Bioorganic & Medicinal Chemistry Letters, Vol.2, No.2, pp. 127-130, 1992
Printed in Great Britain

0960-894X/92 $3.00 + .00

THE SYNTHESIS AND EVALUATION OF DIACYLGLYCEROL ANALOGUES AS POTENTIAL SECOND-MESSENGER ANTAGONISTS

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(*Received* 1 *October* 1991)

Abstract: Structural analogues of diacylglycerol have been synthesised in an attempt to discover antagonists of protein kinase C with the aim of developing new agents for preventing cell proliferation.

The receptor mediated hydrolysis of membrane-bound inositol phospholipids generates[1] D-*myo*-inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG, *e.g.* **1**). Both of these compounds act as intracellular second messengers, DAG in combination with calcium ions and phosphatidyl serine activating protein kinase C (PKC).[2] A range of DAG analogues, e.g. *sn*-1,2-dioctanoylglycerol (**2**)[3] and *sn*-1-oleoyl-2-acetyl-glycerol (OAG, **3**),[4] have been prepared and shown to activate PKC.[2] In addition, and of particular relevance to this work, is the fact that tumour promoters such as the phorbol esters have been shown to mimic the effect of DAG in activating PKC.[2,5] Very few PKC antagonists have been reported,[2,6,7] and although to date antagonists based on DAG analogues which incorporate the 1,2-diacyl functionality of this second messenger have not been discovered, 1,2-acylalkylglycerols and 1,2-dialkylglycerols have been shown to act as competitive inhibitors of PKC.[6,7] We are interested in DAG analogues as potential PKC antagonists for the inhibition of cell proliferation. This preliminary communication describes the preparation of a series of DAG analogues, all in racemic form, and bioassay of these compounds as inhibitors of PKC and as inhibitors of the growth of certain cell lines. The following paper[8] is concerned with conformationally-restricted diacylglycerol analogues.

1,2-Diacylglycerol analogues **4-7** were prepared by acylation of the appropriate diol[9] with octanoyl chloride in pyridine and 1,2-dialkyl analogues were synthesised by alkylation of the parent diol with sodium hydride-octyl iodide in 1,2-dimethoxyethane in the case of **8** and **9** and with octyl iodide-silver oxide in the case of **10**. 1,2-di-*O*-octylglycerol (**11**) was obtained by alkylation of 1-*O*-benzylglycerol[11] followed by hydrogenolysis of the product.

The bis-amide analogue of DAG, compound **12**, was prepared by acylation of the *O*-2-tetrahydropyranyl derivative of 2,3-diaminopropan-1-ol[12] followed by acidic

hydrolysis of the product. The disulphonate **13** and the bis-urethane **14** were prepared by reaction of 1-*O*-trityl-[13] and 1-*O*-benzyl-glycerol with n-butanesulphonyl chloride and n-butylisocyanate, respectively, followed by removal of the protecting 1-*O*-alkyl moieties. Analogue **15**, which differs from the diacyl glycerol **2** in that the oxygen atoms and methylene groups alpha to the carbonyl groups in both ester groups are interchanged, was synthesised according to Scheme 1 in which the key step is oxidative cleavage of 4-benzyloxymethylcyclohexene[14] (**16**) to the dicarboxylic acid (**17**). Esterification of the latter afforded the *O*-benzyl diester **18**, catalytic hydrogenolysis of which gave **15.**

A diketone analogue of 1,2-di-*O*-octanoylglycerol, compound **19** and the corresponding acetate and methyl ether, **20** and **21**, respectively, were synthesised from t-butyl nonanoate (**22**) as shown in Scheme 2. Diester **23**, prepared by treatment of **22** with lithium diisopropylamide and iodine, was reduced to diol **24** which was converted via the dimesylate **25** and diiodide **26** to the 1,3-diene **27**. Diels-Alder reaction of **27** with ethyl acrylate afforded cyclohexene **28**, reduction of which gave the alcohol **29**. The latter was convered to the corresponding acetate and methyl ether, **30** and **31**, respectively. Ozonolysis of compounds **29**, **30**, and **31** then gave the diketones **19**, **20**, and **21**, respectively.

Biological Assays. Protein kinase C activity was measured using rat-brain supernatant as the source of the enzyme and enzymic activity was measured by incorporation of ^{32}P from γ-^{32}P-ATP into Histone IIIS in the presence of phosphatidyl serine and calcium and magnesium ions. In the control system, OAG was used to activate PKC. DAG analogues were tested as possible PKC activators by substituting an analogue for OAG in the assay procedure. To test for activity as PKC inhibitors, an analogue was added to the enzyme system in the presence of OAG. Of the analogues prepared in this work, only 1,2-di(octanamido)propan-3-ol (**12**) activated PKC, but not as effectively as OAG. Only the diketone analogues **19**, **20**, and **21** inhibited PKC significantly, concentrations giving 50% inhibition (I_{50} values) being approximately 0.01, 0.01, and 0.1 mM, respectively.

