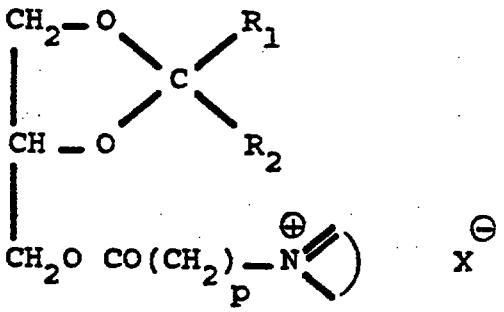
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Aminoacylates of glycerol acetal

DESCRIPTION:

The invention relates to aminoacylates of glycerol acetal, to a process for their preparation and to therapeutic compositions containing them.

The invention provides aminoacylates of glycerol acetal of the general formula I



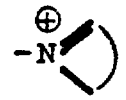
I

wherein

R_1 represents a substituted phenyl group or a group of the formula C_mH_{2m+1}, m being an integer of from 9 to 25,

R_2 represents a hydrogen atom, a phenyl group or a group of the formula C_nH_{2n+1}, n being an integer of from 1 to 10,

p is an integer of from 3 to 10,

 represents a nitrogen containing heterocyclic group, and

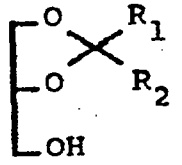
$X^{\ominus}$ represents the anion of a pharmaceutically acceptable inorganic or organic acid.

The nitrogen containing heterocyclic group is preferably a pyridinium, 3-thiazolinium, quinolinium, isoquinolinium, imidazolium or pyrazinium group.

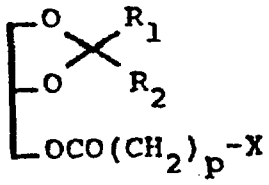
The anion $X^{\ominus}$ is preferably a halide anion, such as chloride, bromide or iodide, or a benzoate, acetate, methanesulphonate or tartrate anion.

The compounds of the invention are interesting as anti-PAF agents (PAF means Platelets Activating Factor), with corresponding activity as anti-anaphylactic, anti-thrombotic, anti-platelet aggregant, anti-broncho constrictor, normo-tensive and anti-ischemic agents, immunodepressors, and active also against immune alteration of kidney, against various shocks, against skin allergies and intestinal ulcers induced by endotoxine for instance.

The invention further provides a process for the preparation of the aminoacylates of glycerol acetal having the general formula I as above defined, the process comprising reacting an aldehyde or ketone of the formula R_1COR_2 wherein R_1 and R_2 are as above defined with glycerol, reacting the resultant compound of the formula



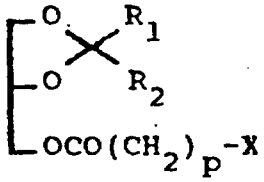
wherein R_1 and R_2 are as above defined with an alkanoyl chloride derivative of the formula $ClCO(CH_2)_pX$ wherein p and X are as above defined, and reacting the resultant compound of the formula



with a nitrogen containing heterocyclic compound of the formula



The reaction of the aldehyde or ketone with the glycerol is preferably effected under reflux in a non-polar solvent in the presence of p-toluenesulphonic acid, using a slight stoichiometric excess of the aldehyde or ketone. The resultant dioxolan derivative is suitably reacted with the alkanoyl chloride derivative at room temperature in the presence of an organic base, such as triethylamine. The reaction of the compound of the formula

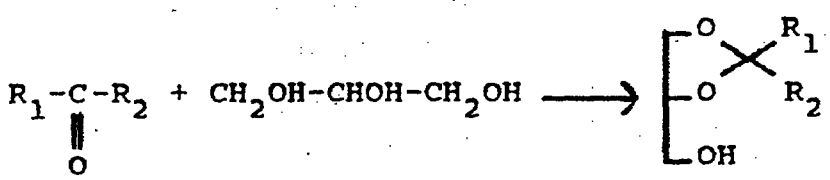


with the heterocyclic compound may be carried out at from 50^{o} to 80^{o} under nitrogen circulation.

The compounds of the invention are obtained as non-separated mixtures of diastereoisomers, but may be separated by the usual methods.

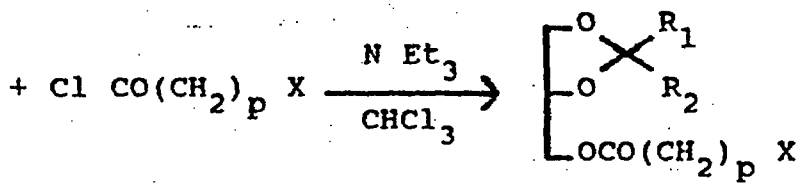
This process is illustrated by the following reaction scheme.

steps



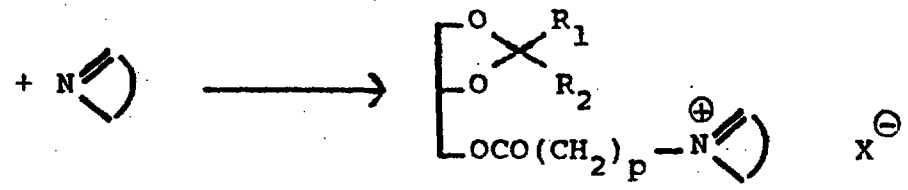
a

2



b

3



c

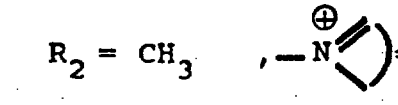
I

The invention also provides a pharmaceutical composition comprising a compound of the general formula I as above defined in admixture with a pharmaceutically acceptable diluent or carrier.

The invention is illustrated by the following examples. Example 1 is set out in full ; in the subsequent examples, only the characteristics of the intermediates and of the resulting compounds are given (Unless otherwise specified, TLC is performed on silica gel plates).

EXAMPLE 1

2-heptadecyl 2-methyl 4-[4'-(N-pyridinium) butyryloxy methyl]-1,3-dioxolan chloride

$R_1 = C_{17}H_{35}$, $R_2 = CH_3$,  = pyridinyl, X = Cl, p = 3.

