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1-ALKYL ANALOGUES OF AMINOGLUTETHIMIDE

COMPARATIVE INHIBITION OF CHOLESTEROL SIDE CHAIN CLEAVAGE AND AROMATASE AND METABOLISM OF THE 1-PROPYL DERIVATIVE, A HIGHLY SELECTIVE INHIBITOR OF AROMATASE

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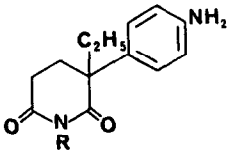
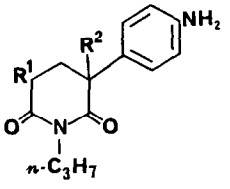
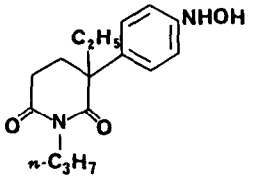
Abstract—A homologous series of 1-*n*-alkyl-derivatives of aminoglutethimide (AG) has been synthesised and tested for inhibitory activity towards the cholesterol side chain cleavage enzyme (desmolase) from bovine adrenals and human placental aromatase in an attempt to find a selective aromatase inhibitor. Activity against desmolase declined from an IC_{50} value of 30 μM for the parent drug to 220 μM for the *n*-propyl derivative but increased again thereafter. Against aromatase, activity was least for the methyl and ethyl derivatives and highest (IC_{50} = 1.6 μM) for the hexyl and octyl analogues. The optimal ratio IC_{50} (desmolase): IC_{50} aromatase of 44 was found for the *n*-propyl derivative, which was therefore selected for preliminary metabolism studies using rat and mouse liver microsomes and hepatocytes and in these species *in vivo*. There were parallels with AG, most notably in the analogous formation from the *n*-propyl derivative of an arylhydroxylamine in the mouse.

Aminoglutethimide [3-ethyl-3-(4-aminophenyl)-piperidine-2,6-dione] [1] is established in clinical use for the treatment of hormone-dependent breast cancer in post-menopausal women [1]. Analogues in which the 3-ethyl moiety is replaced by other alkyl substituents (C-alkyl series) [2] and some having N-alkyl substituents in the glutarimide (piperidinedione) ring (*N*-alkyl series) [3] have been described. Compound (**1**) acts principally by inhibiting the aromatase enzyme complex which converts the androgens androstenedione and testosterone into the oestrogens oestrone and oestradiol [3] but a major and unwanted additional action is inhibition of the cholesterol side chain cleavage enzyme, desmolase [4]. Consequently corticosteroids are depleted and replacement therapy must be given to patients treated with (**1**). The segregation of desmolase and aromatase inhibitory activities and the design of selective inhibitors of aromatase has therefore become a major goal in the therapy of hormone-dependent breast cancer. Examples of such "pure" aromatase inhibitors are the steroidal drug 4-hydroxyandrostenedione [5] and the aminoglutethimide analogue "pyridoglutethimide" [3-ethyl-3-(4-pyridyl)piperidine-2,6-dione] [6].



R	R	R
(**1**) H	(**5**) n-C_4H_9	(**9**) n-C_8H_{17}
(**2**) CH_3	(**6**) n-C_5H_{11}	(**10**) $CH_2CH_2CH_2OH$
(**3**) C_2H_5	(**7**) n-C_6H_{13}	(**11**) CH_2COCH_3
(**4**) n-C_3H_7	(**8**) n-C_7H_{15}	(**12**) $CH_2CH(OH)CH_3$





(**13**) R^1 = OH, R^2 = C_2H_5
(**15**) R^1 = R^2 = H
(**16**) R^1 = H, R^2 = $CH(OH)CH_3$
(**17**) R^1 = H, R^2 = CH_2CH_2OH

(**14**)

Here, we report the first such study of 1-alkyl derivatives (**2–9**), together with some investigations into the metabolism of one such N-alkyl derivative, the N-propyl analogue (**4**), found to have the most favourable ratio between aromatase and desmolase inhibitory activity.

MATERIALS AND METHODS

Animals. Male Wistar rats (200–250 g) and C57B1/6 mice (25–30 g) were obtained from Olac (Oxon, U.K.) and maintained on standard laboratory diets.

