Conformational Analysis of the Aromatase Inhibitor 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione (Rogletimide) and Discovery of Potent 5-Alkyl Derivatives

Raymond McCague† and Martin G. Rowlands*

Drug Development Section, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, England

Received April 15, 1992

Analysis of the proton NMR spectra of 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione (rogletimide, 1) shows that it exists in solution with the aromatic ring in an axial position; the same conformation was found for aminoglutethimide. Excess lithium diisopropylamide treatment of 1 formed a dianion which methylated at C-5. The major product with the methyl group trans to the pyridyl ring retained this ring in an axial position and had higher aromatase inhibitory potency than 1. The minor diastereoisomer with an equatorial pyridyl ring had low potency. Upon elongating the alkyl chain, particularly high inhibitory activity was found for the major product isomer having a C-5 octyl, coinciding with the high activity in C-3 and N-1 octyl derivatives of 1, but there was only a small difference in the activity between the enantiomers of 5-octyl-1 and activity was reduced rather than increased when octyl also replaced ethyl at C-3. The results partially support a previously described model comparing binding of androstenedione to aromatase in as much as an axial pyridyl ring is needed to mimic the axial C-19 methyl group of the steroid and bind to the heme component of the enzyme, but for the derivatives bearing a C-5 octyl, the function of the glutarimide ring seems to be simply as a spacer between the hydrophobic chain and the pyridyl ring.

3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione (rogletimide, 1) is an inhibitor of the enzyme aromatase that converts androgens into estrogens. It was developed[1-3] as an analogue of aminoglutethimide with comparable potency but having the advantages of (i) not additionally inhibiting the cholesterol side-chain cleavage enzyme, so not causing a requirement for corticosteroid replacement therapy, (ii) being devoid of sedative action, and (iii) having a more favorable metabolic profile. This compound has been entered into clinical trial for the treatment of hormone-dependent breast cancer.

In order to understand its mode of binding to aromatase and hence to be able to use it as a basis on which to rationally design improved inhibitors, it is necessary to establish its preferred conformation. Two fundamentally different conformations may be considered, these have the pyridyl ring adopting either an axial or an equatorial position (Figure 1, **1A** and **1B**, respectively). Early conformational dynamic studies had not shown a clear preference; indeed previous modeling studies for aminoglutethimide and 1 used the conformation with an equatorial aryl ring.[4,5] More recently, crystal-structure analysis showed that in the solid phase, the pyridyl ring

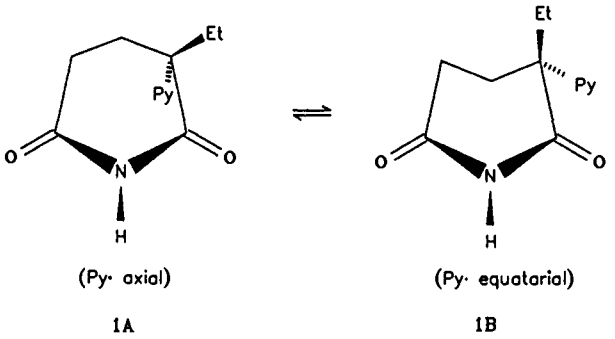


Figure 1. Conformers of 1.

of 1 adopts an axial position, and this conformation has been used to provide a model for binding to aromatase that effectively explained the aromatase inhibitory activity of N-1-alkylated derivatives.[6] The essence of this model is that the pyridyl ring occupies a position analogous to the axial C-19 methyl group of the steroid, binding to the heme while the glutarimide ring mimics the steroidal A ring, and that substitution providing an alkyl group that can occupy a postulated hydrophobic pocket in the aromatase in a position adjacent to C-4 of a bound steroid increases activity. We describe here the successful conformational analysis of 1 in solution by NMR spectroscopy which shows a preferred axial configuration of the pyridyl ring. Also, 5-alkyl derivatives, which were initially prepared to assist in the conformational study, are shown to have considerably increased potency against the enzyme, the results of which can be partially explained in terms of the more recently postulated model.[6]

† Present address: Enzymatix Ltd., Cambridge Science Park, Milton Road, Cambridge CB4 4WE, U.K.

(1) Foster, A. B.; Jarman, M.; Leung, C.-S.; Rowlands, M. G.; Taylor, G. N.; Plevey, R. G.; Sampson, P. Analogues of Aminoglutethimide: Selective Inhibition of Aromatase. *J. Med. Chem.* **1985**, *28*, 200-204.

(2) Leung, C. S.; Rowlands, M. G.; Jarman, M.; Foster, A. B.; Griggs, L. J.; Wilman, D. E. V. Analogues of 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione as Selective Inhibitors of Aromatase: Derivatives with Variable 1-Alkyl and 3-Alkyl Substituents. *J. Med. Chem.* **1987**, *30*, 1550-1554.

