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Original paper

New chemical aspects of primidone metabolism

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Summary — Primidone is metabolized either into phenylethylmalondiamide or phenobarbital. 2-Hydroxyprimidone was synthesized and tested as a potential intermediate common to these two biodegradation pathways in dogs as well as *in vitro*. On the other hand, the mechanism of the formation of α-phenyl-γ-butyrolactone during intoxication was investigated and the role of precursor played by the phenobarbital generated *in vivo* was shown.

Résumé – **Nouveaux aspects chimiques du métabolisme de la primidone.** *La primidone est métabolisée aussi bien en phényléthylmalondiamide qu'en phénobarbital. La 2-hydroxyprimidone a été synthétisée et essayée comme intermédiaire commun à ces deux voies de biodégradation chez le Chien ainsi qu'*in vitro. *Par ailleurs, le mécanisme de la formation d'α-phényl-γ-butyrolactone au cours d'intoxications a été étudié et le rôle de précurseur joué par le phénobarbital apparu* in vivo, *mis en évidence.*

primidone / 2-hydroxyprimidone / phenobarbital / metabolism / antiepileptic drug

Introduction

Primidone(5-ethyl-5-phenyl-4,6(1-*H*, 5-*H*) dihydropyrimidinedione) **1** is commonly used as anticonvulsant [1–3]. This drug is extensively metabolized in humans and in animals [4–10] into an active metabolite, phenobarbital **2** and into 2-ethyl-2-phenylmalondiamide **3**, which does not have any antiepileptic activity [11].

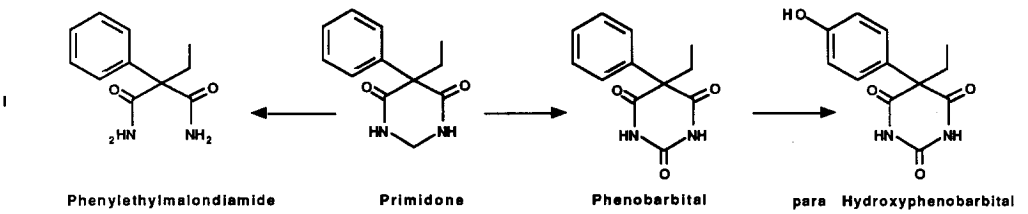
A metabolite of phenobarbital, *p*-hydroxyphenobarbital **4** (and/or its glucuronide) has also been identified from urine in rabbits [12], rats and humans [13].

The major known pathways of primidone metabolism, following the administration of therapeutic levels of the drug, are reported in Scheme 1.

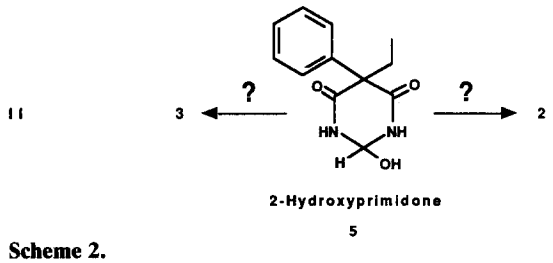
If one considers that phenylethylmalondiamide is never a metabolite of barbiturates, the mechanism of its formation *in vivo* from primidone must involve an intermediate compound which cannot be obtained from barbiturates *via* usual biodegradation pathways.

On the other hand, the only way to explain the formation of phenobarbital from primidone is that it consists of an oxidation of the methylene group situated between the two nitrogen atoms. This kind of oxidation is generally performed *via* a secondary alcohol *in vivo* as well as *in vitro*.

This suggested that a hydroxylated derivative of primidone, **5**, could be a common intermediate compound for the biodegradation of primidone into phenobarbital as well as into phenylethylmalondiamide.

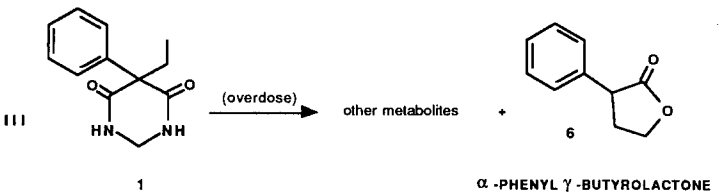


Scheme 1.