Enzyme assays. The *in vitro* assays for the determination of the aromatase and desmolase inhibitory activity of these compounds were carried out as

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described previously [7] using a substrate concentration of 1.5 μM for aromatase ([^{3}H]-testosterone) and 14 μM for desmolase ([^{14}C]-cholesterol).

Chromatography. Silica gel TLC was carried out on 5 × 20 cm or 20 × 20 cm (0.25 mm) silica gel 60 F_{254} (Merck) glass plates with chloroform–methanol (19:1, v/v) as the developing solvent (Solvent I). Reversed phase TLC (RP-TLC) employed 5 × 20 cm (0.20 mm) Whatman $KC_{18}F$ glass plates with acetonitrile–5% aqueous sodium chloride (3:7, v/v) as the solvent (Solvent II). UV absorbing compounds were detected using a Hanovia Chromatolite at 254 nm. R_f values refer to TLC on silica gel unless otherwise indicated.

Nuclear magnetic resonance spectroscopy. ^{1}H NMR spectra were determined either at 60 MHz using a Perkin Elmer R12 instrument [compounds (**5**) and (**6**)] or at 250 MHz using a Bruker AC 250 instrument. The solvent was $CDCl_3$ unless stated otherwise and the internal standard was tetramethylsilane.

Mass spectrometry. Samples were removed from developed TLC plates and eluted with methanol, or collected from the HPLC column, and concentrated on the direct insertion probe. Mass spectra were determined in the electron impact (EI) mode (ionising voltage 70 eV, trap current 100 μA) at an ion-source temperature of 180° using a VG 7070H spectrometer and 2235 data system. Accurate mass measurements, by peak matching at a resolution of 2500 gave results accurate to between 4 and 8 ppm.

Deuteroacetylation of metabolites for mass spectral analysis. In vitro metabolism extracts and *in vivo* urine extracts (mouse) in dichloromethane (20 ml) were treated with d_6-acetic anhydride (50 μl) and immediately concentrated on a rotary film evaporator at 35° as described previously [8]. The method converts arylamines and their N-acetyl derivatives having corresponding substitutions elsewhere in the molecule into a chromatographically indistinguishable mixture of N-trideuteroacetyl and N-acetyl derivatives, thus simplifying the analysis whilst enabling the free amine and its N-acetyl derivative to be discriminated by mass spectrometry. Labile N-arylhydroxylamines are also thereby converted into stable N,O-*bis* (trideuteroacetyl) derivatives.

In vivo *metabolism studies.* Animals received 3-(4-aminophenyl)-3-ethyl-1-propylpiperidine-2,6-dione [1-propylaminoglutethimide, (**4**)] orally in corn oil at 24 hr intervals (20 mg/kg; 10 mg/ml); controls received corn oil alone. The rat was dosed daily for two days and mice daily for three days. The animals were placed in metabowls (one rat per cage; six mice per cage) and the urine and faeces collected over 24 hr periods; samples were stored at −20° prior to extraction. Substrate and metabolites were extracted from the urine samples (8–10 ml, rat; 8 ml/6 mice) with dichloromethane (2 vol., ×3) at physiological pH, the organic extract dried (anhydrous Na_2SO_4), concentrated and the residue taken up in dichloromethane (200 μl) for TLC examination. Rat urine extracts were run on RP-TLC (Solvent II) and the deuteroacetylated mouse urine extracts on silica TLC (Solvent I). UV absorbing material not present in control urine extracts was removed and eluted for mass spectrometry.

In vitro *metabolism studies.* Washed hepatic microsomes were prepared from sodium-phenobarbital pretreated male rats and male C57B1/6 mice, as described previously [9]. Incubations were carried out in stoppered 25 ml Ehrlenmeyer flasks at 37°, initially for 60 min; microsomes (1 ml, equivalent to 1 g liver, 18.0 mg protein/ml; 0.95 nmol $CytP_{450}$/mg protein) were incubated with substrate (0.5 mg in 50 μl methanol) in the absence and presence of an NADPH-regenerating system, as described previously [9]. A cofactor supplementation study [10], was also carried out using rat hepatic microsomes. In controls, substrate was added at the end of the incubation period. The reaction was terminated by placing the flasks on ice, and following addition of sodium chloride (1 g), substrate and metabolites were extracted with ethyl acetate (3 × 7 ml). The combined extracts were concentrated to dryness and the residues dissolved in dichloromethane and deuteroacetylated and examined by TLC as described above.