(3) Seago, A.; Goss, P. E.; Griggs, L. J.; Jarman, M. Pyridoglutethimide [3-ethyl-3-(4-pyridyl)piperidine-2,6-dione], an analogue of Aminoglutethimide. Metabolism and Pharmacokinetics. *Biochem. Pharmacol.* **1986**, *35*, 2911-2916.

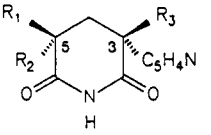
(4) Banting, L.; Smith, H. J.; James, M.; Jones, G.; Nazereth, W.; Nicholls, P. J.; Hewlins, M. J. E.; Rowlands, M. G. Structure-Activity Relationships for Non-Steroidal Inhibitors of Aromatase. *J. Enzyme Inhib.* **1988**, *2*, 215-229.

(5) Neidle, S.; Jarman, M. Influence of the Ethyl Group on the Conformational Flexibility of Aminoglutethimide. *Biochem. Pharmacol.* **1988**, *37*, 143-145.

(6) Laughton, C. A.; McKenna, R.; Neidle, S.; Jarman, M.; McCague, R.; Rowlands, M. G. Crystallographic and Molecular Modelling Studies on 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione and its Butyl Analogue, Inhibitors of Mammalian Aromatase. Comparison with Natural Substrates: Prediction of Enantioselectivity for N-Alkyl Derivatives. *J. Med. Chem.* **1990**, *33*, 2673-2679.

0022-2623/92/1835-3699$03.00/0

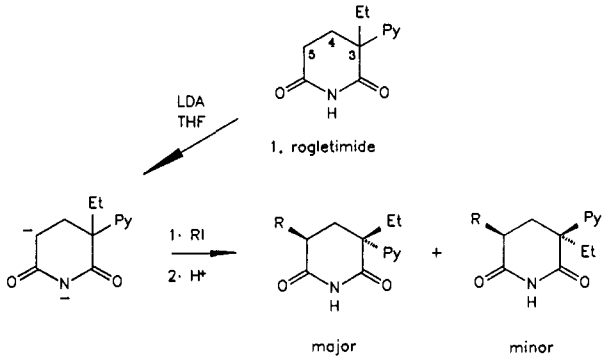
Table I. Physical Data and Aromatase Inhibitory Activity of 5-Alkyl Derivatives of 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione[a]



no.	R_1	R_2	R_3	% yield of 5-alkylation[b]	form[c]	mp, °C	analysis	aromatase inhibitory activity: IC_{50} (95% confidence limits)
1	H	H	Et		FB			63 (46–80)
2	Me	H	Et	58[d]	FB	155–157	C,H,N	23 (16–30)
3	H	Me	Et	13[d]	FB	135–137	C,H,N	93 (85–101)
4	Me	Me	Et	62[e]	FB	161–162	C,H,N	>200
5	$CH_3(CH_2)_3$	H	Et	18	HCl	194–196	C,H,N,Cl	8.7 (7.9–9.5)
6	$CH_3(CH_2)_7$	H	Et	29	HCl	212–214	C,H,N,Cl	2.8 (2.2–3.4)
7	$CH_3(CH_2)_8$	H	Et	61	HCl	180–182	C,H,N,Cl	4.1 (3.7–4.5)
8	H	H	$CH_3(CH_2)_7$		HCl			1.6 (1.2–2.0)
9	$CH_3(CH_2)_7$	H	$CH_3(CH_2)_7$	37	HCl	165–167	H,N[i]	26 (22–30)
6a	$CH_3(CH_2)_7$ (3*R*-enantiomer)	H	Et	57	FB	oil[f,g]	C,H,N	2.4 (1.6–3.2)
6b	$CH_3(CH_2)_7$ (3*S*-enantiomer)	H	Et	58	FB	oil[f,h]	C,H,N	3.0 (3.3–4.5)

[a] Results are for the racemate except where stated otherwise. Note that compound 1 and 8 have been described previously.[1,2] [b] Hexamethylphoric triamide (1 equiv) was present to prepare **7**, **9**, **6a**, and **6b**. [c] FB = free base. [d] Obtained from the same alkylation reaction. [e] Prepared from compound **2**. [f] Hydrochloride was also an oil. [g] Optical rotation $[\alpha]^{20}_D = +52.4°$ (c = 0.65 in MeOH). [h] Optical rotation $[\alpha]^{20}_D = -52.7°$ (c = 0.62 in MeOH). [i] C required, 69.2; found, 70.1.

