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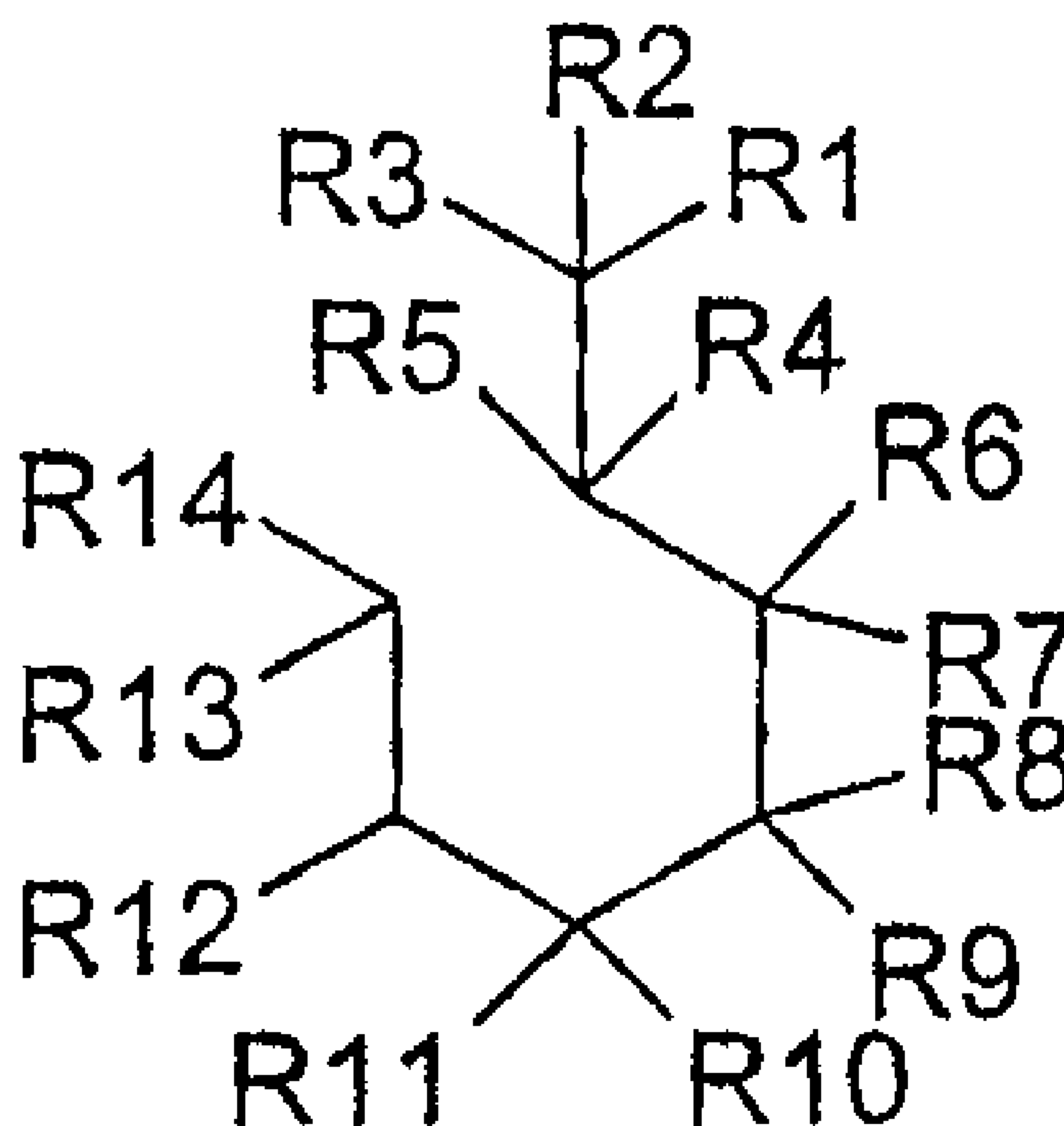
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(54) Titre : LINOLEATES CONJUGUES

(54) Title: CLA-ESTERS



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(57) Abrégé/Abstract:

The invention concerns with esters from conjugated linoleic acid and a food allowable alcohol from the group of terpene alcohols and sesquiterpene alcohols, these esters have good taste and display the health effects from the CLA part and from the food allowable alcohol part of the molecule.



Abstract

The invention concerns with esters from conjugated linoleic acid and a food allowable alcohol from the group of terpene alcohols and sesquiterpene alcohols, these esters have good taste and display the health effects from the CLA part and from the food allowable alcohol part of the molecule.