SYNTHESIS

The methyl (**2**), ethyl (**3**), and *n*-propyl (**4**) derivatives are reported [3]. The following method was used for the synthesis of homologous 1-*n-alkyl*-3-(4-*aminophenyl*)-3-*ethylpiperidine*-2,6-*diones* having substituents butyl (**5**)–octyl (**9**). Aminoglutethimide [(**1**), 1 mmole, 232 mg)], caesium carbonate (1 g) and the corresponding alkyl bromide (excess, 2 mmole) in acetonitrile (5 ml) were heated under reflux for 30 min. Following filtration and concentration on a rotary film evaporator the crude product was chromatographed on a silica column (2.9 × 16 cm, Merck 9385, $CHCl_3$). The product was located in eluant fractions by TLC and concentrated. Products were generally yellow oils and were characterised by their molecular ions in their electron-impact mass spectra (all compounds) and by ^{1}H NMR spectroscopy (butyl and pentyl derivatives). NMR: butyl derivative (**5**); δ 0.83 (t, $C\underline{H}_3CH_2$), 0.92 (t, $C\underline{H}_3(CH_2)_3$), 1.10–2.75 (m, $CH_3C\underline{H}_2$, H-4, H-5, $CH_3C\underline{H}_2C\underline{H}_2$), 3.68 (br s, exchange D_2O, NH_2), 3.83 (t, $CH_3(CH_2)_2C\underline{H}_2$), 6.8 (ABq, arom. H): pentyl derivative (**6**); δ 0.84 (t, $C\underline{H}_3CH_2$), 0.89 (t, $C\underline{H}_3(CH_2)_4$), 1.07–2.71 (m, $CH_3C\underline{H}_2$, H-4, H-5, $CH_3C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.68 (br s, exchange D_2O, NH_2), 3.83 (t, $CH_3(CH_2)_3C\underline{H}_2$), 6.80 (ABq, arom. H). The hexyl derivative (**7**) was obtained as colourless crystals from cyclohexane, m.p. 57–58°; Anal. Calc. for $C_{18}H_{26}N_2O_2$: C, 72.12; H, 8.92; N, 8.85, Found: C, 72.17; H, 9.16; N, 8.90%. To obtain 3-(4-aminophenyl)-1-(3-hydroxypropyl)-3-ethylpiperidine-2,6-dione (**10**) the reaction was carried out using 1-bromopropan-3-ol: ^{1}H NMR spectrum; δ 0.84 (t, *J* 7.4 Hz, $C\underline{H}_3CH_2$), 1.77–2.12 (m, $CH_3C\underline{H}_2$ and $CH_2C\underline{H}_2CH_2$), 2.14–2.30 (m, H-4), 2.57–2.67 (m, H-5), 3.36 (dt, one of $NC\underline{H}_2$), 3.47 (dt, other of $NC\underline{H}_2$), 3.97 (dt, $C\underline{H}_2OH$), 6.80 (ABq, *J* 6.9 Hz, arom. H-3, H-5), 7.10 (ABq, arom. H-2, H-6), mass spectrum M^+ at *m/z* 290 (100%; accurate mass measurement, calc. for $C_{16}H_{22}N_2O_3$: 290.1630, Found: 290.1650).

3-(4-*Aminophenyl*)-3-*ethyl*-1-(2-*oxopropyl*)*piperidine*-2,6-*dione* (**11**)

A solution of (**1**) (0.464 g, 2 mmol) in acetonitrile

Table 1. Inhibition of bovine adrenal desmolase and human placental aromatase by 1-alkyl-3-(4-aminophenyl)-3-ethylpiperidine-2,6-diones

Compound	IC_{50}* (μM) Aromatase	IC_{50} (μM) Desmolase	Compound	IC_{50} (μM) Aromatase	IC_{50} (μM) Desmolase
(1)	7	30	(6)	6.6	73
(2)	32	40	(7)	1.6	20
(3)	28	92	(8)	2.4	27
(4)	5	220	(9)	1.6	19
(5)	2.8	114	(10)	None	†
			(11)	15	n.d.

* IC_{50} is the concentration of inhibitor in μM required to reduce the activity of the enzyme to 50% of the control value. "None" signifies no inhibition at maximum concentration tested: n.d. signifies not determined.

