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Synthesis and Distribution of Tritiated *N,N'*- Dibenzoyl-1,3-diaminopropan-2-ol.

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SUMMARY

Tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol, a compound mimicking a diacylglycerol moiety used as a lipid drug carrier was prepared from *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol by isotopic exchange in the presence of rhodium chloride. Preliminary preparation of the deuterated analog was made in order to assess the position of the substitution. A biodistribution study was carried out in mice after intravenous administration. Five minutes after administration, the level found in the brain was about 9 % of the injected dose per g organ. This value decreases to 1 % 3 hours after administration while in the same time radioactive levels measured in the urine increased.

The results are in accordance with the pharmacological evaluation : *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol exhibits an anticonvulsant activity at 30 minutes in the maximal electroshock seizure test, but was found inactive at 3 hours.

KEY-WORDS : drug carrier, ^{3}H, rhodium trichloride, isotopic exchange

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INTRODUCTION

The conjugation of pharmacological agents to glycerides is a chemical drug delivery strategy known to modify the relative distribution of the active agent in the body by conferring some physiological and metabolic properties of the glycerides. This approach was initially designed to enhance the gastric tolerance to non-steroid anti-inflammatory drugs and lately, to take advantage of the lymphatic pathways to bypass the liver metabolism and/or to target the lymphatic compartment. The ultimate application was to use diacylglycerols as cephalotropic vectors (for a review, see ref. 1). For instance, GABA pseudotriglycerides demonstrate an ability to significantly increase GABA brain penetration (2-5). This latter approach however, suffers from the short plasmatic half-lives of lipid conjugates, which are comparable to those of natural triglycerides. In order to overcome this limitation, we introduced two modifications to the structure of the pseudoglycerides. The first was the bioisosteric substitution of the 1,3-ester bonds by amide bonds (6-7), presumably more resistant to chemical and enzymatic hydrolyses; secondly, non natural fatty acid chains or synthetic substituents were introduced.

In this connection, *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol (figure, 1) was synthesized in order to mimic the lipid drug carrier. However, we surprisingly found that *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was itself pharmacologically active and in particular, exhibits an anticonvulsant activity in the maximal electroshock seizure test. In this model, its ED_{50} is 0.12 mmol/kg (0,049-0.285, 95 % confidence intervals), 30 min after administration to mice. In strychnine-induced seizures, *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol significantly increases the latency to elicit tonic seizures (8-9).

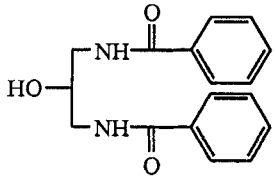


Figure 1 : Structure of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol

The aim of the present study was to synthesize the radiolabelled *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol by catalytic exchange in order to explore its *in vivo* distribution and to determine to what extent it crosses the blood-brain barrier.

EXPERIMENTAL SECTION

Materials

Chemicals were obtained from commercial sources at the highest purity available. Thin layer chromatography (TLC) was run using silicagel 60F254 thin layer plates (Merck, 0.25 mm). Spots were visualized under UV light and iodine vapours.

Radio-thin layer chromatography was carried out using a radio-TLC scanner (Bioscan, Auto changer 300, system 200 Imaging Scanner) with similar silicagel plates.

Radioactivity was measured in a liquid scintillation spectrometer (Liquid scintillation counter-Pharmacia Wallac 1410). An automatic correction of quenching and chemoluminescence was included in the counting program.

Synthesis of N,N'-dibenzoyl-1,3-diaminopropan-2-ol

25 g of 1,3-diaminopropan-2-ol (263 mmol, Aldrich-Chemie, 95 %) were dissolved in 100 ml acetic acid (Union Chimique Belge, p.a.) in a 250 ml Erlenmeyer flask. This dissolution is highly exothermic. 131 g of benzoic anhydride (580 mmol, Merck-Schuchardt) was added with vigorous stirring. After cooling, a precipitate was formed, washed with water and then with a saturated solution of sodium carbonate until the effervescence ceased. The precipitate results in a mixture of triacylated and diacylated products. Alkaline hydrolysis (20 % sodium hydroxide, 5 min., room temperature) in methanol generates only the desired diacylated compound. Recrystallization from a mixture of water/ethanol (9:1, v/v) affords 55.6 g of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol.

