Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 59–64

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Monoacetyldiglycerides as New Ca^{2+} Mobilizing Agents in Rat Pancreatic Acinar Cells

So-Yeop Han,* So-Hye Cho, So-Yeon Kim, Jeong-Taeg Seo,[a] Seok-Jun Moon,[a] and Gil-Ja Jhon

Department of Chemistry, Research Institute for Basic Sciences, and Research Institute for Life Science, Ewha Womans University, Seoul 120-750, Korea.

[a]Department of Oral Biology, Yonsei University College of Dentistry, Seoul, Korea.

Received 14 September 1998; accepted 17 November 1998

Abstract

Several monoacetyldiglycerides were synthesized from glycerol in search for new Ca^{2+} mobilizing agent *in vitro*. All monoacetyldiglycerides except linolenoyl and phenlycyclopropylcarbonyl derivatives showed activity toward Ca^{2+} release in pancreatic acinar cells. Linoleoyl and docosahexaenoyl derivatives were chosen for further test and exhibited unique activity.

Monoacetyldiglycerides are known to exist in *Celastraceae* seed oil and bovine milk fat.[1,2] The presence of monoacetyldiglyceride analogs in deer antler[3] has led us to design and synthesize derivatives of monoacetyldiglycerides, and test them *in vitro* for Ca^{2+} releasing activity in rat pancreatic acinar cells to search for new biological activity of the monoacetyldiglyceride derivatives.

The existence of monoacetyldiglycerides in nature has been proved by spectroscopic studies using GC-MS,[2,4,5] FABMS,[6] NMR,[7] and FT-IR[8] spectrometers, but the positions of

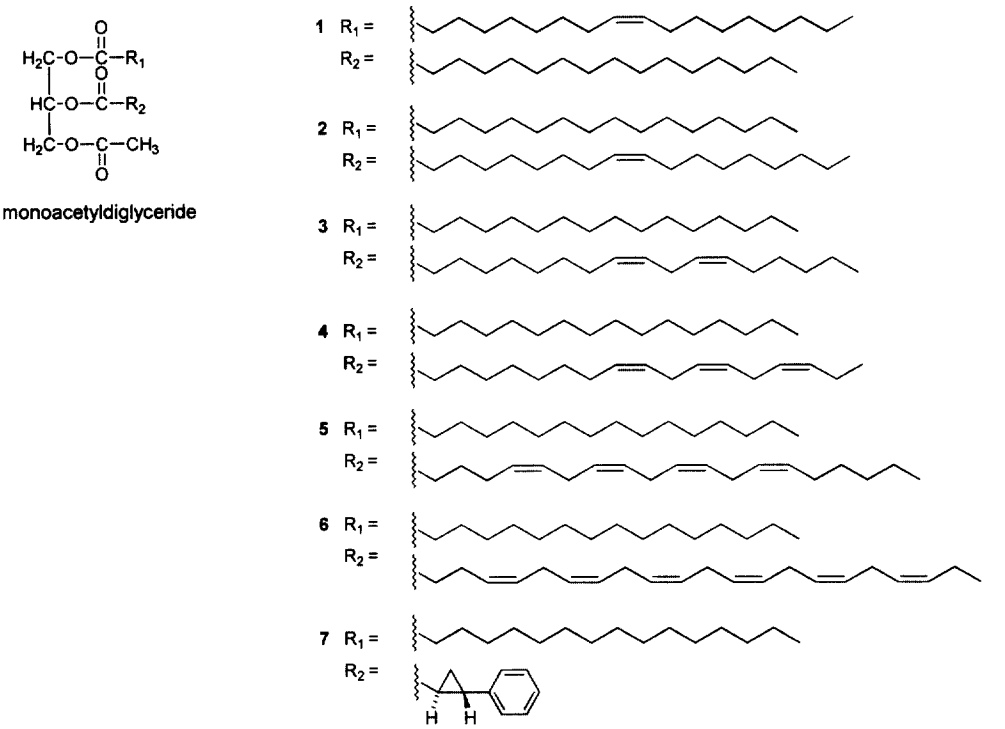
* Fax: 82-2-360-2384, e-mail: syhan@mm.ewha.ac.kr

0960-894X/99/$ - see front matter
PII: S0960-894X(98)00690-8

esters in many compounds are uncertain. Therefore, we systematically synthesized seven monoacetyldiglycerides and confirmed their structures by spectroscopic methods.