† Compound (**10**) gave 20% inhibition of desmolase activity at a concentration of 50 μg/ml.

(5 ml) containing caesium carbonate (1 g) and chloroacetone (Aldrich, technical grade, 90%; 200 μl, 232 mg, *ca.* 2.5 mmol) was heated under reflux for 30 min, treated again with chloroacetone (200 μl) and heated for a further 30 min. The mixture was concentrated, the residue triturated with dichloromethane (40 ml), the filtered organic phase concentrated and the residue crystallised from ethanol to give the title compound (445 mg, 77%). Recrystallisation from ethyl acetate gave almost white crystals, m.p. 147°, NMR: δ 0.85 (t, *J* 7.3 Hz, $C\underline{H}_3CH_2$), 1.87 (dq, one of $CH_3C\underline{H}_2$), 2.03 (dq, other of $CH_3C\underline{H}_2$), 2.23 (s, $C\underline{H}_3CO$), 2.2–2.4 (m, H-4), 2.5–2.7 (m, H-5), 3.67 (s, NH_2), 4.63 (s, one of $C\underline{H}_2N$), 4.65 (s, other of $C\underline{H}_2N$), 6.65 (ABq, *J* 8.7 Hz, arom. H-2, H-6), 7.04 (ABq, arom. H-3, H-5); mass spectrum M^+ at *m/z* 288 (100%; accurate mass measurement, calc. for $C_{16}H_{20}N_2O_3$: 288.1474. Found: 288.1466; Anal. Calc. for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.68; H, 7.11; N, 9.88%).

3-(4-*Aminophenyl*)-3-*ethyl*-1-(2-*hydroxypropyl*)*piperidine*-2,6-*dione* (**12**)

A solution of the foregoing 2-oxopropyl derivative (300 mg) in ethyl acetate (20 ml) was stirred with Adams platinum oxide catalyst (500 mg; n.b., 10% Pd/C was ineffective) in an atmosphere of hydrogen for 5 days at room temperature. Reduction was still incomplete: the product was separated from residual starting material by preparative TLC on Kieselgel 60 (two 20 × 20 cm plates, 2 mm layer) by two developments in dichloromethane–ethanol, 19:1. The lower R_f component was eluted with this solvent (50 ml) to give the title compound as a colourless oil (100 mg), NMR: δ 0.84 (t, *J* 7.4 Hz, $C\underline{H}_3CH_2$), 1.22 (dd, $C\underline{H}_3CHOH$), 1.87 (ddq, one of $CH_2C\underline{H}_2CH_3$), 2.00 (dq, other of $CH_2C\underline{H}_2CH_3$), 2.10–2.30 (m, H-4), 2.46–2.73 (m, H-5), 3.69 (s, NH_2), 3.83–4.01 (m, $NC\underline{H}_2C\underline{H}OH$), 6.80 (ABq, *J* 7.35 Hz, H-2, H-6), 7.01 (ABq, H-3, H-5); mass spectrum M^+ at *m/z* 290 (100%; accurate mass measurement, calc. for $C_{16}H_{22}N_2O_3$: 290.1630. Found: 290.1650), 261 (96), 233 (47), 203 (8), 187 (35), 160 (31), 132 (65).

Samples of N-acetyl-(**10**), -(**11**) and -(**12**) were prepared by dissolution of *ca.* 1 mg of each in dichloromethane (0.5 ml), addition of acetic anhydride (50 μl) and concentration to dryness after 5 min.

3-(4-*Acetylaminophenyl*)-3-*ethyl*-5-*hydroxy*-1-*propylpiperidine*-2,6-*dione* [*N-acetyl*-(13)]