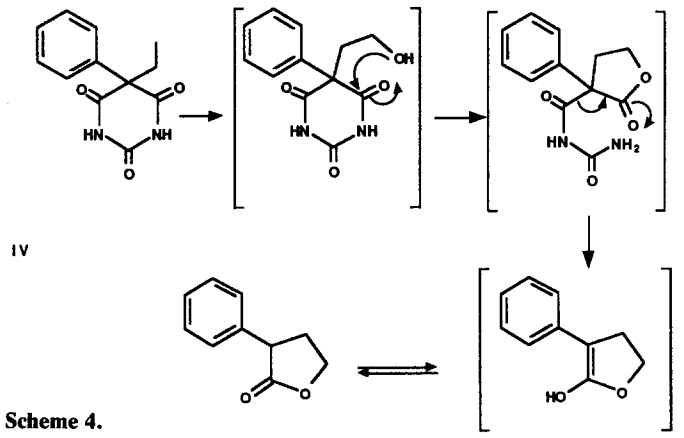
Scheme 2.

Furthermore, another metabolite, α-phenyl-γ-butyrolactone **6** has been isolated from urine samples of patients severely intoxicated by primidone as well as by glutethimide or phenobarbital [14]. This lactone on its own caused severe toxicity, even at moderate dose levels [15] (Scheme 3).



Scheme 3.

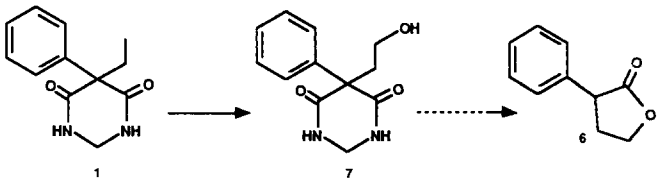
The formation of this toxic metabolite **6** from phenobarbital was observed in dogs and rats even at therapeutic dose levels, and was explained by a β-hydroxylation of the ethyl side chain in the liver, followed by an alcoholysis of the pyrimidinetrione ring and a chemical transformation into **6** [16, 17] (Scheme 4).



Scheme 4.

According to these previous findings, two hypotheses can be made about the mechanism of the biodegradation of primidone **1** into α-phenyl-γ-butyrolactone **6:**

1) The antiepileptic drug could undergo a β-hydroxylation of the ethyl side chain, followed by an alcoholysis of the heterocycle (Scheme 5).



Scheme 5.

2) The γ-lactone **6** could also be produced *via* the oxidation of primidone into phenobarbital **2**, the latter then undergoing a transformation into **6** *via* the mechanism described in Scheme 4.

In order to test all these hypotheses, in this paper we describe the synthesis of the potential intermediates and the study of their behaviour *in vitro* under biomimetic conditions.

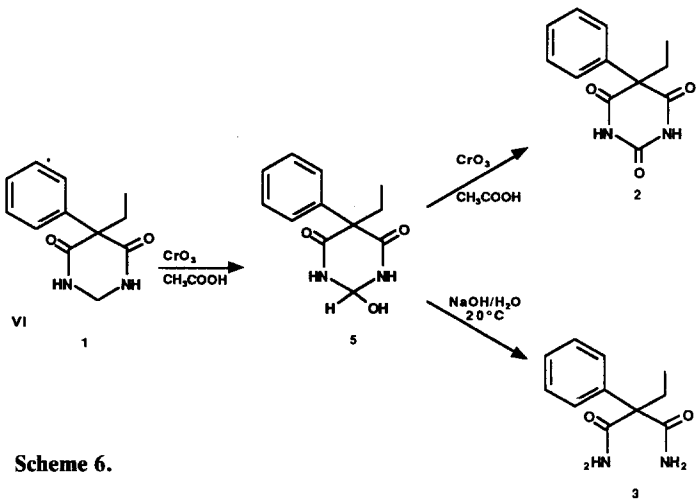
We also report the administration of primidone **1** as well as its potential hydroxymetabolite **5** to dogs, at therapeutic and toxic dose levels and the isolation of their metabolites from urine and their identification and quantitation.