Step a : Preparation of 2-heptadecyl 2-methyl 4-hydroxy-methyl 1,3-dioxolan. 2

In an appropriate reactor were poured 11.1 g (40 mmol) of 2-nonadecanone, 6 g - which represents an excess with regard to the stoichiometric proportions - of twice distilled glycerol and 0.6 g of p-toluenesulphonic acid dissolved in 120 ml of dry toluene. The reaction mixture was refluxed under stirring for 12 hours and the resulting water was eliminated using a Dean Stark apparatus. After cooling, the organic phase was washed with 30 ml of 5% by weight aqueous solution of potassium hydroxide and then three times with the minimum amount of water. After drying over anhydrous sodium sulphate, the solvent was removed under reduced pressure. The residue was then purified by chromatography on a silica gel column. There were successively eluted the fraction of the ketone not involved in the reaction (petroleum ether : diethyl ether, 95:5 by volume), and then the dioxolan a (petroleum ether / diethyl ether 85:15 by volume). the dioxolan is a viscous product, partially crystallized.

Yield 11 g (78 %)

rf : 0.18 (petroleum ether / diethyl ether 70:30 by volume)

IR 3500 cm^{-1} (OH)

1100 - 1060 cm^{-1} (C-O-).

Step b : 2-heptadecyl 2-methyl 4-(4'-chlorobutylryloxy-methyl) 1,3-dioxolan. 3

A mixture of 6.4 g (18 mmol) of 2-heptadecyl 2-methyl 4-hydroxymethyl 1,3-dioxolan , prepared as described in step (a) above, and 6 ml (45 mmol) of triethylamine in 15 ml of dry chloroform was added dropwise to a solution of 6.25 g (22 mmol) of 4-chlorobutyryl chloride in 10 ml of dry chloroform at 0°C. The mixture was stirred for 12 hours at room temperature. After addition of 20 ml of chloroform, the mixture was washed with 1N aqueous solution of sodium hydroxide and then with water until the pH was 7. The elimination of the solvent left a residue which was chromatographed on a silica gel column with petroleum ether : diethyl ether as eluent, successively 95:5, then 90:10 and finally 80:20, by volume. There was thus obtained 7.6 g (yield 82 %) of an oily product which crystallized ; melting point 39-40°C. This product is a mixture of the two cis and trans isomers (separation of the same is uneasy).

rf : 0.45 and 0.37 (petroleum ether / diethyl ether 80:20 by volume)

IR 1740 cm^{-1} (C=O)

1180 - 1160 cm^{-1} (C-O-C ether and ester)

NMR 60 MH_z, $CDCl_3$, (TMS.)

δ : 0.9 (triplet, 3H, CH_3 of the chain)

1.3 (mult., 35H, 32H + CH_3)

2.1 (mult., 2H, $CO-CH_2-\underline{CH_2}$)

2.5 (triplet, 2H, $CO-\underline{CH_2}$)

3.6 (mult., 4H, CH_2-O and CH_2Cl)

4.2 (mult., 3H, CH_2OCO and $C\underline{H}-O$).

Step c : 2-heptadecyl 2-methyl 4-[4'-(N pyridinium)-butyryloxymethyl] 1,3-dioxolan chloride. I

5 g of the compound obtained in the previous step (b) above, dissolved in 30 ml of pyridine, was stirred at 80°C, under nitrogen for 24 hours. Excess pyridine was then eliminated under reduced pressure and the residue was purified by chromotography on silica gel. Elution with chloroform (for the recovery of starting material not involved in the reaction) and then with chloroform / methanol, first 90:10, then 80:20 by volume gave 3.80 g of the title product. This product is a mixture of the two cis and trans isomers (separation of the same is uneasy), yield 40 % overall (65 % for this step). Melting point 84-85°C.

rf : 0.28 ($CHCl_3$ / MeOH 70:30, by volume)

IR (nujol) 1740 cm^{-1}

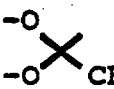
1630 cm^{-1} (pyridine)

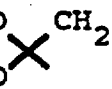
1200, 1180 cm^{-1} (C-O-C)

NMR, 250 MH_z, $CDCl_3$

δ : 0.9 (triplet, 3H, CH_3)

1.2 (sing. large 30H)

1.4 (doublet, 3H, ) (the 2 CH_3 of the two diastereoisomers)

1.55 (mult., 2H, )

2.35 (mult., 2H, CO CH_2-CH_2)

2.6 (triplet, 2H, CO CH_2)

3.9 (mult., 1H, CH-O)

4 (mult., 2H, CH_2O CO)

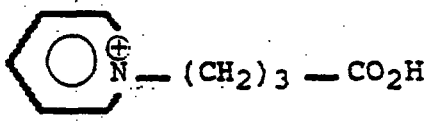
4.2-3.6 (2 mult., 2H, CH_2O) (of the two diastereoisomers)

5.10 (mult., 2H, CH_2N)

pyridinium { 8 (mult., 2H, H_β) ; 8.4 (mult., 1H, H_γ) ; 9.6 (doub., 2H_α) }

Mass spectrum :

M - Cl m/z : 504

 : 166

EXAMPLE 2

2-heptadecyl 2-methyl 4-[4'(N-quinolinium) butyryl oxymethyl] 1,3-dioxolan chloride.

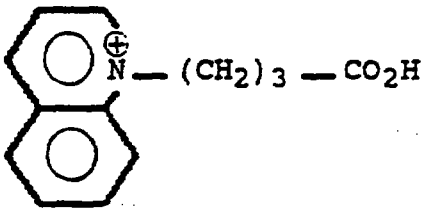
Step c : 5 g of the compound obtained in example 1 (step b) was dissolved in a mixture of 20 ml of quinolin and 20 ml of DMSO and heated at 80°C under nitrogen for 4 days. Quinolin and DMSO were distilled under reduced pressure and the residue was chromatographed on a silica gel column. (eluent successively $CHCl_3$, then $CHCl_3$ / MeOH 95:5). 2.5 g of the desired product were obtained as a mixture of the two diastereoisomers.

rf : 0.58 ($CHCl_3$ / MeOH, 70:30)

IR ν quinolin 1650, 1630 and 1600 cm^{-1}

Mass spectrum :

M - Cl m/z : 554

 : 216.

NMR quinolinium instead of pyridinium.
δ ppm : 7.90 - 8.10 (4H), 8.70 (1H), 8.90 (1H), 10.90 ($1H_\alpha$).

EXAMPLE 3

2-heptadecyl 2-methyl 4-[4'-(N-thiazolinium) butyryl oxymethyl] 1,3-dixolan chloride.

