Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1981–1984

Synthesis and transdermal penetration-enhancing activity of carbonic and carbamic acid esters—Comparison with transkarbam 12

Jana Klimentová, Alexandr Hrabálek,* Kateřina Vávrová, Tomáš Holas and Aleš Kroutil

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

Received 3 November 2005; revised 20 December 2005; accepted 20 December 2005
Available online 30 January 2006

Abstract—Transkarbam 12 (T12) is a novel transdermal penetration enhancer with high activity. Its polar head group is formed by carbamic acid salt that is unstable in acidic environment and releases CO_2. To find out whether this property influences its high activity, two series of compounds with CO_2 stronger bound in the polar head have been prepared—carbonic and carbamic acid esters. The carbamate salt in the polar head was found to be essential for the enhancing activity and its decomposition in an acidic environment suggested relating to the mode of action of T12.

Over the past few years major advances have been made in the field of transdermal drug delivery (TDD). Increasing numbers of drugs are being added to the list of therapeutic agents that can be delivered to the systemic circulation via skin.[1–3] The advantages of TDD are well documented and include the following: avoidance of first-pass metabolism by the liver, reduction of side effects, extended duration of activity, reduction of fluctuations of drug concentration in the blood and convenient termination of drug administration.[4]

The major limitation to TDD is the skin itself. The barrier function of the skin is firmly attributed to the stratum corneum (SC), the outermost layer of the skin that is formed from several layers of dead cells embedded in a lipid matrix.[5] The use of chemical penetration enhancers seems to be an effective way to reduce the barrier properties of SC. Current research focuses particularly on non-irritant, non-toxic[6], and biodegradable[7] enhancers. The activity of some of these compounds arises from their interactions with the lipids of the SC, especially with ceramides;[8,9] however, the detailed mechanisms are not properly elucidated yet.

ω-Amino acid derivatives form an important group of transdermal penetration enhancers,[10,11] for a review of the structure–activity relationships, see Vávrová et al.[12] It has been found that a group of carbamic acid salts derived from ω-amino acid esters (so-called transkarbams) have very high transdermal penetration-enhancing activity. The most active substance amongst this group was a carbamic acid salt formed by reaction of 6-aminohexanoic acid dodecylester (**DDEAC**) with CO_2, termed transkarbam 12[13] (**T12**, Fig. 1). Mode of its enhancing action is unclear and the aim of this study was to contribute to its elucidation.

We assume that the mechanism of action of **T12** is more specific than simply increasing the drug partitioning into the SC or drug solubility in the vehicle. Being a carbamic acid salt, **T12** is very unstable in a mildly acidic environment. Thus, we hypothesize that in the SC, where fatty acids comprise approximately 10% of the SC lipids and the average pH is 5.5, its polar head would be

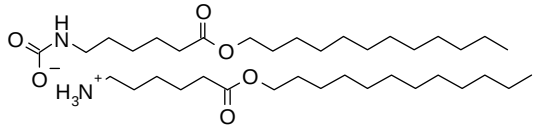


Figure 1. Transkarbam 12.

Keywords: Percutaneous absorption; Penetration enhancers; Carbonate; Carbamate; Carbon dioxide.
* Corresponding author. Tel.: +420 495 067 402; fax: +420 495 514 330; e-mail: alexandr.hrabalek@faf.cuni.cz

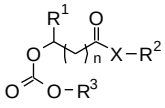
0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.086

decomposed releasing a molecule of CO_2.[7] The CO_2 release in the SC could cause the disruption of the highly ordered lipid lamellae: (a) by changing the hydrogen bonding within the polar heads of the lipids and (b) by conformational changes in the molecule of **T12**. Such disordering of the lipid lamellae would consequently result in an easier drug permeation through the skin barrier. This theory is further supported by the fact that the free amine **DDEAC** was inactive. For the conformational changes and phase behaviour of **T12**, see Zbytovská et al.[14]

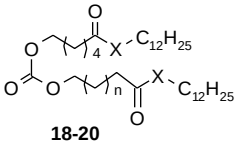
To confirm that the lability of the CO_2-containing polar head of **T12** is the reason for its exceptional activity, two series of compounds with CO_2 covalently bound in the polar head, namely carbonic acid esters **7–20** (Fig. 2) and carbamic acid esters **21–26** (Fig. 3), were prepared from their precursors **1–6** and **DDEAC**. Their transdermal penetration-enhancing activities were evaluated in vitro and compared with that of **T12**. The structures were designed to resemble that of **T12**; the most similar were compounds **19**, **20**, **25** and **26**.

