Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 19 (2009) 3141–3144
Bioorganic & Medicinal Chemistry Letters

NO donors. Part 18: Bioactive metabolites of GTN and PETN—Synthesis and vasorelaxant properties☆

Kathrin Lange, Andreas Koenig, Carolin Roegler, Andreas Seeling and Jochen Lehmann*

Lehrstuhl für Pharmazeutische/Medizinische Chemie, Institut für Pharmazie, Friedrich-Schiller-Universität Jena, Philosophenweg 14, D-07743 Jena, Thuringia, Germany

Received 18 February 2008; revised 23 April 2008; accepted 23 April 2008
Available online 26 April 2008

Abstract—The vasodilators glyceryl trinitrate (GTN) and pentaerythrityl tetranitrate (PETN) are supposed to be degraded in vivo to the lower nitrates PETriN, PEDN, PEMN, 1,2-GDN, 1,3-GDN, 1-GMN, and 2-GMN. We synthesized these bioactive metabolites as reference compounds for pharmacokinetic studies. The use of HPLC-methods for monitoring the stepwise reduction of PETN to lower nitrates and the syntheses of the glyceryl dinitrates proved advantageous. Furthermore, we measured the vasorelaxant properties of all metabolites by performing organ bath experiments with porcine pulmonary arteries. In general, the vasodilator potency increases with the number of nitrate moieties in the compound.

Glyceryl trinitrate (GTN), pentaerythrityl tetranitrate (PETN), isosorbide dinitrate (ISDN), and isosorbide-5-mononitrate (5-ISMN) are used in the therapy of cardiovascular diseases. These compounds are supposed to be enzymatically bioactivated in order to liberate nitric oxide as the vasorelaxant factor. Not only this bioactivation process but also chemical or enzymatic degradation in vivo may generate lower bioactive nitrates from GTN, PETN, and ISDN.[2,3]

Accordingly, the clinical profiles may result not only from the initially administered oligonitrates; but also and even predominantly[4] from the action of the metabolites. Many studies were carried out with PETN and GTN without looking at the bioactive metabolites. The very limited availability of the metabolites for pharmacological and analytical purposes may be one of the reasons for this restriction. We therefore synthesized and characterized pharmacologically the potential bioactive metabolites of GTN and PETN in order to estimate their possible contributions to the overall effect of the substances (Fig. 1).

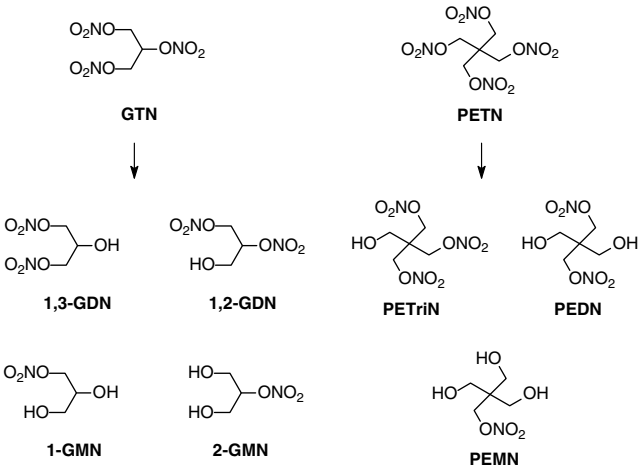


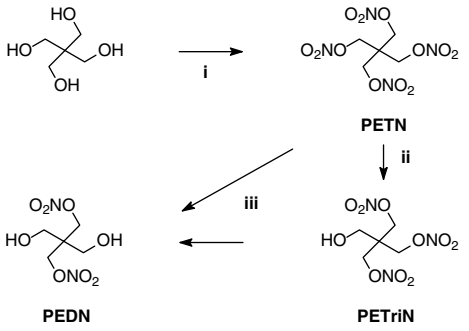
Figure 1. Potential bioactive metabolites of PETN and GTN.

Several strategies were applied to synthesize organic nitrates.[5,6] We performed the synthesis of the PETN-metabolites pentaerythrityl trinitrate (PETriN) and pentaerythrityl dinitrate (PEDN) similarly to Hess[12] by reductively degrading PETN with different molar amounts of hydrazine hydrate (2:1 for PETriN, 8:1 for PEDN) in boiling mixtures of ethanol and dioxane (Scheme 1).