Scheme I



Py = 4-Pyridyl

Results and Discussion

Conformational Analysis. Initial attempts to analyze the part of the proton NMR spectrum of rogletimide (1) containing the glutarimide ring protons using chloroform-*d* as solvent were unsuccessful since three of the proton signals merge, forming a complex pattern. Initial clues to solving the spectrum came from a 5-hydroxy derivative, prepared as a possible metabolite by formation of a dianion of rogletimide with 2 equiv of strong base and then treatment with the oxomolybdenum complex MoOPh. A major diastereoisomer was obtained and found from the coupling constants of its easily analyzable spectrum to have the pyridyl ring in an axial position trans to the hydroxyl function.[14] This compound was inactive against aromatase perhaps due to the high polarity of the hydroxyl function. Therefore the 5-methyl derivative was made with the hope that aromatase activity could be retained in a derivative conformationally constrained by the preference of the methyl group to adopt an equatorial position. Accordingly (Scheme I), 1 was converted into its dianion by treatment with 2 equiv of lithium diisopropylamide and then treated with iodomethane. Previously, dianion formation from glutarimide with sodamide and alkylation on carbon has been demonstrated as a route to anticonvulsant 2-benzylglutarimides.[7]

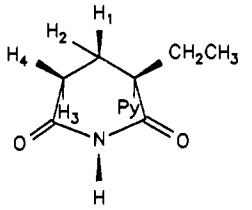
Two diastereoisomeric products were obtained (see Table I), separable on silica gel chromatography, in yields of 58% and 13%. Their proton NMR spectra could be fully analyzed, and confirmation of the analysis was made using an iterative spectrum-simulation program. Values of chemical shifts and coupling constants obtained are given in Table II. Both diastereoisomers exist in a conformation with the introduced methyl group in an equatorial position as evident from the high value (>12 Hz) of one of the coupling constants between protons on C-4 and that on C-5, showing that the C-5 proton is an axial position. Nuclear Overhauser enhancement studies were required to distinguish the relative positions of the C-3 pyridyl and ethyl groups. In the major reaction product, irradiation of the protons meta to the nitrogen atom in the pyridyl ring enhance the proton at C-5, so in this product the pyridyl ring is axial and trans to the methyl group. In the minor reaction product, both the protons at C-4 were enhanced, to a similar extent, showing here that the pyridyl ring is equatorial and therefore in a cis relationship to the C-5 methyl group.

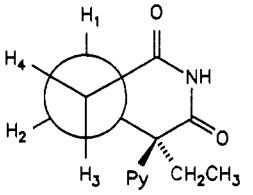
An assumption that the parent compound 1 might exhibit similar coupling constants as in the methyl-substituted derivatives was used as a basis to solve the spectrum of 1. This was still not achieved when chloroform-*d* was the solvent. In order to spread the chemical shifts of the glutarimide ring protons, the spectrum was recorded in benzene-d_6 as solvent to take advantage of "aromatic-solvent-induced shifts" (ASIS) caused by the magnetic anisotropy of the benzene ring.[8] Now, the four signals appeared as separated multiplets and in particular one of these (the C-5 equatorial proton) was at markedly higher frequency. Its appearance as a doublet of double doublets allowed three coupling constants to be estimated directly. The remainder were worked out starting from values obtained for the 5-methyl derivatives. Rogletimide 1 was insufficiently soluble in benzene-d_6 to enable satisfactory NOE data to be obtained. Solubility in pyridine-d_5 was however good. Although the ASIS shifts were reduced in this solvent, it was used for the NOE studies, and the results obtained are presented in Figure 2, which shows also the spectrum simulation (trace B)

(7) Goehring, R. R.; Greenwood, T. D.; Nwokogu, G. C.; Pisipati, J. S.; Rogers, T. G.; Wolfe, J. F. Synthesis and Anticonvulsant Activity of 2-Benzylglutarimides. *J. Med. Chem.* **1990**, *33*, 926–930.

(8) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: Oxford, 1987; pp 32–40.

Table II. Selected Proton NMR Data of Aminoglutethimide, 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione, and 5-Alkyl Derivatives





compound	solvent	chemical shifts, ppm				coupling constants, Hz						% NOE enhancements from meta protons to pyridyl-N					
		H_1	H_2	H_3	H_4	J_{12}	J_{13}	J_{14}	J_{23}	J_{24}	J_{34}	H_1	H_2	H_3	H_4	CH_2	CH_3
rogletimide, 1	$CDCl_3$	2.13	2.24	2.21	2.51	−14.1	13.4	4.4	4.9	3.1	−18.2						
rogletimide, 1	benzene-d_6	1.22	1.29	1.70	1.95	−14.2	13.4	4.1	4.6	3.4	−17.9						
rogletimide, 1	pyridine-d_5	2.20	2.32	2.42	2.68	−14.0	12.9	4.2	4.8	3.8	−18.2	nd	4.6	1.6	nd	2.2 and 1.0%	0.6
N-octyl-1	$CDCl_3$	1.96	2.06	2.11	2.41	−14.5	13.4	4.8	5.1	2.9	−18.2						
5-methyl-1 = **2**	$CDCl_3$	2.08	2.341	2.344		−14.1	13.7		4.7			nd	4.2	4.2	nd	2.1	nd
5-butyl-1 = **5**	$CDCl_3$	2.01	2.37	2.19		−14.2	13.4		4.4			nd	5.7	3.8	nd	3.0	nd
aminoglutethimide	pyridine-d_5	2.12	2.26	2.58	2.64	−14.3	13.5	4.0	5.3	1.9	−17.0	nd	5.0	1.9	nd	2.0	nd
5-methyl-1, minor isomer **3**	$CDCl_3$	2.03	2.10	2.74		−14.3	12.5		5.6								