The target esters of carbonic and carbamic acid were prepared by the reactions of hydroxy (**1–6**) or amino (**DDEAC**) compounds with the corresponding chloroformates.

Scheme 1 shows the preparation of the precursors **1–6**. The hydroxy amides (**1–4**) were prepared by the aminolysis of the appropriate lactones in the presence of catalytic amount of sodium methoxide.[15,16] The hydroxy esters (**5, 6**) were prepared by the reaction of sodium salt of the corresponding ω-hydroxy acid with dodecyl bromide in the presence of tetrabutylammonium iodide (TBAI) as a phase transfer catalyst.[17,18] The sodium salts of ω-hydroxy acids were prepared by the hydrolysis of their lactones. The commercially unavailable ω-heptanolide was obtained by the oxidation of cycloheptanone by H_2O_2.[19]



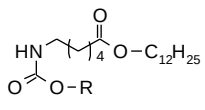
7-17



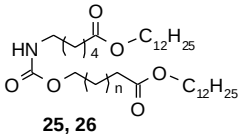
18-20

No.	X	n	R^1	R^2	R^3
7	NH	4	H	decyl	ethyl
8	NH	4	H	decyl	octyl
9	NH	4	H	decyl	decyl
10	NH	4	H	decyl	dodecyl
11	NH	3	H	nonyl	ethyl
12	NH	2	CH_3	dodecyl	ethyl
13	NH	2	CH_3	dodecyl	octyl
14	NH	2	CH_3	dodecyl	decyl
15	NH	2	CH_3	dodecyl	dodecyl
16	O	4	H	dodecyl	ethyl
17	O	4	H	dodecyl	dodecyl
18	NH	3			
19	O	3			
20	O	4			

Figure 2. Carbonic acid esters.



21-24

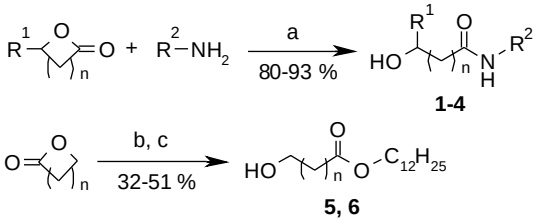


25, 26

No.	R
21	ethyl
22	octyl
23	decyl
24	dodecyl

No.	n
25	3
26	4

Figure 3. Carbamic acid esters.



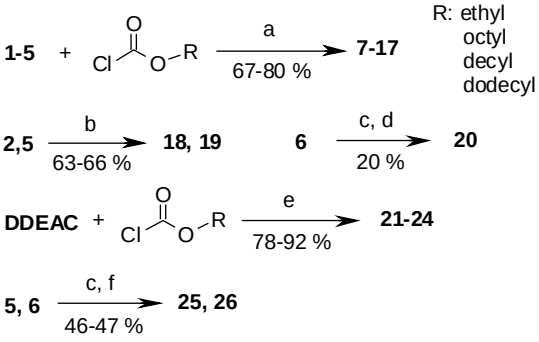
No.	n	R^1	R^2
1	4	H	decyl
2	4	H	dodecyl
3	3	H	nonyl
4	2	CH_3	dodecyl

No.	n
5	4
6	5

Scheme 1. (a) MeO^-Na^+, 120 °C, 1–2 h, (b) 1 M NaOH, reflux, 2 h, (c) TBAI, dodecylbromide, 120 °C, 2 h.

Scheme 2 shows the synthesis of the products **7–26**. The carbonic acid esters **7–17** were prepared by adding the commercially available alkyl chloroformate to the pyridine solution of the hydroxy compounds **1–5**.[20] The preparation of the carbamic acid esters **21–24** from **DDEAC** was similar. **DDEAC** was prepared by decomposition of **T12** in $CHCl_3$ prior to the reaction.[21]

Symmetrical carbonates **18** and **19** were prepared by adding twofold molar excess of the hydroxy compound **2** or **5** to the solution of trichloromethyl chloroformate (diphosgene) in anhydrous tetrahydrofurane (THF). Unsymmetrical carbonate **20** was prepared in two steps. First, the commercially unavailable chloroformate was prepared by adding **6** to the THF solution of an equimolar amount of diphosgene[22] and the resulting chlorofor-