Yield : 72 %

Melting point (uncorrected) : 159-160 °C

Rf (TLC) : 0.38 (eluent : acetone-methylene chloride 1 : 1)

Mass spectra (FAB, Kratos instrument) : [M+1] = 299

RMN ^{13}C (d, ppm, $CDCl_3$) : 43.2 (CH_2), 70.6 (CH-OH), 127.2 (CH arom.), 128.7 (CH arom.), 131.8 (CH arom.), 133.9 (C arom ipso), 169.2 (NH-CO).

An. El. : Calculated C 68.44; H 6.08; N 9.39

Found C 68.58; H 6.15; N 9.19

Synthesis of deuterated N,N'-dibenzoyl-1,3-diaminopropan-2-ol

The procedure used to prepare deuterated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was adapted from the method of Hesk (10). Briefly, using a microkit (Aldrich-Chemie), 0.18 mmol of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol and 0.18 mmol of rhodium trichloride (Aldrich-Chemie) were placed in a round-bottom flask. 0.75 ml of anhydrous dimethylformamide (Aldrich-Chemie) and 0.75 ml of deuterated water (Aldrich-Chemie) were added. The reaction mixture was heated at 108° C for 18 h. After cooling, the reaction mixture was poured into 20 ml of ethyl acetate and successively washed with a solution of saturated sodium hydrogencarbonate (3 x 2 ml), 3M HCl (3 x 2 ml) and deionised water (3 x 2 ml). The different aqueous phases were re-extracted with 10 ml of ethyl acetate. The organic phases were combined, dried over sodium sulfate, filtered and concentrated under reduced pressure. The solid was purified on a silicagel TLC with a 1:1 mixture ethylene chloride-acetone as eluent, giving a compound which elutes at the same time as unlabelled *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol.

Rf (TLC) : 0.38 (eluent : acetone-methylene chloride 1 : 1)

Mass spectra (FAB, Kratos instrument) : [M+1] = 303

RMN ^{13}C (d, ppm, $CDCl_3$) : 43.2 (CH_2), 70.6 (CH-OH), 127.2 (CH arom.), 128.7 (CH arom.), 131.8 (CH arom.), 133.9 (C arom ipso), 169.2 (NH-CO).

Synthesis of tritiated N,N'-dibenzoyl-1,3-diaminopropan-2-ol

Tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was prepared as above replacing deuterated water with tritiated water. 30 mg of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol and 30 mg of rhodium chloride were added to 0.5 ml of anhydrous dimethylformamide, followed by 10 µL of tritiated water

(Amersham Belgium, 185 GBq/ml) and the reaction mixture heated at 108°C for 18 h. After work-up as described above, the product was purified on a silicagel TLC plate with a 1:1 mixture of methylene chloride-acetone as eluent. The spot corresponding to the desired product was isolated and extracted with 10 ml chloroform-ethanol (1:1).

Distribution in mice

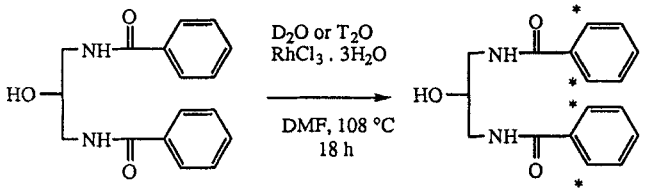
NMRI male mice (15-20 g) from the University facilities were used. Each experiment was repeated three times and all radioactive countings were performed in triplicate.

