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Esters of 6-dimethylaminohexanoic acid as skin penetration enhancers

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We prepared a series of five esters of 6-dimethylaminohexanoic acid, and characterised the compounds by their NMR and IR spectra. Their ability to function as transdermal penetration enhancers was subsequently evaluated using excised human skin as a membrane, modified Franz diffusion cells, and theophylline as a model permeant. The penetration – enhancing efficiency of esters **1**–**5** was studied in the donor media of propylene glycol and isopropyl myristate, and expressed as the corresponding enhancement factors (EF). All the esters increased the penetration of theophylline through the skin. The enhancement factor for the most active substance, undecyl 6-dimethylaminohexanoate, was 118.5 (± 19) from propylene glycol.

1. Introduction

There are a number of potent transdermal penetration enhancers among the derivatives of ω-amino acids, such as amides, esters, cyclic amides and carbamates [1]. In our previous communications, we described notable penetration-enhancing activities of some esters derived from linear ω-amino acids [2, 3].

Amine-based enhancers have also been reported. These are cyclic amines, often described as the analogues of Azone, where the nitrogen atom is built in a ring, e.g. 1-dodecylazetidine, 1-dodecylpyrrolidine, 1-dodecylpiperidine and N-dodecylmorpholine, [4] analogous to its amide, and also substituted tertiary amines of various polarities, given by the presence of at least one hydroxy alkyl residue in their molecules. The results of the evaluation of their activities show that cyclization and the removal of the carbonyl group give rise to compounds with substantially lower penetration-enhancing efficiency and higher irritability [5].

In this work we decided to increase the basicity of the lead compounds [2] of the linear amino acid ester type by the introduction of a tertiary amine moiety into their molecules while preserving their high lipophilicities, and to study the influence of this modification on their activity.

2. Investigations, results, and discussion

2.1. Synthesis of the target compounds

6-Dimethylaminohexanoic acid was prepared by the Eschweiller-Clarke protocol according to Fusco *et al.* [6] from 6-aminohexanoic acid, which was treated with formaldehyde and formic acid in an alkaline medium. The crude product was purified on a Dowex 50 column. The esters were synthesized as described previously [3] by the reaction of the hydrochloride of the acid chloride with the corresponding alcohol. The resultant hydrochloride of the ester was dissolved in chloroform and filtered through a column of alumina. The first fractions were separated in order to remove the recovered primary alcohol. The subsequent fractions were combined and concentrated *in vacuo*. The residue was allowed to stand over a solid paraffin under vacuum for 24 h in order to remove traces of chloroform, dissolved in water, and diethylamine was added to the solution. The resultant oily ester was extracted into diethyl ether, the organic solution was concentrated and allowed to stand over sulphuric acid for several days. The product was then purified on a column of alumina in the same way, and the compounds were characterised by their NMR and IR spectra.

2.2. Penetration-enhancing activity

The experiment was carried out *in vitro* using the modified Franz cells. For all esters, theophylline was used as a permeant of medium polarity, and its concentration in the acceptor phase was determined by HPLC. The results of the 48 h measurements were expressed as the corresponding enhancement factors (EFs). EF for a compound is defined as the ratio of the penetration of theophylline from the donor sample containing 5% of the compound to the penetration from the control sample (without the enhancer, using the skin from the same donor). Presented results are averages of 3–4 experiments.

Substances **1**–**5** were tested in the propylene glycol vehicle. Owing to a good solubility of the esters in this solvent, their 5% solutions were used. The mean values of EF indicate that all compounds showed a high penetration-enhancing effect for theophylline from propylene glycol. The value of 118.5 (±19) represents a more than 100-fold increase as compared to the control sample, which appears to be an almost unique result under the given circumstances. The quantity of the effect is then related to the length of the molecule.

Only the activities of compounds **1, 3,** and **5**, i.e. the first, the last and the middle one from the homologous series were evaluated in the medium of isopropyl myristate. The structure-activity relationship seems probably the same in this case, even though the effect of the enhancers is lower by a few orders. The decrease of activity in a hydrophobic donor medium can be explained by both the enhancer and the permeant possessing a substantially higher affinity towards isopropyl myristate due to their properties. The results of the tests are depicted in Figs. 1 and 2.