Our synthetic targets of monoacetyldiglycerides are shown in Figure 1.

Figure 1 Synthetic Targets of Monoacetyldiglycerides

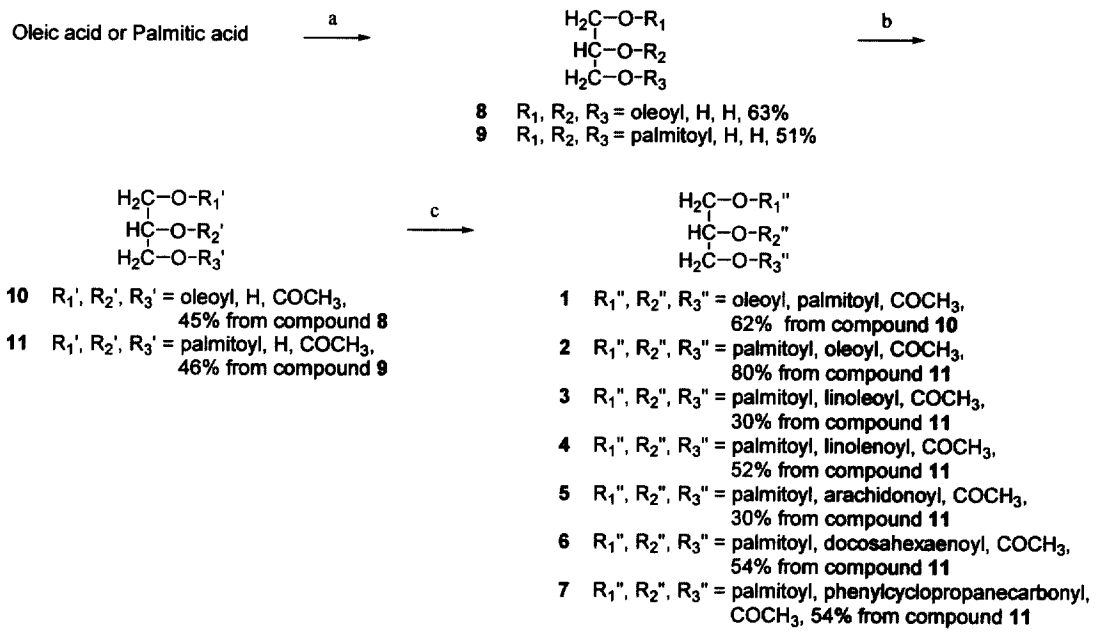


Compounds **1** and **2** differ at C1 and C2 positions of the fatty acid moiety, whereas compounds **2-6** differ from each other in their chain length as well as a degree of unsaturation of the fatty acids at C2 in the glycerol skeleton. Compound **7** was chosen as our target to see if non-linear short-chain could interfere the biological activity. Since the unsaturation may affect the biological function, the study of our system should provide valuable informations on cell biology. The presence of compounds **1-4** in nature was reported without detailed spectroscopic data.[1,2]

Our research has focused on the synthesis of monoacetyldiglyceride derivatives and the structure-activity relationship(SAR) study of them toward Ca^{2+} releasing bioactivity in the cells. Scheme 1 shows an overview of the synthetic pathways employed. Synthesis began with appropriate fatty acids. Oleic or palmitic acid was treated with glycerol in the presence of