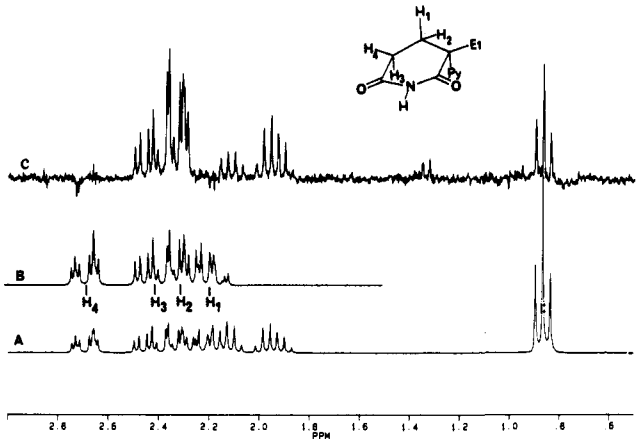


Figure 2. Part of the proton NMR spectrum of the pyridylglutarimide 1 in pyridine-d_5: A is the experimentally obtained normal spectrum, B is a computer simulation giving the assignments and data used in Table II, and C is a NOE difference spectrum after preirradiation of the pyridyl protons meta to the nitrogen atom.

that confirms the assignments. In the NOE difference spectrum obtained after preirradiation of the protons meta to nitrogen on the aromatic ring (trace C), of the glutarimide ring protons, only the equatorial C-4 proton and the axial C-5 proton were enhanced (H_2 and H_3 in the figure at 4.6% and 2.6%, respectively). Therefore the pyridyl ring must lie on the side of these protons, i.e. in an axial position. Similar analysis of aminoglutethimide, where the protons meta to the amino function were preirradiated, gave a closely analogous result, showing too that the aromatic ring lies in an axial position for this compound.

Performing the analysis in other solvents is necessary to ensure that the preference for an axial aromatic ring is not a consequence of the solvent chosen. Given the coupling constants obtained using a pyridine solution of 1, together with ^1H–^{13}C correlation data (see Experimental Section for details) that identify the individual proton chemical shifts, it was now possible to analyze the deuteriochloroform solution spectrum, and the values were similar (Table II).

The data discussed above show the preference for the aromatic ring in an axial position but do not quantify the extent of preference for this conformation. Both 5-methyl diastereoisomers have the methyl group in an equatorial position and thus differ only by the relative positions of the pyridyl and ethyl groups at C-3. Coupled with the consideration that the methyl group in its equatorial position cannot sterically conflict with either C-3 substituent, it is a reasonable assumption that the relative thermodynamic energies of these diastereoisomers should represent the difference in energy between the two conformers of 1. The 5-methyl derivatives **2** and **3** could be separately epimerized by treatment with the base 1,8-diazabicyclo[5.4.0]undec-7-ene in toluene at reflux. Either diastereoisomer led to a 7:1 mixture of compounds **2** and **3** according to NMR analysis and the equation $\Delta G = -RT \ln k_{eqm}$ gives a value of 1.5 kcal mol^{-1} as the free energy preference for an axial pyridyl ring. In comparison the difference in energy between the energy minima of the two forms was calculated[6] to be 2.5 kcal mol^{-1}.

Aromatase Inhibitory Activities. A range of compounds with differing *n*-alkyl substituents were prepared (Table I) by alkylation of the dianion from 1 in THF with the appropriate iodoalkanes. Other than with iodomethane, yields were poor unless hexamethylphosphoric triamide was present to encourage substitution over elimination of hydrogen iodide. In each case, the major isomer was isolated by chromatography on silica. A longer alkyl chain did not seem to influence the preferred conformation of the glutarimide ring substituents as determined from the ^1H NMR spectrum of the butyl derivative **5**.

Aromatase inhibitory potencies (IC_{50}) were determined as the concentration of compound required to halve the rate of tritiated water released upon aromatization of [1-^3H]androstenedione to estrone by a human placental microsome preparation.[2] In this respect it should be noted that the aromatization of androstenedione (chosen to provide the best comparison with the more recent data on related derivatives of 1) is less efficiently inhibited by 1 than is the aromatization of testosterone, for which 1 gives a value of 14 μM. The results using the present system are provided in Table I.

