1

5-Ethyl-5-(3-methylbutyl)-2-thiobarbituric acid **A1** and 5-propyl-5-(3-methylbutyl)-2-thiobarbituric acid **B1** were prepared according to the standard method: condensation of thiourea with the appropriate diethyl dialkylmalonate in alkaline medium [14].



Scheme 5.



Scheme 6.

A1. Yield: 60%; mp 170°C; anal (C, H, N) $C_{11}H_{18}N_2O_2S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.78 (t, 3H, CH_3); 0.88 (d, 6H, $2CH_3$ *i*-amyl); 1.8 (q, 2H, CH_2); 1–2 (m, 5H, $2CH_2$ and CH *i*-amyl); 12.1 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\cdot}$ at *m/z* 242, and other fragments at 213, 172, 157 (100% base peak), 98, 69, 55, 41.

B1. Yield: 50%; mp: 138°C; anal (C, H, N) $C_{12}H_{20}N_2O_2S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (d, 6H, $2CH_3$); 0.87 (t, 3H, CH_3); 1–2.1 (m, 9H, $4CH_2$ and CH *i*-amyl); 12.6 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\cdot}$ at *m/z* 256, and other fragments at 214, 187, 171, 157 (100% base peak), 144, 116, 98, 69, 55.

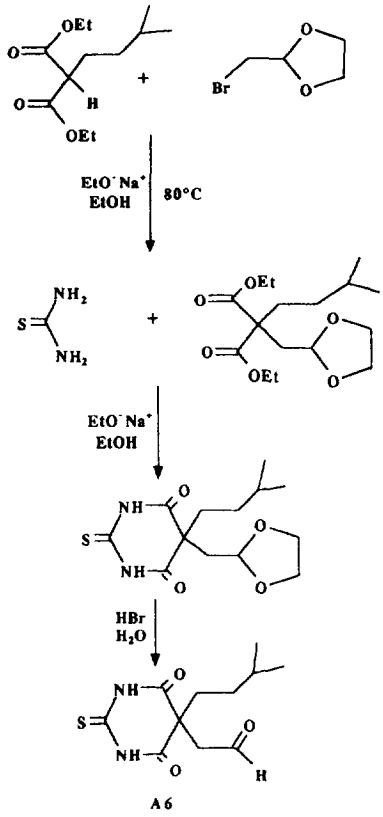
Chromic oxidation of A1 and B1

Amobarbital **A2** and 5-propyl-5-(3-methylbutyl) barbituric acid **B2** were prepared from **A1** and **B1**, respectively, according to the method described in [9]. **A2**: yield: 63%; **B2**: yield: 69%. Analytical data were identical to those already published [8, 15].

Synthesis of hydroxymetabolite 5-ethyl-5-(3-hydroxy-3-methylbutyl)-2-thiobarbituric acid A3. Method 1

This synthesis was performed in five steps.

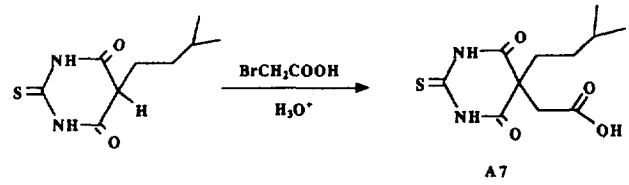
4-Bromo-2-methylbutan-2-ol. Dry magnesium (7.2 g, 0.3 mol), one crystal of iodine and 50.33 g (0.3 mol) of methyliodide were dissolved in 180 ml anhydrous diethylether. After the magnesium had completely dissolved, 25 g (0.146 mol) of 3-bromopropionyl chloride was added. The mixture was stirred at 20°C for 12 h. Then, 50 ml hydrochloric acid (10%) was added dropwise. The organic solution was extracted with diethylether. The solvent was evaporated and the alcohol 4-bromo-2-methylbutan-2-ol was purified by column chroma-



Scheme 7.

tography (silica: Kieselgel 60 Merck; solvent: ethylether/petroleum ether 5:100). Yield: 79%. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.1 (s, 6H, $2CH_3$); 1.6 (t, 2H, CCH_2C); 3.6 (t, 2H, CH_2Br); 4.6 (m, H exch D_2O, OH).