Chemistry

*Synthesis, stability and chemical transformation of **5** into **2** or **3***

The synthesis of 2-hydroxyprimidone **5** was carried out by chromic oxidation of primidone **1** in acetic acid, at room temperature, over a period of 10 min (Scheme 6). Its stability under biomimetic conditions at pH 1 was controlled.

The role of **5** as a chemical precursor of **2** and **3** was then investigated. A new chromic oxidation of **5** yielded phenobarbital **2** (Scheme 6). An alkaline hydrolysis of **5** was then carried out by sodium hydroxide at 20°C, converting this hydroxy compound into phenylethylmalondiamide **3** (Scheme 6).



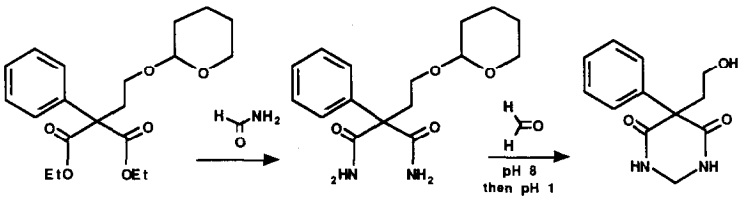
Scheme 6.

2-Hydroxyprimidone **5** can then be considered as a chemical precursor of phenobarbital **2** as well as of phenylethylmalondiamide **3**.

Table I. Compounds isolated from urine.

Experiment	ADMINISTRATION		COMPOUNDS ISOLATED FROM URINES mg				
	Compound	Total mg	Primidone 1	Phenylethyl malondiamide 3	Phenobarbital 2	parahydroxy phenobarbital 4	Phenylbutyro lactone 6
1	1	3780	2192	857	302	108	0
2	1	3420	2030	581	310	105	0
3	1	4180	2269	882	387	148	0
4	1	2600	505	502	385	435	23
5	1	4000	856	652	590	747	33
6	1	4800	1080	718	773	990	40
7	5	3240	0	1420	210	712	0
8	5	3080	0	1440	139	538	0
9	5	3000	0	1070	257	758	0
10	5	4700	0	850	715	1855	40
11	5	4400	0	780	700	1650	30
12	5	2200	0	410	375	840	20

Synthesis, stability and chemical transformation of ***7*** *into* ***6***
The synthesis of β-hydroxyprimidone **7** was performed in a two-step procedure. Pyranether of ethyl 5-phenyl-5-(2-hydroxyethyl) malonate was treated by formamide to yield 5-phenyl-5-[(2-tetrahydropyranyl) oxy-2-ethyl] malondiamide and the ring was then closed using formaldehyde at pH 8 and pH 1 (Scheme 7).

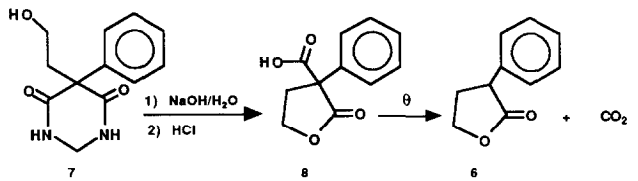


VII

Scheme 7.

The stability of **7** was studied under biomimetic conditions. After seven days at pH 7.40 and 37°C in aqueous solution the compound was recovered unchanged.

At room temperature, alkaline hydrolysis of **7** yielded a carboxylic lactone **8** which underwent decarboxylation by heating (Scheme 8).



VIII 7 8 6

Scheme 8.

β-Hydroxyprimidone **7** can then be considered as a chemical precursor of the toxic α-phenyl-γ-butyrolactone **6**.

Biological results

The amounts of **1, 2, 3, 4,** and/or **6** isolated from urine after administration of either **1** or **5** to dogs are shown in Table I.

