

TITLEGinkgolide DerivativesDESCRIPTION

The invention relates to Ginkgolide derivatives, to methods for their preparation, and to pharmaceutical compositions containing them.

The invention provides 1-alkoxy and 10-alkoxy derivatives, separately or in admixture, of any one of the Ginkgolides A,B,C,J and M. The preferred alkoxy groups are methoxy and ethoxy groups.

The invention also provides a method for the preparation of 1-alkoxy derivatives of Ginkgolides A,B,C,J and M and of 10-alkoxy derivatives of the said Ginkgolides, the method comprising reacting, in solution, Ginkgolide A,B,C,J or M with an excess of diazoalkane and separating the resultant mixture of 1-alkoxy-Ginkgolide and 10-alkoxy-Ginkgolide.

In a preferred procedure, the selected Ginkgolide is dissolved in dioxan, suitably at a concentration of 1g per 100 ml, and the selected diazoalkane is dissolved in diethyl ether. The solution containing the diazoalkane is slowly added to that containing the Ginkgolide, allowing ten equivalents of diazoalkane per equivalent of Ginkgolide. The mixed solution is stood at ambient temperature for from 3 to 8 hours, yielding a mixture of 1-alkoxy-Ginkgolide and 10-alkoxy-Ginkgolide. The separation of the two products from the remaining non-reacted Ginkgolide may suitably be achieved by evaporating off the solvents and eluting the residue through a silica gel column using ethyl acetate: hexane 1:1 by volume as eluent. The resulting solution is evaporated off and treated with chloroform which dissolves

the 10-alkoxy derivative. The said 10-alkoxy derivative is recovered from this chloroformic solution and the remaining solution is then treated with diethyl ether which gives the 1-alkoxy derivative.

The alkoxy-Ginkgolides of the invention are of interest in the treatment of PAF-Acether induced maladies, and the invention accordingly also provides a pharmaceutical composition comprising a 1-alkoxy or 10-alkoxy derivative of one of the Ginkgolides A,B,C,J and M or a mixture of the 1-alkoxy and 10-alkoxy derivatives of one of the said Ginkgolides in admixture with a pharmaceutically acceptable diluent or carrier.

The invention is illustrated by the following examples:

Example 1

1-Methoxy-Ginkgolide B and 10-Methoxy-Ginkgolide B

To a solution of Ginkgolide B in dioxan (10g/l) was slowly added 10 equivalents of a solution of diazomethane in diethyl ether. The mixture was stood at ambient temperature for 4 hours, and then separated following the preferred separation procedure described above. 1-methoxy-Ginkgolide B, the structure of which was confirmed by HPLC, was obtained in 66.1% yield and 10-methoxy-Ginkgolide B was obtained in 24.4% yield.

	1 - methoxy	10 - methoxy
Ginkgolide A	56.3 %	13.2 %
Ginkgolide C	49.1 %	16.7 %

Example 2

1-Ethoxy-Ginkgolide B and 10-Ethoxy-Ginkgolide B

Following the procedure described in Example 1, but using diazoethane in place of diazomethane, and allowing the reactants to stand for six hours, 1-ethoxy-Ginkgolide B was obtained in 63.2% yield and 10-ethoxy-Ginkgolide B was obtained in 25.7% yield.

Proceeding as above, but with Ginkgolides A and C, the following yields were obtained:

	1 - ethoxy	10 - ethoxy
Ginkgolide A	72.8 %	20.1 %
Ginkgolide C	59.2 %	30.4 %

TOXICITY

The toxicity of the compounds of the invention has been measured on mice by the oral route. No death was noticed at the maximum administration dose.

PHARMACOLOGY

The pharmaceutical interest of the compounds of the invention is shown by the following pharmaceutical experiments.

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 detail 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.

The values for IC_{50} on platelets have been found as follows :

Ginkgolide type and substitution position		- OCH ₃		- OC ₂ H ₅	
B	1-	6.6	10 ⁻⁷	1.1	10 ⁻⁶
B	10-	2.9	10 ⁻⁷	7.2	10 ⁻⁶
C	1-	4.2	10 ⁻⁶	8.5	10 ⁻⁶
C	10-	3.0	10 ⁻⁶	9.3	10 ⁻⁶
A	1-	4.6	10 ⁻⁶	8.7	10 ⁻⁶
A	10-	1.3	10 ⁻⁵	6.2	10 ⁻⁴

2) - Anaphylactic bronchoconstriction of a passively sensitized guinea-pig

Passive heterolog sensitizing

Male Hartley guinea-pigs (400-500g) were sensitized by an intravenous injection (IV) of an antiovalbumin immune-serum rabbit (Cooper Biomedical, U.S.A.). To obtain a satisfactory anaphylactic response, 24 hours later, the following conditions of use were fixed : injection into the penis of a diluted serum (to half concentration 0.05 ml/100 g).