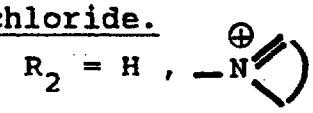
Step c : 850 mg of the product obtained in example 1 (step b) in a mixture of thiazole (4 ml) and DMSO (6 ml) were heated at 80°C for 24 hours. The reaction mixture was evaporated in vacuo and the residue was chromatographed on a silica gel column, eluent $CHCl_3$ / MeOH 50:50 giving a viscous product.

rf : 0.34 ($CHCl_3$ / MeOH 70:30)
NMR thiazolinium instead of pyridinium.
δ ppm : 8.19 (d : 1H), 8.4 (d : 1H), 10.90 (sing. 1H)

Mass spectrum : M - Cl : 510 m/z.

EXAMPLE 4

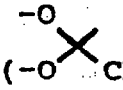
2-heptadecyl 4-[4'-(N-pyridinium) butyryloxymethyl] 1,3-dioxolan chloride.

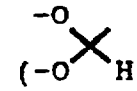
$R_1 = C_{17}H_{35}$, $R_2 = H$,  = pyridinyl , X = Cl, p = 3.

2 rf : 0.17 (petroleum ether / diethyl ether 30:70 by volume)

3 rf : 0.37 (petroleum ether / diethyl ether 70:30 by volume)

I rf : 0.16 (chloroform / methanol 70:30 by volume)

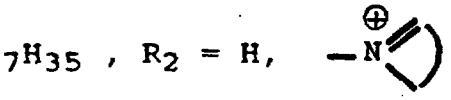
I NMR instead of CH_3 at 1.4 ()

2 multiplets centred on 4.9 ppm ()

Mass spectrum : M - Cl : 490 m/z.

EXAMPLE 5

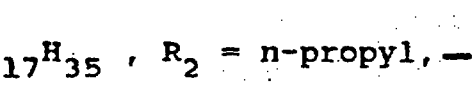
2-heptadecyl 4-[4'-(N-quinolinium) butyryl oxy methyl] 1.3 dioxolan chloride.

$R_1 = C_{17}H_{35}$, $R_2 = H$,  = quinolinium, X = Cl , p = 3.

rf : 0.48 (chloroform / methanol 70:30 by volume).

EXAMPLE 6

2-heptadecyl 2-(n-propyl) 4-[4'-(N-pyridinium) butyryl oxy methyl] 1,3-dioxolan chloride.

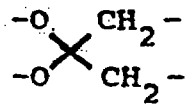
$R_1 = C_{17}H_{35}$, R_2 = n-propyl,  = pyridinyl, X = Cl, p = 3.

2- rf : 0.23 (petroleum ether / diethyl ether 70:30 by volume)

3 rf : 0.38 (petroleum ether / diethyl ether 80:20 by volume)

I rf : 0.20 (chloroform / methanol 70:30 by volume)

I NMR instead of CH_3 at 1.4



1.55 (mult. 4H -O- CH_2 - O- CH_2 -)

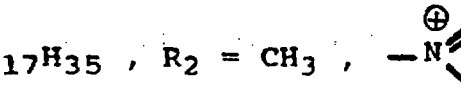
1.2 (32H)

0.9 (triplet, 6H, $2CH_3$)

Mass spectrum : M - Cl : 532 m/z.

EXAMPLE 7

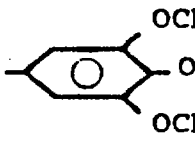
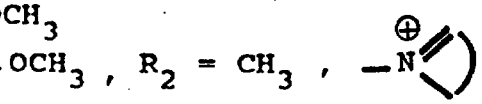
2-heptadecyl 2-methyl 4-[5'-(N-pyridinium) pentanoyl oxymethyl] 1,3-dioxolan chloride.

$R_1 = C_{17}H_{35}$, $R_2 = CH_3$,  = pyridinyl, X = Cl, p = 4.

rf : 0.23 (chloroform / methanol 70:30).

EXAMPLE 8

2-(3,4,5-trimethoxy phenyl) 2-methyl 4-[4'-(N-pyridinium) -butyryloxymethyl] 1,3-dioxolan chloride.

R_1 =  , $R_2 = CH_3$,  = pyridinyl, X = Cl, p=3.

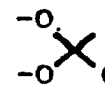
2 rf : 0.32 (chloroform / methanol 98:2 by volume)

3 rf : 0.61 (chloroform / methanol 98:2 by volume)

I rf : 0.34 (chloroform / methanol 60:40 by volume)

IR 1735 (C=O)
1610 (pyridine)
1590 (benzene)
2740 (OCH_3)

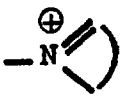
I NMR instead of the chain R_1

1.5 ppm (m, 3H,  for R_2
3.7 (sing., 9H, OCH_3)
6.8 (2H arom.)

Mass spectrum : M - Cl : 432 m/z.

EXAMPLE 9

2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide.

$R_1 = C_{17}H_{35}$, $R_2 = CH_3$,  = pyridinyl, X = Br, p = 5.

step b : 2-heptadecyl 2-methyl 4-(6'-bromo hexanoyloxymethyl) 1,3-dioxolan bromide.

Following the procedure described in exemple 1, using 6-bromo-hexanoyl chloride, the desired product was obtained as a viscous product (yield 82 %).

rf : 0.57 (petroleum ether / ether 80:20).

step c : Following the procedure described in example 1, the desired product was obtained as a viscous product (yield 70 %).

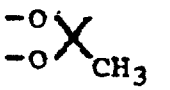
rf : 0.36 ($CHCl_3$ / MeOH 70:30)

IR 3450 cm^{-1} (residual water)

ν_{CO} 1740, ν_{C-O-C} 1180, $\nu_{pyridine}$ 1640 cm^{-1}

NMR 500 MH_z $CDCl_3$, δ (TMS)

0.85 (triplet, 3H, CH_3) ; 1.25 (sing. large 30H) ;

1.35 (d 3H,  ,Z + E) ; 1.45 (mult., 2H, $CH_{2\delta}$) ;

2.1 (mult., 2H, $COCH_2\underline{CH_2}$) ; 2.35 (mult., 2H, $COCH_2$) ; 3.70 and 4.35 (mult., 2H, CH_2O, Z + E) ; 4(mult. 3H, CH_2OCO and H-C-O) ;

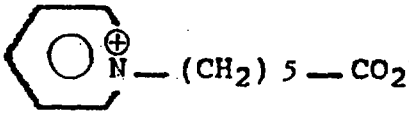
5.05 (triplet, 2H, $\overset{\oplus}{C}H_2N$).

pyridinium 8.1 (triplet, 2H, H_β) ; 8.6 (triplet, 1H, H_γ) ; 9.5 (d : 2H, H_α).

