Antiallergic Activity of ω-Nitroacetophenones

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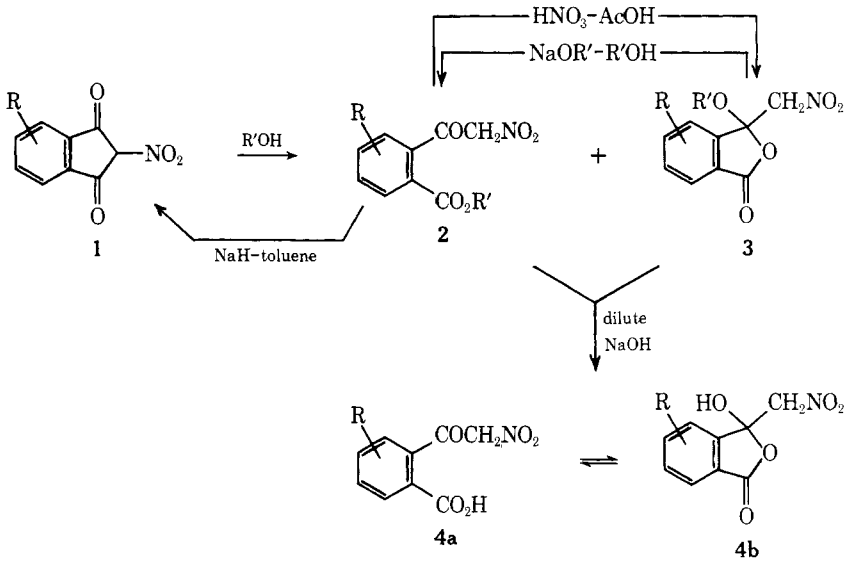
Selected ω-nitroacetophenones, formed by the alcoholic cleavage of 2-nitroindandiones, have been shown to inhibit the homocytotropic antibody–antigen induced passive cutaneous anaphylaxis reaction in the rat. The enzymatic cyclization of these derivatives to the parent nitroindandione has been demonstrated both in vivo and in vitro and this process is suggested as a possible prerequisite to biological activity.

We have previously reported good antiallergic activity in a series of 2-nitroindan-1,3-diones (1)[1] as measured by the rat passive cutaneous anaphylaxis or PCA test. We now report on the biological activity of a selection of alcoholysis products (Scheme I) in which the diketo nitro ring has been cleaved to the ω-nitroacetophenone **2**. Concomitant products, the phthalides **3**, showed no antiallergic activity in our tests although mild hydrolysis of either of the two alcoholysis products **2** or **3** afforded the appropriate 2-carboxy-ω-nitroacetophenones **4a,b** which also showed noticeable activity in the rat PCA test.

In addition, we have chemically cyclized the ω-nitroacetophenones **2** back to the parent indandiones **1** by mild base treatment. The in vivo conversion of **2j** to its parent indandione (Scheme III) has led us to suggest that this is an important factor for biological activity.

Scheme I



Chemistry. Alcoholysis of 2-nitroindan-1,3-dione (I, R = H) with lower alcohols has been shown by Zalukaev[2] to yield mixtures of two isomeric compounds **2** and **3** (Scheme I). The interconvertibility of **2** and **3** under varying pH has also been shown.[2] Thus under acid conditions compound **2** smoothly converts to its cyclic form **3**, a process which may be reversed by treating **3** with a mixture of sodium alkoxide and alcohol.

We have further investigated this reaction with 2-nitroindan-1,3-dione itself and with its 4-phenyl and 5,6-dimethyl analogs. As expected the phenyl derivative **1** (R = 4-Ph) on methanolysis afforded two isomeric 2-carbomethoxy-ω-nitroacetophenones (**2**, R = 3-Ph and R = 6-Ph) with the latter predominating. Fractional recrystallization of this mixture gave a pure sample of the larger component (**2q**). Only one cyclic ester (**3**, R = 7-Ph) was formed, however, possibly due to steric hindrance at the C-4 site. In addition, the methanolysis of 2-alkyl- and 2-aryl-2-nitroindandiones (**8**) was carried out with the addition of a catalytic amount of sodium methoxide to ensure sole formation of the acylic products **2d–f,o** (Scheme II).