For the mono 5-methyl diastereoisomers **2** and **3**, it was found that the major isomer formed (**2**) is more potent then the parent compound, and the other isomer (**3**) is less potent. In **2**, the introduced methyl group, by adopting a preferred equatorial position, encourages more strongly the trans-related pyridyl to be axial whereas in **3** the methyl group tends to make the pyridyl ring equatorial. These

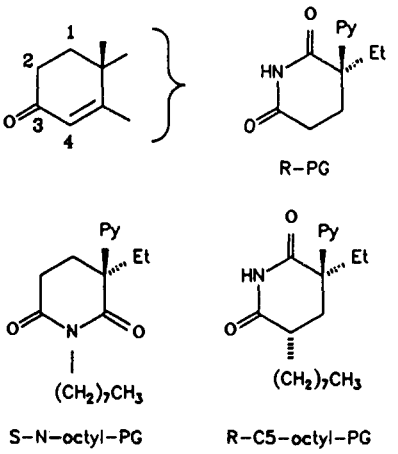


Figure 3. Proposed comparisons with androstenedione binding to aromatase.

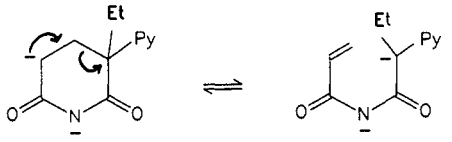


Figure 4. Potential mechanism for racemization during the alkylation reaction.

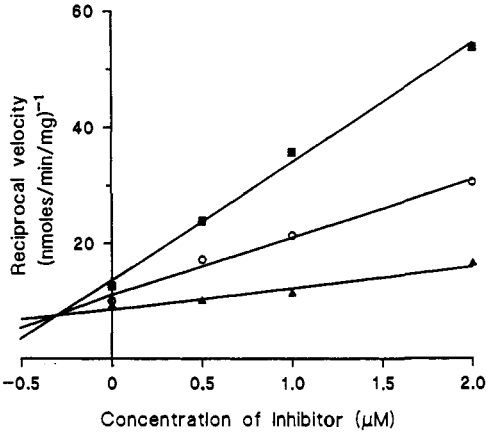


Figure 5. Dixon plot (reciprocal velocity against inhibitor concentration) of the inhibition of the aromatization of testosterone by the C-5 octyl analogue of rogletimide. The substrate concentration was ▲ = 1.5 μM, o = 0.75 μM, and ■ = 0.375 μM. Each point is the mean (±10%) of triplicate determinations.

results support the model previously put forward[6] that the pyridine ring needs to be axial to mimic the C-19 methyl group of the steroid, and so bind to the heme. The 5,5-dialkyl compound **4** prepared by further methylation of **2** lacked activity, indicating that the enzyme pocket cannot accommodate the spacial bulk generated by a quaternary center at C-5, or that the requirement for an axial methyl group influences the configuration at C-3 by making the pyridyl less able to occupy an adjacent axial position.

Upon proceeding to longer alkyl chains, activity increases before decreasing at more than eight carbon atoms. The 5-octyl derivative is some 20-fold more potent than the parent compound, paralleling high activity in the C-3 octyl (in place of C-3 ethyl) and N-1 octyl analogues, both of which are 30-fold more potent than 1.[2] Kinetic analysis of the 5-octyl analogue **6**, using the Dixon plot, demonstrated that the inhibition was of a competitive nature (Figure 5); a similar result was observed for rogletimide and C-3 octyl and N-1 octyl derivatives.[1,2] Inhibitor constants (K_i values) for **6** of 0.26 and 0.18 μM were obtained for the aromatization of androstenedione and testosterone, respectively. The corresponding values for rogletimide were 14 and 1.1 μM.[2]