U. V. (Ethanol) logϵ 3.65 λ 258.8 nm.

Mass spectrum :

M - Br m/z : 532

 : 194.

EXAMPLE 10

2-heptadecyl 2-methyl 4-[6'-(N-quinolinium) hexanoyloxymethyl) 1,3-dioxolan bromide.

rf : 0.60 ($CHCl_3$ / MeOH 70:30)

IR Quinolinium 1650, 1630, 1600 cm^{-1}

U. V. (Ethanol) loge 4.53 λ 235.4 nm

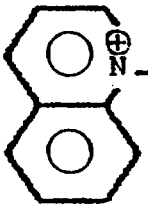
2.18 λ 316.5 nm

NMR quinolinium instead of pyridinium.

δ : 5.5 (2H, $\overset{\oplus}{C}H_2N$) , 7.90 - 8.40 (5H) , 9.10 (1H) , 10.70 (1H, Hα).

Mass spectrum :

M - Br m/z : 582



: 244;

EXAMPLE 11

2-heptadecyl 2-methyl 4-[6'-(N-isoquinolinium) hexanoyloxymethyl) 1,3-dioxolan bromide.

step c : Using isoquinoline instead of quinoline.

rf : 0.55 ($CHCl_3$ / MeOH 73:30)

IR isoquinolin 1650, 1610, 1590 cm^{-1}.

NMR isoquinolinium instead of quinolinium.

δ : 5.15 (triplet, 2H, $\overset{\oplus}{C}H_2N$) ; 7.95 - 8.40 (4H) ; 8.80 (2H) ; 11.05 (1H).

Mass spectrum : M - Br : 582 m/z.

EXEMPLE 12

2-heptadecyl 2-methyl 4-[11'-(N-pyridinium undecanoyloxymethyl) 1,3-dioxolan bromide.

$R_1 = C_{17}H_{35}$, $R_2 = CH_3$,  = pyridinyl, X = Br, p = 10.

step b : 2-heptadecyl 2-methyl 4-(11'-bromo undecanoyloxymethyl) 1,3-dioxolan.

Following the procedure described in exemple 1, using 11-bromo undecanoyl chloride, the desired product was obtained.
Melting point : 52°C (yield 60 %)
rf : 0.26 (petroleum ether / ether 85:15).

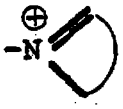
step c

Melting point : 92°C
rf : 0.45 ($CHCl_3$ / MeOH 70:30)

Mass spectrum : M - Br : 602 m/z.

EXEMPLE 13

2-heptadecyl 2-(n-propyl) 4-[6'-(N-pyridinium) hexanoylmethyl] 1,3-dioxolan bromide.

$R_1 = C_{17}H_{35}$, R_2 = n-propyl,  = pyridinyl, X = Br, p = 5.

rf : 0.44 ($CHCl_3$ / MeOH 70:30)
Melting point : 76°C

Mass spectrum : M - Br : 560 m/z.

EXAMPLE 14

2-nonyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl) 1,3-dioxolan bromide.

$R_1 = C_9H_{19}$, $R_2 = CH_3$,  = pyridinyl, X = Br, p = 5.

step b

rf : 0.50 (petroleum ether / ether 80:20)

step c

rf : 0.30 ($CHCl_3$ / MeOH 70:30)

Mass spectrum : M - Br : 420 m/z.

TOXICOLOGY

The compounds of the invention have been administered to mice for determination of acute LD_{50}. For all the compounds of the invention LD_{50} was over 300 mg/Kg (IP or SC) and 600 mg/Kg (PO).

PHARMACOLOGY

A proof of the pharmaceutical interest of the compounds of the invention has been established by the following pharmaceutical experimentations :

1°)- Inhibition of the platelets aggregation on New Zealand rabbits.

The experimentation was conducted on platelets with plasma of New Zealand rabbits.

Blood samples were taken from auricular artery and placed in a citrate buffer (3.8 % ; pH 7.4) ; blood was further centrifugated for 15 mn at 1200 RPM.

The tested sample was prepared in DMSO, then poured on platelets rich plasma for 1 mn, then a dose of 2.5 nM of PAF was added.

The determination is made on a Cronolog Coultronics apparatus which determines the transmission percentage corresponding to the maximum height of the peak before the desaggregation.

The percentage of variation of the inhibition with respect to the transmission percentage is calculated (control : pure DMSO).

This method was described in details in LABORATORY INVESTIGATIONS, Vol. 41, No. 3, p. 275, 1979, JEAN-PIERRE CAZENAVE, Dr. MED. , JACQUES BENVENISTE, Dr. MED., AND J. FRASER MUSTARD, M. D., "Aggregation of Rabbits Platelets by Platelet-Activating Factor Is Independent of the Release Reaction and the Arachidonate Pathway and Inhibited by Membrane-Active Drugs".

The results demonstrate that the compounds inhibit the aggregation induced by 2.5 nM of PAF. Five tests made on 5 different rabbits allowed us to calculate the IC_{50} of the various compounds using the linear regression test.

Inhibition of PAF induced platelet aggregation in platelet rich plasma from rabbits.

Example No.	IC_{50} PRP (M)
No. 1	$2.2 \cdot 10^{-6}$
No. 2	$1.2 \cdot 10^{-6}$
No. 3	$2.15 \cdot 10^{-5}$
No. 4	$7 \cdot 10^{-6}$
No. 5	$3.1 \cdot 10^{-6}$
No. 6	$3.04 \cdot 10^{-6}$
No. 7	$5 \cdot 10^{-6}$
No. 8	$1.13 \cdot 10^{-5}$
No. 9	$4.04 \cdot 10^{-7}$
No. 10	$3.03 \cdot 10^{-7}$
No. 12	$3.12 \cdot 10^{-5}$
No. 13	$1.73 \cdot 10^{-6}$
No. 14	$1.46 \cdot 10^{-6}$

2 - BINDING

MATERIAL AND METHOD

Synthetic tritiated [^{3}H]-PAF used was in ethanol solution with a specific activity of 59.5 ci/mmols. Unlabelled PAF and lyso-PAF were solubilized in ethanol solution and stored at - 80°C. The compounds were solubilized in DMSO.