Treating an organic nitrate with hydrazine hydrate results among other compounds in the formation of inor-

Keywords: Organic nitrates; Metabolites; GTN; PETN.
☆ See Ref. 1.
* Corresponding author. Tel.: +49 3641 949803; fax +49 3641 949 802; e-mail: j.lehmann@uni-jena.de

0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.04.057



Scheme 1. Synthesis of PETN, PETriN, and PEDN. Reagents and conditions: (i) nitric acid (100%), 4–5 h, −5 to −10 °C; (ii) hydrazine hydrate, dioxane, ethanol, 3 h, reflux; (iii) hydrazine hydrate, dioxane, ethanol, 6.5 h, reflux.

ganic nitrite, nitrogen, nitric oxide, ammonia, and the corresponding alcohol.[7] Monitoring the reaction is advantageous and was accomplished by HPLC-method A.

To prepare PEDN, we used HPLC-method A. We measured the degradation of PETN to PETriN, PEDN, and PEMN (Fig. 2; data from PEMN not shown) and identified a suitable end point after 6.5 h (Fig. 2).

PETriN was obtained after 3 h with a 2-fold excess of hydrazine hydrate. Purification was accomplished by column chromatography.

Reductive degradation was shown to be less advantageous for the preparation of pentaerythrityl mononitrate (PEMN), 1,2-glyceryl dinitrate (1,2-GDN), 1,3-glyceryl dinitrate (1,3-GDN), and 1-glyceryl mononitrate (1-GMN). We preferred reacting the corresponding bromides with silver nitrate in acetonitrile (Schemes 2 and 3) and, if necessary, using a 'fractionated extraction' to remove byproducts. This procedure resulted in purer compounds and higher yields than did purification by column chromatography. Progress of the reaction was monitored using the HPLC-method B for GDN.

A 4-step synthesis of 2-GMN was performed by acetylation of dihydroxy acetone, reduction of **1**, and nitration

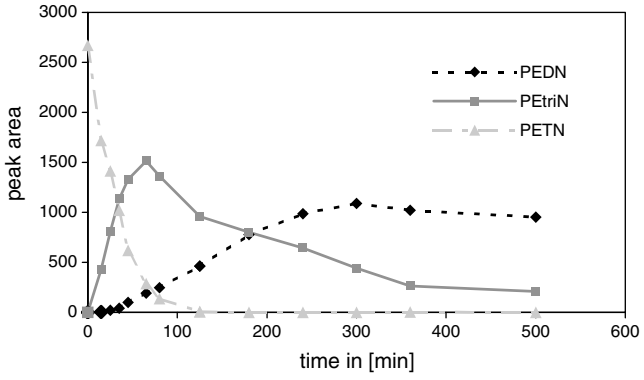
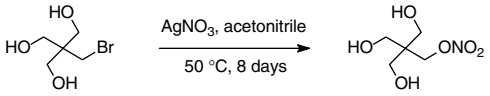
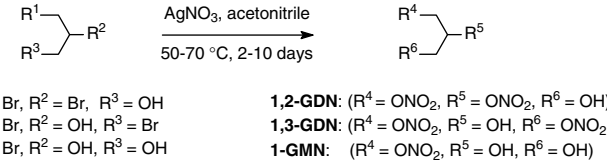


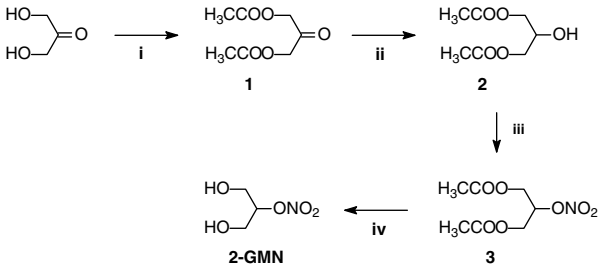
Figure 2. Synthesis of PEDN by reductive degradation with an 8-fold excess of hydrazine hydrate, monitored by HPLC.



Scheme 2. Synthesis of PEMN.



Scheme 3. Synthesis of 1,3-GDN, 1,2-GDN, and 1-GMN.



Scheme 4. Synthesis of 2-GMN. Reagents and conditions: (i) pyridine, acetic anhydride, 1.5 h, rt, lit.[11]; (ii) BH_3 * THF, THF, 7 h, 3 °C; (iii) urea, nitric acid (100%), acetic anhydride, 12 h, 3 °C; (iv) sodium hydroxide, dichloromethane, 7 min, rt.

of **2** with fuming nitric acid in the presence of acetic anhydride. After hydrolysis of **3** with sodium hydroxide, 2-GMN was obtained initially together with ~30% 1-GMN. The isomerization of 2-GMN to 1-GMN under basic conditions has been already described by Capellos et al.[8] and was explained by a higher stability of the ONO_2-group in position 1. Less basic conditions and shorter reaction times resulted in higher yields of 2-GMN (Scheme 4). Chemical protocols are given in reference.[13–24]

As described previously,[2] organ bath experiments were performed using $PGF_{2\alpha}$-precontracted porcine pulmonary arteries. The vasorelaxant responses of PETN, GTN, the synthesized bioactive of metabolites, and of compound **3** are given in Table 1.

