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A CONCISE SYNTHESIS OF RACEMIC PYRIDOGLUTETHIMIDE AND ITS RESOLUTION USING CHIRAL STATIONARY PHASE HPLC

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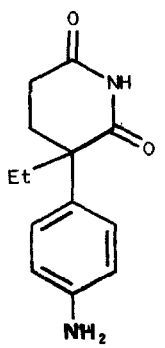
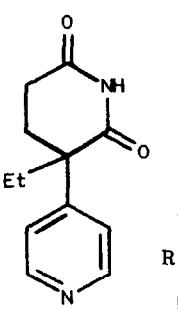
Abstract-We describe a 'one-pot' synthesis of the aromatase inhibitor pyridoglutethimide [3-(4-pyrido)-3-ethylpiperidine-2,6-dione] and its 3-octyl-analogue, together with details of the resolution of the former using chiral stationary phase hplc.

Over a quarter of a million new cases of breast cancer are diagnosed each year in North America and Europe, so there is a continuing need for effective chemotherapeutic agents. Aminoglutethimide [1] is such an agent, and is especially useful for the treatment of hormone dependent tumours, acting through inhibition of the enzyme aromatase (inhibition of estrogen biosynthesis)[1,2]. It produces, however, neurological side-effects, and also interferes with general steroid biogenesis through inhibition of the enzyme desmolase (inhibition of side-chain cleavage in cholesterol). The analogue, pyridoglutethimide [2] [3-(4-pyrido)-3-ethylpiperidine-2,6-dione], first synthesised by Foster, Jarman and coworkers[3] is apparently not neurotoxic and does not inhibit desmolase; but the published synthesis of [2] is not practical on a large scale. In this paper we describe the development of a new route that has been used to prepare the compound on the kilogramme scale.

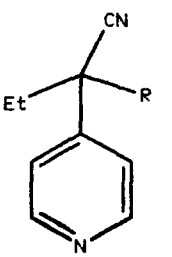
[1]



[2]

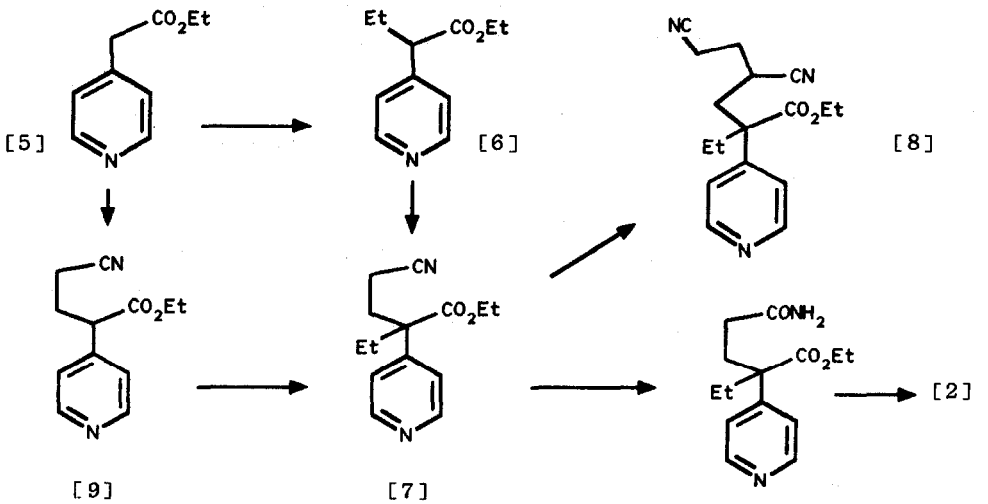


[3]
R = CO_2Et
[4] R = H



Our initial efforts involved reaction of 4-chloropyridine with ethyl,2-cyanobutanoate in the presence of a variety of bases to produce the desired adduct [3] in variable yield (10-50%). Attempted decarboethoxylation to provide the nitrile [4] using acid, base, or NaCl in DMSO, failed; and this approach was abandonded in favour of an alternative route commencing with ethyl 4-pyridylethanoate [5] (Scheme One).