Synthesis of the nitro derivatives **8** was by the direct nitration of the appropriate 2-alkyl- or arylindandiones (**7**), which for R_1 = alkyl, were prepared by Claisen condensation of phthalic esters **5** with esters **6**.

Both the open chain **2** and cyclic **3** esters were saponified on mild aqueous alkali treatment to the corresponding acids **4a,b**. Usually these acids were isolated in the acyclic form (**4a**) but the ability of 2-acylbenzoic acids to tautomerize to hydroxy lactones of type **4b** is well known.[3] More vigorous or prolonged exposure to alkali resulted in increasing amounts of the derived phthalic acids.

The reverse procedure (**2** → **1**) has also been effected by

Scheme II

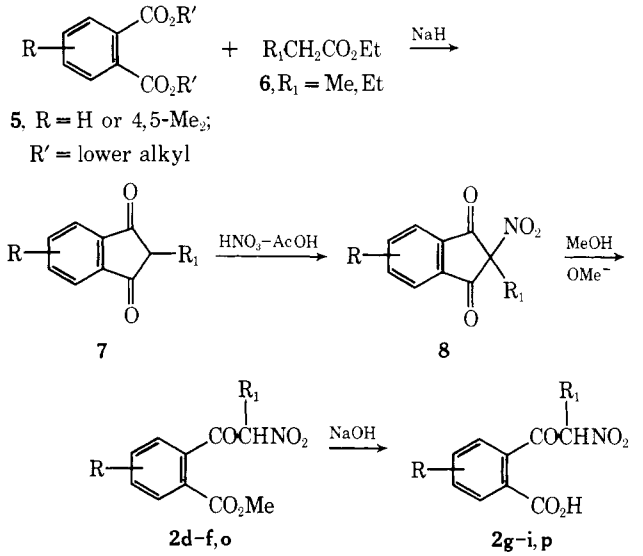
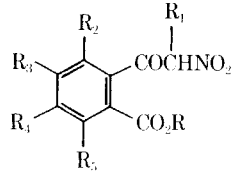


Table I. ω-Nitroacetophenones



Compd	R	R_1	R_2	R_3	R_4	R_5	Mp, °C	Lit. mp, °C	Formula	Analyses	Yield, %	Act. in rat PCA test, ED_{50},[a] mg/kg sc at T_{max}[b]
	Disodium cromoglycate											6.7 (4.9–9.3, 147, 56)
9	5,6-Dimethyl-2-nitroindandione											0.17 (0.08–0.40, 60, 108)
2a	Me	H	H	H	H	H	108–109 (EtOH)	108–109[c]	$C_{10}H_9NO_5$	C, H, N	26	>50
2b	Et	H	H	H	H	H	81 (EtOH)	82–83[c]	$C_{11}H_{11}NO_5$	C, H, N	31	>50
2c	H	H	H	H	H	H	131–132 (PhH)	133–134[c]	$C_9H_7NO_5$	C, H, N	75	>50
2d	Me	Me	H	H	H	H	59 (MeOH)	62–63[d]	$C_{11}H_{11}NO_5$	C, H, N	72	Inactive[e]
2e	Me	Et	H	H	H	H	52 (MeOH)		$C_{12}H_{13}NO_5$	C, H, N	78	Inactive[e]
2f	Me	Ph	H	H	H	H	100 (MeOH)		$C_{16}H_{13}NO_5$	C, H, N	60	Inactive[e]
2g	H	Me	H	H	H	H	104 (PhH)		$C_{10}H_9NO_5$	C, H; N[f]	35	Inactive[e]
2h	H	Et	H	H	H	H	78 (PhH–petroleum ether)		$C_{11}H_{11}NO_5$	C, H, N	40	Inactive[e]
2i	H	Ph	H	H	H	H	118 (PhH)		$C_{15}H_{11}NO_5$	C, H, N	71	Inactive[e]
2j	Me	H	H	Me	Me	H	138–139 (EtOH)		$C_{12}H_{13}NO_5$	C, H, N	36[g]	0.7 (0.04–6.9, 50.6, 24)
2k	Et	H	H	Me	Me	H	102–103 (EtOH)		$C_{13}H_{15}NO_5$	C, H, N	39	1.5 (0.5–3.9, 87, 24)
2l	*n*-Pr	H	H	Me	Me	H	94–94.5 (*n*-PrOH)		$C_{14}H_{17}NO_5$	C, H, N	39	7.0 (*h*, –, 18)
2m	*i*-Pr	H	H	Me	Me	H	129–130 (*i*-PrOH)		$C_{14}H_{17}NO_5$	C, H, N	40	>50
2n	H	H	H	Me	Me	H	165–167 (PhH)		$C_{11}H_{11}NO_5$	C, H, N	96	17.6 (9.4–33.1, 87.5, 18)
2o	Me	Me	H	Me	Me	H	102–103 (MeOH)		$C_{13}H_{15}NO_5$	C, H, N	73	Inactive[e]
2p	H	Me	H	Me	Me	H	132 (PhH)		$C_{12}H_{13}NO_5$	C, H, N	36	Inactive[e]
2q	Me	H	Ph	H	H	H	132 (EtOH)		$C_{16}H_{13}NO_5$	H, N; C[i]	16[j]	>50
2r	H	H	H	H	H	Ph	107 (PhH)		$C_{15}H_{11}NO_5$	C, H, N	50	>50