Table 1. Concentration–response curves and vasodilatory effects (EC_{50}-values) of PETN and GTN, their active metabolites, and compound (**3**)

Compound	EC_{50}[a] (nM)
PETN	7*
PETriN	30*
PEDN	2100*
PEMN	72,000*
GTN	27*
1,3-GDN	523
1,2-GDN	1930
1-GMN	56,300
2-GMN	115,000
3	1230

[a] Values are means of three to nine experiments.
* Taken from a previous publication.[3]

Table 1 demonstrates that the vasodilator potencies of PETN, GTN, and their metabolites correlate with the number of nitrate groups. But the decrease of potency is more than a stoichiometric one and previous investigations have shown that not only the number of nitrate groups; but also the 'nitrate carriers' structure significantly influences the vasorelaxant activity.[2] Here too, acetylation of the mononitrate 2-GMN to the mononitrate **3** enhances the vasodilator potency dramatically. Furthermore, quite significant differences in vasodilator potency were found between the isomeric 1,3-GDN and 1,2-GDN and between 1-GMN and 2-GMN, respectively. In general, a terminal location of the nitrate group (1,3-GDN, 1-GMN vs 1,2-GDN, 2-GMN) increases the vasoactivity. We conclude that these differences in vasorelaxant potency are due to different affinities and reactivities toward the nitrate bioactivating enzymes. After all it should be mentioned that the in vitro results given in Table 1 are not simply transmittable to the in vivo situation due to very different bioavailabilities of the compounds. For example, the clinical dosages of PETN have to be ~80-fold higher than those of GTN, although PETN is superior to GTN in vitro (Table 1).[3]

Supplementary data

Spectral data of the synthesized compounds (^{1}H NMR, ^{13}C NMR, and IR). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.057.