Alkylation of this ester with ethyl iodide could be accomplished in near quantitive yield using hindered bases (lithium diisopropylamide or potassium tertiary butoxide) or KF supported on alumina[45]. The resultant ethyl 2-(4-pyridyl)propanoate [6], underwent a Michael addition when treated with acrylonitrile in the presence of either lithium diisopropylamide or KF/alumina, to yield some of the expected product [7], but mostly the dinitrile [8]. However, when the order of reactions was reversed, viz. ester [5] and acrylonitrile in conjunction with triton B, followed by reaction of the product [9] with lithium diisopropylamide and ethyl iodide, the desired adduct [7] was obtained in an overall yield of ca. 25% for the two steps. Reaction of [7] with a mixture of glacial acetic acid and sulphuric acid yielded amide [10], which cyclised to produce pyridoglutethimide [2] upon treatment with potassium tertiary butoxide (ca. 75% overall). This route, though successful, is multi-step and low-yielding, and the alternative 'one-pot' process was subsequently developed.

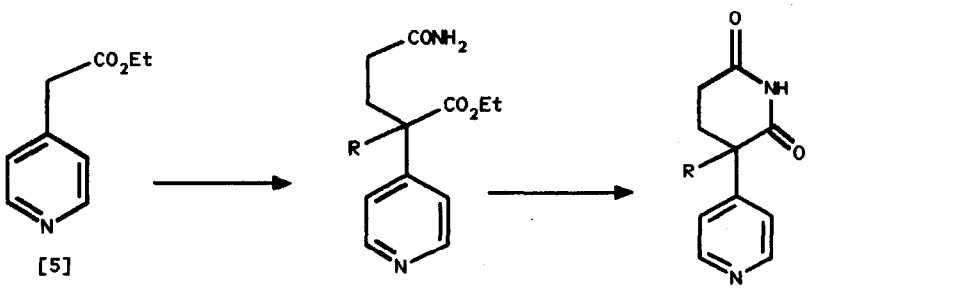


SCHEME ONE

Reaction of the ester [5] with potassium tertiary butoxide and ethyl iodide (in tertiary butanol), was followed 30 minutes later by addition of a further equivalent of the base and an excess of acrylamide. Subsequent neutralisation, and extraction of the mixture with ethyl acetate provided crude pyridoglutethimide, which could be recystallised to homogeneity using 2-propanol. Yields ranged from ca. 65% on a 10 gramme scale to around 45% on a 100 gramme (or greater) scale. A similar 'one-pot' procedure was used to prepare the 3-octyl analogue of pyridoglutethimide [11] (previously prepared by Jarman and coworkers[6]) though better yields (up to 70%) were obtained if the intermediate ester, amide [12] was purified prior to cyclisation (Scheme Two).

Finally, it was necessary to resolve racemic pyridoglutethimide in order to determine the biological potency of the individual enantiomers. To this end a chiral stationary phase derived from (R,R)-tartramide [13] was prepared according to the methods described by Hara and Dobashi[7], and used to effect an HPLC separation of the two enantiomers. The effectiveness of this stationary phase can be seen in the Figure, and it is interesting to note that neither Pirkle-type columns (chiral phenylglycine derivatives attached to aminopropylated silica)[8] nor columns using β-cyclodextrin as chiral stationary phase[9] produced any separation of the enantiomers.

Contemporaneous with these studies, the group of Jarman and McCague had effected a resolution of the enantiomers of pyridoglutethimide by chemical means[10], and there was a perfect matching of their samples with our own (by HPLC, n.m.r., and optical rotation). Subsequent biological evaluation was carried out by their group using human placental aromatase (and will be reported in due course); but the key results are presented in ref.10. As with aminoglutethimide[2], the (+)-enantiomer is the most active, but is not spectacularly more active than the racemate.