Platelet membrane preparations

Rabbit whole blood (6 volumes) was drawn from the central ear artery into 1 volume of ACD solution (citric acide 1.4 g, sodium citrate 2.5 g, Dextrose 2 g per 100 ml of H_2O) and centrifuged at 150 g for 15 minutes. The platelet-rich plasma (PRP) was carefully removed and centrifuged for 15 minutes at 1000 g. The platelet pellet was then washed 3 times : twice in Tris-HCl buffer 10 mM, pH 7.4 containing NaCl 150 mM, $MgCl_2$ 5 mM, EDTA 2 mM and the last time in the same but sodium-free buffer.

The platelet pellet was resuspended in this latter buffer, quickly frozen in liquid nitrogen and slowly thawed at room temperature for at least 3 times as described by T. Y. Shen et al.. The lysed platelets were centrifuged at 100 000 g for 30 minutes in a BECKMAN (RTM) model L8.55 ultra centrifuge (rotor 50.2 Ti). Platelet membrane homogenate was stored at -80°C and used within two weeks without noticeable changes in PAF-acether binding characteristics.

Protein contain was determined by the Lowry method using bovine serum albumine as standard.

Binding assay

60 to 100 μg of membrane proteins were added to a final volume of 1 ml in plastic tubes containing 1 nM [^{3}H]-PAF in Tris-HCl 10 mM pH 7 buffer containing 0.025 % bovine serum

albumin and incubated with or without unlabelled PAF or PAF-antagonists. The incubation was carried out for 1 h 30 at 0° C. The bound [^{3}H]-PAF was separated from the free [^{3}H]-PAF by immediate filtration through whatman GF/C glass fiber filters under vacuum (Brandel system). The reaction, and the filters were washed 3 times with 5 ml of ice-cold buffer. The filters were then placed into polyethylene phials filled up with 10 ml of liquid scintillation fluid and the radioactivity was measured by an LKB β counter with 45 % efficiency.

The non specific binding was determined in the presence of 10^{-6} M of unlabelled PAF. The specific binding was calculated by substracting non specific binding from the total binding. The inhibition by compounds on the specific [^{3}H]-PAF binding was determined as the percent inhibition by the equation:

$$\% \text{ inhibition} = \frac{(^3\text{H-PAF total bound}) - \left\{\begin{matrix}(^3\text{H-PAF bound in presence} \\ \text{of compounds})\end{matrix}\right\}}{^3\text{H-PAF specifically bound}} \times 100$$

Example No.	IC_{50}
No. 1.....	$4.5 \cdot 10^{-7}$
No. 9.....	$3.5 \cdot 10^{-8}$
No. 10.....	$3.5 \cdot 10^{-8}$
No. 14.....	$6.1 \cdot 10^{-7}$

3 - BRONCHOCONSTRICTION, LEUKOPENIA AND THROMBOCYTOPENIA INDUCED BY PAF IN THE GUINEA PIG

MATERIALS AND METHODS

Male Guinea-pig (400 - 500 g) were anesthetized with urethane (1.5 g/kg : IP) prior to be tracheotomized and ventilated with a respiratory pump (UGO BASILE) (70 - 80 breath/minute, 1 ml air / 100 g / breath) to abolish spontaneous respiration a pneumothorax was performed. The resistance to inflation was measured with a pressure transducer (UGO BASILE) against an initial pressure of 10 cm H_2O according to Konzett and Rossler. Animals were allocated into groups and were treated or not by the compounds of the invention (0.05, 0.1, 0.5, 1 and 5 mg/kg).The products were given IV 1 hour before challenge with PAF.

The animals were challenged with various doses of PAF (30 - 100 n/kg) given intravenously, and the variations in the resistance to inflation were recorded.

Blood samples were collected prior to and 1,5 and 10 minutes following to PAF-challenge. Forty μl of blood were diluted in 10 ml isoton (Coultronics, France); erythrocytes were lysed with Zapoglobin (France) prior leukocytes were counted in a Coulter counter. 'Coulter' is a Trade Mark. To determine platelet number, 40 μl of blood were diluted in 2 ml isoton and centrifuged at 100 runs per minute for 30 secondes, 100 μl of the supernatant were collected and further diluted in 10 ml isoton prior counting in a Coulter counter.

Table No. 1 : Effects of 2 - heptadecyl 2 - methyl 4 - [6' - (N - pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced bronchoconstriction.

	Doses mg/kg IV	n	Percentage Bronchoconstriction	Percentage Variation
Control	-	8	79.8 +/- 3.22	-
Compound of example No. 9	5	3	3.5 +/- 1.53	- 95.6 ***
	1	4	19.1 +/- 10.51	- 76.1 ***
	0.5	6	23.6 +/- 7.15	- 70.4 ***
	0.1	6	43.8 +/- 14.77	- 45.1 **
	0.05	3	79.0 +/- 9.27	- 1.0 NS

LEGEND OF ALL THE TABLES :

NS : Non Significative
* : $p < 0.05$
** : $p < 0.01$
*** : $p < 0.001$

Table No. 2 : Effects of 2 - heptadecyl 2 - methyl 4 - [6' - (N - pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced leukopenia.

