SYNTHESIS AND BIOLOGICAL EVALUATION OF p,p'-DIDEUTERIOPHENYTOIN.

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SUMMARY

A reaction sequence is described to synthesize p,p'-dideuteriophenytoin in three steps from commercially available starting materials. The deuterium content at best reached 99.2% as measured by mass spectrometry. For materials with isotopic content up to 95%, excellent agreement was found between the data obtained by mass spectrometry and ^{13}C-nuclear magnetic resonance operated in a NOE-suppression mode. p,p'-Dideuteriophenytoin was evaluated comparatively with phenytoin and p-deuteriophenytoin in the maximal electroshock test. While all three compounds exhibited a high degree of antiepileptic activity, none of them demonstrated significant superiority over the others.

Key words : phenytoin, 5,5-diphenylimidazolidine-2,4-dione, deuterium, isotopic effects, MS, ^{13}C-NMR, antiepileptic activity.

Phenytoin (5,5-diphenyl-2,4-imidazolidinedione) is a widely prescribed antiepileptic drug, commonly employed in the treatment of tonic-clonic seizures ("grand mal") and elementary partial (focal motor) seizures (1). In 1978, the Epilepsy Branch of the National Institute of Neurological and Communicative Disorder and Stroke of the N. I. H. (Bethesda, Maryland, U. S. A.) evaluated 16 commercially available antiepileptic drugs. Phenytoin ranked first in the maximal electroshock seizure (MES) test, but surprisingly was

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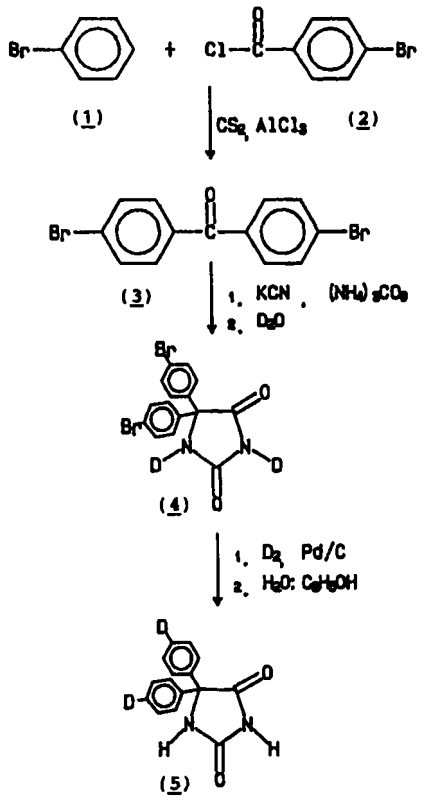
found totally inactive in the subcutaneous metrazol (scMET) test (2). In view of its continuing therapeutic interest, this compound has received considerable attention both on the point of view of its metabolic fate and pharmacokinetic behaviour (3). For these studies, there is an ever-growing need for tracers of suitable isotopic content and purity. This is exemplified by numerous papers dealing with the synthesis of phenytoin or its metabolites bearing various isotopic labels : ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{15}N (4-16). Recent works have shown the potentialities of a deuterated analogue of phenytoin, namely racemic p-deuteriophenytoin, as a molecular probe to detect and quantitate the extent of arene oxide formation during the hepatic metabolization of phenytoin (17-18). However, as the hydroxylation metabolic process of phenytoin is stereospecific with the predominant production of (S)-(-)-p-hydroxyphenytoin in man and laboratory animal species (rat, mouse) (19), the use of a racemic substrate appears to be a major drawback to demonstrate whether there is a differential metabolism for the pro-(R) and pro-(S) phenyl moieties of phenytoin (20). This could be realized only by having an achiral molecular probe. It is noteworthy also that the discovery of an antiepileptic drug selectively active in the maximal electroshock test (MES test) with a high therapeutic index represents a real challenge for the pharmacochemist. So far, this has only been achieved with phenytoin and clobazam (21). As a consequence, there was also a real interest for the pharmacological evaluation of the title compound.

This article describes a straightforward preparation of p,p'-dideuteriophenytoin obtained with high isotopic purity in three steps from commercially available starting materials and its spectroscopic (MS and ^{13}C-NMR) and biological evaluation in comparison with phenytoin and p-deuteriophenytoin in the MES test.

CHEMISTRY

At first, different synthetic schemes were explored : selective deuteriolysis of p,p'-dichlorobenzophenone or p,p'-dibromobenzophenone, deuteriolysis of p,p'-dichlorophenytoin. None of these approaches turned out to be satisfactory in terms of isotopic purity. We therefore focused our attention on the deuteriolysis of p,p'-dibromophenytoin (Reaction Scheme).