[5] [12] [2] R=Et

[11] R=octyl

SCHEME TWO

Experimental

I.r. spectra were recorded with a Perkin-Elmer 881 double beam grating spectrophotometer; n.m.r. spectra were recorded with a Perkin-Elmer R34 (220MHz) or Varian T60 (60MHz) instruments using tetramethylsilane as internal standard; flash chromatography was performed using Crossfield Sorbsil C60 silica gel (40-60μm); and petrol refers to petroleum ether b.pt. 40-60°.

Ethyl,4-cyano-2-(4-pyridyl)butanoate [9]

Ethyl-(4-pyridyl)ethanoate (10g, 60mmol) was added to tert-butanol (20ml) and the solution cooled to 0° prior to the addition of Triton B (0.32ml of a 40% w/w methanol solution) and acrylonitrile (5.2ml, 120mmol). The resultant reaction mixture was then stirred for 48hr at RT. After concentration, and addition of water (100ml), the product was extracted into chloroform. Purification was effected by flash chromatography using ethyl acetate as elutant, to yield the ester [9] as a light yellow oil (12.4g, 87%). I.r. (neat) 2200 (CN), 1740 (ester), 1610 cm^{-1}; n.m.r.($CDCl_3$, 60MHz) 1.3 (t, J 7Hz, ester Me), 2.5 (m, other aliphatic H), 4.0 (q, J 7Hz, ester CH_2), 7.1 and 8.7 (2m, pyridyl H)ppm; m/z 218.

Ethyl,4-cyano-2-ethyl-2-(4-pyridyl)butanoate [7]

The ester [9] (3.4g, 15mmole) dissolved in anhydrous THF (10ml) was added to lithium diisopropylamide (from 12ml diisopropylamine in 5ml THF treated with 12ml of 1.6M n-butyl lithium) at -78°. To this solution was added iodoethane (2.5ml), and the reaction mixture was then allowed to warm to RT over a period of one hour. After addition of water, the products were extracted into dichloromethane, and subsequently purified by flash chromatography using ethyl acetate as elutant. In this way 1.1g (30%) of the desired ester [7] was obtained as a light yellow oil. I.r. (neat) 2220 (CN), 1735 (ester), 1610 cm^{-1}; n.m.r. ($CDCl_3$, 60MHz) 0.9 (t, Me), 1.3 (t, ester Me), 2.0-2.5 (m, other aliphatic H), 4.0 (q, ester CH_2), 7.1 and 8.5 (2m, pyridyl H)ppm; m/z 232.

3-(4-Pyrido)-3-ethylpiperidine-2,6-dione (pyridoglutethimide) [2]

The ester [7] (250mg, 1mmol) was reacted at 100° for 2 hr with a mixture of glacial acetic acid (0.8ml) and conc. sulphuric acid (0.35ml). At the end of this time the mixture was poured onto ice, and the pH adjusted to 7-8 with $NaHCO_3$. The product was then extracted into dichloromethane, and after washing with water, drying and concentration provided 300mg. of a yellow solid. This was dissolved in tert-butanol (10ml) containing dry THF (0.5ml) and treated with potassium tert-butoxide (0.125g, 1.1mmol) at RT.

After stirring for 2 hr, the reaction mixture was acidified with 2M. HCl, and the product extracted into dichloromethane. This yielded 170mg of pyridoglutethimide as an off-white solid, which provided 145mg (66%) of pure product after one recrystallisation from isopropanol. M.pt. 135-7°; and spectroscopic data identical to those described in full below.