	Doses mg/kg IV	n	Time (mn)	Percentage Decrease of leukocyte number	Percentage Variation
Control	-	8	1	40.8 +/- 7.18	-
		8	5	29.2 +/- 10.89	-
		8	10	33.5 +/- 5.04	-
Compound of example No. 9	5	3	1	16.5 +/- 2.42	- 59.6 *
		3	5	15.9 +/- 0.99	- 45.5 NS
		3	10	18.7 +/- 5.08	- 44.2 NS
	1	4	1	3.5 +/- 12.60	- 91.4 **
		4	5	2.9 +/- 10.51	- 90.1 *
		4	10	1.0 +/- 22.62	- 97.0 **
	0.5	6	1	34.6 +/- 6.35	- 15.2 NS
		6	5	19.0 +/- 6.42	- 34.9 NS
		6	10	14.6 +/- 5.36	- 56.4 NS
	0.1	6	1	41.8 +/- 6.50	+ 2.5 NS
		6	5	31.9 +/- 4.88	- 9.2 NS
		6	10	33.5 +/- 4.65	0.0 NS
	0.05	3	1	45.3 +/- 5.70	+ 11.0 NS
		3	5	21.6 +/- 3.42	- 26.0 NS
		3	10	14.2 +/- 17.30	- 57.6 NS

Table No. 3 : Effects of 2 - heptadecyl 2 - methyl 4 - [6'- (N - pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced thrombocytopenia

	Doses mg/kg IV	n	Time (mn)	Percentage Decrease in leukocyte number	Percentage Variation
Control	-	8	1	68.6 +/- 6.79	-
		8	5	42.4 +/- 2.71	-
		8	10	42.5 +/- 3.14	-
Compound - of example No. 9	5	3	1	4.2 +/- 2.35	- 93.9 ***
		3	5	5.6 +/- 1.84	- 86.8 ***
		3	10	1.1 +/- 1.15	- 97.4 ***
	1	4	1	11.5 +/- 6.21	- 83.2 ***
		4	5	22.5 +/- 3.27	- 46.9 *
		4	10	21.0 +/- 3.87	- 50.6 *
	0.5	6	1	19.3 +/- 10.19	- 71.9 ***
		6	5	9.6 +/- 12.15	- 77.4 ***
		6	10	10.9 +/- 9.94	- 74.4 ***
	0.1	6	1	30.1 +/- 13.72	- 56.1 **
		6	5	34.7 +/- 3.60	- 18.2 NS
		6	10	42.3 +/- 4.79	- 0.5 NS
	0.05	3	1	45.2 +/- 1.20	- 34.1 NS
		3	5	43.0 +/- 4.80	+ 1.4 NS
		3	10	36.1 +/- 2.41	- 15.1 NS

Table No. 4 : Effects of 2 - heptadecyl 2 - methyl 4 - [6'- (N - quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced bronchoconstriction.

	Doses mg/kg IV	n	Percentage Broncho-constriction	Percentage Variation
Control	-	6	90.8 +/- 0.95	-
Compound of Example No. 10	1	5	8.1 +/- 2.91	- 91.1 ***
	0.5	5	25.1 +/- 6.53	- 72.4 ***
	0.1	5	80.4 +/- 13.78	- 11.5 NS

Table No. 5 : Effects of 2 - heptadecyl 2 - methyl 4 - [6'- (N - quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced leukopenia

	Doses mg/kg IV	n	Time (mn)	Percentage Decrease in leukocyte number	Percentage Variation
Control	-	6	1	41.4 +/- 8.0	-
		6	5	28.7 +/- 6.9	
		6	10	26.9 +/- 10.1	
Compound of example No. 10	1	5	1	24.1 +/- 6.8	- 41.0 NS
		5	5	9.3 +/- 9.4	- 67.6 **
		5	10	8.9 +/- 13.2	- 66.9 **
	0.5	5	1	17.0 +/- 12.9	- 58.9 *
		5	5	9.0 +/- 11.8	- 68.6 **
		5	10	- 7.6 +/- 17.5	+ 128.3 NS
	0.1	5	1	38.8 +/- 6.3	+ 6.3 NS
		5	5	38.9 +/- 5.7	+ 35.5 NS
		5	10	41.2 +/- 6.5	+ 53.2 NS

Table No. 6 : Effects of 2-heptadecyl 2-methyl 4 - [6'- (N-quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide on PAF-induced thrombocytopenia

	Doses mg/kg IV	n	Time (mn)	Percentage Decrease in leukocyte number	Percentage Variation
Control	-	6	1	65.3 +/- 8.5	-
		6	5	49.1 +/- 4.8	
		6	10	48.5 +/- 0.5	
Compound - of example No. 10	1	5	1	19.3 +/- 1.4	- 58.3 ***
		5	5	22.1 +/- 2.5	- 55.0 **
		5	10	19.4 +/- 3.6	- 60.0 **
	0.5	5	1	26.2 +/- 6.3	- 59.9 **
		5	5	29.8 +/- 1.7	- 39.3 *
		5	10	32.1 +/- 5.8	- 33.8 *
	0.1	5	1	51.1 +/- 12.1	- 21.7 NS
		5	5	49.1 +/- 8.8	0 NS
		5	10	45.0 +/- 9.7	- 7.2 NS

RESULTS

2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyl-oxymethyl] 1,3 dioxolan bromide (0.5, 1 and 5 mg/kg IV) and 2-heptadecyl 2-methyl 4-[6'-(N-quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide (0.5 and 1 mg/kg IV) inhibit the PAF-induced bronchoconstriction and the PAF-induced thrombocytopenia in the guinea-pig.

4 - ANTIGEN-INDUCED BRONCHOCONSTRICTION, LEUKOPENIA AND THROMBOCYTOPENIA IN GUINEA-PIGS PASSIVELY SENSITIZED WITH HETEROLOGOUS SERUM

Intravenous injection of ovalbumin to guinea-pigs passively sensitized with rabbit anti-ovalbumin antiserum induces a bronchoconstriction associated with a leukopenia and a thrombocytopenia. This anaphylactic reaction appears to be due to the release of histamine and the generation of arachidonic acid metabolites (prostaglandins, thromboxane A_2 and leukotrienes). In the guinea-pig, the release of these autacoids is primarily mediated via immunoglobulins of the IgG class.

METHOD

- Animals :

. Hartley male guinea-pigs, Charles River (450 - 550 g)

- Immunisation procedure :

. Intravenous injection 18 h prior to challenge with the antigen of 0.5 ml/kg of 1/2 dilution of rabbit anti-ovalbumin antiserum.

- Challenge is obtained by :

  . Ovalbumin, 0.75 mg/kg,

  . Intravenous injection of the antigen in a final volum of 1 ml/kg,

  . Vehicle, 0.15 M NaCl.

- Compounds are administered by oral route in a volume of 2.5 ml/kg, 1 h prior to challenge with the antigen. After anesthesia by intraperitoneal injection of ethyl carbamate (1.5 g/kg) in a volume of 10 ml/kg. Parameters monitored and expression of the data :

  a - Bronchoconstriction

  . Antigen-induced bronchoconstriction in mm (A),

  . Maximal bronchoconstriction in mm (B)

  The percentage of bronchoconstriction is calculated as follows :

$$\frac{A \times 100}{B}$$

  Neutrophil and platelet counts were performed 1 minute prior to and 1,5 and 10 minutes after challenge. The changes were expressed in percentages calculated over the values obtained one minute prior to challenge.