Scheme 2. (a) Pyridine, −20 °C for 1 h, room temperature (rt) overnight, (b) diphosgene, THF, pyridine, −20 °C for 2 h, rt overnight, (c) diphosgene, THF, activated charcoal, rt, 1.5 h adding **6** to diphosgene, 1 h stirring, (d) **5**, pyridine, −20 °C for 1 h, rt overnight, (e) $CHCl_3$, pyridine, room temperature, 3 h, (f) **DDEAC**, $CHCl_3$, pyridine, rt overnight.

mate reacted with the pyridine solution of **5** immediately without any purification following the same procedure as in the carbonates **7–17**.

The preparation of carbamates **25** and **26** was similar to the previous one. In the first step, the unavailable chloroformate was prepared from hydroxy esters **5** or **6** and added to freshly prepared **DDEAC** in chloroform with pyridine.

All these reactions were carried out under nitrogen atmosphere. Yields, melting points, FT-IR, ^{1}H NMR and ^{13}C NMR spectra of the products and intermediates are available as supporting information.

The transdermal penetration-enhancing activity was evaluated in vitro using Franz diffusion cell and theophylline as a model permeant. Porcine ear skin of full thickness was employed. Donor samples (200 μL/cm^2) were prepared as 5% w/w suspensions of theophylline with 1% w/w of the suspended enhancer in three donor vehicles of different polarity: (a) water, (b) mixture of propylene glycol/water 6:4 (PG/W) and (c) isopropylmyristate (IPM). Control samples were prepared likewise without the addition of enhancer. Theophylline in the acceptor phase was determined by HPLC. The detailed preparation of the skin, donor samples and the theophylline determination have been described elsewhere.[23]

Cumulative amounts of theophylline (μg/cm^2) were plotted against time and fluxes were calculated (μg/cm^2/h). The transdermal penetration-enhancing activity was then expressed as the enhancement ratio (ER), which is the ratio of flux of theophylline from the donor sample with the addition of the enhancer and without the enhancer (control).

Table 1 shows the ER values of selected compounds in three vehicles of different polarity compared with **T12**.

Using porcine skin, **T12** was only slightly active (ER = 1.9) when applied in an aqueous suspension. On the contrary, when PG/W was used as the vehicle, the ER value was an order of magnitude higher (22.8). The large difference between its activities from these two vehicles was surprising; no such difference was observed in the previous study using the human cadaver skin (ERs were 39.3 and 35.0, when applied in water and PG/W, respectively).[10] The compounds **7**, **16**, **17** and **25** were more active than **T12** from aqueous suspensions ($p < 0.05$).

Table 1. Enhancement ratios of selected compounds and **T12**

Compound	ER ± SD		
	Water	PG/W	IPM
7	3.37 ± 0.66*,†	1.36 ± 0.31	1.75 ± 0.91
10	1.13 ± 0.14	2.66 ± 0.74*	1.48 ± 0.22*
12	1.48 ± 0.50	1.45 ± 0.89	1.77 ± 0.54
15	1.70 ± 0.48*	1.11 ± 0.19	1.15 ± 0.17
16	2.40 ± 1.15†	2.13 ± 0.03*	1.07 ± 0.37
17	2.49 ± 1.13*,†	1.13 ± 0.36	1.29 ± 0.57
18	0.91 ± 0.24	3.29 ± 1.24*	1.23 ± 0.36
19	1.76 ± 0.63	3.70 ± 1.14*	1.39 ± 0.38
20	0.80 ± 0.03	1.24 ± 0.81	1.49 ± 0.68
21	1.83 ± 0.37*	1.52 ± 0.50	1.01 ± 0.12
24	0.77 ± 0.16	0.47 ± 0.10	0.57 ± 0.20
25	2.27 ± 0.36*,†	2.63 ± 0.06*	1.29 ± 0.56
26	1.22 ± 0.72	1.02 ± 0.46	1.07 ± 0.47
T12	1.90 ± 0.27*	22.80 ± 1.08*	6.56 ± 0.52*

* $p < 0.05$ versus control.
† $p < 0.05$ versus **T12**.