4-Bromo-2-methyl-2-butyltetrahydropyranylether. 4-Bromo-2-methylbutan-2-ol (19.2 g, 0.115 mol) was dissolved into 15 g (0.179 mol) of dihydropyran. One drop of hydrochloric acid



Scheme 8.

was added. The mixture was stirred at 20°C for 6 h, 20 ml sodium hydroxide (10%) was added. The organic solution was separated and purified by column chromatography (silica: Kieselgel 60 Merck; solvent: ether/petroleum ether 3:100). Yield: 60%. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.09 (s, 6H, $2CH_3$); 1.6 (t, 2H, CCH_2C); 1.7 (m, 6H, $3CH_2$ cycle); 3.8 (m, 4H, CH_2O and CH_2Br); 4.8 (m, 1H, OCHO).

Alkylation of diethyl 2-ethylmalonate. Sodium (1.92 g) was dissolved in 74 ml ethanol. After cooling, 15.74 g of diethyl 2-ethylmalonate was added. The mixture was stirred at room temperature for 1 h. 4-Bromo-2-methyl-2-butyltetrahydropyranylether (21 g) was added. The mixture was stirred at 80°C for 3 h. After cooling, the solvent was evaporated. The residue was dissolved in water. The organic phase was extracted with diethylether and dried (sodium sulfate). The solvent was evaporated and the residue was purified by column chromatography (silica: Kieselgel 60 Merck; solvent: diethylether/petroleum ether 10:100). Yield: 51%. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.1 (s, 6H, $2CH_3$); 1.25 (t, 6H, $2CH_3$ from Et); 1–2.2 (m, 6H, $3CH_2$ ring); 1.8 (m, 4H, CH_2); 3.4 (t, 2H, CH_2O); 4.2 (q, 4H, $2CH_2$ from Et); 4.4 (t, 1H, CH).

Tetrahydropyranylether of 5-ethyl-5-(3-hydroxy-3-methylbutyl)-2-thiobarbituric acid. The tetrahydropyranylether of 5-ethyl-5-(3-hydroxy-3-methylbutyl)-2-thiobarbituric acid was prepared according to the standard method [8]. Yield: 21%; anal (C, H, N) $C_{16}H_{26}N_2O_4$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.9 (s, 6H, $2CH_3$); 1–2.2 (m, 15H, $6CH_2$ and CH_3 from Et); 3.4 (t, 2H, CH_2O); 4.4 (t, 1H, CH); 12.3 (s, 2H exch D_2O, 2NH).

Table I. Compounds isolated from urine and faeces.

Experiment	*Administered compound*		*Compounds isolated from urine (mg)*				*Compounds isolated from faeces (mg)*	
	Compound	*Dose (mg)*	***A1***	***B3***	***A3***	***B10***	***A1***	***B3***
1	**A1**	3030	85		1747		39	
2	**A1**	3280	97		1835		66	
3	**A1**	3074	76		1655		42	
4	**B1**	3027		1590		290		6.2
5	**B1**	2917		1380		220		3.4
6	**B1**	2788		1470		270		5.3

Hydrolysis of tetrahydropyranylether of 5-ethyl-5-(3-hydroxy-3-methylbutyl)-2-thiobarbituric acid. Pyranylether (2.5 g, 7.31 mol) was dissolved into 147 ml dry ethanol. Sulfuric acid (86.2 ml) was then added to the mixture, which was heated (20°C) without stirring for 24 h. Then 50 ml water was added. The mixture was cooled at 4°C for 48 h. Compound **A3** was filtered and then dried.

A3. Yield: 45%; mp: 170°C; anal (C, H, N) $C_{11}H_{18}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.75 (t, 3H, CH_3); 1 (s, 6H, $2CH_3$); 1.2 (m, 2H, CH_2); 1.8 (m, 4H, $2CH_2$); 4.4 (s, 1H, exch D_2O, OH); 12.6 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 258, and other fragments at 241, 188, 170, 158 (100% base peak), 112, 98, 69, 55, 41.

Synthesis of hydroxymetabolite 5-ethyl-5-(3 hydroxy-3-methylbutyl)-2-thiobarbituric acid A3. Method 2

5-Ethyl-5-(3-methylbuten-2-yl)-2-thiobarbituric acid. 5-Ethyl-5-(3-methylbuten-2-yl)-2-thiobarbituric acid was prepared according to the standard method: condensation of thiourea with diethyl 2-ethyl-2-(3-methylbuten-2-yl)malonate in alkaline medium [8]. Yield: 60%; mp: 152°C; Anal (C, H, N) $C_{11}H_{16}N_2O_2S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (t, 3H, CH_3 from Et); 1.6 (s, 6H, $2CH_3$); 1.85 (q, 2H, CH_2 from Et); 2.6 (d, 2H, CH_2); 5 (m, 1H, =CH); 12.2 (m, 2H exch D_2O, 2NH).