Table 1: Prepared compounds

$(CH_3)_2N—(CH_2)_5COO—R$

Compd.	R
1	oktyl
2	nonyl
3	decyl
4	undecyl
5	dodecyl

Table 2: Data for compounds 1–5:

Compd.	Formula M.W.	Yield [%]*	IR ($CHCl_3$) [cm^{-1}] νC=O	ν–O–CH_2
1	$C_{16}H_{33}NO_2$ 271.5	63	1725	1467
2	$C_{17}H_{35}NO_2$ 309.6	65	1725	1467
3	$C_{18}H_{37}NO_2$ 299.5	52	1725	1467
4	$C_{19}H_{39}NO_2$ 313.5	50	1725	1467
5	$C_{20}H_{41}NO_2$ 327.6	39,3	1725	1467

* The yield is related to the starting amino acid

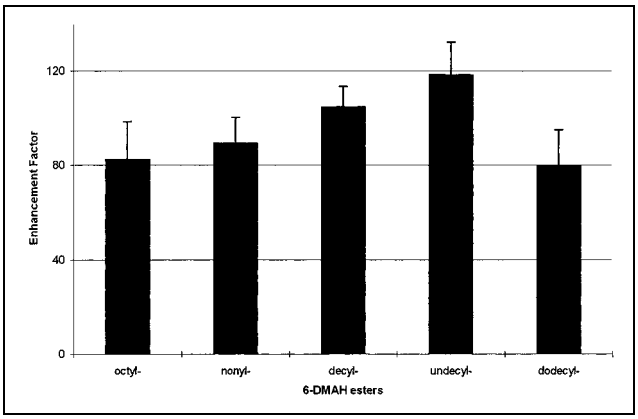

Fig. 1: EF values of 5 % 6-dimethylaminohexanoic (6-DMAH) esters in propylene glycol at *in vitro* skin permeation of theophylline (3 to 5 replicants)

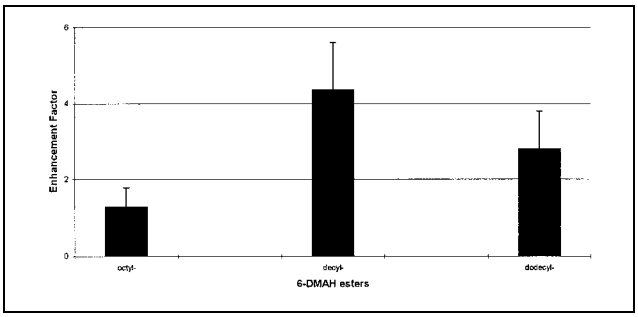

Fig. 2: EF values of 5 % 6-dimethylaminohexanoic (6-DMAH) esters in isopropyl myristate at *in vitro* skin permeation of theophylline (3 to 5 replicants)

2.3. Structure – penetration-enhancing activity relationships

The quantity of the effect of the esters of 6-dimethylaminohexanoic acid is probably a function of the overall length of the molecule. In case of a hydrophilic medium, the relationship has a bell-like shape with a maximum of penetration-enhancing efficiency corresponding to 19–20 atoms. The activity of the most active substance **4** was unusually high 118,5 (±19). We thus conclude that upon increasing the basicity of the terminal amino group by the substitution of two hydrogens of the primary amino group with two methyl moieties, transdermal penetration enhancers of high efficiency can be prepared.

Similar structure-activity relationships hold for a hydrophobic medium, although the activities are about ten times lower.

3. Experimental

3.1. Starting materials

6-Aminohexanoic acid, 40% aqueous formaldehyde solution, formic acid, alkanoles, propylene glycol, isopropyl myristate, and aluminium oxide were commercially available (Merck, Darmstadt). The alkanoles were dried over 4 Å molecular sieves and redistilled before use. Methanol for HPLC was supplied from Sigma.