The following equipement was used :

- Ugo Basile, Comerio ITALY,
  . Rodent ventilator ref. 7025
  . Bronchospam transducer ref. 7020
  . Two-Channel Recorder "GEMINI" ref. 7070

- Coultronics, FRANCE,
  . Coulters Counter Z B I

Table No. 7 : Effects of 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on antigen-induced bronchoconstriction

	Dose mg/kg IV	n	Percentage Broncho-constriction	Percentage Variation
Control	-	6	71.9 +/- 14.2	-
Compound of example No. 9	5	8	51.3 +/- 14.2	- 28.7 NS

Table No. 8 : Effects of 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on antigen-induced leukopenia

	Dose mg/kg IV	n	Time (mn)	Percentage Decrase in leukocytes number	Percentage Inhibition
Control	-	6	1	- 11.8 +/- 5.1	-
		6	5	- 39.6 +/- 9.5	
		6	10	- 53.9 +/- 7.1	
Compound of example No. 9	5	8	1	- 15.6 +/- 6.6	+ 32.0 NS
		8	5	- 8.7 +/- 10.7	- 78.0 *
		8	10	- 12.3 +/- 14.1	- 77.2 *

Table No. 9 : Effects of 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide on antigen-induced thrombocytopenia

	Dose mg/kg IV	n	Time (mn)	Percentage Decrease in leukocytes number	Percentage Inhibition
Control	-	6	1	- 6.8 +/- 2.7	-
		6	5	- 39.7 +/- 12.5	
		6	10	- 26.1 +/- 8.1	
Compound of example No. 9	5	8	1	+ 0.3 +/- 7.9	- 104.4 NS
		8	5	- 3.5 +/- 3.1	- 91.2 **
		8	10	- 15.7 +/- 6.2	- 39.8 NS

RESULTS

2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyl-oxymethyl] 1,3 dioxolan bromide (5 mg/kg IV) antagonizes antigen-induced leukopenia and thrombocytopenia without significantly reducing antigen-induced bronchoconstriction.

5 - ACTION ON PAF-INDUCED HYPOTENSION IN THE ANESTHETIZED RAT

MATERIALS AND METHOD :

Male Sprague Dawley Rats (Charles River Breeding Station) (250 - 300 g) were anesthetized with ethyl carbamate at the dose of 1.2 g/kg IP.

Intravenous injection of PAF provokes a dose-dependent hypotension. The effects of 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyloxymethyl] 1,3 dioxolan bromide and 2-heptadecyl 2-methyl 4-[6'-(N-quinolinium hexanoyloxymethyl] 1,3 dioxolan bromide were sought against PAF-induced hypotension, in curative treatments. Hypotension was provoked by 1 single dose of PAF (1 µg/kg) in an intravenous injection, directly into the penis vein, in a volume of 0.1 ml/100g. At the maximum hypotension, i. e. 3 minutes after PAF injection, drugs were administered.

- Parameters measured and expression of the results :

The systolic and diastolic arterial pressures (mmHg) were measured. The values were expressed as a mean +/- SEM in the drawings.

- Measurement apparatus :

. Gould P_{50} transducer to measure arterial pressure,
. Braun (R-M) perfuser,
. Gould 8000 S polygraphic recorder.

'Gould' is a Trade Mark.

6 - ANTAGONISM OF THE POLYMORPHONUCLEAR LEUKOCYTE AGGREGATION INDUCED BY PAF

Polymorphonuclear leukocytes (PMNs) were obtained as described by A. W. Ford-Hutchinson & al. (Br - J. Pharmacol. 76, 367 - 371, 1982). Cell suspensions (< 90% PMNs) were prepared from peritoneal exudates obtained after a 5 ml IP injection of sodium caseinate 12 % (w/v) to male Wistar rats, 300 g body weight.

After centrifugation, cells were washed with Hanks and resuspended in MEM/Hepes 30 mM medium (PM = 7.4) at a concentration of 10^7 cells/ml. Cytochalasin B (5 µg/ml) was added 10 minutes before trigering aggregation to amplify the PMNs aggregation. The light transmittance through the cell suspension (400 µl) was measured under a 900 R/mn magnetic stirring, with a dual beam aggregometer (Chronolog Corp. Coultronics).

The control cuvet is the cell suspension diluted to 20 % of the cell count with MEM and the difference represents 100 % transmittance. Change in transmittance, which occurs when PAF is added, was continuously recorded.

Drugs solubilized in MEM/Hepes were added (4 µl) to PMNs suspension 2 minutes before PAF addition.

RESULTS

PAF 10^{-8} M induced 55 to 70 % aggregation of this PMNs suspension.

2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyl-oxymethyl] 1,3 dioxolan bromide and 2-heptadecyl 2-methyl 4-[6'-(N-quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide

dose-dependently inhibit this aggregation with respective $IC_{50}s$:

. 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyl-oxymethyl] 1,3 dioxolan bromide : 5×10^{-7} M

. 2-heptadecyl 2-methyl 4-[6'-(N-quinolinium) hexanoyl-oxymethyl) 1,3 dioxolan bromide : 10^{-6} M

7 - <u>INHIBITION OF INTRACYTOSOLIC FREE CALCIUM MOBILIZATION INDUCED BY PAF IN WASHED RABBIT PLATELETS</u>

Blood was taken from the auricular artery of the rabbit on citric acid/sodium citrate/dextrose and anticoagulan and the washed platelets were prepared according to Ardlie & al., Brit. J. Pharmacology, <u>19</u>, 7-17, 1970. They were incubated with acetylsalicylic salt (100 μM, 30 minutes) to avoid thromboxane induced aggregation. They were then loaded with the fluorescent calcium indicator dye quin 2 by incubation with quin 2 aceto-methylester (15 μM in DMSO, 20 minutes at room temperature) which penetrates the cell membrane and after hydrolysed is trapped in the platelet. Drugs were preincubated with platelets for 15 minutes simultaneously to CP/CPK mixture which destroys secreted ADP.