[a]Figures in parentheses are 95% confidence limits, slope of inhibition/log dose line, number of animals used. [b]*T* max is the time between sc administration of the drug and challenge to give maximum activity. For all compounds with an ED_{50} < 50 this was 10 min. [c]L. P. Zalukaev, *J. Gen. Chem. USSR (Engl. Transl.)*, **26**, 1039 (1956). [d]J. Freimanis and G. Vanags, *Zh. Obshch. Khim.*, **31**, 1945 (1961); *Chem. Abstr.*, **55**, 27229 (1961). [e]No activity shown by a dose of 100 mg/kg given subcutaneously just before and 30 min before antigen challenge for soluble compounds and when given just before and 60 min before challenge for suspensions. [f]N: calcd, 6.28; found, 5.07. [g]88% yield by isomerization of the ester **3**. [h]Nonlinear dose response. [i]C: calcd, 64.21; found, 64.65. [j]A 16% yield as a mixture of R_2 = Ph and R_5 = Ph with the former predominating.

refluxing with sodium hydride in either benzene or toluene for several hours and by aqueous bicarbonate treatment at 60°.

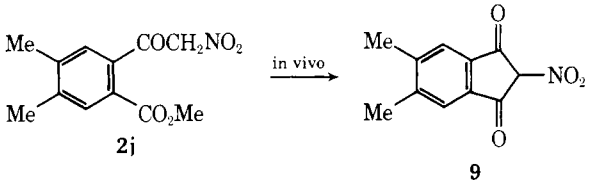
Results and Discussion

Selected ω-nitroacetophenones (**2a–r**) were screened for antiallergic activity as measured by their ability to inhibit homocytotropic antibody–antigen induced passive cutaneous anaphylaxis in the rat (rat PCA test) and their effectiveness is reported in Table I. We noted that whereas the methyl and ethyl esters of 2-carboxy-ω-nitroacetophenone, **2a** and **2b,** respectively, required subcutaneous doses greater than 50 mg/kg to give 50% inhibition of the rat PCA response, the 4,5-dimethyl homologs **2j** and **2k** showed markedly greater efficacy. These results are perhaps not surprising in view of the enhanced activity of 5,6-dimethyl-2-nitroindandione (**9**) over that of the parent 2-nitroindandione.[1] More surprising was the difference in activity between the *n*-propyl and isopropyl esters of 2-carboxy-5,6-dimethyl-ω-nitroacetophenone (**2l** and **2m,** respectively). This difference possibly reflects steric crowding in the isopropyl isomer. The phenyl derivative **2q** and the isopropyl ester **2m** showed only marginal activity. The 2-carboxy-ω-nitroacetophenones **2c** and **2n** in general showed a reduced activity over their respective methyl esters **2a** and **2j.**

The failure to demonstrate activity in the rat PCA test by using ω-nitroacetophenone itself and the facile chemical conversion of **2** to **1** led us to suspect that a similar cyclization might occur in vivo and that the nitroacetophenones reported here owe their activity to their ability to regenerate nitroindandiones.

In order to test this hypothesis we treated rats with a 50 mg/kg subcutaneous dose of the acyclic derivative **2j** and assayed the serum from groups of three 60 min after dosing in an attempt to detect the potential cyclization product 5,6-dimethyl-2-nitroindandione (Scheme III).