In order for the 5-alkyl derivatives to be used to provide meaningful three-dimensional structure–activity data, it is necessary to consider the separate enantiomers. In the recent modeling studies[6] the pyridylglutarimides have been proposed to mimic the A-ring part of the natural substrate as shown in Figure 3. Here *R*-PG is considered to be a better enzyme inhibitor than the *S*-enantiomer since a –CONH– unit is better tolerated at a position corresponding to C-1–C-2 of the steroid than at C-5–C-4. This corresponds with recent findings with human aromatase where a glutamate residue in the vicinity of C-2 of the bound steroid[9] could form a hydrogen bond to the NH functionality of **1** only in the *R*-enantiomer. In the case of N-alkylation, where such a hydrogen-bond is prevented, the preferred enantiomer becomes *S*, which could be explained by the postulated presence of a hydrophobic pocket in the enzyme at a position corresponding to C-4 of the steroid. Indeed, (*R*)-*N*-octyl-**1** has been shown to be inactive.[6] On the basis of these results and the modeling, it would be predicted that, for C-5 alkylated derivatives of **1**, activity would reside solely in the 3*R*-enantiomer once the chain had reached sufficient length, as only in this enantiomer might the alkyl chain project into the putative hydrophobic pocket while the glutarimide ring occupies a position corresponding to that proposed for the *R*-enantiomer of **1**. The separate enantiomers were prepared for the C-5-octyl compound **6** by starting with the separate enantiomers of **1** that were available in small quantity from a chemical synthesis using a camphor-derived auxiliary.[10] Surprisingly, although the *R*-enantiomer was more potent, it was only just so. The *S*-enantiomer had a markedly high activity not explainable on the basis of the model. One consideration was that the alkylation might have been accompanied by partial racemization; a possible mechanism could be via a reversible ring opening reaction that would momentarily leave charge in a highly stabilized position α to the pyridine ring (Figure 4). To test this possibility in the absence of an enantiomeric excess assay for **6**, a sample of optically pure **1** was treated with 2 equiv of base to form the dianion and then quenched simply with water. The resulting sample of **1** was found by analysis by chiral-column HPLC[11] to have not racemized, and therefore the activity of the (*S*)-5-octyl derivative **6b** is real. These results indicate the presence of more than one binding mode to the aromatase enzyme in which the glutarimide may act as a spacer between the pyridine ring that binds to the heme and the alkyl chain that binds into a hydrophobic pocket. In this respect it is worthwhile to compare a series of alkyl 4-pyridylacetates which are potent aromatase inhibitors having a similar molecular arrangement.[12] Presumably in certain derivatives such as (*S*)-*N*-octyl-**1** the arrangement of atoms in the glutarimide ring is such as to prevent

(9) Graham-Lorence, S.; Khalil, M. W.; Lorence, M. C.; Mendelson, C. R.; Simpson, E. R. Structure-Function Relationships of Human Aromatase Cytochrome P-450 using Molecular Modelling and Site-directed Mutagenesis. *J. Biol. Chem.* **1991**, *266*, 11939–11946.

(10) McCague, R.; Jarman, M.; Rowlands, M. G.; Mann, J.; Thickitt, C. P.; Clissold, D. W.; Neidle, S.; Webster, G. Synthesis of the Aromatase Inhibitor, 4-Ethyl-3-(4-pyridyl)piperidine-2,6-dione and its Enantiomers. *J. Chem. Soc. Perkin Trans. 1* **1989**, 196–198.

(11) A Daicel Chiracel-OJ column was used.

(12) McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. Inhibition of Enzymes of Estrogen and Androgen Biosynthesis by Esters of 4-Pyridylacetic Acid. *J. Med. Chem.*, **1990**, *33*, 3050–3055.

this type of binding although without knowing the structure of the enzyme cavity it is not possible to explain why this might be.

A further interesting result concerns the derivative with octyl at both C-3 and C-9 (**9**). Replacement of the C-3 ethyl group of rogletimide **1** by octyl[2] gave a compound (**8**) with over 20-fold greater potency against aromatase. On the basis of previous modeling, alkyl chains at C-3 and C-5 would be predicted to perform distinct binding functions, the former mimicking the B-/C-/D-rings of the steroid and the latter entering the hydrophobic pocket discussed above. Thus their effects should be additive and lead to an especially potent compound. However, the dioctyl compound **9** was 6-fold *less* potent than that with a shorter chain (ethyl) at C-3 and over 10-fold less potent than that with no C-5 substituent (i.e. **8**). This might indicate that the two long chains oppose in their action by competing for the same binding site. Another possibility though is that a certain narrow range of lipophilicity is necessary for optimal activity and compound **9** is too lipophilic.

Conclusions

The conformational NMR study has given valuable insight into the preferred conformation of the drug in solution; in this case showing the preferred axial configuration of the aromatic ring. In as much as this is a feature of aminoglutethimide as well as **1**, it may be a relevant factor governing the sedative action of glutethimide and related compounds. It is suggested that such NMR study be carried out wherever practical in addition to any crystal-structure analysis aimed at rationalizing structure–activity relationships.

The results of the aromatase inhibition determinations only partially support the previously forwarded model for binding to aromatase, indicating that there is a likelihood of more than one possible mode of binding in aromatase inhibitors that bear the pyridyl ring. Thus for compound **1** itself, there is a mode in which the glutarimide ring functionality is important for binding, but for the C-5 alkylated derivatives, the effect of the alkyl function can be paramount and the glutarimide ring acts simply as a spacer between the pyridyl ring that binds to the heme and the alkyl function that binds into a hydrophobic pocket in the enzyme.