5-(3-Bromo-3-methylbutyl)-5-ethyl-2-thiobarbituric acid. 5-Ethyl-5-(3-methylbuten-2-yl)-2-thiobarbituric acid (1 g, 4.17 mol) was added to 30 ml of hydrobromic acid (47%). The mixture was stirred at 20°C for 12 h. The residue was filtered and washed with water. The crystals were then dried. Yield: 92%; mp: 169°C; anal (C, H, N) $C_{11}H_{17}N_2O_2SBr$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (t, 3H, CH_3 from Et); 1.6 (m, 4H, $2CH_2$); 1.7 (s, 6H, $2CH_3$); 1.9 (q, 2H, CH_2 from Et); 2.9 (m, 2H, CH_2); 12.7 (s, 2H exch D_2O, 2NH).

γ-Hydroxy-2-thioamobarbital A3. This was obtained by dissolving 0.5 g (1.56 mol) of 5-(3-bromo-3-methylbutyl-5-ethyl-2-thiobarbituric acid in water (15 ml) at pH 5 (HCl) the medium was refluxed for 30 min. After cooling the residue was filtered and dried. Yield: 65%; physical data were the same as above.

5-(3-Hydroxy-3-methylbutyl)-5-propyl-2-thiobarbituric acid B3
Compound **B3** was prepared according to the sequence described in *Method 2* of the synthesis of compound **A3**. This synthesis was performed in three steps: (i) synthesis of 5-(3-methylbuten-2-yl)-5-propyl-2-thiobarbituric acid by condensation of thiourea with diethyl 2-(3-methylbuten-2-yl)-2-propylmalonate in alkaline medium [8] (yield: 81%); (ii) synthesis of 5-(bromo-3-methylbutyl)-5-propyl-2-thiobarbituric acid (yield: 70%); and (iii) synthesis of 5-(hydroxy-3-methylbutyl)-5-propyl-2-thiobarbituric acid **B3**.

B3. Yield: 75%; mp: 164°C; anal (C, H, N) $C_{12}H_{20}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.84 (t, 3H, CH_3); 0.99 (s, 6H, $2CH_3$); 1.15 (m, 4H, $2CH_2$); 1.81 (m, 4H, $2CH_2$); 4.28 (s, 1H exch D_2O, OH); 12.58 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 272, and other fragments at 212, 186, 157, 111, 98, 59 (100% base peak).

5-(3-Hydroxypropyl)-5-(3-methylbutyl)-2-thiobarbituric acid B4
Compound **B4** was prepared according to the last four steps of the sequence described in *Method 1* of the synthesis of compound **A3**, using 3-bromopropanol (0.115 mol) instead of 4-bromo-2-methylbutanol (0.115 mol). The sequence was as follows: (i) synthesis of 3-bromopropyltetrahydropyranylether (yield: 90%); (ii) alkylation of diethyl 2-(3-methylbutyl)malonate with 3-bromopropyltetrahydropyranylether (yield: 89%); and (iii) condensation with thiourea in alkaline medium. Compound **B4** was purified by column chromatography (silica: Kieselgel 60 Merck; solvent: chloroform/acetone 100:10).

B4. Yield: 20%; mp: 119°C; anal (C, H, N) $C_{12}H_{20}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (d, 6H, $2CH_3$); 1–2 (m, 9H, $4CH_2$ and CH *i*-amyl); 3.4 (t, 2H, CH_2O); 5.6 (s, 1H, OH); 12.07 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 272, and other fragments at 213, 202, 184 (100% base peak), 157, 113, 98, 55.

5-(2-Hydroxyethyl)-5-(3-methylbutyl)-2-thiobarbituric acid A5
Compound **A5** was prepared according to the last four steps of the sequence described in *Method 1* of synthesis of compound **A3**, using 2-bromoethanol (0.115 mol) instead of 4-bromo-2-methylbutan-2-ol (0.115 mol). The sequence was as follows: (i) synthesis of 2-bromoethyltetrahydropyranylether (yield: 80%); (ii) alkylation of diethyl 2-(3-methylbutyl)malonate with 2-bromoethyltetrahydropyranyl ether (yield: 72%); and (iii) condensation with thiourea in alkaline medium (yield: 40%). Hydrolysis was then performed as follows. Tetrahydropyranylether of β-hydroxy-2-thioamobarbital (5.3 g, 0.0155 mol) and 310 ml absolute ethanol were placed in a 1 l flask. Sulfuric acid (128 ml) was then added dropwise; after 24 h at room temperature, 160 ml water was added. The mixture was cooled at 4°C for 48 h. The precipitated alcohol **A5** was collected by filtration and dried.