The title compound was prepared on a small scale suitable for mass spectrometry by stirring 3-(4-acetylaminophenyl)-3-ethyl-5-hydroxypiperidine-2,6-dione [11] (1 mg) in acetonitrile (0.1 ml) containing *n*-propyl bromide (10 μl) and caesium carbonate (10 mg) for 16 hr. TLC (chloroform–methanol, 9:1) showed complete conversion into a product of higher R_f value which on elution gave NMR and mass spectra consistent with the proposed structure: ^1H NMR spectrum (solvent d_6DMSO); δ 0.73 (t, *J* 7.3 Hz, propyl CH_3), 0.76 (t, *J* 7.3 Hz, ethyl CH_3), 1.30 (sextet, $J_{2,1}$ and $J_{2,3}$ 7.3 Hz, propyl $C\underline{H}_2CH_3$), 1.89 (dq, ethyl $C\underline{H}_2CH_3$), 2.20 (s, $C\underline{H}_3CO$), 2.63 (dd, $J_{4,4}$, 10.7 Hz, $J_{4,5}$ 6.1 Hz, one of H-4), 2.73 (dd, $J_{4,5}$, 8.0 Hz, other of H-4), 2.93 (dq, $NC\underline{H}_2$), 4.90 (dd, H-5), 7.33 (ABq, *J* 8.8 Hz, arom. H-2, H-6), 7.51 (ABq, H-3, H-5), 9.78 (s, NH); mass spectrum M^+ at *m/z* 332 (100%; accurate mass measurement, calc. for $C_{18}H_{24}N_2O_4$ 332.1732, Found 332.1763), 303 (38), 290 (15), 261 (18), 218 (37), 43 (64).

RESULTS

The *in vitro* aromatase and desmolase inhibitory activities for the series of 1-*n*-alkylaminoglutethimide derivatives presently described are given in Table 1; inhibitory activity is expressed as IC_{50} values.

Reversed-phase TLC of extracts from the urine of a rat dosed with (**4**) revealed unchanged substrate (R_f 0.03) together with several UV absorbing bands not present in extracts of urine from untreated rats. Two products were identified as the N-acetyl derivatives of (**12**) (R_f 0.13) and the corresponding ketone (**11**) (R_f 0.08) by comparison of their mass spectra with those of synthesised material (see Synthesis above). Principal and structurally diagnostic ions of these and other metabolites described here are listed in Table 2. On incubation of (**4**) with a phenobarbital-induced rat hepatic microsomal system the substrate was rapidly N-acetylated, independent of an NADPH-regenerating system. Following incubation for 60 min in the presence of an NADPH-regenerating system there was a reduction in the proportion of metabolically N-acetylated product

Table 2. Major ions in the mass spectra of metabolites of 1-propylaminoglutethimide (**4**) after deuteroacetylation

Compound*	Major or structurally diagnostic ions only are given. Relative abundance (%) and structural assignments are given in parenthesis ( ) and, where available, corresponding values for deuteroacetylated variants [ ]
(**10**)†	332 (79, M^+), 303 (25, $M^+ - C_2H_5$), 275 (50), 261 (16), 229 (22), 203 (36), 189 (100)
(**11**)†	330 (100, M^+), 301 (41, $M^+ - C_2H_5$), 288 (28, $M - CH_2CO$), 273 (31), 259 (67), 230 (55)
(**12**)	332 (29, M^+), 288 (77), 275 (51), 259 (20), 231 (31), 203 (23), 189 (62), 43 (100, CH_3CO^+)
(**13**) + (**15**) or (**16**)	332 (16, M^+) and 335 [10], 288 (37) and 291 [31], 246 (24), 218 (13), 161 (50) and 164 [34], 119 (77), 43 (100, CH_3CO^+) and 46 [92]
(**14**)	380 [3, M^+], 336 [59, $M^+ - CD_2CO$], 292 [59, $M^+ - 2CD_2CO$], 187 [69], 145 [100], 46 [54, CD_3CO^+]
(**15**) or (**16**)	288 (48, M^+) and 291 [23], 246 (22, $M^+ - CH_2CO$), 161 (45) and 164 [23], 43 (100, CH_3CO^+) and 46 [59]
(**17**)	332 (21, M^+) and 335 [4], 314 (39, $M^+ - H_2O$), and 317 [9], 285 (56) and 288 [18], 229 (46), 187 (63), 172 (35), 130 (44), 43 (100, CH_3CO^+) and 46 [32]
Unidentified mouse urinary metabolite	335 [28, M^+, CD_3CO deriv], 291 [48, $M^+ - CD_2CO$], 262 [33], 43 [100]

* Formula numbers refer to the free amine.

