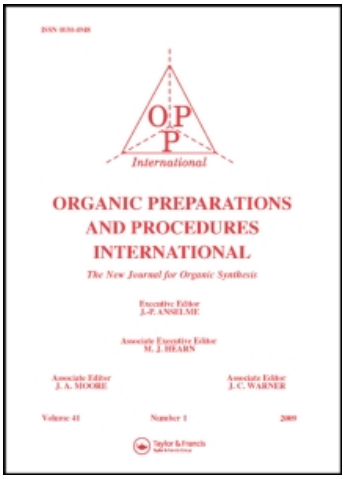

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A SELECTIVE SYNTHESIS OF 5-*p*-AMINOPHENYLBARBITURIC ACID

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A SELECTIVE SYNTHESIS OF 5-*p*-AMINOPHENYLBARBITURIC ACID

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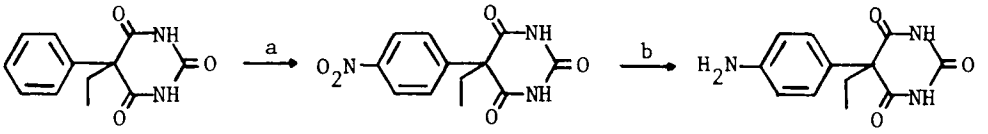
We recently needed samples of fluorescently labelled phenobarbital for the development of a fluoroimmunoassay for this barbiturate.[1] Since this technique relies upon competitive binding of labelled and unlabelled drug to an antibody, the fluorescent tag must

not sterically interfere with the binding site on the barbituric acid moiety. Thus, introduction of an amino group on the *para* position of the aromatic ring, for attachment of the label, is critical in optimizing the dynamic range of the immunoassay.[2]

Early approaches[3] to the synthesis of 5-ethyl-5-*p*-aminophenylbarbituric acid involving initial nitration with HNO_3/H_2SO_4 were later shown to yield mixtures containing in excess of 50% of the *meta* isomer.[4] Purification of these mixtures was tedious and invariably resulted in poor yields of the *para* nitrated product. Through the use of a nitronium salt, however, these obstacles have been overcome and we describe here a simple,



1 2 3

a) NO_2BF_4, CH_3CN, 0° b) H_2, 5% Pd/C, 5% HOAc/EtOH, 60°

inexpensive and selective route to higher yields of 5-ethyl-5-*p*-aminophenylbarbituric acid. Subsequent reaction of this compound with commercially available fluorescein and rhodamine isothiocyanate derivatives then yields useful analytical reagents.[2,5]

The synthesis employed is depicted in the Scheme. Phenobarbital (**1**) was selectively nitrated in 96% yield by treatment with one molar equivalent of nitronium tetrafluoroborate[6,7] in acetonitrile at 0°. Removal of approximately 5% of the *meta* isomer was effected at this stage by two recrystallizations from a minimum of hot absolute ethanol to give 81% of the pure *para* product. Experimentally, it was observed that only white, free-flowing nitronium tetrafluoroborate, as obtained from the Ozark-Mahoning Co.,[7] resulted in high selectivity. This salt purchased from other commercial sources often appeared discolored (tan) or wet and selectivity using such material was greatly reduced. Catalytic hydrogenation of the resulting 5-ethyl-5-*p*-nitrophenylbarbituric acid (**2**) using 5% Pd/C in acidic ethanol at 60° under 3 atm of hydrogen then produced the title compound (**3**) in nearly quantitiative yield.

The source of the electrophilic nitronium ion appears to be critical to the selectivity of the process. While the classical method yields a 40:60 mixture of *para:meta* product, the nitronium salt affords a 95:5 ratio in favor of the *para*. Positional selectivity favoring *ortho-para* substitution has been previously noted and rationalized;[6] in the present case, steric factors may also contribute to the preponderance of *para* product.

EXPERIMENTAL SECTION

Phenobarbital (Sigma), nitronium tetrafluoroborate (Ozark-Mahoning)[7] and all other reagents and solvents were used directly from newly opened bottles. Melting points were obtained on a Thomas Cooper melting point apparatus and are uncorrected. IR spectra were recorded with a PE-681 instrument and are referenced to polystyrene. ^{1}H-NMR spectra were measured as solutions in $CDCl_3$ at 300 MHz using a Varian XL-300 superconducting FT instrument; chemical shifts are reported in δ units relative to internal TMS. UV spectra were recorded in ethanol using a Hitachi 100-80A spectrophotometer. High resolution mass spectra (HRMS) were recorded using a CEC double focusing mass spectrometer.

5-Ethyl-5-*p*-nitrophenylbarbituric Acid (2). - To a stirred 100-mL CH_3CN solution of 10 g (43 mmol) of phenobarbital (**1**) under N_2 at 0° was slowly added a solution of 5.7 g (43 mmol) of NO_2BF_4 in 50 mL of CH_3CN. The reaction was stirred at 0° for 10 hrs, then added to 500 g of crushed ice and refrigerated at -20° for 8 hrs. The crude product was filtered cold and dried under vacuum at 23° to give 11.4 g (96%) of a 95:5 *para:meta* product mixture. Two recrystallizations from a minimum of hot absolute ethanol yielded 9.65 g (81%) of pure **2** as light yellow crystals, mp. 216-217°, lit.[4] mp. 216°; IR (KBr): 3500, 1730 cm^{-1}; ^{1}H-NMR: δ 11.91 (s, 2H), 8.31 (d, 2H, A of A_2B_2, J = 12), 7.70 (d, 2H, B of A_2B_2, J = 12), 2.38 (q, 2H, J = 8), 0.91 (t, 3H, J = 8); UV λ_{max}: 254 nm (ε 5330); mass spectrum (70 eV): m/e 277 (parent), 231 (base); HRMS: calcd for $C_{12}H_{11}N_3O_5$, m/e 277.0699; found m/e 277.0732.

5-Ethyl-5-*p*-aminophenylbarbituric Acid (3). - A solution of 10 g (36 mmol) of **2** in 100 mL of 95:5 absolute ethanol:acetic acid was shaken with 0.1 g of 5% Pd/C under 3 atm of H_2 at 60° until the initial rapid uptake of H_2 subsided (about 40 min). The mixture was filtered through Celite®, the solvent was removed under vacuum at 40° using a rotovap and the product was recrystallized twice from a minimum of hot absolute ethanol to

yield 8.36 g (94%) of **3** as white crystals, mp. 187-188°, lit.[4] mp. 187°; IR (KBr): 3510, 1735 cm^{-1}; ^1H-NMR: δ 11.86 (s, 2H), 8.34 (d, 2H, A of A_2B_2, J = 12), 7.76 (d, 2H, B of A_2B_2, J = 12), 3.62 (bs, 2H), 2.41 (q, 2H, J = 8), 0.90 (t, 3H, J = 8); UV λ_{max}: 261 nm (ε 4720); mass spectrum (70 eV): m/e 247 (parent), 231 (base); HRMS: calcd for $C_{12}H_{13}N_3O_3$ m/e 247.0957; found m/e 247.0971.

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