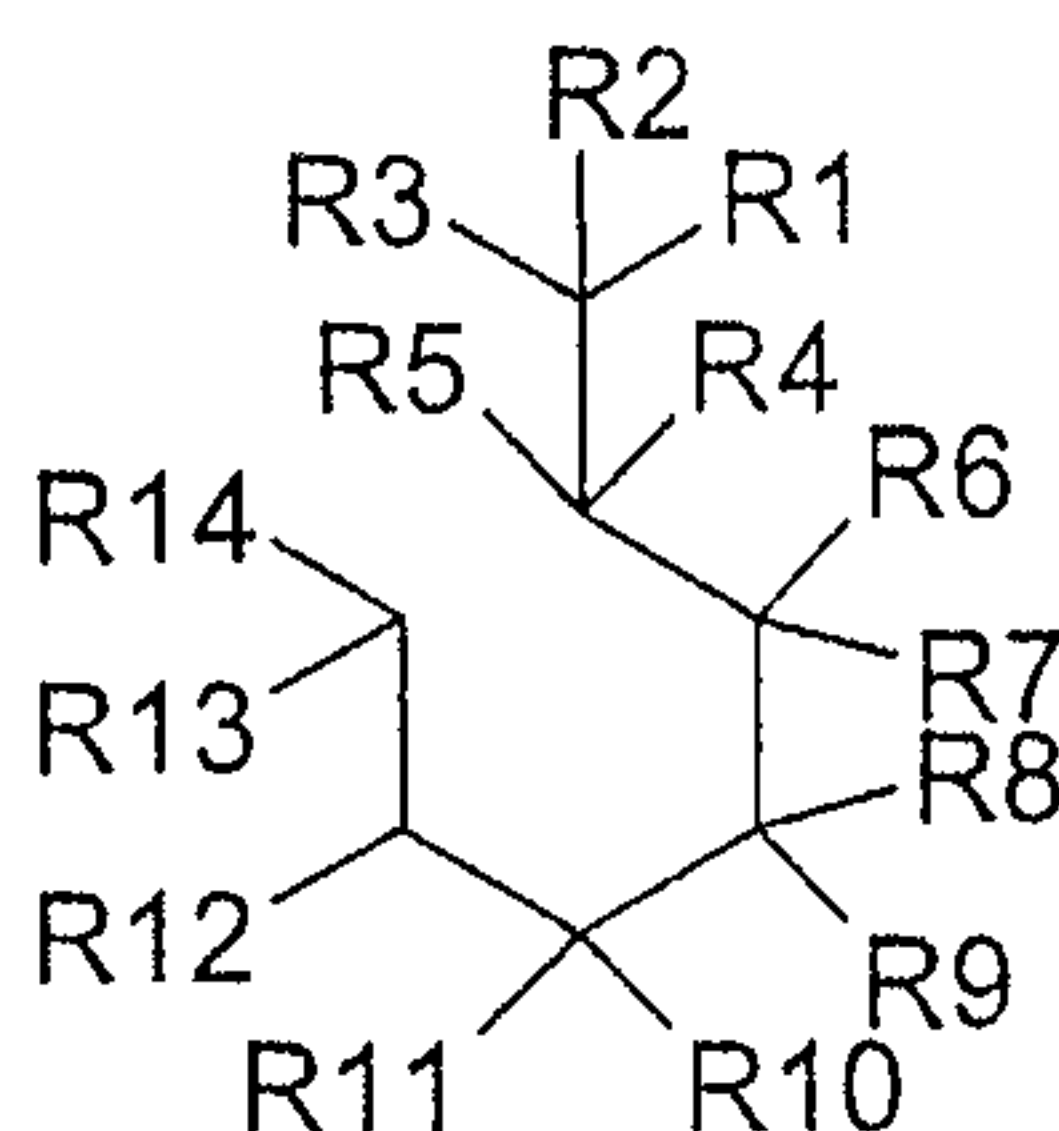
CLA-ESTERS

Esters from CLA (=reaction product of conjugated linoleic acid and an alcohol) are known from eg WO 97/18320 or
5 WO 99/32105. However the esters disclosed in WO 97/18320 are mainly the octylesters and these esters have the disadvantage that the alcohol residue is not food allowable and that therefore the use of these esters in foods is a problem. In this same document also glycerol esters of CLA
10 isomers are disclosed which can be made from glycerol and free CLA by esterification. However during this esterification the enrichment in a specific CLA isomer (in general c9t11 or t10c12 CLA) is low or the reaction rate and or yield for this conversion is very low. Moreover the
15 products so obtained often are not very active for their desired health effect. Another problem is that the esters disclosed in WO '105 often are derived from an alcohol that, although being food allowable is not readily available or is difficult to remove from the reaction
20 mixture (eg tocopherol alcohol or ascorbyl alcohols or retinyl alcohols).

We studied whether we could find a solution for above problems. This study resulted in the finding of new esters
25 of CLA isomers that can be made easily in good yields in relatively short times while the products obtained often displayed high enrichment rates in specific CLA-isomers (in particular in c9t11 and/or t10c12 isomers). Moreover the alcohols used herefore are food allowable and easily
30 available and can be separated easily from the crude reaction mix resulting from the partial conversion of the free CLA with the alcohol.