3.2. Preparation of 6-dimethylaminohexanoic acid

6-aminohexanoic acid (50,0 g 0,44 mol) was dissolved in a 25% sodium carbonate solution (100 ml), and 85% formic acid (41.6 ml, 0.92 mol) followed by 36% formaldehyde solution (73 ml, 0.92 mol) were added. The resultant mixture was heated at reflux until carbon dioxide ceased to evolve, the same amounts of formaldehyde and formic acid of the same concentrations were added again, and the mixture was again heated until the evolution of carbon dioxide ended. The mixture was concentrated *in vacuo*, the residue was dissolved (concentration 10%), and the solution was transferred onto a column of freshly activated Dowex 50 in the H-cycle. Following the anchoring of the amino acid to the ionex, it was eluted with a 1 M ammonia solution. The solution was evaporated to dryness, and the resultant ammonium salt allowed to stand over sulphuric acid under vacuum for ten days with occasional stirring. The crude acid was crystallised from the propanol/aceton mixture. The yield of 6-aminohexanoic acid was 45 g (62%), melting point 107–9 °C, which was in agreement with the literature [6].

3.3. Preparation of esters of 6-aminohexanoic acid hydrochlorides (general procedure)

6-Aminohexanoic acid hydrochloride (2.0 g, 10.2 mmol) was dissolved in thionyl chloride (10 ml) at 40 °C. After 20 minutes, the solution was concentrated *in vacuo*, toluene (5 ml) was added to the residue, and the resultant mixture was concentrated again to afford dry, colourless crystals of the acid chloride.

A solution of the corresponding alkanol (10.2 mmol) in chloroform p.a. (10 ml) was then added to the above intermediate, the reaction mixture was stirred at 70 °C for 60 minutes, and evaporated to dryness. Hydrogen chloride was removed from the residue upon standing over potassium hydroxide under vacuum. In order to remove the unreacted alcohol, the crude product was subsequently dissolved in a minimum amount of chloroform, and transferred onto a column (5 × 300 mm) with 10 g of alumina. Ester-containing fractions were concentrated, the residue was dissolved in water and diethyl amine (1 ml) was added. The resultant emulsion was extracted with diethyl ether (3 × 10 ml), the extracts were combined, dried over sodium sulfate, and the solvent removed. The residue was allowed to stand over sulphuric acid under vacuum in order to remove the traces of diethyl amine, dissolved in chloroform and purified on a column of alumina in the same fashion as the hydrochloride of the ester.

The IR spectra of $CHCl_3$ solutions were recorded on a Nicolet Impact 400 spectrometer. Wavenumbers are given in cm^{-1}. The NMR spectra were recorded for $CDCl_3$ solutions on a Varian Mercury-Vx BB 300 instrument, operating at 300 MHz for ^1H, 75 MHz for ^{13}C. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^1H and 77.0 ppm for ^{13}C).

1: Yield: 63%. IR ($CHCl$)(cm^{-1}): 1725 (C=O), 1467 (–O–CH_2). ^1H NMR (300 MHz, ($CDCl_3$) δ 4.03t 2 H J = 6.73 (CH_2), 2.28t overlaped 2 H J = 7.56 (CH_2), 2.26–2.19m 2 H (CH_2), 2.18s 6 H (CH_3), 1.68–1.52m 4 H (CH_2), 1.52–1.39m 2 H (CH_2), 1.38–1.19m 12H (CH_2), 0.86t 3 H J = 6.73 (CH_3). ^{13}C NMR 173.8, 64.4, 59.6, 45.4, 34.3, 31.7, 29.2, 29.1, 28.6, 27.4, 27.0, 25.9, 24.9, 22.6, 14.0.
$C_{16}H_{33}NO_2$ (271.5)

