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Transkarbams with terminal branching as transdermal permeation enhancers

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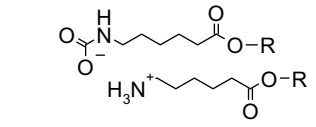
Abstract—Transkarbams (T) represent novel group of highly active, non-toxic transdermal permeation enhancers. This study was focused on the influence of small symmetrical terminal branching on their enhancing activity. Series of T with terminal methyl or ethyl branching was prepared and their permeation-enhancing activity was compared to that of their linear analogues. The results showed completely a different behaviour from similarly branched alcohols, supporting the key role of the ammonium-carbamate polar head in the enhancing activity of T.

One of the approaches used to enlarge the number of transdermally applicable drugs is the employment of permeation enhancers. These compounds facilitate drug absorption through the skin by a reversible decrease of the barrier resistance; however, the exact mechanism of action of most enhancers has not been elucidated yet. They can act by one of these alternative ways: interaction with the stratum corneum intercellular lipids, interaction with protein structures and partitioning modification; combination of these effects is possible as well.[1–4]

A novel group of transdermal permeation enhancers, the so-called transkarbams (carbamic acid salts derived from ω-amino acid esters, T), has been reported in our previous studies.[5,6] These compounds were specifically designed as open-cycle analogues of Azone® (1-dodecylazacycloheptan-2-one).[7,8] One of these substances, namely 5-(dodecyloxycarbonyl)pentylammonium-5-(dodecyloxycarbonyl)pentylcarbamate (Transkarbam 12, **T12**, Fig. 1) has shown an exceptional enhancing activity for numerous drugs with a wide range of physico-chemical properties,[9,10] displaying low toxicity and



R: linear		branched	
T8	octyl	**2a**	7-methyloctyl
T9	nonyl	**2b**	8-methylnonyl
T10	decyl	**2c**	9-methyldecyl
T11	undecyl	**2d**	10-methylundecyl
T12	dodecyl	**2e**	11-methyldodecyl
		2f	8-ethyldecyl
		2g	10-ethyldodecyl

Figure 1. Studied linear transkarbams **T8**–**T12** and their methyl- and ethyl-branched analogues **2a**–**2g**.

no dermal irritability.[9] Its high activity seems to be closely related to the structure of its polar head (ammonium carbamate salt) that can be decomposed easily in mildly acidic environment releasing a molecule of CO_2.[11,12] The free amino ester[9] as well as compounds with CO_2 covalently bound in the polar head[13] appeared to be inactive. Moreover, ester group in the lipid chains exhibited a key influence on the enhancing activity as well.[14,15]

Previously, the influence of terminal methyl and ethyl branching on the activity of fatty alcohols with the chain length of 8–12 carbons was studied.[16] In 12C alcohols,

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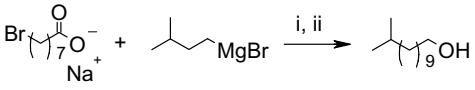
the methyl branching did not significantly change the activity, whereas the ethyl branching increased the enhancement potency by approximately 50%.

This study attempts to elucidate the influence of terminal branching in the alkanol moiety on the transdermal permeation-enhancing activity of T. The previously studied branched alcohols were incorporated into the molecules of newly synthesized T (compounds **2a**–**2g**, Fig. 1) in order to find whether such structural change will increase their enhancing activity in comparison with the linear T (**T8**–**T12**, Fig. 1).

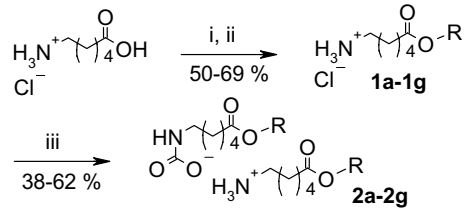
For the preparation of the branched carbamates **2a**–**2g**, the branched alcohols were prepared first by a procedure described previously.[16] First, branched carboxylic acid salts were obtained by linking ω-bromo acid sodium salts of a suitable chain length with the corresponding branched alkylmagnesium bromides in the presence of Li_2CuCl_4. Without isolation, the crude product was reduced with $LiAlH_4$ to yield the branched alcohols (Scheme 1).

Then 6-aminohexanoic acid hydrochloride was converted to the corresponding acyl chloride that further reacted with the appropriate branched alcohol to form ester.[17] The amino group was liberated with triethylamine (TEA) and the basic amino ester then reacted with CO_2 to form the target carbamate salts[18] (Scheme 2). The yields, melting points and spectral data of the newly prepared compounds **2a**–**2g** and their precursors **1a**–**1g** are available in the Supporting information.

The presence of the ammonium carbamate salt was confirmed by NMR and IR spectroscopy. The ^{13}C NMR spectrum in toluene showed a signal at 163 ppm, attributed to the carbonyl carbon of the carbamate salt, and two resonances at 42 and 41 ppm, corresponding to the methylene carbons next to NH_3^+ and $NHCOO^-$, respectively. The ^1H NMR spectrum further confirmed



Scheme 1. Synthesis of 11-methyldodecanol. Other branched alcohols were prepared likewise using different combinations of the chain lengths and type of branching of the precursors (described in detail in Ref. 16). Reagents and conditions (i) Li_2CuCl_4, THF, −10 °C, 4 h; (ii) $LiAlH_4$, THF, reflux, 1 h.