When primidone **1** was administered at therapeutic levels (20 mg kg^{-1} d^{-1} *per os*), (Expts 1, 2 and 3), for one week or more, 57.21 ± 1.5% of the drug was recovered unchanged in the urine. The main metabolite was phenylethylmalondiamide **3** (21.43 ± 1.8%). The group consisting of phenobarbital **2** (8.2 ± 0.37%) and the *para*hydroxyphenobarbital **4** (2.77 ± 0.18%) did not represent much more than 10% of the drug administered.

When a single dose of primidone **1** (200 mg kg^{-1} *per os*) was administered (Expts 4, 5 and 6), 21.12 ± 0.89% of the drug was recovered unchanged in the urine. The percentage of phenylethylmalondiamide **3** decreased slightly (17.83 ± 1.36%), while phenobarbital **2** (14.30 ± 0.42%) and *para*hydroxy-phenobarbital **4** (16.41 ± 0.99%) increased.

When 2-hydroxyprimidone **5** was administered, neither **1** nor **5** were recovered from the urine. When the dose was 20 mg kg^{-1} d^{-1}, over a period of more than 5 days (Expts 7, 8 and 9), the main metabolite was once again phenylethylmalondiamide **3** (47.80 ± 3.77%). The group consisting of phenobarbital **2** (6.58 ± 1.18%) and the *para*hydroxyphenobarbital **4** (20.35 ± 2.13%) increased due to the formation of a significant quantity of **4**.

When a single dose of 2-hydroxyprimidone **5** (200 mg kg^{-1} *per os*) was administered (Expts 10, 11 and 12), the group consisting of phenobarbital **2** (15.43 ± 0.31%) and the *para*hydroxyphenobarbital **4** (34.59 ± 1.34%) became more important than phenylethylmalondiamide **3** (19.67 ± 0.51%).

Phenyl-γ-butyrolactone **6** appeared only with high doses (200 mg kg^{-1} *per os*) of primidone **1** or 2-hydroxyprimidone **5**, i.e. respectively 1.14 ± 0.03 (Expts 4, 5 and 6) and 1.12 ± 0.10 (Expts 10, 11 and 12).

β-Hydroxyethylprimidone **7** was not detected in the urine.

Discussion

Whatever the drug administered at therapeutic levels is (primidone **1** or 2-hydroxyprimidone **5**), the ratio between phenylethylmalondiamide **3** and the group consisting of phenobarbital **2** and its *para*hydroxymetabolite **4** is the same (about 2).

On the other hand, the amounts of isolated metabolites increase when the precursor **5** is administered instead of **1** (75% instead of 32% of administered drug.)

These results show that 2-hydroxyprimidone **5** which is a chemical precursor of either phenylethylmalondiamide **3** or phenobarbital **2** has the same role in dogs as *in vitro*.

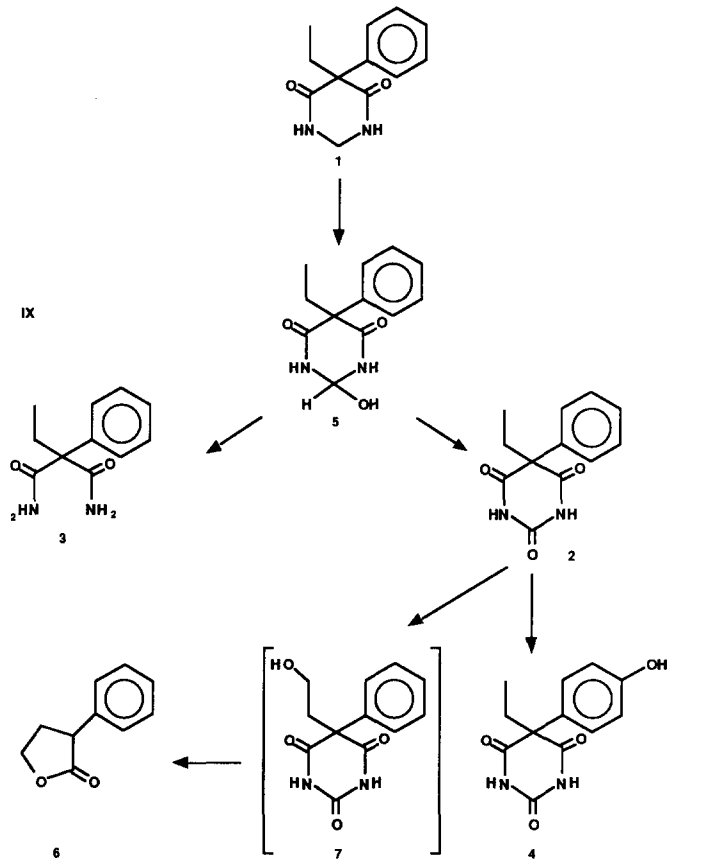
The increase of *para*hydroxyphenobarbital **4** can be explained by the better transformation of phenobarbital **2** which appears in the medium more rapidly when **5** rather than **1** is administered. **2** then has enough time to be hydroxylated into **4** before being excreted.

