

TITLE:

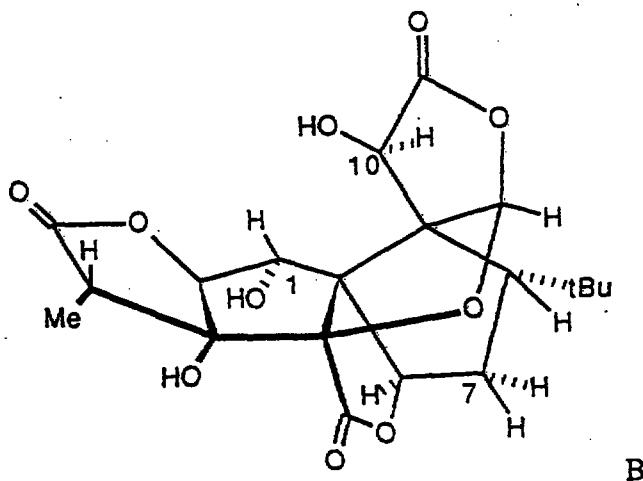
Conversion of Ginkgolide C to Ginkgolide B

DESCRIPTION:

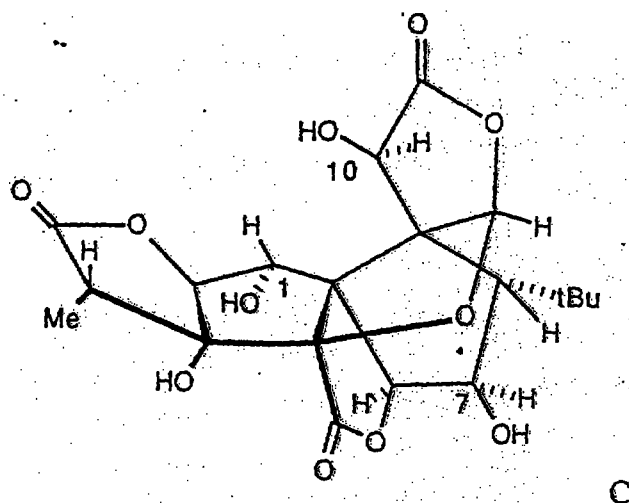
The invention relates to a process for the preparation of ginkgolide B from ginkgolide C.

Ginkgolides B and C are diterpene lactones which are isolated from leaves and roots of the ginkgo biloba tree in generally comparable proportions (the proportions vary according to the material and to the source of the same and also to the extraction method used). Ginkgolide B has been found to be a potent inhibitor of PAF-acether (cf for instance US Patent 4734280) whereas ginkgolide C is less active in this regard. As ginkgolides are extracted in generally comparable proportions, it was interesting to convert ginkgolide C into ginkgolide B, insofar that starting from a costless product renders the conversion process definitely cheaper than the extraction.

The invention provides a process for the preparation of ginkgolide B, of the formula B



from ginkgolide C, of the formula C



the process comprising the following steps:

- protecting the 10-hydroxy group of ginkgolide C by conversion to an alkyl ester, the reaction being effected in dimethylformamide at a temperature of from 15 to 50°C for from 4 to 10 hours;

- activating the 7-hydroxy group of the resultant 10-protected ginkgolide C by conversion to a (R)thiocarbonyl ester wherein R represents a phenoxy group, a phenoxy group substituted by one or more halogen atom(s), or an imidazolyl group; the activation being effected by treating the 10-protected ginkgolide C with a phenoxythiocarbonyl chloride, the phenoxy group being optionally substituted by one or more halogen atom(s), or with 1,1'-thiocarbonyldiimidazole in basic conditions at a temperature of from 0 to 40°C for from 1 to 24 hours;

- deoxygenating the 7-activated group of the resultant 10-protected 7-activated ginkgolide C by treating it with tributyltin hydride or tris-(trimethylsilyl)silane, in an aprotic solvent, in the presence of a free-radical generator, the reaction being effected at a temperature of from 70 to 110°C for from 15 minutes to 4 hours under an inert atmosphere; and

- cleaving the protecting group from the 10-hydroxy group of the resultant 10-protected ginkgolide B.

In the first step, the C-10 hydroxy group of the ginkgolide C is preferably protected as an acetyl, n-butyryl or n-valeryl ester, the protecting agent for such conversion being respectively acetic anhydride, n-butyric anhydride and n-valeric anhydride. Preferably, the reaction is

carried out at a temperature of from 20 to 40°C for from 7 to 9 hours.