DCC and DMAP to afford a C1-monoacylated glycerol(compound **8** or **9**) as the major product, along with 1,3-di- and 1,2,3-triacylated glycerols as minor products. The reaction was conducted carefully, and monitored closely by TLC in order to obtain C1-monoacylated derivative with high regio-selectivity. Octadecenoate **8** and hexadecanoate **9** were converted to their C3-monoacetates **10** and **11** by treatment with acetic anhydride in the presence of DMAP at -78 °C. Under the same reaction conditions, 2,3-diacetylated byproducts could also be obtained in small amounts. Compounds **10** and **11** were then reacted with corresponding fatty acids to produce the target molecules, monoacetyldiglycerides **1-7**, in good to moderate yields, along with trace amounts of acyl group migrated byproducts between C2 and C3.[9] The C2,C3-acyl migrated byproduct showed a unique NMR behavior due to its pseudo C_2-symmetric chemical environment.

Scheme 1



[a] glycerol, DCC, DMAP, acetone, 0 °C; [b] Ac_2O, DMAP, CH_2Cl_2, -78 °C; [c] palmitic acid, oleic acid, linoleic acid, linolenic acid, arachidonic aicd, docosahexaenoic acid or phenylcyclopropanecarboxylic acid, DCC, DMAP, CH_2Cl_2, 0 °C.

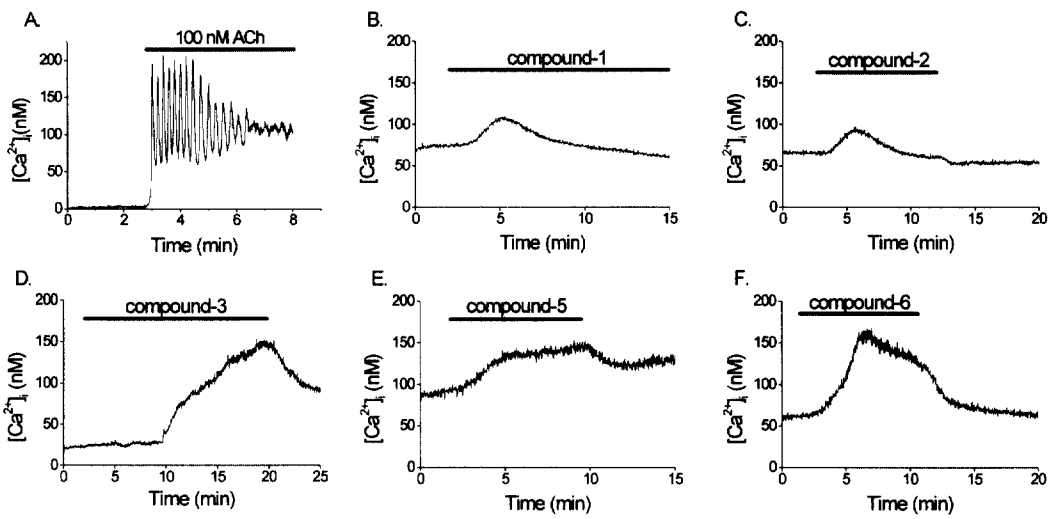
We could also prepare compounds **1** and **3** from corresponding phophatidyl cholines by acetolysis.[4] Phosphatidyl cholines were either purchased from Sigma or isolated from egg yolk. They were reacted with acetic anhydride in the presence of acetic acid at reflux to

afford compounds **1** and **3** in 70% yield.

The effect of synthetic compounds **1-7** on the Ca^{2+} mobilization was investigated in the fura-2 loaded pancreatic acinar cells (Figure 2). Pancreas were removed from rats and acini were prepared by enzymatic digestion with collagenase in a HEPES-buffered physiological solution. Isolated acini and single cells were loaded with fura-2 by incubation with 2 μM fura-2/AM in HEPES-buffered solution for 30 min at room temperature under 100% O_2. They were washed twice and resuspended in a HCO_3^--buffered solution. The cells were allowed to attach to a coverslip, and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by spectrofluorometry with excitation at 340 nm and 380 nm and emission measured at 510 nm. The values of $[Ca^{2+}]_i$ were calculated from the ratio of fluorescence intensities ($F_{340/380}$) according to Grinkiewicz.[10]