However, the activities of the prepared compounds were significantly lower than those of **T12** in both PG/W and IPM on porcine skin. PG and IPM are used widely in dermatological formulations as solvents or co-solvents for a wide range of drugs and they can influence the activity of the enhancer[24] as shown for **T12**. However, no such effect was observed in the prepared compounds. Their lack of activity in IPM and approximately an order of magnitude lower ERs in PG/W supports our hypothesis about the importance of the labile carbamate group for the activity of transkarbams.

Figure 4 represents cumulative amounts of theophylline permeated through the porcine skin plotted against time. In this case, PG/W was used as a donor medium and **T12** and two compounds with the most similar structure (**19** and **25**) were used as enhancers. The permeation profiles clearly showed the difference between an ammonium-carbamate salt (**T12**), carbonate (**19**) and carbamate (**25**).

The ERs of the prepared compounds ranged from 0.77 to 3.37 and 0.47 to 3.70 in water and PG/W, respectively. Using IPM as a donor medium, no significant enhancing activity was detected. Because of the low activities, there were no relevant structure–activity relationships within the group of the prepared enhancers. The maximum activities were observed in the compounds, where both chains contained ester or amide group in contrast to those with one simple alkyl chain, either carbonates (**18**, **19**) or carbamates (**25**). On contrary, the lowest enhancing effect was achieved in the compound **24** (carbamate with one simple dodecyl chain), which could be considered to be a permeation retardant with theophylline flux significantly lower than

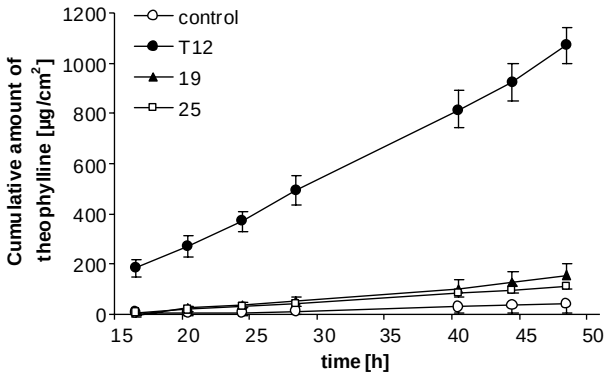


Figure 4. Example permeation profiles of theophylline through porcine skin using PG/W as a donor medium and **T12** or its analogues (**19** and **25**) as enhancers.

that of control (ER < 1 in all three vehicles). However, the mechanism of its retarding activity is not known and the effect should be evaluated for a wider spectrum of permeants.

In conclusion, this study showed that the permeation-enhancing activity of **T12** is connected with the ammonium-carbamate polar head; the most similar carbonates **19** and **20** and carbamates **25** and **26** did not reach its activity. The results suggest that the exceptional activity of **T12** is connected with the lability of its polar head and very probably arises from its ability to release CO_2 in SC. Further studies are to be done to describe the detailed interaction of **T12** with SC lipids.

Acknowledgments

The work was financially supported by the Ministry of Education of the Czech Republic (Project Nos. 715/G6/2005 and MSM0021620822).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.12.086.