Distribution of tritiated N,N'-dibenzoyl-1,3-diaminopropan-2-ol

0.4 mmol of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol containing tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol affording ~ 10^6 dpm /animal was administered as a dimethyl sufoxide solution (volume : 2 ml/kg) intravenously to mice via one of the tail veins. Two minutes before the required time (5 min., 30 min. and 180 min.) the mice were anaesthetised with diethyl ether and sacrificed by cardiac puncture. Several organs and biological fluids were collected : brain, liver, kidneys, spleen, lungs, heart, tail (site of injection), bone, muscle, blood and urine. Three tissue aliquots were collected, carefully washed with a saline solution and then dissolved during 18 h at 40° C in 1 ml Soluene 350 ® (Canberra-Packard). Samples were discolored by addition of 1 ml hydrogen peroxide (UCB) to small portions (0.1-0.2 ml) and then diluted with 10 ml of scintillation liquid Hionic fluor ® (Canberra-Packard). Radioactive samples were counted 24 h after this addition in order to avoid chemiluminescence problems. The radioactivity of the injected solution was measured with the counting of three aliquots and the volume injected was determined by weighing. The results are expressed as corrected injected radioactivity i.e. the administered radioactivity minus that precipitated at the site of injection.

RESULTS

The synthesis of deuterated and tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was performed according to the method of Hesk et al. (11), namely rhodium trichloride catalysed isotopic exchange (Scheme 1).



Scheme 1. Synthesis of deuterated and tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol, * indicates the labelled positions

The radiochemical and chemical yields are presented in Table I.

Chemical yield	57 %
Radiochemical yield	98 %
Specific activity	89.7 MBq/mmol

Table I. Purity and yields of tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol

The number and the position of the aromatic hydrogens isotopically substituted were determined by NMR and mass spectra of the deuterated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol. The ortho aromatic peak is reduced and gave a triplet in the ^{13}C-NMR while in the FAB mass spectrum, the deuterated compound presents a [M+1] of 303 compared to a 299 value for the unlabelled compound. These results indicate that four ortho aromatic hydrogens have been substituted (Figure 2). Radio-TLC as well as ordinary TLC conducted with dichloromethane/acetone (1:1) and hexane/propan-2-ol (1:1) as eluents indicate spot identity between unlabelled *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol and deuterated or tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol.

The *in vivo* distribution of tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was carried out at 5 min., 30 min. and 3 h after intravenous administration to mice, 30 min. is the time peak for optimal anticonvulsant activity (9-10). The results presented in Table II are expressed as percentages of corrected injected radioactivity both per organ and per g of organ.

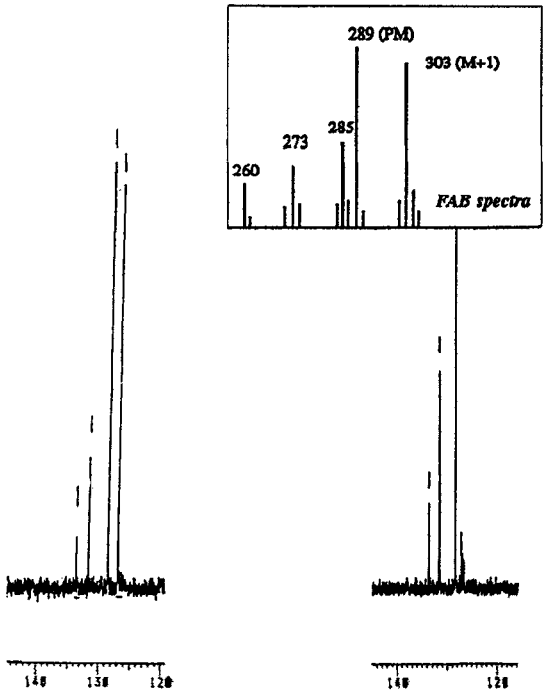


Figure 2 : ^{13}C-NMR signals of the aromatic carbons of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol (to the left) and its deuterated analog (to the right). In the window, the mass spectrum of deuterated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol is presented.

