Synthesis of the Aromatase Inhibitor 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione and Its Enantiomers

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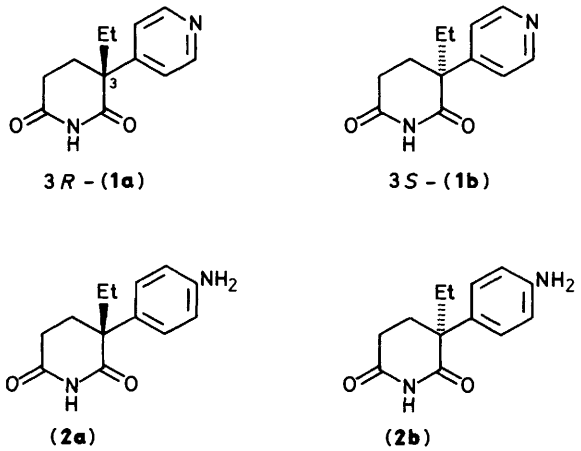
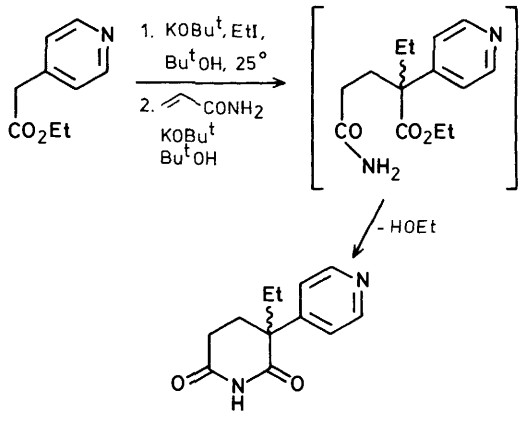
Convenient syntheses of (*R/S*)-3-ethyl-3-(4-pyridyl)piperidine-2,6-dione (pyridoglutethimide) and its enantiomers are described; aromatase inhibitory activity was almost entirely confined to the 3*R*-enantiomer.

3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione (**1**) (pyridoglutethimide) is an analogue of aminoglutethimide (**2**) that is a selective inhibitor of the enzyme aromatase which converts androgens into oestrogens.[1–3] It is presently undergoing clinical trial for the treatment of hormone-dependent breast cancer. The previously reported three-stage synthetic procedures[1] give only modest overall yields (20%[1] and 25%[2]) from 4-pyridylacetonitrile and are, therefore, not ideally suited to the production of substantial quantities of the drug for clinical use. In a new 'one-pot' procedure more amenable to large-scale work (Scheme 1) commercially available ethyl 4-pyridylacetate



(**1**), (**2**) = racemic mixtures



Scheme 1.

was monoethylated by treatment with potassium t-butoxide (1.1 equiv.) and iodoethane (1.0 equiv.) in t-butyl alcohol at ambient temperature followed by the addition of acrylamide (1.5 equiv.) and a further equivalent of base to give, after a simple work-up

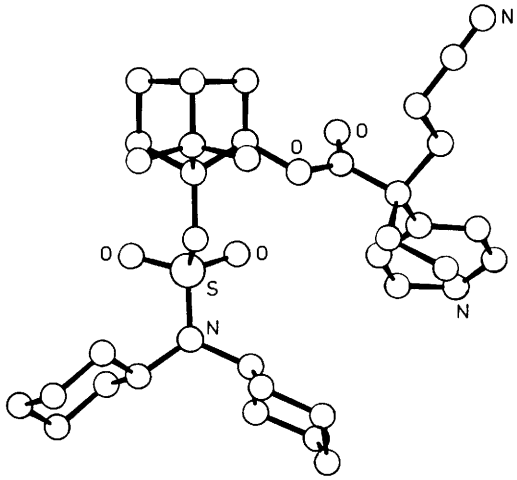


Figure. A computer plot of the crystal structure of the precursor (**5a**) of (*R*)-(+)-pyridoglutethimide

procedure and recrystallisation twice from isopropanol, pyridoglutethimide, m.p. 134—136 °C (lit.,[1] 138—139 °C) in 56% overall yield. The extra equivalent of base was necessary to promote cyclisation of the reversibly formed Michael adduct.