References and notes

1. Konter, J.; Moellmann, U.; Lehmann, J. *Bioorg. Med. Chem.* **2008**, *16*, 8294.
2. Koenig, A.; Roegler, C.; Lange, K.; Daiber, A.; Glusa, E.; Lehmann, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5881.
3. Koenig, A.; Lange, K.; Konter, J.; Daiber, A.; Stalleicken, D.; Glusa, E.; Lehmann, J. *J. Cardiovasc. Pharmacol.* **2007**, *5*, 68.
4. Weber, W.; Michaelis, K.; Luckow, V.; Kuntze, U.; Stalleicken, D. *Arzneim.-Forsch.* **1995**, *45*, 781.
5. Boschan, R.; Merrow, R. T.; Van Dolah, R. W. *Chem. Rev.* **1955**, *55*, 485.
6. Berthmann, A.; Ratz, H.. In *Houben-Weyl – Methoden Houben-Weyl—Methoden der Organischen Chemie*; Bayer, E., Berthmann, A., Hausweiler, A., Eds.; Stuttgart: Thieme, 1963; 6, p 329.
7. Merrow, R. T. *J. Am. Chem. Soc.* **1956**, *78*, 1297.
8. Capellos, C.; Fisco, W. J.; Ribaudo, C.; Hogan, V. D.; Campisi, J.; Murphy, F. X.; Castorina, T. C. *Int. J. Chem. Kinet.* **1982**, *14*, 903.
9. Brandstaetter-Kuhnert, M.; Kuhnert, G. *Sci. Pharm.* **1960**, *28*, 287.
10. Korolev, A. M.; Eremenko, L. T.; Meshikhina, L. V. *Russ. Chem. Bull.* **2002**, *51*, 2306, (Translation of *Izv. Akad. Nauk, Ser. Khim.* **2002**, *51*, 2141).
11. Bentley, P. H.; McCrae, W. *J. Org. Chem.* **1970**, *35*, 2082.
12. Hess, U. U.S. Patent 6180664, 1997, 10.
13. *HPLC-methods:* (A) column: Chromolith® Performance RP-18 100 to 4.6 mm (5 μm); eluent: methanol/water 1:1; flow: 0.3 mL/min (isocratic); detection: UV, 215 nm; retention factors: 22.3 min PETN; 13.5 min PETriN; 7.6 min PEDN; 5.8 min PEMN. (B) column: Chromolith® Performance RP-18 100 to 4.6 mm (5 μm); eluent: methanol/water 30:70; flow: 0.3 mL/min (isocratic); detection: UV, 215 nm; retention factors: 11.7 min 1,3-GDN; 16.8 min 1,2-GDN.
14. *Pentaerythrityl tetranitrate (PETN):* Pentaerythritol (0.50 g, 3.67 mmol) was dissolved dropwise under stirring in 2 mL of concentrated nitric acid (100%) at −5 to −10 °C. After 2–3 h 1 mL of water was added and the mixture maintained for 2 more hours. The voluminous white precipitate was filtered off, washed carefully with water, dissolved in 20 mL of acetone, and filtered again. The filtrate was diluted with 20 mL of water and the acetone cautiously evaporated. The white precipitate was separated and dried in a desiccator under reduced pressure. Yield 0.51 g (44%), white solid, mp 142.2 °C (lit.[9] 142 °C). Anal. ($C_5H_8N_4O_{12}$) C, H, N.
15. *Pentaerythrityl trinitrate (PETriN):* Similarly to Hess[12] pentaerythrityl tetranitrate (2.25 g, 7.12 mmol) was dissolved under stirring in a boiling mixture of 50 mL of dioxane and 50 mL of ethanol. Hydrazine hydrate (0.72 g, 14.4 mmol) in 30 mL of water was added dropwise to the stirred solution over 1 h. The mixture was refluxed for 2 h and cooled to room temperature. The progress of the reaction was monitored with HPLC-method A. The solution was diluted with 100 mL of water and extracted with 6× 50 mL of dichloromethane. The combined organic phases were dried ($MgSO_4$) and evaporated. The crude oil was purified by column chromatography on silica gel using hexane/ethylacetate (40:60) as eluent and obtained as a light yellow oil. Yield 0.98 g (51%), light yellow oil. Anal. ($C_5H_9N_3O_{10}$) C, H, N.
16. *Pentaerythrityl dinitrate (PEDN):* Similarly to Hess[12] pentaerythrityl tetranitrate (2.43 g, 7.69 mmol) was dissolved under stirring in a boiling mixture of 50 mL of dioxane and 50 mL of ethanol. Hydrazine hydrate (3.09 g, 61.7 mmol) in 30 mL of water was added dropwise under stirring for over 1.5 h. The mixture was refluxed for further 5 h and then cooled to room temperature. The progress of the reaction was monitored with HPLC-method A. The solution was diluted with 100 mL of water and extracted with 6× 50 mL of ethylacetate. The combined organic phases were dried ($MgSO_4$) and evaporated, and the resulting oil was purified by column chromatography on silica gel using hexane/ethylacetate (40:60) as eluent. Yield 0.75 g (43%), light yellow oil, later crystallizing to a light yellow solid. Anal. ($C_5H_{10}N_2O_8 \times \frac{1}{5}$ ethylacetate) C, H, N.
17. *Pentaerythrityl mononitrate (PEMN):* A solution of 2-bromomethyl-2-hydroxymethyl-1,3-propandiol (6.61 g, 33.2 mmol) and silver nitrate (12.41 g, 73.0 mmol) in 50 mL of dry acetonitrile was heated at 50 °C under stirring and protection from light. The precipitated silver bromide was filtered off repeatedly. After 8 days the mixture was cooled to room temperature and saturated sodium chloride solution was added. The filtrate of this mixture was extracted with 10 × 100 mL of ethylacetate. The combined organic phases were dried ($MgSO_4$) and evaporated. One hundred microliters of water was added and the mixture extracted with 3× 100 mL of *n*-hexane, 4× 100 mL of diethylether, 4× 100 mL of dichloromethane, and 6× 100 mL of ethylacetate. The *n*-hexane, diethylether and dichloromethane phases were discarded and the ethylacetate phases were analyzed for byproducts by HPLC. The pure ethylacetate phases were combined, dried ($MgSO_4$), and evaporated. The remaining precipitated white solid was recrystallized from diethylether. Yield

1.35 g (22%), white solid, mp 78 °C (lit.[10] 78–79 °C). Anal. ($C_5H_{11}NO_6$) C, H, N.