Bronchoconstriction measure

Guinea-pigs were anesthetized with urethan (2 g/kg IP), then tracheotomized and ventilated by mean of a respiratory pump (UGO BASILE) : stroke volume 1 ml/100 g, 60 strokes/mn.

A pneumothorax was done to abolish spontaneous respiration. The initial resistance was kept constant at 10 cm water pressure according to the method of Konzett and Rössler and the excess of air volume was measured with a bronchospasm transducer (UGO BASILE) connected to a UGO BASILE recorder "Gemini". The jugular vein was catheterized for intravenous injections. The anaphylactic shock was induced by an intravenous injection of 0.75 mg/kg of heterolog passive of ovalbumine. Products were given by oral route, 1 hour before the antigenic stimulation in the form of a gummy water suspension, at the dose of 25 mg/kg.

Results

The bronchoconstriction induced by ovalbumin was expressed in percentage of maximal bronchoconstriction given by clamping of the trachea. The results are reported in the following table :

Ginkgolide type and substitution position		- OCH ₃	- OC ₂ H ₅
B	1-	- 49.7 ***	- 38.3 **
B	10-	- 54.9 ***	- 36.2 **
C	1-	- 40.1 **	- 39.8 **
C	10-	- 30.6 *	- 23.1 *
A	1-	- 32.0 *	- 15.1 NS
A	10-	- 25.2 *	- 12.2 NS

NS : Non Significant
 * : Significant
 ** : Very Significant
 *** : Highly Significant

POSODOLOGY

In human therapy, usual doses for per as administration are 0.5 to 1 g per diem, in tablets or gelatine capsules for one month.

In I.V. administration, three weekly injections at 0.05 to 0.2 g in isotonic solution, for one month are recommended.



CLAIMS

1. A 1-alkoxy or 10-alkoxy derivative of one of the Ginkgolides A,B,C,J or M or a mixture of the 1-alkoxy and 10-alkoxy derivatives of one of the said Ginkgolides.
2. A Ginkgolide derivative according to claim 1 or mixture of Ginkgolide derivatives according to claim 1 in which the or each alkoxy group is a methoxy or ethoxy group.
3. A process for the preparation of a Ginkgolide derivative according to claim 1 or a mixture of Ginkgolide derivatives according to claim 1, the process comprising reacting one of the Ginkgolides A,B,C,J or M with an excess of a diazoalkane in a solvent, and optionally separating the resultant mixture of 1-alkoxy-Ginkgolide and 10-alkoxy-Ginkgolide.
4. A process according to claim 3 in which the diazoalkane is diazomethane or diazoethane.
5. A process according to claim 3 or claim 4 in which approximately 10 equivalents of the diazoalkane are employed per equivalent of Ginkgolide.
6. A process according to any of claims 3 to 5 in which a diethyl ether solution of the diazoalkane is mixed with a dioxan solution of the Ginkgolide and the mixture is allowed to react for from 3 to 8 hours at ambient temperature.
7. A process according to any of claims 3 to 6 in which the mixture is separated by evaporating off the solvent, eluting the residue through a silica gel column using ethyl



acetate: hexane 1:1 by volume as eluent, evaporating off the solvent from the eluate, taking up the residue in chloroform, recrystallising the 10-alkoxy-Ginkgolide from solution and adding diethyl ether to the remaining solution to obtain the 1-alkoxy-Ginkgolide.

8. A process for the preparation of a Ginkgolide derivative according to claim 1 or a mixture of Ginkgolide derivatives according to claim 1, the process being substantially as described herein with reference to either of the Examples.

9. A pharmaceutical composition comprising a 1-alkoxy or 10-alkoxy derivative of one of the Ginkgolides A,B,C,J and M or a mixture of the 1-alkoxy and 10-alkoxy derivatives of one of the said Ginkgolides in admixture with a pharmaceutically acceptable diluent or carrier.