The second step in the process of the invention is the activation of the 7-hydroxy group of ginkgolide C. The said 7-hydroxy group is converted to a (R)thiocarbonyl ester wherein R preferably represents a phenoxy, 2,4,6-trichlorophenoxy, pentafluorophenoxy or imidazolyl group, by treating it with 1 to 3 equivalents of an activating agent selected from phenoxythiocarbonyl chloride, 2,4,6-trichlorophenoxythiocarbonyl chloride, pentafluorophenoxythiocarbonyl chloride and 1,1'-thiocarbonyldiimidazole. The reaction is preferably carried out in a mixed solvent one component of which is pyridine in an amount of from 1 to 90% by volume, or in acetonitrile containing from 2 to 4 equivalents of 4-(dimethylamino)-pyridine.

In the third step, that is the deoxygenation of the 7-activated group, the solvent is preferably acetonitrile, benzene, toluene or a mixture of two or more thereof. The preferred free-radical generators are α , α' -azoisobutyronitrile and ter-butylhydroperoxide. 2 to 4 equivalents of tributyltin hydride or tris-(trimethylsilyl)silane in suitable solvents can be used in proportions wherein the derivative is soluble in the presence of 0.01 to 0.1 equivalent of the free-radical generator. The inert atmosphere may be nitrogen or argon.

In the fourth step of the process of the invention, the cleavage of the 10-protecting group may be effected by any conventional method such as treatment by a concentrated ammonia solution; due to the opening of the lactone rings in basic conditions, acidification to a pH below 3 to remove excess ammonia and keep the lactone rings closed should follow. In some cases, it is not necessary to carry out this step as a separate step, since the desired cleavage may have taken place under the operating conditions of the third step; when the deoxygenation is carried out with a trace of 4-(dimethylamino)pyridine (DMAP), deprotected ginkgolide B is obtained directly without separate performance of the deprotection step.

The following Examples illustrate the invention.

EXAMPLE 1:

Step 1: preparation of C-10 alkyl ester of ginkgolide C

4.40 g (10mM) of ginkgolide C was dissolved in 20 ml of dimethylformamide (DMF) in a 250 ml round bottom flask. 3.77 ml (40 mM) of acetic anhydride was added and the mixture was stirred overnight (16 hours) at room temperature. The completion of the reaction was checked by HPLC. 10 ml of ethanol was then added and the mixture was evaporated to near dryness under reduced pressure (<25 mmHg, <3333 pascals) on a rotary evaporator over a water bath at 50°C. The residual DMF was removed by adding toluene and evaporating off at about 90°C under reduced pressure. This operation may be repeated several times to ensure that all the DMF has been removed. The residue obtained was dissolved in 20 ml of ethyl acetate and the resultant solution was added dropwise into a beaker containing 100 ml of n-heptane (or n-hexane or petroleum ether) with constant stirring. The acetate precipitated out in flocs which may be filtered off on a sintered glass filter (porosity 4) and dried in a vacuum oven to yield 4.30 g (90%) of C-10 acetate of ginkgolide C.

The corresponding C-10 butyrate and C-10 valerate have been prepared in the same conditions as described above, but using n-butyric anhydride and n-valeric anhydride respectively instead of acetic anhydride (Yields: 4.44 g of C-10 butyrate (87%) and 4.88 g of C-10 valerate (93%)).

EXAMPLE 2:

Step 2: preparation of C-7 phenoxythiocarbonyl ester of C-10 acetate of ginkgolide C wherein the phenoxy group is optionally substituted by one or several halogen atom(s).

482 mg (1 mM) of C-10 acetate of ginkgolide C and 490 mg (4 mM) of 4-dimethylaminopyridine (DMAP) was dissolved in 9.6 ml of acetonitrile. Thereafter 276.6 μ l (2mM) of phenoxythiocarbonyl chloride was added and the mixture was stirred for 20 minutes. The completion of the reaction was checked by HPLC. 20 ml of toluene was then added and the solution was filtered through 10 g of silica gel (Merck 7734). This operation removed DMAP which was absorbed on the silica gel. 20 ml of

toluene/acetonitrile (7/3) was added for elution. The filtrate was evaporated off to near dryness and 40 ml of n-hexane (or n-heptane or petroleum ether) were added to precipitate out the product. The precipitate was filtered off on a G4 sintered glass filter and dried in a vacuum oven at ambient temperature to yield 405 mg (66%).

The corresponding C-7 2,4,6-trichloro- and pentafluoro-phenoxythiocarbonyl esters of C-10 acetate of ginkgolide C have been prepared in the same conditions as described above, but using 2,4,6-trichlorophenoxythiocarbonyl chloride and pentafluorophenoxythiocarbonyl chloride respectively instead of phenoxythiocarbonyl chloride (Yields: 512 mg (71%) and 475 mg (67%) respectively).