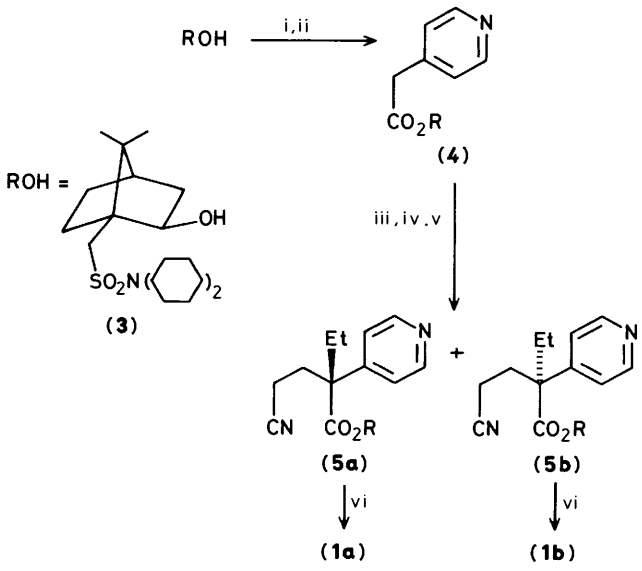
Enantiomers of aminoglutethimide have been separated by fractional crystallisation of tartrate salts[4] and aromatase inhibitory activity reported to be confined almost entirely to one enantiomer[4,5] which has been assigned *R*-stereochemistry (**2a**) on the basis of circular dichroism and optical rotatory dispersion comparisons with related compounds derived from natural amino acids. Enantiomers of pyridoglutethimide were prepared (Scheme 2) from the methyl ester of 4-pyridylacetic acid which was transesterified (78% yield) with Oppolzer's camphor derived chiral auxiliary (**3**)[6] (1 equiv.) and butyllithium (1 equiv.) in tetrahydrofuran (THF).[7] The resulting ester (**4**)*, m.p. 164—165 °C, (26 mmol) was deprotonated by potassium hydride (30 mmol) at −10 °C in THF and the anion monoethylated with iodoethane (26 mmol). After work-up the crude product was treated with acrylonitrile (57 mmol) in t-butyl alcohol containing potassium t-butoxide (0.8 mmol) as catalyst to give two diastereoisomers [(**5a**), m.p. 190—191 °C and (**5b**), m.p. 154—156 °C] which were easily separable by column chromatography on silica gel [eluant 1:25:25 triethylamine–ether–light petroleum (b.p. 60—80 °C)] and were obtained in 51% combined yield with a small diastereoisomeric excess (6%) in favour of (**5a**). On heating the individual isomers (**5a**) and (**5b**) in a mixture of acetic and sulphuric acids (4:1) at reflux for 2.5 h, the (+)- and (−)-enantiomers of pyridoglutethimide (**1a**), m.p. 110—111 °C, $[\alpha]_D^{20}$ +151.0° (*c* 0.6 in EtOH) and (**1b**) m.p. 110—111 °C, $[\alpha]_D^{20}$ −151.6° (*c* 0.6 in EtOH) formed directly (43 and 52% yield respectively).

The enantiomers could be distinguished by n.m.r.spectroscopy in deuterium oxide solution by the addition of β-cyclodextrin as a chiral shift reagent,[8] when there is a pronounced chemical shift difference (0.03 p.p.m.) for one of the prochiral ethyl methylene protons.

The absolute configuration of the enantiomers were assigned by a single crystal *X*-ray diffraction study of the diastereoisomeric precursor (**5a**). A representation of the structure is illustrated in the Figure. Since the configuration of the camphor moiety is known, the (+)-isomer of pyridoglutethimide can be assigned the *R*-stereochemistry, the same relationship as reported for aminoglutethimide.[4]

Aromatase inhibitory activity was determined by the amount of compound required to halve the rate of tritiated water released on aromatisation of [1,2-$^3H_2$]testosterone to oestradiol by a human placental microsome preparation.[9] Racemic pyridoglutethimide ($I.C._{50}$ = 14 μM) was comparably potent to racemic aminoglutethimide ($I.C._{50}$ = 10 μM, *cf.* lit.[5] value 14 μM) in this assay. The *R*-(+)-pyridoglutethimide (**1a**) ($I.C._{50}$ = 10 μM) showed greater potency than the racemate but the *S*-(−)-enantiomer (**1b**) was 20-fold less inhibitory ($I.C._{50}$ = 200 μM). Therefore the *R*-(+)-enantiomer is essentially responsible for the aromatase inhibitory activity of the racemate. These data closely parallel results reported[5] for aminoglutethimide [*R*-(+)-isomer (**2a**) $I.C._{50}$ = 8 μM; *S*-(−)-isomer (**2b**) $I.C._{50}$ = 310 μM]. We can thus confirm the correctness of the assignment of absolute configuration reported for aminoglutethimide, and the close parallelism in the activities provides strong evidence that in binding to the aromatase enzyme, aminoglutethimide and pyridoglutethimide do so in the same manner, the interaction of the amino substituent in the former being replaced by one to the pyridyl in the latter.

The route used to prepare the enantiomers of pyridoglutethimide has the disadvantage that the chiral auxiliary was destroyed in the harsh conditions employed in the final cyclisation. When the ester (**4**) was treated under the one-pot conditions of Scheme 1 the auxiliary was recovered in high yield (93%) and the yield of pyridoglutethimide, obtained with a 22% enantiomeric excess in favour of the *S*-enantiomer (**1b**), was also good (69%), but as no diastereoisomeric intermediates are isolable, this method cannot be used to prepare the pure individual enantiomers. It is noteworthy that this procedure gives an excess of the opposite enantiomer to that obtained by the method of Scheme 2; a possible explanation for the difference is that the acrylamide amino function forms a weak hydrogen bond to the sulphone function of the auxiliary, whereas acrylonitrile can form no such hydrogen bond.



Scheme 2. *Reagents:* i, BuLi, THF, 5 °C: ii, methyl 4-pyridylacetate, −5—20 °C; iii, KH, THF, −5 °C; iv, EtI, −5—20 °C; v, CH_2=CH–CN, KOBu^t, Bu^{t}OH, 25 °C; vi, H_2SO_4, AcOH, reflux

* Satisfactory analytical data and n.m.r. spectra obtained for compounds (**1a**), (**1b**), (**4**), (**5a**), and (**5b**) are available as a Supplementary publication [SUP No. 56735 (2 pp.)]. For details of the Supplementary publications scheme, see 'Instructions for Authors (1989),' *J. Chem. Soc., Perkin Trans. 1*, 1989, Issue 1.

Acknowledgements

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