A5. Yield: 100%; mp: 158°C; Anal (C, H, N) $C_{11}H_{18}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (d, 6H, $2CH_3$); 1.2 (m, 5H, $2CH_2$ and CH *i*-amyl); 2.2 (t, 2H, CH_2); 3.4 (t, 2H, CH_2O); 4.2 (s, 1H exch D_2O, OH); 11.4 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 258, and other fragments at 243, 172 (100% base peak), 157, 97, 69, 59, 41.

5-(2-Hydroxypropyl)-5-(3-methylbutyl)-2-thiobarbituric acid B5
This synthesis was performed in two steps.

5-(3-Methylbutyl)-5-(propen-2-yl)-2-thiobarbituric acid. Diethyl 2-(3-methylbutyl)-2-(propen-2-yl)malonate (28.4 g, 0.105 mol) was added to 10.03 g (0.132 mol) of thiourea in 275 ml ethanol containing sodium ethylate (0.473 mol). The mixture was stirred at 80°C for 18 h. After cooling, the solvent was evaporated and the residue was dissolved in water and hydrochloric acid was added dropwise, the precipitate was filtered and crystallized from water. Yield: 74%; mp: 116°C; anal (C, H, N) $C_{12}H_{18}N_2O_2S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.9 (d, 6H, 2 CH_3 *i*-amyl); 1–2.2 (m, 5H, 2 CH_2 and CH *i*-amyl); 2.64 (d, 2H, CH_2); 5–5.6 (m, 3H); 12.2 (s, 2H exch D_2O, 2NH).

5-(2-Hydroxypropyl)-5-(3-methylbutyl)-2-thiobarbituric acid B5. 5-(3-Methylbutyl)-5-(propen-2-yl)-2-thiobarbituric acid (3 g, 1.18 mol) was dissolved in 10 ml acetic acid. The flask was placed in a boiling water bath. Sulfuric acid (2 ml) was added, and the mixture was stirred for 30 min. Water (30 ml) was added and the mixture was stirred for 30 min. Water (30 ml) was added and the mixture was stirred again for 30 min. After cooling, the solid residue was filtered and crystallized from ethanol/sulfuric acid.

B5. Yield: 47%; mp: 132°C; anal (C, H, N) $C_{12}H_{20}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.8 (d, 6H, 2 CH_3 *i*-amyl); 1.3 (d, 3H, CH_3); 1–2 (m, 5H, $2CH_2$ and CH *i*-amyl); 2.5 (m, 2H, CH_2); 3.4 (m, 1H, CHOH); 4.6 (s, 1H exch D_2O, OH); 12.34 (s, 2H exch D_2O, 2NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 272, and other fragments at 197, 158, 135, 69 (100% base peak).

5-(3-Methylbutyl)-5-(2-oxoethyl)-2-thiobarbituric acid ***A6***
This synthesis was performed in three steps.

Diethyl 2-[methyl-2-(1,3-dioxolyl)]-2-(3-methylbutyl)malonate. Sodium (2.3 g, 0.1 mol) was dissolved in 67 ml ethanol. After cooling, 23 g (0.1 mol) of diethyl 2-(3-methylbutyl)malonate was added. The mixture was stirred at room temperature for 1 h. 2-Bromomethyl-1,3-dioxolane (16.7 g, 0.1 mol) was added. The mixture was stirred at 80°C for 3 h. After cooling, the solvent was evaporated. The residue was dissolved in water. The organic phase was extracted with diethylether and dried (sodium sulfate). The solvent was evaporated and the solid residue was purified by column chromatography (silica: Kieselgel 60 Merck; solvent: diethylether/petroleum ether 20:100). Yield: 89%; ^{1}H-NMR ($CDCl_3$) δ ppm = 0.8 (d, 6H, $2CH_3$); 0.9 (m, 2H, CH_2); 1.25 (t, 6H, $2CH_3$); 1.4 (m, 1H, CH); 1.8 (m, 2H, CH_2); 2.2 (d, 2H, CH_2); 3.71 (s, 4H, $2CH_2$); 4.2 (q, 4H, $2CH_2$); 4.8 (t, 1H, CH).