EXAMPLE 3

Step 2: preparation of C-7 phenoxythiocarbonyl ester of C-10 acetate of ginkgolide C (alternative method)

1446 mg (3mM) of C-10 acetate of ginkgolide C was dissolved in 6 ml of pyridine:chloroform (8:2 by volume). 0.83 ml (2 equivalents) of phenoxythiocarbonyl chloride was added, and the mixture was stirred (or submitted to ultrasonics) until the yellow precipitate had dissolved. The solution was stood at ambient temperature until TLC showed no trace of ginkgolide C acetate (2 to 16 hours). 18 ml of toluene was then added to the reaction mixture, and the solution was filtered through a bed of 10 g of silica gel. The product was eluted with 100 ml of toluene:acetone (7:3 by volume). This removed the red colouration from the product. The filtrate was evaporated to near dryness and the viscous liquid was redissolved in 5 to 10 ml of toluene. This solution was added dropwise under stirring to 100 ml of n-hexane, resulting in buff coloured precipitate in flocs. The product was filtered off, washed with 10 ml of n-hexane and dried under reduced pressure at ambient temperature to yield 1.07 g an off-white product (58%).

EXAMPLE 4:

Step 2: preparation of C-7 imidazolylthiocarbonyl ester of C-10 valerate of ginkgolide C

524 mg (1 mM) of C-10 valerate of ginkgolide C and 244 mg (2 mM) of 4-dimethylaminopyridine (DMAP) was dissolved in 10 ml of acetonitrile. Thereafter 356 mg (2 mM) of 1,1'-thiocarbonyldiimidazole was added and the mixture was stirred for 2 hours at room temperature. The completion of the reaction was checked by HPLC. 15 ml of toluene was then added and the solution was filtered through 10 g of silica gel (Merck 7734). This operation removed DMAP which was absorbed on the silica gel. 25 ml of toluene/acetonitrile (7/3) was added for elution. The filtrate was evaporated off to near dryness and 45 ml of n-hexane (or n-heptane or petroleum ether) were added to precipitate out the product. The precipitate was filtered off on a G4 sintered glass filter and dried in a vacuum oven at ambient temperature to yield 457 mg (72%).

EXAMPLE 5:

Step 3: reaction of C-7 2,4,6-trichlorophenoxythiocarbonyl ester of C-10 acetate of ginkgolide C with tributyltin hydride

In 100 ml crimp vial with teflon-lined septum aluminium cap was dissolved 360 mg (0.5 mM) of C-7 2,4,6-trichloro-phenoxythiocarbonyl ester of C-10 acetate of ginkgolide C and 2.5 mg of α, α' -azoisobutyronitrile (AIBN) in 35 ml of acetonitrile. The solution was flushed with argon and 0.54 ml (2 mM) of tributyltin hydride was added. The vial was crimped and warmed to 75°C for 4 hours in an oil bath. The mixture was analysed by HPLC; 191 mg of C-10 acetate of ginkgolide B was recovered (yield 82%).

EXAMPLE 6:

Step 3: reaction of C-7 imidazolylthiocarbonyl ester of C-10 valerate of ginkgolide C with tris-(trimethylsilyl)silane

The reaction was carried out in the conditions described in Example 5, but reacting C-7 imidazolylthiocarbonyl ester of C-10 valerate of ginkgolide C (191 mg - 0.3 mM) with tris-(trimethylsilyl)silane instead of tributyltin hydride. 119 mg of C-10 valerate of ginkgolide B was recovered (yield 78%).

EXAMPLE 7:

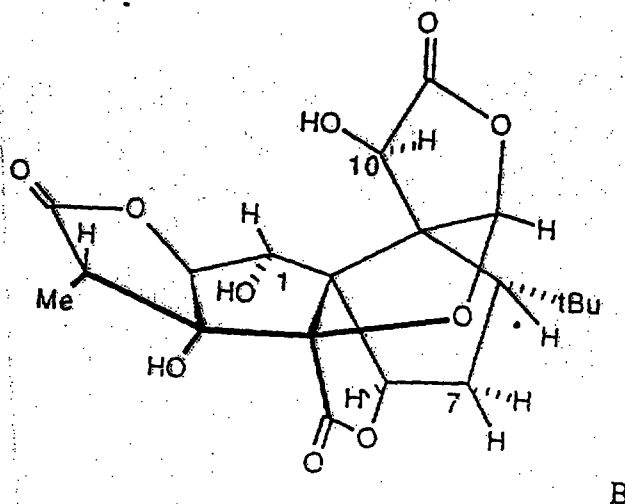
Step 4: deprotection reaction of C-10 acetate of ginkgolide B

A drop of 30% ammonia solution was added to the reaction mixture obtained in Example 5. After acidification to pH 3 with hydrochloric acid, 130 mg of ginkgolide B was recovered (yield 77%).