The study has raised areas where further experimentation is warranted to rationalize the structure–activity relationships, in particular with respect to the curiously low activity of the 3,5-dioctyl compound and thus whether there is an overall lipophilicity requirement for aromatase inhibitors, perhaps to a range about that of the natural steroidal substrates.[2] However, there is a need to consider the value of such work against efforts to establish the 3D structure of the active site of aromatase directly, progress toward which has been made in reported literature.[9,13]

Experimental Section

Chemical Methods. Standard Procedures. ^{1}H NMR spectra (250 MHz) were recorded on a Bruker AC250 spectrometer. The Bruker PANIC software was used to provide simulated spectra. Chromatography refers to column chromatography on silica gel with the solvent indicated applied at a positive pressure of 0.5 atm. Melting points were determined with a Kofler hot-stage and are uncorrected. THF refers to anhydrous tetrahydrofuran. See Table II for selected ^{1}H NMR data of specific compounds and Table I for other physical data.

Diastereoisomers of 3-Ethyl-5-methyl-3-(4-pyridyl)piperidine-2,6-dione (2 and 3). To a stirred solution of racemic rogletimide (**1**, 1.86 g, 8.54 mmol) in THF (20 mL) under argon at ambient temperature was added a solution of lithium diisopropylamide in hexane (1.5 M, Aldrich Chemical Co.; 12.5 mL, 18.8 mmol). After 15 min, iodomethane (1.45 g, 10.2 mmol) in THF (5 mL) was added and after 1 h the mixture was poured into saturated aqueous $NaHCO_3$ (50 mL), and the products were extracted with dichloromethane (3 × 60 mL). The extracts were dried (Na_2SO_4) and concentrated. Chromatography of the residue, eluting with 10:10:1 diethyl ether–petroleum ether (bp 60–80 °C)–triethylamine, gave (i) [3*R**,5*S**]-3-ethyl-5-methyl-3-(4-pyridyl)piperidine-2,6-dione, (**2**) as crystals (1.16 g, 58% yield) [Mp 155–157 °C (from toluene); NMR δ_H ($CDCl_3$) 0.89 (t, J = 7.4 Hz, 3, CH_3CH_2), 1.28 (d, J = 6.5 Hz, 3,5-CH_3), 1.90 (dq, J = 15.7 Hz, 7.4 Hz, 1, one of CH_3CH_2), 2.03 (m, **2**, one of CH_3CH_2 and H-4 trans to pyridyl), 2.26–2.42 (m, 2, H-4 cis to pyridyl and H-5), 7.21–7.24 (m, 2, pyridyl meta to N), 8.12 (br, 1, N*H*), 8.60–8.63 (m, 2, pyridyl ortho to N). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N] and (ii) the [3*R**,5*R**]-diastereoisomer **3** as crystals (0.26 g, 13% yield) [Mp 135–137 °C (from toluene); NMR δ_H ($CDCl_3$) 0.79 (t, J = 7.4 Hz, 3, CH_3CH_2), 1.24 (d, J = 6.9 Hz, 3,5-CH_3), 2.0–2.2 (m, 4, CH_3CH_2 and 2 × H-4), 2.74 (m, 1 H-5), 7.30 (m, 2, pyridyl meta to N), 8.57 (m, 2, pyridyl ortho to N). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N].

Epimerization of 3-Ethyl-5-methyl-3-(4-pyridyl)piperidine-2,6-dione Diastereoisomers. A solution of **2** or **3** in toluene (3 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (0.1 mL) and the mixture refluxed for 45 min. The mixture was then concentrated under vacuum and the residue analyzed by proton NMR spectroscopy, where the isomers differ by the position of the pyridyl-ring protons. The major diastereoisomer from the preparation **2** and the minor diastereoisomer **3** gave a product mixture with compounds **2** and **3** in the ratios 6.2:1 and 7.3:1, respectively.

3-Ethyl-5,5-dimethyl-3-(4-pyridyl)piperidine-2,6-dione (4). The procedure to prepare **2** and **3** was repeated but using the monomethyl compound **2** as the starting material, 2.2 equiv, of lithium diisopropylomide, and 1.2 equiv of iodomethane. The free base was obtained as crystals in 62% yield: mp 161–162 °C (from toluene) NMR δ_H ($CDCl_3$) 0.72 (s, 3, 5-CH_3 cis to pyridyl), 0.91 (t, J = 7.3 Hz, CH_2CH_3), 1.28 (s, 3,5-CH_3 trans to pyridyl), 1.82 (dq, J = 14.1, 7.3 Hz, one of CH_3CH_2), 2.02 (dq, J = 14.1, 7.3 Hz, one of CH_3CH_2), 2.26 (s, 2,4-CH_2), 7.24 (m, 2, pyridyl meta to N), 8.31 (br, 1 N*H*), 8.58 (m, 2, pyridyl ortho to N). Relative assignments of the 5-CH_3 groups were made by an NOE experiment where preirradiation of the signal at δ 0.72 was required to give an enhancement of the protons meta to *N* on the pyridyl ring. Anal. ($C_{14}H_{18}N_2O_2$) C, H, N.