5-[Methyl-2-(1,3-dioxolyl)]-5-(3-methylbutyl)-2-thiobarbituric acid. Diethyl 2-[methyl-2-(1,3-dioxolyl)]-2-(3-methylbutyl)-malonate (28 g, 88.6 mmol) was added to 10.1 g (132.9 mol) of thiourea in 236 ml ethanol containing sodium ethylate (0.354 mol). The mixture was stirred at 80°C for 18 h. After cooling the solvent was evaporated. The residue was dissolved in water. Hydrochloric acid was added until pH = 5. The precipitate was filtered and crystallized from water. Yield: 40%; mp: 198°C; anal (C, H, N) $C_{13}H_{20}N_2O_4S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.78 (d, 6H, 2 CH_3 *i*-amyl); 0.9 (m, 2H, CH_2); 1.4 (m, 1H, CH *i*-amyl); 1.79 (m, 2H, CH_2); 2.2 (t, 2H, CH_2); 3.71 (s, 4H, 2 CH_2O); 4.76 (t, 1H, CH-O); 11.37 (s, 2H exch D_2O, 2NH).

Hydrolysis of 5-[methyl-2-(1,3-dioxolyl)]-5-(3-methylbutyl)-2-thiobarbituric acid. 5-[Methyl-2-(1,3-dioxolyl)]-5-(3-methylbutyl)-2-thiobarbituric acid (2.4 g, 8 mmol) was dissolved into 30 ml hydrobromic acid (47%). The mixture was stirred at room temperature for 24 h. The solid was filtered and washed with water and dried.

A6. Yield: 54%; mp: 194°C; anal (C, H, N) $C_{11}H_{16}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.8 (d, 6H, $2CH_3$ *i*-amyl); 1.2 (m, 5H, $2CH_2$ and CH *i*-amyl) 2.4 (s, 2H, CH_2); 9.6 (s, 1H, CHO); 12.42 (s, 2H, 2NH).

5-(2-Carboxyethyl)-5-(3-methylbutyl)-2-thiobarbituric acid ***A7***
This synthesis was performed in two steps.

5-(3-Methylbutyl)-2-thiobarbituric acid. Diethyl 2-(3-methylbutyl)malonate (20 g, 87 mmol), 8 g (348 mmol) sodium and 9.92 g (130.5 mmol) thiourea were dissolved into 247 ml ethanol. The mixture was stirred at 80°C for 5 h. After cooling, the solvent was evaporated. The residue was dissolved in water. Hydrochloric acid was then added until pH 5. The precipitate was filtered, dried and crystallized from petroleum ether. Yield: 66%; anal (C, H, N) $C_9H_{14}N_2O_2S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.8 (d, 6H, $2CH_3$ *i*-amyl); 1–2 (m, 5H, $2CH_2$ and CH *i*-amyl); 3.7 (t, 1H); 11.1 (s, 2H exch D_2O, 2NH).

Introduction of a carboxyethyl group. 5-(3-Methyl)-2-thiobarbituric acid (5.28 g, 24.7 mmol), 3.43 g (24.7 mmol) of bromoacetic acid were added to a mixture of sulfuric acid (2 ml) and water (50 ml). The mixture was refluxed for 4 h. After cooling, the precipitate was filtered and washed with water.

A7. Yield: 54%; mp: 209°C; anal (C, H, N) $C_{11}H_{16}N_2O_4S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.88 (d, 6H, $2CH_3$ *i*-amyl); 1–2 (m, 2H, CH_2); 1.45 (m, 1H, CH *i*-amyl); 2.3 (t, 2H, CH_2); 3.97 (s, 2H, CH_2); 11.2 (s, 2H exch D_2O, 2NH).

3-Thioallophanyl-3-(3-methylbutyl-2-oxotetrahydrofurane ***A8***
Compound **A5** (4.55 g, 0.0176 mol) was dissolved in 500 ml water, pH 7.5. Chloroform (200 ml) was added. The medium was refluxed for 2 h and after cooling, the organic phase was separated. The solvent was evaporated. The residue was purified by column chromatography (silica: Kieselgel 60 Merck; solvent: chloroform/acetone 100:10) and crystallized from diethylether/petroleum ether 50:50.