Scheme III



2j **9**

Using both uv spectroscopy and high-pressure liquid chromatography (HPLC) it was possible to demonstrate the presence of cyclized material (**9**) in the serum taken from rats that had been dosed previously with **2j.** Moreover, a solution of **2j** treated with freshly homogenized rat liver in 10% sucrose phosphate buffer resulted in a rapid conversion to **9** at room temperature, as evidenced by HPLC, with most acyclic material disappearing within 2 hr, leading to over 90% conversion to **9.** No change was noticed after keeping a solution of **2j** in rat serum or buffer over 3 hr at 37°.

Chemical evidence that in vivo cyclization contributed to activity in the PCA test was sought in the acetophenones (**2d–i,o,p**) derived from **8** (R = Me, Et, or Ph). It has been shown[1,4] that both 2-alkyl- and 2-aryl-2-nitroindandiones show no activity in the rat PCA test and we argued therefore that the alcoholysis products from these (**2d–f,o**) should also show no activity if compounds of type **2** exhibit their action via the cyclic indandione derivatives. Moreover, we reasoned that if compounds of type **2** show intrinsic activity, unrelated to their cyclization, then, especially with small alkyl substituents on the ω-carbon atom, little effect on activity would be incurred by this substitution. A failure under our conditions to demonstrate any activity in the rat PCA test for both the esters **2d–f,o** and their acids **2g–i,p** lent support to our postulation that prior cyclization to nitroindandiones is an important factor in the activity of derivatives of type **2.**

The inactivity of ω-nitroacetophenone itself, which is incapable of forming nitroindandione-like compounds, and the in vivo and in vitro evidence given above lead us to suggest that the cyclization of ω-nitroacetophenones **2** to 2-nitroindandiones is a prerequisite for biological activity.

Experimental Section

Melting points were determined using a Büchi melting point apparatus and are recorded uncorrected. The structures of all compounds were confirmed by their ir and NMR spectra, the latter of which were determined as solutions in either $CDCl_3$ or DMSO-d_6. The analyses of all compounds fell within ±0.4% of the calculated values.

2-Methyl-2-nitroindan-1,3-dione. To a solution of 2-methylindan-1,3-dione (0.960 g, 0.006 mol) in glacial AcOH (10 ml) was added a solution of fuming HNO_3 (2 ml, *d* 1.52) in glacial AcOH (10 ml) and the mixture warmed to 70°. After stirring at this temperature for 15 min the solution was cooled and concentrated in vacuo and 5 *N* HCl was added. The white crystalline nitro derivative was filtered off, washed with 5 *N* HCl, and dried in vacuo over NaOH to give 0.420 g (34%) of material, mp 84° (MeOH) (lit.[5] mp 87°). Anal. ($C_{10}H_7NO_4$) C, H, N.

2-Ethyl-2-nitroindan-1,3-dione. In a similar manner to the methyl homolog, 2-ethylindan-1,3-dione (4.16 g, 0.024 mol) was nitrated to give 2.68 g (51%) of the nitro derivative of mp 78° (EtOH). Anal. ($C_{11}H_9NO_4$) C, H, N.

2,5,6-Trimethylindan-1,3-dione. A mixture of dimethyl 4,5-dimethylphthalate (100 g, 0.45 mol), ethyl propionate (180 ml), and a 60% dispersion of NaH in mineral oil (29.7 g) was heated at 100° for 4 hr, cooled, and triturated with dry ether. The orange-red solid was filtered under vacuum, dried, and added to 5 *N* HCl (400 ml) at 70° with stirring. After decarboxylation was complete (20 min) the brown residue was filtered, washed with water, and dried in vacuo over P_2O_5. Recrystallization from benzene with charcoal added afforded 21.6 g (26%) of the indandione of mp 122°. Anal. ($C_{12}H_{12}O_2$) C, H.

2-Nitro-2,5,6-trimethylindan-1,3-dione. Nitration of 2,5,6-trimethylindan-1,3-dione (2.80 g, 0.015 mol) as described for the monomethyl homolog yielded 1.56 g (40%) of the 2-nitro compound, mp 134° (EtOH). Anal. ($C_{12}H_{11}NO_4$) C, H, N.