	% radioactivity 5 min.		% radioactivity 30 min.		% radioactivity 180 min.	
	per org.	per g org.	per org.	per g org.	per org.	per g org.
Blood	9.27 ± 1.47	7.76 ± 0.86	1.90 ± 0.26	1.15 ± 0.14	0.93 ± 0.18	0.55 ± 0.10
Brain	1.70 ± 0.28	3.75 ± 0.50	0.21 ± 0.04	0.55 ± 0.09	0.12 ± 0.03	0.27 ± 0.05
Liver	12.20 ± 1.4	13.54 ± 1.6	6.49 ± 0.81	5.59 ± 0.26	1.64 ± 0.09	1.39 ± 0.06
Lung	1.8 ± 0.28	10.4 ± 1.71	0.12 ± 0.02	0.73 ± 0.09	0.17 ± 0.02	1.07 ± 0.11
Kidney	3.06 ± 0.06	10.2 ± 2.4	1.21 ± 0.1	4.41 ± 0.62	0.66 ± 0.09	2.32 ± 0.41
Spleen	0.30 ± 0.09	2.60 ± 0.35	0.11 ± 0.03	0.77 ± 0.16	0.07 ± 0.01	0.55 ± 0.12
Heart	0.96 ± 0.14	3.57 ± 0.95	0.10 ± 0.01	0.80 ± 0.12	0.05 ± 0.01	0.47 ± 0.1

Table II : Distribution of radiolabelled *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol. Results are expressed by the mean ± s.e.m. ($n \geq 3$).

N,N'-dibenzoyl-1,3-diaminopropan-2-ol is quickly distributed throughout the entire body, included the brain. The elimination is essentially through the kidneys. The amount found in the urine 30 min. after injection was 69 ± 14 % of the injected dose. As seizures occur in the central nervous system, our attention was focused on the crossing of the blood-brain barrier by *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol. In the five minutes following the iv injection, 1.7 % of the radioactivity of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was found in the brain. The usual way for estimating the penetration through the blood-brain barrier is to determine the Brain Penetration Index (BPI) according to the method of Shashoua *et al.* (2-4, 12). The BPI is defined as the following ratio :

$$BPI = \frac{\text{radioactivity in the brain} \times 100}{\text{radioactivity in the liver}}$$

measured 5 min. after the administration.

The liver was chosen as reference organ as no physiological barrier restricts the hepatic uptake, *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol has a BPI of 13.9 ± 2.1.

DISCUSSION

Hesk and co-workers (11) developed an efficient method of one-step catalytic deuteration and tritiation, using rhodium trichloride catalyst. Applying this method, we describe a new example of regiospecific deuteration and tritiation of the aromatic nucleus of a pharmacological agent. The preparation of tritiated *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was conducted in order to characterize the properties of this « drug carrier ». *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol was initially designed as a potential mimic of a diacylglycerol-acting drug carrier. Glycine and *N*-benzyloxycarbonylglycine were attached to this carrier system and these compounds exhibit some anticonvulsant activities (10). Surprisingly, *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol itself was found to be an anticonvulsant too, but to a lower extent.

We found that *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol is quickly distributed throughout the body and also penetrates the brain rapidly. This result is in accordance with the anticonvulsant activity of *N,N'*-dibenzoyl-

1,3-diaminopropan-2-ol. The BPI measurement is compatible with a transcellular diffusion process; for example glycine and GABA which are two amino-acids known to be unable to significantly cross the blood-brain barrier have BPI values below 2 (2,12). In conclusion, the rationale behind the design of *N,N'*-dibenzoyl-1,3-diaminopropan-2-ol as a potential drug carrier has been described; so also has its ability to confer sufficient lipophilicity to hydrophilic pharmacological agents and its crossing of the blood-brain barrier. However, the discovery of intrinsic pharmacological activity definitively restricts its use as CNS drug carrier.

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