Scheme 2. Synthesis of the branched carbamate salts. Reagents and conditions (i) $SOCl_2$, 40 °C, 30 min; (ii): R–OH, $CHCl_3$, reflux, 1.5 h; (iii): TEA, CO_2. R: as in Figure 1.

the carbamate structure; both NH_3^+ and NH signals were observed at around 5 and 3.3 ppm, respectively, together with two signals of the methylene hydrogens adjacent to ammonium and carbamate nitrogen at around 2.6 and 2.2 ppm, respectively, at the ratio of 1:1. When dissolved in $CHCl_3$ or similar acidic solvents, the products, i.e. ammonium carbamate salts, decomposed into carbon dioxide and the parent amino esters were detected. In the IR spectrum in Nujol, the medium-intensity band at 1634–1652 cm^{-1}, assigned to the amide I band of the carbamic acid salt, was present. When dissolved in $CHCl_3$, the band disappeared, while a new one emerged at 2337 cm^{-1}, corresponding to the released CO_2 (see Supporting information, IR of the compounds in Nujol and $CHCl_3$, respectively). For the detailed information on the behaviour of the long-chain ammonium carbamates, see Refs. 11 and 12.

The transdermal permeation-enhancing activity was evaluated in vitro using Franz diffusion cell, theophylline as a model drug and porcine ear skin of full thickness as a model membrane. Theophylline was selected as a model permeant of medium polarity as it has been widely studied previously in various transdermal drug delivery systems.[19,20] The donor samples were prepared by suspending theophylline (50 mg) and the tested enhancer (10 mg) in 60% propylene glycol (1 mL). The control samples were prepared likewise without addition of the enhancer. This amount of theophylline was well above its solubility limit, i.e. the maximum thermodynamic activity was maintained in all donor samples either with or without the enhancers. In the aqueous donor samples, the ammonium carbamates are stable providing neutral or slightly alkaline pH is maintained.[11,21] Propylene glycol was added to the donor samples according to our previous results showing greater enhancement ability of T in this vehicle. Phosphate buffer saline (pH 7.4) supplemented with 0.03% of sodium azide was used as the acceptor phase. The detailed description of the skin preparation, permeation experiment and HPLC determination of theophylline in the acceptor phase was described elsewhere.[16]

Cumulative amounts of theophylline (μg/cm^2) permeated through the skin were plotted against time and steady state fluxes (μg/cm^2/h) were calculated from the linear region of the plot. The enhancement ratio (ER) was calculated as the ratio of the flux of theophylline with and without the enhancer. The data are presented as means ± SD ($n = 6$–14) obtained from the skin fragments of 4–8 animals (on each skin fragment both enhancer and its respective control were evaluated). The statistical significance of the differences was analyzed with the Student's t-test; value of $p < 0.05$ was considered significant.

The permeation-enhancing activities expressed as ER are summarized in Table 1.

As shown in Table 1, both linear and branched T reflected a parabolic relationship between their chain length and permeation-enhancing activity. The maximum was observed at around 10 carbons in the alkanol

Table 1. Enhancement ratios (ER) of the studied carbamates: R–O–CO–$(CH_2)_5$NHCOO$^-$ $H_3N^+(CH_2)_5$–CO–O–R

Compound	R	ER ± SD[a]
T8	Octyl	10.35 ± 4.95
T9	Nonyl	16.28 ± 5.70
T10	Decyl	33.60 ± 3.38
T11	Undecyl	28.86 ± 9.80
T12	Dodecyl	20.02 ± 3.69
2a	7-Methyloctyl	11.41 ± 5.86
2b	8-Methylnonyl	23.28 ± 8.77
2c	9-Methyldecyl	26.34 ± 11.95
2d	10-Methylundecyl	26.12 ± 9.93
2e	11-Methyldodecyl	18.97 ± 10.15
2f	8-Ethyldecyl	23.19 ± 4.17
2g	10-Ethyldodecyl	18.08 ± 6.12

n = 6–14 (skin fragments from 4 to 8 animals for one compound).
[a] Significantly different from control ($p < 0.05$) for all presented data.

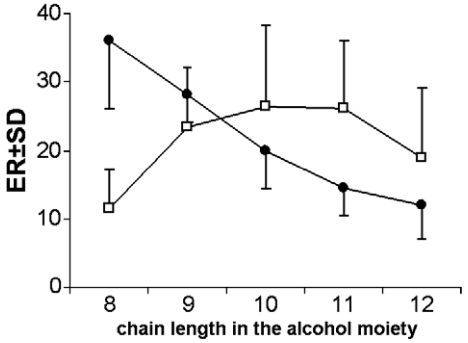


Figure 2. The enhancing activity of methyl-branched fatty alcohols and T plotted against their chain length. Filled circles: alcohols; open squares: T. The ER values of the alcohols were evaluated under the same conditions in our previous study.[16] Data are presented as means ± SD.