2-Nitro-2-phenylindan-1,3-dione. A stirred suspension of 2-phenylindan-1,3-dione (6.6 g, 0.03 mol) in anhydrous Et_2O (50 ml) was cooled below 10° during the dropwise addition of fuming HNO_3 (10 ml, *d* 1.52). After stirring for a further 1 hr at room temperature the clear solution which had formed within 30 min had precipitated the nitro derivative as a white crystalline solid. Filtration and recrystallization gave 7.5 g (93%), mp 112° (EtOH) (lit.[6] mp 122°). Anal. ($C_{15}H_9NO_4$) C, H, N.

Alcoholysis Products. Two general procedures were used for alcoholic cleavage of the 2-nitroindan-1,3-diones depending on the degree of substitution at C-2. The physical and biological data of these derivatives are recorded in Table I.

Method A. For those derivatives having a proton at C-2 the general procedure followed was a modification of that of Zalukaev.[2]

A solution of the nitroindandione **1** (0.10 mol) in the alcohol (100 ml) was heated under reflux for 4 hr and then cooled. At this stage nearly pure ester **2** usually separated and could be filtered off and recrystallized. Dilution of the filtrate with water gave crude cyclic ester **3** which was purified by dissolution in $CHCl_3$, washing with aqueous $NaHCO_3$, evaporation, and recrystallization. Alternatively, the whole alcoholic solution could be diluted with water, the mixture of **2** and **3** taken up in $CHCl_3$, and the acidic **2** removed by extraction with aqueous $NaHCO_3$, leaving **3** in the organic phase. Acidification of the aqueous phase afforded pure **2.**

Method B. With either alkyl or aryl substituents at C-2 a modified procedure was used in which only acyclic ester **2** was isolated. Addition of catalytic amounts of sodium alkoxide to the alcoholic solution prior to reflux not only enhanced the nucleophilicity of the solvent but encouraged the isomerization of potential **3** to **2.** Thus, the nitroindandione **8** (0.10 mol) was dissolved in 100 ml of alcohol to which a little metallic Na had been added and the red solution stirred at room temperature for several minutes. After dilution with water and acidification with 5 *N* HCl the precipitated oil was extracted into $CHCl_3$. After drying ($MgSO_4$) and remov-

al of the solvent in vacuo the residual oil solidified on scratching to give the ω-nitroacetophenone.

Hydrolysis Products. All ω-nitroacetophenones were hydrolyzed with dilute aqueous NaOH at room temperature, a procedure which could equally well be applied to the hydrolysis of **3.**

Method. A mixture of the ester (0.10 mol) and 5% aqueous NaOH (400 ml) was stirred at room temperature for 1 hr, acidified, and allowed to stand in ice for a further 30 min. Those compounds having no alkyl or aryl substituent in the side chain (**2c,n,r**) crystallized at this stage and after filtration were purified by recrystallization. Compounds bearing these substituents in the side chain, however (**2g–i,p**), separated as oils. Isolation in this case was by $CHCl_3$ extraction, drying ($MgSO_4$), and solvent removal in vacuo. After trituration of the oily residue with ligroine the products crystallized and were purified by recrystallization.

Rat PCA Test. The rat PCA test and the evaluation of the results was carried out as previously described.[1]

Compounds **2j, 2k,** and **2l** were injected subcutaneously as a suspension in isotonic saline buffered to pH 7 with phosphate buffer, PBS (Bacto Hemagglutination buffer, Difco Laboratories), and containing 0.5% methylcellulose, PBS/MC. Compound **2n** was given subcutaneously as a solution in PBS neutralized to pH 7 with sodium bicarbonate. The compounds were free of nitroindandiones and these did not form under the condition in which the compounds were dissolved or suspended prior to administration.

Bioconversion of the Methyl Ester of 2-Carboxy-4,5-dimethyl-ω-nitroacetophenone (2j) to 5,6-Dimethyl-2-nitroindandione (9). In Vivo. Groups of three male Wistar rats of 250–350 g were bled by cardiac puncture under halothane anaesthesia 1 hr after receiving a subcutaneous dose of compound **2j** of 50 mg/kg as a suspension in PBS/MC. The blood was allowed to clot over a period of 5 min and the serum was separated by centrifuging. The serum was assayed for the presence of the nitroindandione **9** and compound **2j.**

a. Uv Spectroscopy. 5,6-Dimethyl-2-nitroindandione (**9**) exhibits a pronounced uv absorbance at 353 nm (ϵ^{H_2O} 23,200) and a smaller absorbance at 316 nm (ϵ^{H_2O} 16,300) whereas the acetophenone **2j** shows only a small absorption in this range at 343 nm (ϵ^{EtOH} 2946). By direct comparison of the intensity of the 353 nm peak with that at 316 nm therefore, a qualitative evaluation of the dimethylindandione **9** could be made in the presence of **2j.** The evaluation was facilitated by initial deproteination of the serum with trifluoroacetic acid followed by extraction into butanol. Using this procedure cyclized **9** was detected in the serum taken from rats that had been dosed subcutaneously 60 min previously.