Fluorescent measurement was then performed (excitation λ = 339 nm, emission λ = 492 nm) after PAF addition to platelet suspension. The fluorescence intensity is proportional to the cytosolic free calcium level $[Ca^{2+}]i$ which can be quantified as described by Tsien & al; , J. Cell Biol., <u>94</u>, 325 - 334, 1982.

RESULTS

Platelet stimulation induced by PAF 2 x 10^{-9} M increased the $[Ca^{2+}]i$ level from about 150 nM at resting state to 400 - 600 nM .

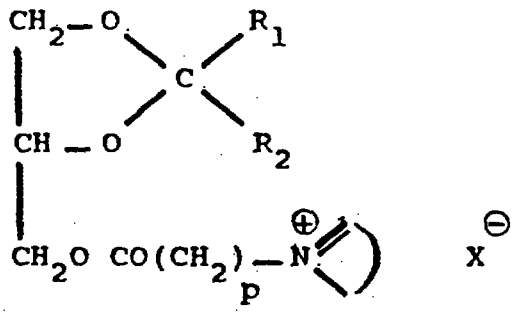
2-heptadecyl 2-methyl 4-[6'-(N-pyridinium) hexanoyl-oxymethyl] 1,3 dioxolan bromide and 2-heptadecyl 2-methyl 4-[6'-(N-quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide at 5 x 10^{-7} M totally inhibit the PAF-induced $[Ca^{2+}]i$ mobilization. At 5 x 10^{-8} M, the inhibition of PAF effect is respectively 44 % for 2-heptadecyl 2-methyl 4-[6'-(N-pyridinium hexanoyloxymethyl] 1,3 dioxolan bromide and 75 % for 2-heptadecyl 2- methyl 4-[6'-(N-quinolinium) hexanoyloxymethyl] 1,3 dioxolan bromide.

POSOLOGY

In human therapy, by oral administration, daily doses are from 0.1 to 0.5 mg in tablets or capsules with an enteric coating ; by IV route, the corresponding daily doses are from 0.01 to 0.05 mg. Treatment is generally from 2 to 6 weeks.

CLAIMS:

1. An aminoacylate of glycerol acetal having the general formula I



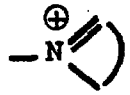
I

wherein

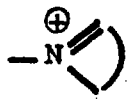
R_1 represents a substituted phenyl group or a group of the formula C_mH_{2m+1}, m being an integer of from 9 to 25,

R_2 represents a hydrogen atom, a phenyl group or a group of the formula C_nH_{2n+1}, n being an integer of from 1 to 10,

p is an integer of from 3 to 10,

 represents a nitrogen containing heterocyclic group, and

$X^{\ominus}$ represents the anion of a pharmaceutically acceptable inorganic or organic acid.

2. An aminoacylate of glycerol acetal according to claim 1 in which  represents a pyridinium, 3-thiazolinium, quinolinium, isoquinolinium, imidazolium or pyrazinium group.

3. An aminoacylate of glycerol acetal according to

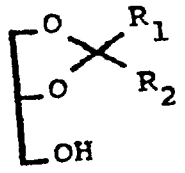
* C L M S *

claim 1 or claim 2 in which $X^{\ominus}$ represents a chloride, bromide, iodide, benzoate, acetate, methanesulphonate or tartrate ion.

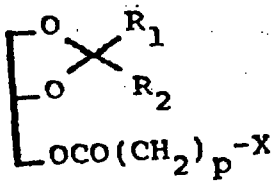
4. Any one of the following compounds:
2-heptadecyl-2-methyl-4-[4'-(N-pyridinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-2-methyl-4-[4'-(N-quinolinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-2-methyl-4-[4'-(N-thiazolinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-4-[4'-(N-pyridinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-4-[4'-(N-quinolinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-2-(n-propyl)-4-[4'-(N-pyridinium)-butyryloxy--methyl]-1,3-dioxolan chloride,
2-heptadecyl-2-methyl-4-[5'-(N-pyridinium)-pentanoyloxy--methyl]-1,3-dioxolan chloride,
2-(3,4,5-trimethoxyphenyl)-2-methyl-4-[4'-(N-pyridinium)--butyryloxymethyl]-1,3-dioxolan chloride,
2-heptadecyl-2-methyl-4-[6'-(N-pyridinium)-hexanoyloxy--methyl]-1,3-dioxolan bromide,
2-heptadecyl-2-methyl-4-[6'-(N-quinolinium)-hexanoyloxy--methyl]-1,3-dioxolan bromide,
2-heptadecyl-2-methyl-4-[6'-(N-isoquinolinium)-hexanoyloxy--methyl]-1,3-dioxolan bromide,
2-heptadecyl-2-methyl-4-[11'-(N-pyridinium)-undecanoyloxy--methyl]-1,3-dioxolan bromide,
2-heptadecyl-2-(n-propyl)-4-[6'-(N-pyridinium)-hexanoyloxy--methyl]-1,3-dioxolan bromide, and
2-nonyl-2-methyl-4-[6'-(N-pyridinium)-hexanoyloxy--methyl]-1,3-dioxolan bromide,

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5. A process for the preparation of an aminoacylate or glycerol acetal according to claim 1, the process comprising reacting an aldehyde or ketone of the general formula R_1COR_2 wherein R_1 and R_2 are as defined in claim 1 with glycerol, reacting the resultant compound of the formula



wherein R_1 and R_2 are as defined in claim 1 with an alkanoyl chloride derivative of the general formula $ClCO(CH_2)_pX$ wherein p and X are as defined in claim 1, and reacting the resultant compound of the formula



with a nitrogen containing heterocyclic compound of the formula



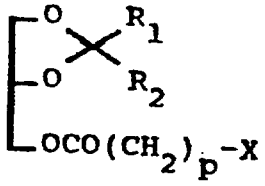
6. A process according to claim 5 in which a slight stoichiometric excess of the aldehyde or ketone is refluxed with the glycerol in a non-polar solvent in the presence of p-toluenesulphonic acid.

7. A process according to claim 5 or claim 6 in which the dioxolan derivative is reacted with the ~~halo~~alkanoyl chloride at room temperature in the presence of an organic base.

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8. A process according to claim 7 in which the organic base is triethylamine.

9. A process according to any of claims 5 to 8 in which the compound of the formula



is reacted with the heterocyclic compound at 50° to 80° under nitrogen circulation.

10. A pharmaceutical composition comprising a compound according to any of claims 1 to 4 in admixture with a pharmaceutically acceptable diluent or carrier.

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