A8. Yield: 22%; mp: 120°C; Anal (C, H, N) $C_{11}H_{18}N_2O_3S$. ^{1}H-NMR (DMSO-d_6) δ ppm = 0.85 (d, 6H, $2CH_3$ *i*-amyl); 1.14 (m, 2H, CH_2); 1.5 (m, 1H, CH *i*-amyl); 1.85 (t, 2H, CH_2); 2.2 (m, 1H, CH_2); 2.7 (m, 1H, CH_2); 4.35 (q, 2H, CH_2O); 9.37 (s, 1H, NH_2); 9.62 (s, 1H, NH_2); 10.3 (s, 1H, NH). The mass spectrum showed the molecular ion peak $M^{+\bullet}$ at *m/z* 258, and other fragments at 188, 170, 158, 142, 113, 99, 77, 69, 60, 41 (100% base peak).

Compounds ***A9, A11, A10, A12 and B10***
These compounds were synthesized according to the procedure described in publications [6–8] and spectral and analytical data were in agreement with the published data.

Biology

Administered compounds
5-Ethyl-5-(3-methylbutyl)-2-thiobarbituric acid **A1** was administered in experiments 1–3, and 5-propyl-5-(3-methylbutyl)-2-thiobarbituric acid **B1** was administered in experiments 4–6.

Formulation
A suspension of **A1** or **B1** was prepared according to the following procedure. To *n* ml of solution (30:70 transcutol/water) (*n* = number of rats x number of days) was added *n'* mg of compound **A1** or **B1** (*n'* = total weight of rats (kg) x daily dose (20 mg/kg)). The medium was homogenized for 15 min using a mixer (Silverson R). A few drops of Tween 80 were then added. Homogenization was again performed for 10 min. The suspension was stored at 4°C for the entire experiment.

Animals
n rats (Sprague Dawley) were placed in Pajon metabolism cages, 3 rats per cage, with free access to food and water.

Administration
A dose of 1 ml of the suspension was administered daily to each rat directly into the stomach, using a curved canula 60/10 (Carrieri). Data concerning conditions of the experiments are summarized in table II.

Collection of urine and faeces samples
Urine and faeces, for all experiments, were collected and frozen (– 18°C) during each administration period and for the following 2 d.

Extraction procedure
Urine samples were allowed to warm to room temperature. The pH was then adjusted to 5 with hydrochloric acid. The samples were divided into 1.5 l aliquots. Each aliquot was extracted three times with ethyl acetate (1 l). Organic solutions were then dried over anhydrous sodium sulfate and filtered. The solvent was then evaporated under reduced pressure. The pH of every urine sample was then lowered to 0.5 and the procedure repeated.

Table II. Experimental conditions for administration of thiobarbiturates **A1** and **B1** and collection of urine.

Experiment	*Rats*			*Administered ration*			*Collection duration (d)*
	Number	*Sex*	*Total weight (kg)*	*Compound*	*Dose (mg)*	*Duration (d)*	
1	33	M	16.83	**A1**	3030	9	11
2	33	M	16.40	**A1**	3280	10	12
3	33	M	17.08	**A1**	3074	9	11
4	33	M	15.135	**B1**	3027	8	10
5	27	M	13.260	**B1**	2917	9	11
6	27	M	12.675	**B1**	2788	9	11

Faecal samples were allowed to warm to room temperature in 2 l ethyl acetate over 12 h. The medium was mixed and the liquid phase was separated. Hydrochloric acid was added to the residue until pH 5. The medium was then extracted with ethyl acetate (2 l). The new residue was stirred and refluxed with ethyl acetate (2 l) for 1 h and the liquid phase was separated. The three organic solutions were collected and dried over anhydrous sodium sulfate and filtered. The solvent was then evaporated under reduced pressure.

Isolation

The solid residues obtained after extraction were treated by column chromatography (silica: Kieselgel 60 H Merck; solvent petroleum ether/ethyl acetate 70:30). Collected fractions were then studied by thin-layer chromatography (silica: Kieselgel 60F 254R Merck; solvent: petroleum ether/ethyl acetate 50:50). All similar fractions were then assembled and the solvents were evaporated under reduced pressure.

Identification and quantification

Compounds isolated from urines or faeces were identified by comparison of their ^{1}H-NMR and mass spectra with those of synthesized models. Quantification was performed either by gravimetry or by a GC-MS procedure.

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