Regarding the formation of α-phenyl-γ-butyrolactone **6** *in vivo* from primidone **1**, owing to the fact that ≈ 1% of this γ-lactone is isolated from the urine when high doses of 2-hydroxyprimidone **5** are administered to dogs (200 mg kg^{-1} *per os*), as well as when primidone **1** on its own is given to animals (200 mg kg^{-1} *per os*) one can conclude that phenobarbital **2** is a precursor of phenyl-γ-butyrolactone **6** *in vivo*.

These results are in agreement with our previous findings [16] on the metabolism of phenobarbital.

The only difference is that here, phenyl-γ-butyrolactone **6** appears with high doses of either primidone **1** or hydroxyprimidone **5**, while this toxic metabolite was isolated even for therapeutic doses of phenobarbital **2**. This can be interpreted by the formation of larger amounts of the group consisting of phenobarbital **2** and *para*hydroxyphenobarbital **4** when **1** or **5** are administered at high doses, which induces the secondary metabolic pathway (*i.e.*, oxidation of the ethyl side chain followed by transformation into **6**).

On the other hand, for therapeutic doses of either **1** or **5** there is not enough phenobarbital generated to induce this oxidation which, of course, can take place when phenobarbital **2** on its own administered at therapeutic doses. The various pathways of primidone metabolism are presented in Scheme 9.



Scheme 9.

Experimental protocols

Primidone **1**, phenobarbital **2**, phenylethylmalondiamide **3** and *para*hydroxyphenobarbital **4** were all obtained commercially and were fully characterized spectroscopically.

^1H NMR spectra were recorded on a Varian T-60 spectrometer using $(CH_3)_4Si$ for reference. Melting points were recorded on a Kofler apparatus and are uncorrected. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values.

Thin-layer chromatograms (TLC) were run on pre-coated silica-gel 60 F 254 plates with either: a) ethyl acetate; or b) chloroform: ethyl acetate (80:20) as the mobile phase.

Chemistry

Synthesis of 2-hydroxyprimidone: 5-ethyl-2-hydroxy-5-phenyl-1,3-diazinane-4,6-dione

This was performed according to an adaptation of the procedure described by Kato and Kohketsu [18] for the synthesis of γ-hydroxyamobarbital from amobarbital.

In a 500-ml Erlenmeyer flask 8 g of primidone (0.0367 mol), 150 ml of acetic acid and 18 g of chromium trioxide (0.18 mol) were added. The mixture was stirred at 20°C for 10 min, and then poured into 1 l of ethyl ether. After cooling and stirring, the organic solution was separated in a separatory funnel, washed in small amounts of water, and dried over anhydrous magnesium sulfate. The solvent was then evaporated under reduced pressure. The solid residue was collected and crystallized from ethanol.