Reaction Scheme

Thus,Friedel-Crafts reaction of p-bromobenzoyl chloride (2) with bromobenzene (1) in carbon disulfide in the presence of aluminium trichloride yields p,p'-dibromobenzophenone (3) in 65% yield, after extensive recrystallization from ethanol to remove some o,p'-dibromobenzophenone present in the reaction products. The purification was monitored by HPLC. The purified p,p'-dibromoketone (3) was then treated by potassium cyanide and ammonium carbonate in dimethylformamide:water (90:10, v/v) solution at 125°C (oil bath) for 72 h to afford the highly insoluble hydantoin in 82% yield, after recrystallization from ethanol (a sohxlet device was required) (9). This material was extensively dried in vacuo over P_2O_5, finely powdered and recrystallized twice from anhydrous dioxane:D_2O (70:30, v/v) to yield the N(1), N(3)-dideuteriohydantoin (4), which was then submitted to deuteriolysis over 10% palladium on charcoal in anhydrous dioxane:D_2O (95:5, v/v). Exchangeable deuterium was equilibrated with protium by two recrystallizations from ethanol: water (80:20, v/v). This material (5) was pure as judged from melting point, TLC and HPLC.

MEASUREMENT OF ISOTOPIC PURITY

Measurement of deuterium content in the para- and para'-positions was carried out by two independent methods, i.e. mass spectrometry (MS) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR). While MS may not be capable of ensuring that some deuterium scrambling has not occurred during the deuteriolysis of the dibromo-precursor, ^{1}H-NMR, even at high field, does not provide sufficiently resolved signals and ^{13}C-NMR, at least in the broad-band proton-decoupling mode, faces the problem of inaccurate quantitative measurement due to the perturbation produced by different NOE (Nuclear Overhauser Enhancement) factors for the various signals concerned. We therefore set up an "inverse-gated" proton-decoupling experiment to obtain a NOE-free ^{13}C-spectrum. This was made possible by our prior knowledge of the T_1 of the carbons involved in our measurement (22).

The principle in the determination of the deuterium content in a specific position is based on the well-established behaviour of deuterium, which induces a shielding effect of 0.1 ppm on the adjacent β-carbon (22). Accordingly, the presence of deuterium in both para-positions produces a line at 128.25 ppm for the meta-carbon, which is then compared to the corresponding peak of the mono-deuterio phenytoin. As no d_0 impurity was detected by MS, the deuterium isotopic content of phenytoin-d_2 could be easily calculated. The table 1 provides the chemical shifts of phenytoin-d_0, d_1 and d_2 for the ipso(i), ortho(o), meta(m) and para(p) carbons.

Table 1. ^{13}C-Chemical shifts.

carbons	d_0 [a]	d_1 [a]	d_2 [a]
i	139.81	139.81	139.81
o	126.57	126.56	126.56
m	128.35	128.35 [b]	128.25
		128.25 [c]	
p	127.93	d	d

a. d_0, d_1 and d_2 denote phenytoin, p-deuteriophenytoin and p,p'-dideuteriophenytoin, respectively.

b. for the unsubstituted phenyl group.

c. for the p-deuteriophenyl group

d. This signal should appear as a triplet. Due to low NOE factor and very long T_1, this signal cannot be discriminated from the background noise.

Classical measurement of deuterium content by MS on different samples with isotopic content ranging from 86 to 95% produced figures in excellent agreement with those obtained by the ^{13}C-NMR, provided the NOE-suppression technique was applied. For example, a sample having 86+0.2% by MS had 85.5±0.8% by the ^{13}C-NMR technique. When the deuterium content was higher than 95%, the signal of the monodeuterio impurity could not be accurately discriminated from the noise.

When the reaction of deuteriolysis was carried with all the precautions described above, a material with an isotopic purity of 97.2±0.2% was obtained; another run in which the palladium on charcoal had been equilibrated with D_2O overnight yielded a material with an isotopic purity of 99.2±0.2%. When, however, the recrystallizations in dioxane:D_2O were omitted, the isotopic purity dropped down to 93.6±0.2%. When commercial anhydrous dioxane was used and no D_2O was added to the deuteriolysis medium, the deuterium content was as low as 86%.

PHARMACOLOGICAL ACTIVITY

The most significant test to evaluate the antiepileptic activity of phenytoin is the maximal electroshock test (MES test). Each compound was administrated orally to male NMRI mice (20-25g) as a suspension in a solution of tragacanth (1%). The dose-effect behaviour of the three products tested was examined by administration of five different doses of each compound, treating ten mice at each dose. One hour after administration of the drug, the animals were submitted to the MES test. Maximal electroshock seizures were elicited with a 50 Hz alternating current of 30 mA delivered for 0.2 sec via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes. Abolition of the hind limb tonic extension component of the seizure was defined as protection and results were expressed as ED_{50}'s in µmol/kg computed by the method of Litchfield and Wilcoxon.

Table 2. ED_{50} of phenytoin (d_0), p-deuteriophenytoin (d_1) and p,p'-dideuteriophenytoin (d_2) in the MES test.