Cell culture studies were made on HL60, HT29, and MR4 cells, these being derived, respectively, from a human leukemia, a human colo-rectal tumour, and a murine line possessing an active human oncogene *ras* T24. Cells were grown in Dulbeccos minimal essential medium containing 10% foetal calf serum until they reached a density of 1.5×10^4 to 2×10^5 cells/mL. After addition of the test compounds, cytotoxic effects and cell growth were measured after 72 h using, respectively, the FRAME toxicity test [15] and by measurement of Kenacid Blue R binding.[16] Of the compounds showing inhibitory activity towards PKC, compound **19** was found to be more effective than **21** at inhibiting the growth of MR4 and HT29 cells, I_{80}

concentrations being 0.05 - 0.1 mM for **19** and 0.1 - 1.0 mM for **21.** Initial studies indicate that compound **20** has a potency similar to that of **19** on MR4 cells.

Acknowledgments. This work was financially supported by the Medical Research Council and the British Technology Group.

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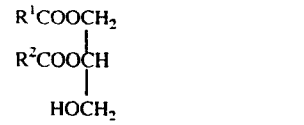
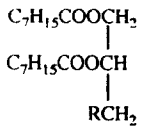
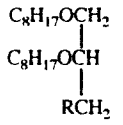


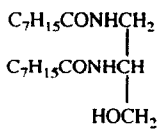
1 $R^1 = CH_3(CH_2)_{16}$
$R^2 = C_5H_{11}(CH{=}CHCH_2)_4(CH_2)_2$
2 $R^1 = R^2 = CH_3(CH_2)_6$
3 $R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7$
$R^2 = CH_3$



4 R = H
5 R = Cl
6 R = F
7 R = OCH_3



8 R = H
9 R = OCH_3
10 R = F
11 R = OH



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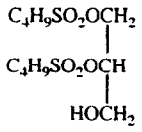

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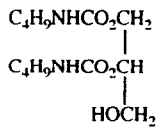

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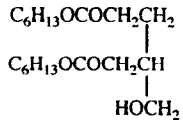

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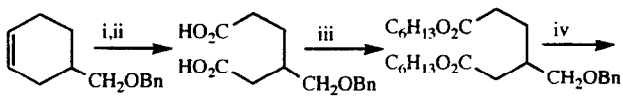
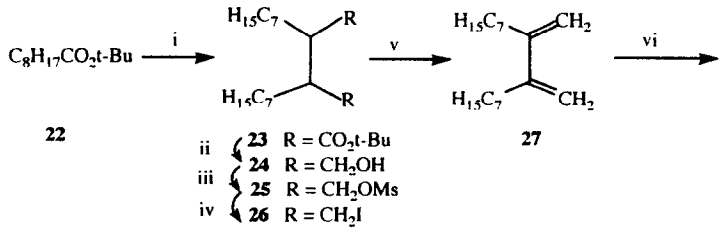
Scheme 1



16 **17** **18** **15**

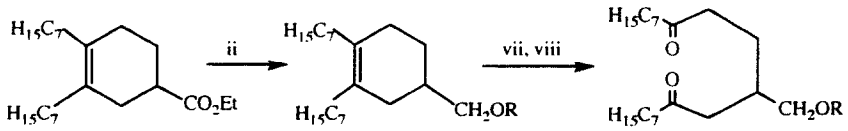
Reagents: (i) O_3/O_2 in MeOH (ii) H_2O_2/H_3O^+ (iii) $C_6H_{13}OH/H^+$ (iv) H_2-Pd/MeOH

Scheme 2



22 **27**

ii **23** R = CO_2t-Bu
iii **24** R = CH_2OH
iv **25** R = CH_2OMs
26 R = CH_2I



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29 R = H
30 R = Ac
31 R = Me

19 R = H
20 R = Ac
21 R = Me

Reagents: (i) $LiN(iPr)_2/I_2$; (ii) $LiAlH_4/Et_2O$; (iii) $MsCl/Et_3N/THF/CH_2Cl_2$; (iv) $NaI/CH_3COCH_2CH_3$
(v) t-BuOK/n-BuOH; (vi) $CH_2{=}CHCO_2Et$/PhMe; (vii) O_3-O_2/EtOAc; (viii) Me_2S