† Contaminants contribute to characteristic ions of lower m/z value, so these are not recorded: synthetic N-acetyl-(**10**) showed m/z 43 (60), synthetic N-acetyl-(**11**) m/z 43 (47).

present. This was determined by mass spectrometry by a comparison of the intensity of the molecular ions at 316 and 319 daltons [respectively N-acetyl and d_3-N-acetyl-(**4**)] after extracts were treated with d_6-acetic anhydride prior to TLC. Concomitant with this change was the appearance on silica TLC of three additional UV absorbing metabolites. Comparison with mass spectra of synthesised materials identified the N-acetyl derivatives of (**12**) (R_f 0.17) and (**10**) (R_f 0.15). All products had undergone complete metabolic N-acetylation since following deuteroacetylation, no deuteroacetylated products were obtained. However, addition of further cofactors at 0 and 20 min after the initial 60 min incubation period [10] promoted deacetylation of the metabolites to (**11**) and (**12**) as evidenced, following deuteroacetylation, by the increased ratio of deuteroacetylated to acetylated species from, respectively, 1:1.6 and 1:5.25 at time 20 min to 1:0.15 and 1:1.14 at 60 min after the final addition of cofactor.

For C57B1/6 mice, the major component isolated after deuteroacetylation of extracts of urine was d_3-acetyl-(**4**) (R_f 0.40) indicating (**4**) to be excreted largely unchanged, a contrast with the rat. A *bis*-trideuteroacetylated product derived from a hydroxylamino derivative (**14**) (R_f 0.65) was also detected. The EI mass spectrum of this compound was analogous to that of the corresponding N,O-di(trideuteroacetylated) derivative of hydroxylaminoglutethimide formed [8] in the C57B1/6 mouse in having a weak molecular ion (here m/z 380) giving abundant fragmentations by successive losses of 2 CD_2CO residues. Also formed from this mouse urine extract was a deuteroacetylated monohydroxylated product (R_f 0.43) but not the acetylated counterpart, showing the metabolite to have a free amino function. From the mass spectrum, this metabolite was a C-hydroxy derivative but it has not been further characterised.

N-Acetylation of (**4**) by phenobarbital-induced C57B1/6 mouse liver microsomes was rapid and independent of an NADPH-regenerating system, but in contrast to the rat was not complete since some d_3-acetyl-(**4**) was detected after trideuteroacetylation. Use of an NADPH-regenerating system afforded three products (R_f 0.26, 0.22, 0.17) all of which were monohydroxylated and partially N-acetylated, as evidenced by EI mass spectrometry. However, this hydroxylation was not in the propyl moiety, since the mass spectra lacked the ion at m/z 275, characteristic of the N-acetylated hydroxypropyl derivatives. With the C57B1/6 microsomal system no N-hydroxylation of substrate could be detected. Of the possible structures having hydroxy groups at C-4 or C-5 of the glutarimide residue, or at C-1 or C-2 of the ethyl residue only the C-5 hydroxy derivative N-acetyl-(**13**) was readily available for comparison. Two of the bands from TLC contained compounds giving molecular ions at m/z 288 (291 on deuteroacetylation) consistent with de-ethylation of (**4**) and N-acetyl-(**4**) to give (**15**) and N-acetyl-(**15**). One band (R_f 0.26) contained no other component, but the other band (R_f 0.22) was a mixture of the component of MW 288 (291) with another having a mass spectrum appropriate to the aforementioned N-acetyl-(**13**). In particular the ion m/z 218 is characteristic for this compound and probably identical in structure to that found [11] in the mass spectrum of the corresponding metabolite of (**1**). It has been shown both by Egger *et al.* [12] and by ourselves [11] that 1-hydroxylation in the ethyl moiety of aminoglutethimide gives a product which is thermally unstable, decomposing into acetaldehyde and the desethyl derivative. Hence by analogy one of the components of MW 288 was N-acetyl-(**15**), whilst the other was the 1-hydroxyethyl analogue N-acetyl-(**16**). Unfortunately these could not be discriminated since attempts to synthesise (**15**) from the known [11] desethyl derivative of (**1**) were unsuccessful.

A further mouse microsomal metabolite (R_f 0.17 after deuteroacetylation and TLC) gave a mass spectrum in which loss of H_2O from the molecular ion was abundant. No such abundant loss has been noted for any of the hydroxylated metabolites of AG

[11, 12]. However, it is possible that the present metabolite is a 2-hydroxyethyl analogue (**17**). The corresponding urinary metabolites derived from (**1**) and its N-acetyl derivative from the human and rat respectively rearrange spontaneously to lactones [11, 12] but the presence of the *n*-propyl residue may hinder this rearrangement, in which case the elimination of H_2O to form a vinyl derivative would be an alternative pathway.