[3*R*,5*S*]-3-Ethyl-5-octyl-3-(4-pyridyl)piperidine-2,6-dione (6a). A stirred solution of the *R*-(+)-enantiomer of rogletimide (34.8 mg, 0.16 mmol) in THF (1.5 mL) under argon at 20 °C was treated with a solution of lithium diisopropylamide in cyclohexane (1.5 M; 0.23 mL, 0.35 mmol). After 15 min, a mixture of iodooctane (45.8 mg, 0.19 mmol) and hexamethylphosphoric triamide (56.9 mg, 0.32 mmol) in THF (0.5 mL) was added. After 1.5 h, the mixture was worked up as described in the preparation of **2** and **3**. Chromatography used elution with 1:1 diethyl ether–petroleum ether (bp 60–80 °C) containing 5% triethylamine to give **6a** as an oil (30:1 mg, 57% yield); see Table I for physical properties; compounds **6b**, **7**, and **9** were prepared in the same manner using appropriate starting materials. For compound **9** (as free base): NMR δ_H ($CDCl_3$) 0.83–0.94 (m, 6, 2 × CH_3), 1.17–1.40 (m, 24, octyl CH_2), 1.40–1.58 (m, 2, CH_2 of 5-octyl), 1.68–1.86 (m, 1, one of CH_2 of 3-octyl), 1.88–2.06 (m, 1, one of CH_2 of 3-octyl), 2.04 (t, J = 13.4 Hz, H-4 trans to pyridyl), 2.12–2.28 (m, 1, H-5), 2.42 (dd, J = 2.8, 13.5 Hz, H-4 trans to pyridyl), 2.12–2.28 (m, 1, H-5), 2.42 (dd, J = 3.8, 13.5 Hz, H-4 cis to pyridyl), 7.19–7.23 (m, 2, pyridyl meta to N), 8.00 (br, 1, N*H*), 8.60–8.63 (m, 2, pyridyl ortho to N).

(13) Kellis, J. T.; Childers, W. E.; Robinson, C. H.; Vickery, L. E. Inhibition of Aromatase Cytochrome P-450 by 10-oxirane and 10-Thiirane Substituted Androgens. *J. Biol. Chem.* **1987**, *262*, 4421–4426.

(14) A description of this compound and its NMR spectral data are being submitted separately as part of a metabolism study of rogletimide.

Determination of Proton NMR Chemical Shift Positions of Rogletimide (1) Using ^1H–^{13}C 2D-Correlation Spectroscopy. Correlation spectroscopy was carried out using the following parameters[1] on a 10% solution of rogletimide in $CDCl_3$: ^1H data points = 1024 over the range δ 0–2.85 and ^{13}C data points = 2048 over the range δ 0–41; data collected by 512 increments of the delay between the ^1H and ^{13}C pulses, with decoupling of the proton signals from the carbon signals. Under these conditions projections of the carbon signals in the proton domain give a singlet, except for CH_2 groups where an AB quartet is observed. In the region of interest the following signals were observed:

^{13}C domain	^1H domain	assignment
δ 8.8	singlet δ 0.72	CH_3CH_2
δ 26.4	AB q for chem shifts at δ 2.13, 2.24	4-CH_2
δ 28.9	AB q for chem shifts at δ 2.21, 2.51	5-CH_2
δ 32.3	AB q for chem shifts at δ 1.76, 1.90	CH_3CH_2

The positions thus obtained for proton chemical shifts were combined with the coupling constants obtained using pyridine-d_5 as solvent (see Figure 2) and iterative spectrum simulation used to provide the data are presented Table II (first row).

Determination of Aromatase Inhibition. The reagents and conditions for the assay of aromatase activity are those previously described.[1,2] The enzyme was obtained from the microsomal fraction of human placenta. Activity was monitored by measuring the tritiated water released during the conversion of [1β-^3H]-labeled androstenedione into estrogens. Separation of the tritiated water from the steroid was achieved by addition of activated charcoal followed by centrifugation. The K_m value for androstenedione is 0.038 μM; therefore the IC_{50} value is the concentration of inhibitor required to reduce the enzyme activity to 50% of the control value at a substrate concentration of 0.38 μM. The results are expressed as IC_{50} values with 95% confidence limits. Kinetic analysis was carried out using [1β-^3H]androstenedione and [1β,2β-^3H]testosterone (K_m = 0.13 μM) as substrates. The inhibitor constants (K_i values) were obtained from Dixon plots of reciprocal velocity against the concentration of inhibitor with the method of least-squares analysis being used to obtain a linear fit to the data.

Acknowledgment. This investigation was supported by a grant to the Institute of Cancer Research (Royal Cancer Hospital) from the Cancer Research Campaign and Medical Research Council. We thank Professor M. Jarman for encouraging this study.