Yield = 22%; mp: 152°C; TLC: solvent a; Anal. (C, H, N) $C_{12}H_{14}N_2O_3$; ^1H NMR (DMSO–d_6) δ ppm: 0.9 (t, 3H, CH_3); 2.25 (9, 2H, CH_2); 4.9 (s, 1H, C*H*OH); 5.2 (s, 1H, exch. D_2O, OH); 7.2 (s, 5H, C_6H_5); 10.7 (s, 2H, exch. D_2O, 2 NH).

Synthesis of β-hydroxyprimidone: 5-(2-hydroxyethyl)-5-phenyl-1,3-diazinane-4,6-dione

This synthesis was performed in two steps.

First step. 2-Phenyl-2-[(2-tetrahydropyranyloxy)-2-ethyl] malondiamide was first synthesized from corresponding tetrahydropyranyloxymalonate using the procedure described by Alvin and Bush for the synthesis of phenylethylmalonamide from ethyl phenylethylmalonate and formamide [8].

Yield: = 66%; mp: 134°C; TLC: solvent b; Anal. (C, H, N) $C_{16}H_{22}N_2O_4$; ^1H NMR ($CDCl_3$) δ ppm: 1.6 (m, 6H, $3CH_2$ cycle); 2.3 (m, CH_2–CH_2–O); 3.2–4.0 (m, 4H, 2 CH_2–O); 4.6 (m, 1H, O–CH–O); 6.0–6.8 (4H, exch. D_2O, 2 NH_2); 7.3 (m, 5H, C_6H_5).

Second step. In an Erlenmeyer flask, with a magnetic stirrer, 500 ml of water was added to 9.18 (0.03 mol) of 2-phenyl-2-[(2-tetrahydropyranyloxy)-2-ethyl] malondiamide. Sodium hydroxide was added until the mixture attained pH 8. 30 ml of an aqueous solution of formaldehyde (35%) were added dropwise to the mixture which was then heated at 40°C for 48 h. Hydrochloric acid was then added until pH 1, and the mixture was heated at 80°C for 1 h. After cooling, the mixture was concentrated to 100 ml. The precipitate of β-hydroxyprimidone was collected by filtration and crystallized from ethanol–water (60–40).
Yield: 41%; mp: 141°C; TLC: solvent a; Anal. (C, H, N) $C_{12}H_{14}N_2O_3$; ^{1}H NMR (DMSO–d_6) δ ppm: 2.5 (t, 2H, CH_2 α to cycle); 3.5 (t, 2H, CH_2OH); 4.1 (m, 2H, CH_2 cycle); 5.1 (1H, exch. D_2O, OH); 7.35 (m, 5H, C_6H_5); 8.6 (2H, exch. D_2O, 2 NH).

Synthesis of phenobarbital from 2-hydroxyprimidone
2.34 g (0.01 mol) of 2-hydroxyprimidone were added to 50 ml of acetic acid. 6 g of chromium trioxide (0.06 mol) were then added to the mixture which was stirred at 20°C for 10 min. The mixture was then poured into 1 l of ethyl ether. After cooling and stirring, the organic solution was separated in a separatory funnel, washed with small amounts of water, and dried over anhydrous sodium sulfate. The solvent was then evaporated under reduced pressure. The solid residue was triturated with ethyl ether, collected by filtration, and crystallised from water.
Yield: 38% (phenobarbital); (checked by ^{1}H NMR, analysis, TLC: solvents a and b).

Synthesis of phenylethylmalondiamide from 2-hydroxyprimidone
In an Erlenmeyer flask with a magnetic stirrer, containing 200 ml of water, sodium hydroxide was added until the mixture reached pH 10. 2.34 g (0.01 mol) of 2-hydroxyprimidone were then added and the mixture was stirred at 20°C for 8 h. The solution was adjusted to pH 7 with hydrochloric acid and was extracted 3 times with 50 ml of dichloromethane. Organic solutions were mixed, dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The solid residue was triturated with ethyl ether, filtered and crystallized from ethanol.
Yield: 68%, (phenylethylmalondiamide checked by ^{1}H NMR, Analysis, TLC: solvents a and b).