One-pot synthesis of pyridoglutethimide [2]

Ethyl-(4-pyridyl)ethanoate [5] (100g, 0.6mol) was dissolved in tert-butanol (1L), and potassium tert-butoxide (80g, 0.66mol) was added portionwise to the stirred solution. Ethyl iodide (48ml, 0.6mol)was then added dropwise over a period of ca. 10 minutes, and the temperature rose to about 45°. The mixture was stirred in all for about 1.5 hr, prior to the addition of acrylamide (64g, 0.9mol) dissolved in tert-butanol (500ml). This was followed by addition of potassium tert-butoxide (80g, 0.66mol). The reaction mixture was then stirred for a period of 2.5hr. Water (400ml) was added, and the mixture was buffered to pH 7-8 with 4M. HCl, before being continuously extracted with hot toluene for 20 hr. The toluene extract was reduced in volume to induce crystallisation, and the crude product (80g) was recrystallised twice from isopropanol to yield pure pyridoglutethimide (61g, 48%). M.pt. 136-8° [Lit. 138-9° (ref.3)]; i.r. (nujol mull) 3185 1785, 1720, 1605 cm^{-1}; n.m.r ($CDCl_3$, 200MHz) 0.85 (t, J 7Hz, Me), 1.80-2.80 (m,3xCH_2), 7.15 and 8.55 (2xd, J 4.5Hz, pyrido H) ppm.

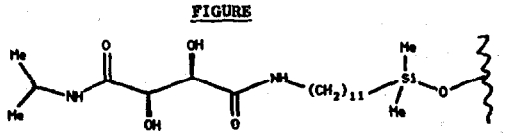
3-Octyl-3-(4-pyrido)piperidine-2,6-dione [11]

Ethyl-(4-pyridyl)acetate [5] (5g, 30mmol) and 1-bromooctane (7.9g, 33mmol) were dissolved in tert-butanol (100ml), and treated with potassium tert-butoxide (4.02g, 33mmol). An exothermic reaction was noted, and the temperature rose to ca. 50°. After 40 minutes, acrylamide (33.2g, 45mmol) and potassium tert-butoxide (4.02g, 33mmol) were added, and the mixture stirred for a further hour, prior to work-up (as described for pyridoglutethimide). A yellow oil was obtained and this was purified by passage through flash chromatography grade silica, using ether:petrol (19:1) as initial elutant to remove unreacted starting materials, then methanol to produce the methyl ester of [12] by transesterification. The neat ester was dissolved in dry DMF (50ml), then reacted with potassium tert-butoxide (44.02g, 33mmol), with stirring at RT overnight. After adjustment of the pH to 5-6 with 2M HCl, water was added, and the product extracted into ethyl acetate. Crystallisation of the initial yellow gum (from pentane) provided [11] as a white powder (6.2g, 68%).M.pt. 58-59° [Lit. 60-62° (ref. 5)]; n.m.r. ($CDCl_3$, 200MHz) 0.87 (t, J7Hz, Me), 1.24 (s, side-chain CH_2), 1.80-2.80 (m, other CH_2), 7.26 and 8.64 (2xm,pyrido-H)ppm.

Resolution of Racemic Pyridoglutethimide using HPLC and a Chiral Stationary Phase

The chiral stationary phase based on the (R,R)-tartramide shown below was prepared exactly as described by Dobashi and Hara[7]. A column of 250x4.9mm dimensions was employed, with hexane:isopropanol (3:1) as eluent, and a flow rate of 1ml/min. Multiple semi-preparative separations were effected on solutions of 0.3mg of racemate in 200μl of eluent, and the separations obtained are shown in the Figure. The (-)-enantiomer eluted first, $[\alpha]_D^{20}$ -150.5° (*c* 0.8 in EtOH) [Lit. -151.6° (ref. 10)], then the (+)-enantiomer, $[\alpha]_D^{20}$ +149.8° (*c* 0.8 in EtOH) [Lit. +151.0° (ref. 10)]. These enantiomers co-chromatographed with samples provided by McCague[10].

FIGURE



[13]

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b. Castrol (U.K.) Ltd., Pangbourne, Berks.

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