moiety in both series (ER 33.60 ± 3.38 and 26.34 ± 11.95 in **T10** and **2c**, respectively). This finding was consistent with those made by Hrabálek et al.[6] and Kanikkannan et al.[22]

Nevertheless, there was no significant difference between the linear and the methyl-branched group. The ethyl branching even caused a decrease in activity (see compounds **T10** and **2f** with the same chain length; $p < 0.05$). On the other hand, when comparing **2f** and **T12**, i.e. derivatives with the same carbon number and approximately the same lipophilicity, their potencies were comparable. In compounds **T12** and **2g** (the ethyl-branched analogue of **T12**), the activity remained almost the same. These findings differ from those made in the group of fatty alcohols where the same ethyl branching clearly increased their potency.[16]

In the fatty alcohols, introduction of the terminal branching probably changed their mode of action (or the prevailing mode of action).[16] No such change was observed in the studied linear and branched T, suggesting that the mechanism of action of T was completely different from that of the corresponding fatty alcohols. This hypothesis is further supported by the difference in the chain length-activity relationship between the groups of the fatty alcohols and T—while the branched T showed a parabolic relationship, the activity of the branched alcohols decreased with increasing chain length[16] (see Fig. 2).

These results strongly contrast with our previous study, where branching of similar size was situated close to the ester group of T. Unlike with the terminal branching, this resulted in an order-of-magnitude lower activity compared to their linear counterparts.[23] One of the reasons for such decrease of activity was probably the steric hindrance of the alkyl group preventing formation of a stable carbamate salt.[11] The terminal methyl and ethyl branching, on the other hand, allowed formation of crystalline carbamates. This comparison points out the importance of the carbamate polar head in the mode of action of T: a structural change that did not influence the stability of the carbamate salt did not alter the activity of T and vice versa. This result is consistent with that obtained previously where the decarboxylated free amino ester derivative of **T12** tested under argon completely lost its permeation-enhancing activity.[9] On the other hand, the activity of the fatty alcohols was highly dependent on the structure of their lipophilic chain.

Yet another conclusion can be drawn from these results. Being esters, T undergo enzymatic hydrolysis by esterases. In **T12**, second-order degradation by porcine esterase has been described in vitro.[9] As esterase activity is present also in the stratum corneum,[24] an additional hypothesis must be taken into account that the high activity of **T12** might arise from the activity of free dodecanol released after its hydrolysis. However, the results of this study disproved this hypothesis as the behaviour of T and the corresponding alcohols differed significantly. Hence, T are not carriers or 'pro-enhancers' of the fatty alcohols. The enzymatic hydrolysis is likely to take place in the deeper skin layers, where the released alcohols cannot exert their permeation-enhancing properties.

In conclusion, although the activities of the newly prepared compounds were comparable to the parent ones, the comparison revealed interesting relationships. We can reject the hypothesis that T act via the released alcohols. Moreover, the differences in the structure-activity relationships and the relative insensitivity of T towards terminal branching support our hypothesis that the mode of action is directed by the unusual polar head of these compounds, the ammonium carbamate. Further studies aiming at elucidating the importance of this polar head group by physico-chemical methods are in progress.

Acknowledgments

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Supplementary data

Supporting information contain the melting points and spectral data of the synthesized compounds and the yields of the reactions. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.040.

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17. General procedure of the preparation of 5-alkoxycarbonylpentylammonium-chlorides **1a**–**1g**: A mixture of 5-carboxypentylammonium chloride (6.16 mmol) and $SOCl_2$ (2.5 mL) was stirred at 40 °C for 30 min and then concentrated under reduced pressure. Anhydr. toluene was added and the mixture was concentrated again to remove the residual thionyl chloride. The corresponding branched alcohol (5.54 mmol) in anhydr. chloroform (20 mL) was added and the mixture was heated to reflux for 1.5 h. The solution was then evaporated and the yellowish waxy crude product was dried in vacuum over NaOH. Then it was suspended in hexane and purified by column chromatography on cellulose powder (ca 15 g) using hexane to hexane/chloroform 7:3 as a mobile phase. The products were dried in vacuum over P_4O_{10}.
18. General procedure of the preparation of branched carbamates **2a**–**2g**: Ammonium chloride **1a**–**1g** (3.40 mmol) was dissolved in water (30 mL), alkalized by TEA (10.20 mmol) and extracted with diethyl ether (5×20 mL). Combined ethereal extracts were washed with water (2×10 mL), dried with Na_2SO_4, filtered (the drying agent was washed properly with $CHCl_3$), concentrated and dried in vacuum. The resulting yellow oil was dissolved in small amount of diethyl ether and CO_2 was slowly bubbled through the solution for 20 min to yield the carbamate salt as white crystals. The suspension was left to crystallize at −20 °C for two days. The crystals were filtered off and dried in vacuum over P_4O_{10}. In the case of compounds **2a**, **2f** and **2g** the carbamate formed unfilterable fine crystals. Thus, the supernatant over the precipitate was removed at −20 °C, replaced by new portion of diethyl ether and the procedure was repeated until the supernatant was clear. Then it was evaporated and dried over P_4O_{10}.
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