In pancreatic acinar cells, neurotransmitters such as acetylcholine evoke an increase in $[Ca^{2+}]_i$ which plays a central role in the exocytosis of secretory granules. Figure 2A shows a typical trace of $[Ca^{2+}]_i$ change in response to 100 nM acetylcholine. Exposure of single pancreatic acinar cells to 100 nM acetylcholine caused a rapid increase in $[Ca^{2+}]_i$ followed by the spontaneous $[Ca^{2+}]_i$ oscillations which lasted for a few minutes.

Figure 2 The Effect of Acetylcholine and Monoacetyldiglycerides on Ca^{2+} Mobilization in Pancreatic Acinar Cells

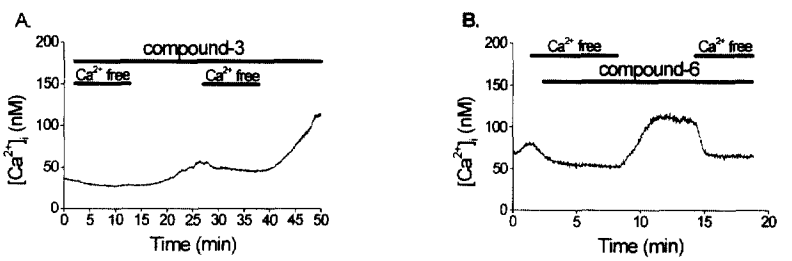


Although the response was slower, some synthetic compounds evoked a significant incrase in $[Ca^{2+}]_i$. As shown in Figure 2B-2F, compounds **3** and **6** at a concentration of 0.01

mg/mL induced increases in $[Ca^{2+}]_i$ by 84.9 ± 15.0 nM (n = 6) and 75.2 ± 10.6 nM (n = 3) respectively while compounds **1**, **2** and **5** caused smaller increases in $[Ca^{2+}]_i$ (31.2 nM, 26.6 nM and 32.8 nM respectively). There was a time lag of 10.2 ± 1.1 min before $[Ca^{2+}]_i$ started to increase in response to compound **3** although the lag time of the other compounds was less than 1 min. The rates of $[Ca^{2+}]_i$ increases evoked by compounds **3** and **6** were 9.8 ± 2.1 nM/min and 26.7 ± 3.2 nM/min, respectively. Among seven monoacetyldiglycerides tested in this study, compounds **4** and **7** induced little change in $[Ca^{2+}]_i$. These data suggested that synthesized monoacetyldiglycerides variably increased $[Ca^{2+}]_i$ in pancreatic acinar cells but there seemed to be no direct correlation between their biological effect on Ca^{2+} mobilization and the degree of unsaturation of the fatty acids at C2 in the glycerol skeleton.

Free calcium is one of the most important intracellular second messengers. Increases in $[Ca^{2+}]_i$ can be achieved both by the mobilization of Ca^{2+} from intracellular stores and by the Ca^{2+} entry across the plasma membrane.[11,12] Our results showed that $[Ca^{2+}]_i$ increases evoked by the synthetic compounds in pancreatic acinar cells were strongly dependent on the presence of extracellular Ca^{2+}. As shown in Figure 3, compounds **3** and **6**, which had been shown to evoke larger increases in $[Ca^{2+}]_i$ than the other compounds, did not induce prominent increase in $[Ca^{2+}]_i$ in the absence of perfusate Ca^{2+}. $[Ca^{2+}]_i$ only increased by re-addition of Ca^{2+} to the perfusate in the continued presence of each compound. This result strongly suggested that the compounds directly activated Ca^{2+} entry pathway on the plasma membrane.