18. Glyceryl trinitrate (GTN) was purchased from Merck, Darmstadt, Germany.
19. *1,3-Glyceryl dinitrate (1,3-GDN):* A solution of 1,3-dibromo-2-propanol (0.51 g, 2.3 mmol) and silver nitrate (1.42 g, 8.4 mmol) in 8 mL of dry acetonitrile was heated at 50 °C for 7 days under stirring and protection from light. The precipitated silver bromide was separated repeatedly. Progress of the reaction was monitored with HPLC-method B. The solution was then cooled to room temperature and saturated sodium chloride solution was added repeatedly. The precipitated silver chloride and silver bromide were removed and the filtrate was extracted with 3× 100 mL of chloroform. The combined organic phases were dried ($MgSO_4$) and evaporated to give 1,3-dinitrooxy-2-propanol as a light yellow oil. Yield: 0.40 g (94%). Anal. ($C_3H_6N_2O_7$) C, H, N.
20. *1,2-Glyceryl dinitrate (1,2-GDN):* Prepared as described above for 1,3-GDN from 2,3-dibromo-1-propanol (1.00 g, 4.6 mmol) and silver nitrate (7.80 g, 45.9 mmol) for 2 days. Extraction with 6× 100 mL of ethylacetate. The crude oily product was diluted with 60 mL of water and extracted with 3× 60 mL of *n*-hexane and 4× 60 mL of diethylether. The *n*-hexane phases were discarded and the diethylether phases were analyzed for byproducts with HPLC-method B. The pure diethylether phases were combined, dried ($MgSO_4$), and evaporated. Yield: 0.51 g (61%), colorless oil. Anal. ($C_3H_6N_2O_7$) C, H, N.
21. *1-Glyceryl mononitrate (1-GMN):* Prepared as described above from 3-bromo-1,2-propandiol (1.00 g, 6.5 mmol) and silver nitrate (1.96 g, 11.6 mmol) in 12 mL of dry acetonitrile, 70 °C for 10 days. Extraction with 4× 60 mL of ethylacetate, the combined organic phases were dried ($MgSO_4$), evaporated, the crude oily product was diluted with 80 mL of water and extracted with 3× 80 mL of *n*-hexane, 3× 80 mL of diethylether, 3 × 80 mL of dichloromethane and 5 × 80 mL of ethylacetate. The hexane, diethylether, and dichloromethane phases were discarded. The pure ethylacetate phases were combined, dried ($MgSO_4$), and evaporated. Yield: 0.33 g (37%), light yellow oil. Anal. ($C_3H_7NO_5 \times \frac{3}{11}$ water $\times \frac{1}{22}$ ethylacetate) C, H, N.
22. *Glyceryl 1,3 diacetate (2):* 1 M-BH_3 * THF (3.40 mL, 3.40 mmol) was added dropwise under nitrogen and stirring to 1,3-dihydroxypropane-2-one 1,3 diacetate **(1)** (0.25 g, 1.44 mmol), synthesized as described previously,[11] in 4 mL of dry THF for over 7 h at 3 °C (monitoring by GC/MS). The mixture was diluted with 30 mL of water, extracted with 3× 20 mL of chloroform, and the combined organic phases were dried ($MgSO_4$) and evaporated. Yield: 0.085 g (34%), colorless oil. Anal. ($C_7H_{12}O_5$) C, H, N.
23. *2-Glyceryl mononitrate 1,3 diacetate **(3)**:* A solution of **(2)** (0.50 g, 2.84 mmol) and urea (0.004 g, 0.067 mmol) in 15 mL of dichloromethane was cooled to 3 °C and concentrated nitric acid (100%) (0.36 g, 5.70 mmol) was added dropwise at <10 °C under stirring. After cooling to 3 °C, acetic anhydride (0.58 g, 5.70 mmol) was added dropwise at <10 °C, the mixture stirred overnight at room temperature and then diluted with 20 mL of water. The organic phase was separated and washed once with 30 mL of water and 30 mL of saturated sodium bicarbonate solution and twice with 30 mL of water. The organic phase was dried ($MgSO_4$) and evaporated. Yield: 0.60 g (96%), colorless oil. Anal. ($C_7H_{11}NO_7$) C, H, N.
24. *(2-GMN):* A solution of sodium hydroxide (0.13 g, 3.25 mmol) in 0.19 mL of water was added dropwise under stirring to 1,3-dihydroxy-2-nitrooxypropane 1,3 diacetate (0.37 g, 1.67 mmol) dissolved in 2.4 mL of dichloromethane. The mixture was maintained for 7 more minutes, then neutralized with concentrated hydrochloric acid; and the solvent evaporated under reduced pressure. The residue was extracted with 3× 10 mL of diethylether and the combined organic phases were dried ($MgSO_4$) and evaporated. Yield: 0.12 g (53%), light yellow oil. Anal. ($C_3H_7NO_5$) C, H, N.