References and notes

1. Smith, E. W.; Maibach, H. I. In *Percutaneous Penetration Enhancers*; Smith, E. W., Maibach, H. I., Eds.; CRC Press: Boca Raton, 1995; pp 1–4.
2. Benson, H. A. E. *Curr. Drug Deliv.* **2005**, *2*, 23.
3. Prausnitz, M. R.; Mitragotri, S.; Langer, R. *Nat. Rev. Drug Discov.* **2004**, *3*, 115.
4. Büyüktimkin, N.; Büyüktimkin, S.; Rytting, J. H. In *Transdermal and Topical Drug Delivery Systems*; Ghosh, T. K., Pfister, W. R., Eds.; Interpharm Press: Buffalo Grove, IL, 1997; pp 357–476.
5. Suhonen, T. M.; Bouwstra, J. A.; Urtti, A. *J. Control Release* **1999**, *59*, 149.
6. Akimoto, T.; Nagase, Y. *J. Control Release* **2003**, *88*, 243.
7. Vávrová, K.; Hrabálek, A.; Doležal, P.; Holas, T.; Klimentová, J. *J. Control Release* **2005**, *104*, 41.
8. Barry, B. W. *J. Control Release* **1987**, *6*, 85.
9. Williams, A. C.; Barry, B. W. *Adv. Drug Deliv. Rev.* **2004**, *56*, 603.
10. Doležal, P.; Hrabálek, A.; Semecký, V. *Pharm. Res.* **1993**, *10*, 1015.
11. Hrabálek, A.; Doležal, P.; Farsa, O.; Šklubalová, Z.; Kuneš, J. *Die Pharmazie* **2000**, *55*, 759.
12. Vávrová, K.; Zbytovská, J.; Hrabálek, A. *Curr. Med. Chem.* **2005**, *12*, 2273.
13. Hrabálek, A.; Doležal, P.; Farsa, O.; Krebs,A.; Roman, M.; Šklubalová, Z. U.S. Patent 6,187,938, 2001.
14. Zbytovská, J.; Raudenkolb, S.; Wartewig, S.; Hübner, W.; Rettig, W.; Pissis, P.; Hrabálek, A.; Doležal, P.; Neubert, R. H. H. *Chem. Phys. Lipids* **2004**, *129*, 97.
15. Mizushima, H.; Takahashi, M.; Yamamuro, A.; Matsuo, T.; Yahagi, K. U.S. Patent 5,599,483, 1997.
16. General procedure for the preparation of the hydroxyamides **1–4**: To a mixture of lactone (17.5 mmol) and *n*-alkyl amine (19.3 mmol), 1 M methanolic solution of MeONa (0.1–0.2 mL) was added. The mixture was heated to 120 °C for 1–2 h. After cooling to room temperature, the products were purified by precipitating the methanolic or ethanolic solution with 10% HCl.
17. Barry, J.; Bram, G.; Decodts, G.; Loupy, A.; Pigeon, P.; Sansoulet, J. *Tetrahedron Lett.* **1982**, *23*, 5407.
18. General procedure for the preparation of the hydroxyesters **5** and **6**: sodium salt of ω-hydroxyacid (6.5 mmol) and TBAI (0.65 mmol) were suspended in dodecylbromide (8.3 mmol) and heated to reflux for 2 h. After cooling to room temperature, the mixture was diluted with diethyl ether (10 mL) and filtered. The filtrate was dried with Na_2SO_4, filtered and evaporated. The resulting yellowish oil was chromatographed on SiO_2 (*n*-hexane/EtOAc 3:2).
19. Heyne, H. W.; Jones, H. *J. Am. Chem. Soc.* **1951**, *73*, 1361.
20. General procedure for the preparation of the compounds **7–17**: a solution of hydroxy compound **1–5** (1 mmol) in anhydrous pyridine (5 mL) was cooled to −20 °C and alkyl chloroformate (1.25 mmol) was added dropwise. The mixture was stirred at −20 °C for 1 h and warmed to room temperature overnight. Then it was diluted with $CHCl_3$ (10 mL), washed with water (3× 5 mL) and the organic layer was dried over Na_2SO_4 and filtered. The pyridine was removed by azeotropic distillation with toluene in vacuo. The residue was dried in vacuo over H_2SO_4 for 1 day. The crude material was chromatographed on SiO_2 (mixtures of *n*-hexane/EtOAc) to give the desired products.
21. General procedure for the preparation of the compounds **21–24**: to a solution of **T12** (0.6 mmol) in dry $CHCl_3$ (5 mL) and pyridine (1 mL) alkyl chloroformate (1.5 mmol) was added drop wise. The mixture was stirred at room temperature for 3 h. Then it was diluted with $CHCl_3$ (10 mL), washed with water (3× 5 mL) and the organic layer was dried over Na_2SO_4 and filtered. The pyridine was removed by azeotropic distillation with toluene in vacuo. The residue was dried in vacuo over H_2SO_4 for 1 day. The crude material was chromatographed on SiO_2 (mixtures of *n*-hexane/EtOAc) to give the desired products.
22. Pei-Lin, Wu; Chia-Hao, Su; Yi-Jeng, Gu *J. Chin. Chem. Soc.-Taip.* **2000**, *47*, 271.
23. Vávrová, K.; Hrabálek, A.; Doležal, P.; Šámalová, L.; Palát, K.; Zbytovská, J.; Holas, T.; Klimentová, J. *Bioorg. Med. Chem.* **2003**, *11*, 5381.
24. Møllgard, B. In *Pharmaceutical Skin Penetration Enhancement*; Walters, K. A., Hadgraft, J., Eds.; Marcel Dekker: New York, 1993; pp 229–242.