DISCUSSION

As with corresponding C-alkyl analogues of (**1**), there was a trend towards an increase in inhibitory potency towards aromatase with increasing chain length of the alkyl substituent, but no marked increase beyond the butyl derivative (**5**).

In the desmolase assay there was a marked initial drop in inhibitory potency in the homologous series with a nadir at the propyl derivative (**4**) followed by a recovery, so that the last three homologues (**7–9**) were somewhat more potent inhibitors than the parent (**1**). This is in contrast to the C-alkyl series [2] where no clear trend emerged for inhibition of desmolase. The propyl analogue (**4**) showed the best ratio between aromatase and desmolase inhibitory activity and was the nearest approach to a "pure" aromatase inhibitor in the N-alkyl series. This compound was therefore studied further, to compare the metabolism profile with that of (**1**) itself and to examine the susceptibility of the propyl residue to metabolism. Our previous study on the N- and C-octyl analogues of 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione [13] showed these to be highly susceptible to oxidative metabolism in the alkyl residue, negating the advantage of much greater inhibitory potency towards aromatase compared with the parent compound.

We have previously reported [8] that the C57B1/6 strain of mouse shows a metabolism profile for aminoglutethimide which strongly resembles that seen in humans. In the present study, the metabolism of (**4**) has been compared in the rat and mouse. In a previous study [8] we reported the technique of trideuteroacetylation as a method of converting the labile N-hydroxylamino derivative of aminoglutethimide, present as a metabolite in mouse urine into a more stable derivative. Here, we have used it to show that (**4**) also forms a hydroxylamino derivative (**14**) in these mice, and also that much of the substrate is excreted unchanged in mouse urine.

As with aminoglutethimide itself, the urinary metabolites of 1-propylaminoglutethimide in the rat differed from those in the mouse. Analogously [14], there was no evidence for the formation of (**14**) in the rat. The formation of the N-acetylated 2-hydroxypropyl (**12**) and 2-oxopropyl (**11**) derivatives was predictable, since (ω-1) oxidation is the preferred site for metabolic oxidation of shorter linear alkyl chains [15]. Of the free amino-derivatives corresponding to these metabolites the 2-hydroxy derivative (**12**) was inactive against aromatase but the 2-oxo derivative (**11**) was a moderately good inhibitor.

Metabolism of (**4**) *in vitro* with rat hepatic microsomes gave the same metabolites and additionally the N-acetylated 3-hydroxypropyl derivative (**10**) as the product of ω-hydroxylation. In contrast, the metabolism of (**4**) with C57B1/6 hepatic microsomes was not predictive of the *in vivo* situation and none of the microsomal products could be matched with urinary counterparts.

It would appear from the present study that there are major differences in the route of metabolism of (**4**) in the two species investigated. In the rat, oxidation occurs predominantly on the 1-propyl sidechain, both *in vivo* and *in vitro*. In the C57B1/6 mouse, the propyl sidechain is not metabolised *in vitro* or *in vivo*. Instead, the major *in vivo* product results from N-hydroxylation of the amino function. Since this metabolic route appears to require self-induction and therefore cannot occur *in vitro*, alternative sites on the glutarimide ring and ethyl sidechain become the prime sites for oxidation.

In summary, this study of homologous N-alkyl derivatives of aminoglutethimide has revealed one, the *n*-propyl derivative (**4**), having a particularly favourable selectivity for aromatase. Although further quantitative metabolism studies are required the ready formation in the mouse of an induced metabolite, the hydroxylamino-derivative, is indicative of a reasonable and sustained plasma level of unchanged (**4**). Thus aminoglutethimide itself does not induce the formation of hydroxylaminoglutethimide in the rat [14], probably because of its extensive acetylation in this species. Further, in the analogous series of C-alkyl analogues of (**1**), the *n*-propyl derivative was as good an inhibitor of the oestrogen-dependent DMBA tumour in rats as higher homologues which were better inhibitors of aromatase [2]. Hence an alkyl residue of medium chain length could afford the best compromise between potency for the target enzyme and susceptibility to metabolism.

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