Stability of β-hydroxyprimidone under biomimetic conditions
In a 1 l Erlenmeyer flask thermostated at 37°C, and containing 500 ml of a buffer at pH 7.40 (citric acid–disodium phosphate), 500 mg (0.0021 mol) of β-hydroxyprimidone were added. The mixture was stirred only from time to time over a period of 7 days and extracted 3 times with 100 ml of ethyl acetate. The organic solutions were collected, dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. β-hydroxyprimidone (100%) was then recovered unchanged (checked by ^{1}H NMR, analysis, TLC: solvent a).

Synthesis of α-phenyl-γ-butyrolactone from β-hydroxyprimidone
In a 500-ml Erlenmeyer flask containing 100 ml of water 1 g (0.0042 mol) of β-hydroxyprimidone was added. The pH was adjusted to 10 with sodium hydroxide and the mixture was stirred at 60°C for 4 h. After cooling, 50 ml of ethyl ether were added to the mixture, which was adjusted to pH 1 with hydrochloric acid and stirred. The organic phase was separated, dried on anhydrous sulfate and the solvent was evaporated under reduced pressure at room temperature. The solid residue was collected.
Yield: 72%; (phenyl-γ-butyrolactonecarboxylic acid) (checked by ^{1}H NMR, analysis, TLC: solvent a).

This acid was heated at 60°C and decarboxylated into phenyl-γ-butyrolactone. Yield: 100%; (checked by ^{1}H NMR, analysis, TLC: solvents a and b).

Biology

Administered compounds
Primidone **1** was administered in experiments 1–6 and hydroxy-2 primidone **5** in experiments 7–12.

Formulation
1 or **5** powder was placed in hard gelatine capsules for dosing. The weight of powder was adjusted so that each dog received the dose indicated in Tables II or III.

Table II. Conditions of administration of primidone **1** to dogs.

EXPERIMENT	ADMINISTRATION		DOGS	
	Dose mg/kg/day	Time days	Sex	Weight kg
1	20	7	male	27
2	20	7	female	19
3	20	11	female	18
4	200	1	female	13
5	200	1	female	20
6	200	1	female	24

Table III. Conditions of administration of 2-hydroxyprimidone **5** to dogs.

EXPERIMENT	ADMINISTRATION		DOGS	
	Dose mg/kg/day	Time days	Sex	Weight kg
7	20	9	female	18
8	20	11	female	14
9	20	5	male	30
10	200	1	female	23.5
11	200	1	female	22
12	200	1	male	11

Animals
12 male or female dogs, were placed in separate Pajon R metabolism cages with free access to food and water.

Administration
Every morning, each animal received one capsule orally, containing the daily dose.

Collection of urine samples
Urine was collected every morning and evening, filtered and allowed to freeze (–18°C) during the administration period and for the following 2 days.

Extraction procedure
Urine samples from one experiment were allowed to thaw without any heating and collected. The pH was then adjusted to 4.5. The samples were divided into 1.5-l aliquots. Each aliquot was extracted 4 times with ethyl acetate (500 ml). Organic solutions were then dried over anhydrous sodium sulfate (500 g) and filtered. Ethyl acetate was then eliminated by distillation under reduced pressure at room temperature.

The pH of every urine lot was then lowered to 1 and the procedure repeated.

Isolation
All the residues corresponding to the same experiment were collected and treated by column chromatography (Silica: Kieselgel 60 H R; pressure: 4 bars; pump: Duramat R; solvent: ethyl acetate–cyclohexane 50:50 (experiments 1–6) or chloroform–cyclohexane 60:40 (experiments 7–12).

Collected fractions were then studied by thin-layer chromatography (Silica: Kieselgel 60 F 254 R; solvent: ethyl acetate) in order to identify those fractions which contained the same compounds. All similar fractions were then assembled, solvents were evaporated and residues were dried under reduced pressure.

Identification
A sample of each isolated product was dissolved in the appropriate deuterated solvent $CDCl_3$ or DMSO–d_6 and studied by ^{1}H NMR. Spectra of isolated compounds were identical to those of synthesized models (**1, 2, 3, 4** or **6**).

Acknowledgments

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