Figure 3 The Role of Extracellular Ca^{2+} in Compounds **3**- and **6**-Induced $[Ca^{2+}]_i$ Increases



However, the involvement of intracellular Ca^{2+} stores could not be ruled out. As shown in Figure 3A and 3B, re-withdrawal of perfusate Ca^{2+} caused $[Ca^{2+}]_i$ to decrease to the slightly elevated level compared with the baseline concentration. This means that intracellular Ca^{2+} stores may play a role in the increase in $[Ca^{2+}]_i$ evoked by the compounds. In addition, if the compounds slowly deplete the Ca^{2+} stores, and if Ca^{2+} ATPase in plasma membrane is active enough to pump out the released Ca^{2+} from the stores, then we may not be

able to detect $[Ca^{2+}]_i$ increases. Similar observation has recently been made in Dictyostelium discoideum by Schaloske.[13] Therefore, the exact mechanism for the enhancement of Ca^{2+} influx through plasma membrane by these compounds remains to be elucidated.

In summary, we have performed an efficient synthesis of monoacetyldiglycerides **1-7** and confirmed their structures by spectroscopic methods. They were individually synthesized from appropriate fatty acids and glycerol in three steps and 7-19% overall yield. We also discovered that synthetic monoacetyldiglycerides behave as new Ca^{2+} mobilizing agents in rat pancreatic acinar cells, with no direct evidence of correlation between structure and activity in regard to the degree of unsaturation. Calcium(II) influx study suggested that monoacetyldiglycerides might directly activate Ca^{2+} entry pathway on the plasma membrane with little disturbance of intracellular signalling pathway.

Acknowledgements

The financial support for this work from the Korean Ministry of Education through Research Fund (BSRI-97-3422, BSRI-97-3442) and MOST through the Women's University Research Fund (1997) is gratefully appreciated.

References

[1] Spitzer, V.; Aichholz, R. *J. High Resol. Chromatogr.* **1996**, *19*, 497-502.

[2] Myher, J. J.; Kuksis, A.; Marai, L. *J. Chromatogr.* **1988**, *452*, 93-118.

[3] Isolation and biological activity of monoacetyldiacylglyceride analogs from deer antler will be reported elsewhere.

[4] (a) Hasegawa, K.; Suzuki, T. *Lipids* **1973**, *8*, 631-634. (b) Hasegawa, K.; Suzuki, T. *Lipids* **1975**, *10*, 667-672.

[5] Murata, T. *Anal. Chem.* **1977**, *49*, 2209-2213.

[6] Nizigiyimana, L.; Van den Heuvel, H.; Claeys, M. *J. Mass Spectrom.* (Spec. Issue) **1995**, S19-S28.

[7] (a) Zhang, B.-L.; Buddrus, S.; Trierweiler, M.; Martin, G. J. *J. Agric. Food Chem.* **1998**, *46*, 1374-1380. (b) Vlahov, G. *JAOCS* **1996**, *73*, 1201-1203. (c) Klemann, L. P.; Aji, K.; Chrysam, M. M.; D'Amelia, R. P.; Henderson, J. M.; Huang, A. S.; Otterburn, M. S.; Yarger, R. G. *J. Agric. Food Chem.* **1994**, *42*, 442-446. (d) Henderson, J. M.; Petersheim, M.; Templeman, G. J.; Softly, B. J. *J. Agric. Food Chem.* **1994**, *42*, 435-441.

[8] Yano, J.; Kaneko, F.; Kobayashi, M.; Sato, K. *J. Phys. Chem. B.* **1997**, *101*, 8112-8119.

[9] All synthesized compounds **1-7** gave satisfactory spectroscopic data (1D and 2D NMR, IR, FABMS, and/or FABHRMS).

[10] Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440-3450.

[11] Berridge, M. J. *Nature* **1993**, *361*, 315-325.

[12] Clapham, D. E. *Cell* **1995**, *80*, 259-268.

[13] Schaloske, R.; Sonnemann, J.; Malchow, D.; Schlatterer, C. *Biochem. J.* **1998**, *332*, 541-548.