b. High-Pressure Liquid Chromatography (HPLC). A better assessment of 5,6-dimethyl-2-nitroindandione (**9**) in the presence of **2j** could be carried out using HPLC since each compound could be individually measured and quantitated. Under our conditions using a Bondapack phenyl corasil column the cyclic material **9** had a retention time of 7.5 min, compared with 9.0 min for **2j.** Moreover, the need to deproteinate the serum was no longer important since identification of each component could be achieved by scanning the eluent with light at 353 nm. Evidence for up to 16 μg/ml of the cyclic material **9** at 60 min after subcutaneous injection of **2j** to rats was found. Residual **2j** up to levels of 32 μg/ml was also found.

In Vitro. Fresh rat livers, of about 15 g, were washed with 0.1 *M* Tris–HCl buffer, pH 7.9, containing 10% sucrose (Tris–sucrose), and then homogenized at 4° in 10 ml of Tris–sucrose for 2 min using an electric homogenizer (Ultra-turrax). The suspension was then centrifuged for 15 min at 4000 rpm and the layer between the solid debris and the fat was separated to give undiluted liver extract. A diluted liver extract was prepared from this by diluting to four times its own volume with Tris–sucrose, centrifuging for 10 min at 4000 rpm, and collecting the supernatant. Equal volumes of solutions of **2j** or **9** in PBS neutralized with sodium bicarbonate were added to Tris–sucrose, fresh rat serum, diluted liver extracts, and undiluted liver extracts to give a final concentration of **2j** or **9** in solution of 100 mg/ml. The solutions were incubated at 37° or stood at room temperature and then assayed for the presence of **2j** or **9** using HPLC calibrated with standard solutions of **2j** and **9.**

When **2j** was stood in undiluted rat liver extract at room temperature for 2 hr there was a 92% conversion to **9** and a 67% conversion after 1 hr and 13 min. When **2j** was incubated at 37° in the diluted rat liver extracts there was a progressive increase in conversion of **2j** to **9** with time so that there was a 78% conversion after 3 hr. Incubation of **2j** in fresh rat serum or Tris–sucrose for 3 hr at 37° gave no conversion to **9.** Compound **9** was stable to liver extracts.

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References and Notes

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Synthesis of an Active Hydroxylated Glutethimide Metabolite and Some Related Analogs with Sedative–Hypnotic and Anticonvulsant Properties

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Two synthetic pathways are described for the preparation of 4-hydroxy-2-ethyl-2-phenylglutarimide (**2**), an active hydroxylated metabolite of glutethimide (**1**). Fourteen other glutethimide analogs were also synthesized and tested for biological activity. Most of the analogs exhibited sedative–hypnotic properties and compound **2** possessed the greatest activity compared to the parent drug. 4-Amino-2-ethyl-2-phenylglutarimide and 4-hydroxy-2-ethyl-2-phenylglutaconimide (**13**) exhibited the greatest potential as anticonvulsant agents. The structure–activity relationships of the series are discussed.

Glutethimide [(±)-2-ethyl-2-phenylglutarimide, **1**] is a nonbarbiturate sedative–hypnotic agent. The metabolism of **1** has been studied in the rat, dog, guinea pig, and man and has been shown to involve hydroxylation at several sites in the molecule.[1–4] Injection of the glucuronide conjugates of hydroxylated metabolites of **1** into mice produced no sedative effect.[5] In a more recent study, Ambre and Fischer[6] showed that a hydroxylated metabolite of **1** accumulates in the plasma of humans intoxicated by glutethimide overdose. This metabolite was subsequently isolated[7] from the urine of dogs given large doses of **1** and was chemically identified as 4-hydroxy-2-ethyl-2-phenylglutarimide

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