Moreover we found that these esters displayed good health properties and excellent taste properties. In particular the taste of the esters was improved compared to the taste of the free acids.

- 5 Therefore our invention concerns in the first instance with esters of conjugated linoleic acid (= CLA) and a food allowable alcohol wherein the food allowable alcohol is selected from terpene alcohols or sesquiterpene alcohols. In particular the use of the alcohols with general formula I,



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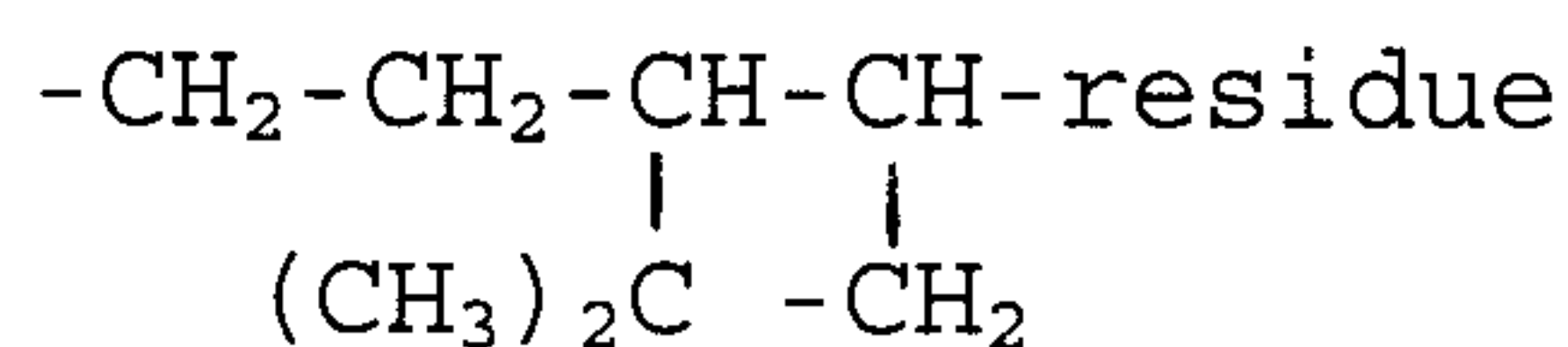
wherein:

R1 = H, or forms together with R4 a CH₂-CH₂ bond,

R2 = H or CH₃

R3 = H, CH₃ or OH

- 15 R4 = H or forms together with R1, or with R6 or with R14 a CH₂-CH₂ bond, or forms together with R9 a -CH₂- bridge or forms together with R9 a



- 20 R5 = H or forms together with R13 or with R14 a CH₂-CH₂ bond

R6 = H or forms together with R4 a CH₂-CH₂ bond

R7 = H, or OH

R8 = H or OH or forms together with R10 a CH₂-CH₂ bond

R9 = H, or forms together with R4 a -CH₂- bridge or a

- 25
$$\begin{array}{c} \text{-CH}_2\text{-CH}_2\text{-CH-CH-residue} \\ | \quad | \\ (\text{CH}_3)_2\text{C} \quad \text{-CH}_2 \end{array}$$

R10 = H or forms together with R8 or with R12 a CH₂-CH₂ bond

R11 = H, i-propyl, i-propenyl, CH₃ or forms together with R4 a -C(CH₃)₂- bridge

- 30 R12 = H or forms together with R10 a CH₂-CH₂ bond

R13 = H, OH, or -C(CH₃)=C-C(OH)- or forms together with R5 a C-C bond

R14 = H, or forms together with R4 or R5 a C-C bond lead to excellent results

5

In particular the invention concerns with esters derived from an alcohol selected from the group consisting of menthol, isopulegol, menthenol, carveol, carvomenthenol, carvomenthol, isobornylalcohol, caryophyllenealcohol, geraniol, farnesol and
10 citronellol, These alcohols are all food allowed, easily available and give high enrichment in specific CLA isomers when converted partially with free CLA (in particular with a 50:50 mix of c9t11. and t10c12 CLA) along an enzymic route, Moreover the esters display good activity with respect to anti
15 inflammatory and anti diabetic or insuline resistance effects and taste far better than the free CLA where they are derived from. The esters can be obtained easily in a form wherein the esters comprise more than 80 wt %, preferably more than 90 wt % and in particular more than 95 wt % of c9t11 plus t10c12 CLA
20 isomers. Depending on the conversion that is applied the esters can be obtained as a mix wherein the c9t11 and t10c12 CLA-isomers are present in a weight ratio of 50:50, or as a mix wherein the c9t11 : t10c12 isomers are present in a weight ratio of more than 70 : 30, in particular more than 80 : 20.
25 The esters of the enriched isomer mix have the advantage that the mix mainly will display the specific health effects from the specific isomer present in high contents while the other specific health effects of the other isomer hardly will be noticed. This enables us to dose more specifically to achieve
30 a specific health effect, while also the dosage of the CLA

compound can be less which is of particular advantage if the esters are applied as food supplement because this will limit the size of the capsules and the smaller the capsules can be, the easier they can be swallowed by the consumer.