Compounds	ED_{50} (µmol/kg)
d_0	27.8 (24.2-31.7)
d_1	25.0 (20.2-30.9)
d_2	29.4 (25.0-33.7)

CONCLUSION

While the deuterated analogues of phenytoin retained a high degree of antiepileptic activity, neither d_1 nor d_2 exhibited a clear-cut superiority over their protio-congener. In view of the excellent agreement found between the isotopic content data by ^{13}C-NMR and MS, it is obvious that the deuteriolysis process presented here is a specific one. Therefore, the molecular probe offered to the toxicologist appears useful to further delineate the stereospecific fate of the (R)-and (S)-arene oxide species of phenytoin.

EXPERIMENTAL SECTION

All reagents were purchased from Aldrich Chemicals and solvents were of Gold Label grade. Deuterium oxide had a deuterium content of 99.8%. p-Deuteriophenytoin was available from previous studies (15,17-19). Parr bottles were dried at 150°C overnight. ^{13}C-NMR spectra were recorded on a Brucker WP-80-SY operating at 20.15 MHz. The compounds were dissolved in DMSO-d_6 to form 0.5M solutions. The probe temperature was maintained at 38°C. The central peak of DMSO-d_6 was positioned at 39.60 ppm and was used as reference. The inverse-gated proton decoupling technique was set up according to Brucker specifications. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are corrected. GC-MS analyses were performed in a LKB model 9000 S instrument; mass spectra were recorded at 70 eV electron energy. Deuterium content determination was carried out on permethylated samples obtained after flash-heater treatment with trimethylanilinium hydroxide. Measurements carried out by the direct inlet technique on underivatized samples gave irreproducible figures. Isotope enrichment calculation was performed according to Millard (23). Merck TLC plates (silicagel 60 F) were eluted with a mixture of chloroform:acetone:benzene:ammonia (66:22:11:1); the spots were detected by iodine vapors or UV light.HPLC analyses were carried out with a Waters Associates model 6000A solvent delivery system, a Rheodyne model 7125 syringe-loading sample injector and a Pye Unicam (Cambridge, England) model LC3 variable wavelength UV detector. Detection was carried out at 220 nm. A Radial Pak (100x8 mm) C-8 10µ reverse phase column from Waters Associates was employed in connection with a methanol:water (70:30, v/v) solvent. IR spectra were recorded on a Perkin-Elmer model 457 spectrometer.

p,p'-Dibromobenzophenone (3) was prepared according to Montagne [24]. This compound was recrystallized three times from 95% ethanol to give a material melting at 175-176°C (lit.(25) 177°C). Yield : 65%.

p,p'-Dibromophenytoin (5,5-di-(4-bromophenyl)hydantoin)(4). A solution of 60 g of p,p'-dibromobenzophenone (0.176 mol) in 300 ml of DMF and a solution of 30 g of potassium cyanide (0.46 mol) were placed in a 500 ml stainless steel pressure bottle. After addition of solid ammonium carbonate (100 g, 1.04 mol), the bottle was closed and immersed in an oil bath thermostatized at 125°C for 72 h. After cooling, the bottle was opened and the reaction mixture was treated with 1.5 l of hot water. The turbid brownish mixture was transfered to a 5 l flask and slowly acidified under stirring by addition of concentrated hydrochloric acid. The crude product was dissolved in 10% sodium hydroxyde and the resulting mixture was extracted with ether to remove unreacted ketone. The aqueous layer was acidified with concentrated hydrochloric acid to give a precipitate which was crystallized from 95% ethanol (a sohxlet was employed) to give a material melting at 307-309°C (lit.(9) mp 308-309°C). Yield : 82%.

p,p'-dideuteriophenytoin (phenytoin-d_2) (5).

The finely powdered dibromohydantoin was dried to constant weight at 100°C under 0.02 mmHg. It was dissolved in dry dioxane and precipitated by addition of D_2O. This process was carried out twice. At this stage, the N-H signal in ^{1}H-NMR had faded completely. Prior use, palladium on charcoal was shaken in a Parr bottle with a ten-fold excess of D_2O. In a Parr bottle attached to a modified Parr hydrogenation apparatus totally dedicated to deuteriolysis, 2.5 g of the dibromoprecursor (6mmol) treated as mentionned above and dissolved in 95 ml of dioxane (redistilled from lithium aluminium hydride) 4 ml of triethylamine (redistilled from potassium hydroxide), and 1 ml of D_2O were shaken for 24 h at room temperature and at an initial pressure of 60 psi in the presence of 250 mg of 10% palladium on charcoal pretreated as above. The reaction mixture was filtered to remove the catalyst and some triethylammonium deuteriobromide. The filtrate was evaporated in vacuo and the residue was triturated with water and filtered. Two crystallizations from ethanol: water (80:20, v/v) yielded analytically pure (5)(HPLC k' 6.0) that cochromatographs with phenytoin. Melting point: 297-298° C. IR (KBr) 1775, 1720 cm^{-1}. MS (m/z) 254, 226, 212, 182, 105.

Yield : 62%.

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