2: Yield: 65%. IR ($CHCl_3$) (cm^{-1}): see compd. **1**. ^1H NMR (300 MHz, ($CDCl_3$) δ 4.03t 2 H J = 6.73 (CH_2), 2.28t overlaped 2 H J = 7.56 (CH_2), 2.26–2.19m 2 H (CH_2), 2.18s 6 H (CH_3), 1.68–1.52m 4 H (CH_2), 1.52–1.38m 2 H (CH_2), 1.37–1.17m 14H (CH_2), 0.85t 3 H J = 6.73 (CH_3). ^{13}C NMR 173.8, 64.4, 59.6, 45.4, 34.2, 31.8, 29.4, 29.2, 29.2, 28.6, 27.4, 27.0, 25.9, 24.9, 22.6, 14.1.
$C_{17}H_{35}NO_2$ (309.6)

3: Yield: 52%. IR: see compd. **1**. ^1H NMR (300 MHz, ($CDCl_3$) δ 4.03t 2 H J = 6.73 (CH_2), 2.28t overlaped 2 H J = 7.42 (CH_2), 2.25–2.19m 2 H (CH_2), 2.18s 6 H (CH_3), 1.69–1.52m 4 H (CH_2), 1.52–1.39m 2 H (CH_2), 1.38–1.16m 16H (CH_2), 0.86t 3 H J = 6.73 (CH_3). ^{13}C NMR 173.8, 64.4, 59.6, 45.4, 34.3, 31.8, 29.5, 29.3, 29.2, 28.6, 27.4, 27.0, 25.9, 24.9, 22.6, 14.1.
$C_{18}H_{37}NO_2$ (299.5)

4: Yield: 50%. IR: see compd. **1**. ^1H NMR (300 MHz, ($CDCl_3$) δ 4.04t 2 H J = 6.73 (CH_2), 2.29t overlaped 2 H J = 7.56 (CH_2), 2.26–2.17m 2 H (CH_2), 2.19s overlaped 6 H (CH_3), 1.69–1.53m 4 H (CH_2), 1.53–1.40m 2 H (CH_2), 1.39–1.17m 18H (CH_2), 0.86t 3 H J = 6.73 (CH_3). ^{13}C NMR 173.8, 64.4, 59.6, 45.5, 34.3, 31.9, 29.6, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 27.0, 25.9, 24.9, 22.6, 14.1.
$C_{19}H_{39}NO_2$ (313.3)

5: Yield: 39.3%. IR: see compd. **1**. ^{1}H NMR (300 MHz, ($CDCl_3$) d 4.03t 2 H J = 6.73 (CH_2), 2.29t overlaped 2 H J = 7.42 (CH_2), 2.26−2.19m 2 H (CH_2), 2.19s overlaped 6 H (CH_3), 1.70−1.53m 4 H (CH_2), 1.53−1.39m 2 H (CH_2), 1.38−1.16m 20H (CH_2), 0.86t 3 H J = 6.73 (CH_3). ^{13}C NMR 173.8, 64.4, 59.6, 45.5, 34.3, 31.9, 29.6, 29.6, 29.5, 29.5, 29.3, 29.2, 28.6, 27.4, 27.0, 25.9, 24.9, 22.7, 14.1.
$C_{20}H_{41}NO_2$ (327.6)

3.4. In vitro permeation experiment

The experiments were designed to imitate the occlusive administration with the use of the modified Franz cells using theophylline as the permeant. The donor samples (0.75 ml) contained 2.5% theophylline suspended in propylene glycol or in isopropyl myristate containing 5% of the tested ester (with the exception of the control samples). The isotonised phosphate buffer (pH 7.4) maintained at a constant temperature of 37 °C was used as the acceptor phase. Samples for the determination of the concentration of the permeant were withdrawn at fixed time intervals during all 48-hour experiments.

3.5. Determination of theophylline

The HPLC apparatus consists of an LCP 4100 high-pressure pump (ECOM, Prague), LiChroCART 125-4 (LiChrospher 100, RP 18, 5 μm, Merck, Darmstadt), an SP 8440 UV detector (Spectra Physics) and CSW version 1.7 of the integrating software. CH_3OH/H_2O (1 : 1) was used as a mobile phase at a flow rate of 1 ml/min. The effluent was monitored at 272 nm. The retention time of theophyllinee was 2.70 ± 0.02 min.

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