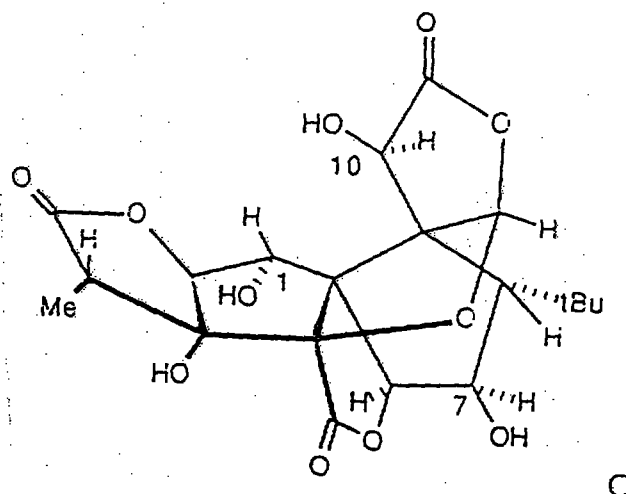


CLAIMS:

1. Process for the preparation of ginkgolide B, of the formula B



from ginkgolide C, of the formula C



the process comprising the following steps:

- protecting the 10-hydroxy group of ginkgolide C by conversion to an alkyl ester, the reaction being effected in dimethylformamide at a temperature of from 15 to 50°C for from 4 to 10 hours;

- activating the 7-hydroxy group of the resultant 10-protected ginkgolide C by conversion to a (R)thiocarbonyl ester wherein R represents a phenoxy group, a phenoxy group substituted by one or more halogen atom(s), or an imidazolyl group; the activation being effected by treating the 10-protected ginkgolide C with a phenoxythiocarbonyl chloride, the phenoxy group being optionally substituted by one or more halogen atom(s), or with 1,1'-thiocarbonyldiimidazole, in basic



conditions at a temperature of from 0 to 40°C for from 1 to 24 hours;

- deoxygenating the 7-activated group in the resultant 10-protected 7-activated ginkgolide C by treating it with tributyltin hydride or tris-(trimethylsilyl)silane, in an aprotic solvent, in the presence of a free-radical generator, the reaction being effected at a temperature of from 70 to 110°C for from 15 minutes to 4 hours under an inert atmosphere, and

- cleaving the protecting group from the 10-hydroxy group of the resultant 10-protected ginkgolide B.

2. A process according to claim 1 in which ginkgolide C is reacted with acetic anhydride, n-butyric anhydride or valeric anhydride to protect the C-10 hydroxy group as an acetyl, n-butyryl or n-valeryl ester respectively.

3. A process according to claim 1 or claim 2 wherein the first step is effected at a temperature of from 20 to 40°C for from 7 to 9 hours.

4. A process according to any preceding claim wherein the 7-hydroxy group of ginkgolide C is converted to a (R)thiocarbonyl ester wherein R represents a phenoxy, 2,4,6-trichlorophenoxy, pentafluorophenoxy or imidazolyl group, by treating the 10-protected ginkgolide C with from 1 to 3 equivalents of phenoxythiocarbonyl chloride, 2,4,6-trichlorophenoxythiocarbonyl chloride, pentafluorophenoxythiocarbonyl chloride, or 1,1'-thiocarbonyldiimidazole.

5. A process according to any preceding claim in which the activation step is effected in a mixed solvent, one component of which is pyridine in an amount of from 1 to 90% by volume, or in acetonitrile containing from 2 to 4 equivalents of 4-(dimethylamino)pyridine.

6. A process according to any preceding claim in which the solvent used in the deoxygenation step is acetonitrile, benzene, toluene or a mixture of two or more thereof.

7. A process according to any preceding claim in which 2 to 4 equivalents of tributyltin hydride or tris-(trimethylsilyl)silane are used in



the oxygenation step.

8. A process according to any preceding claim in which the free-radical generator used in the deoxygenation step is α, α' -azo-isobutyronitrile or t-butylhydroperoxide.

9. A process according to any preceding claim in which the deoxygenation step is effected under nitrogen or argon.

10. A process according to any preceding claim in which the cleavage of the 10-protecting group is effected in the presence of concentrated ammonia solution and is followed by acidification to keep the lactone rings closed.

11. A process according to any of claims 1 to 9 wherein the cleavage of the 10-protecting group is effected simultaneously with the deoxygenation of the 7-activated group.