5

A particular preference exists for esters of CLA and menthol wherein more than 80 wt %, preferably more than 90 wt % of the CLA residue of the esters consists of c9t11-CLA, while less than 2 wt % of the total CLA-residue
10 consists of CLA-isomers other than c9t11 and t10c12 and the esters contain less than 5 wt % preferably less than 3 wt% of free fatty acids. Administering of these products means that potentially negative side effects of other components can be avoided.

15

Our invention further concerns a process for the preparation of esters of conjugated linoleic acid (=CLA) and a food allowable alcohol as defined above, wherein free conjugated linoleic acid is reacted with the food allowable
20 alcohol in the presence of less than 1.8 wt % water and a lipase. This process will result in esters that can be used in food products as both components of the products are food allowable.

In the instance that an ester is desired wherein higher
25 contents of a specific CLA-isomer is present the above process can be adapted to a process wherein a food allowable alcohol with the formula as defined above is reacted with a mix of free CLA with about 50 % of both main isomers (c9t11 and t10c12) and wherein the reaction is
30 performed by partially converting this mixture of c9t11 and t10c12 CLA in the presence of an enzyme than can discriminate between c9t11 and t10c12-CLA isomers and which

enzyme preferably is *Candida rugosa*, whereupon CLA-esters are isolated enriched in c9t11 CLA-isomer and free fatty CLA is isolated as unconverted reactant enriched in t10c12 CLA isomer.

- 5 Other enzymes that can be applied are lipase D; lipase QL; lipase-SL; *Mucor miehei* lipase, optionally on a support such as duolite; *Candida antarctica* lipase and lipozyme.

The different products can be obtained by working up the crude
10 reaction product as follows:

- first add a diluted base to the crude reaction mixture
- then separate the water phase and the organic phase
- followed by washing of the organic phase and adjusting the pH of this phase to pH 5-7
- 15 - and finally by removal of the water from this phase, preferably by distillation.

By this route esters are obtained enriched in the c9t11 CLA isomer.

20 To obtain a free CLA product enriched in the t10c12 isomer the procedure is as follows:

- the water phase of the reaction product obtained according to above process is acidified to a pH < 3.0, preferably < 1.5,
- whereupon the mixture obtained is separated in an upper
25 layer and a residue and
- the upper layer is washed with water and the water is removed therefrom.

According to another aspect of our invention our invention
30 also concerns food products comprising a fat and a good tasting health component. wherein the taste and health

component is an ester according to the invention. The food products are specifically selected from the group consisting of spreads (10-90% fat contents); dressings, mayonnaise, cheese, ice cream, ice cream coatings; 5 confectionery coatings or fillings; sauces and culinary products. These food products suitably contain 0.5 to 20 wt % of our novel CLA esters.

Our novel esters can also be used for the preparation of 10 triglycerides containing CLA residues. Herefore an ester composition according to the invention can be converted with glycerol or with a vegetable oil in the presence of a base or an enzyme while in the instance that glycerol is converted the liberated alcohol residue from the esters of 15 the CLA-esters is removed from the reaction mix during the conversion. This removal of liberated alcohol can be done by vacuum distillation or by molecular distillation.

According to a last embodiment our invention also concerns 20 the use of our CLA esters as a good tasting health agent, in particular having anti-inflammatory or anti-diabetic or insulin resistant properties.

Examples

25

1) CLA menthol esters:

2.5 g CLA 50:50, 80% main isomers (cis9,t11, t10,c12)

0.02 g demin water

0.4 g menthol

30 0.026 g *Candida rugosa* lipase

T 38 °C

Reaction time 22 hours

Mix well 2.5 g CLA with 0.02 g water. Add 0.4 g menthol and mix very well for 30 min in shaker at 38 °C and 200 rpm. Add this mixture to 0.026 g *Candida rugosa* lipase; mix everything very well. Put the mixture in the shaker at 38 5 °C and 200 rpm. After 22 hours add to the reaction mixture 30 ml isooctane/ethanol (1:1) and 33 ml 0.2 N NaOH. Mix very well. Centrifuge for 5 min at 3500 rpm. Separate the organic phase from the aqua phase. Wash the organic phase 3x 100 ml of demin water and evaporate the solvent.

10

Add aqua phase to 50 ml H₂SO₄ (1:10) and 30 ml isooctane. Remove the organic phase and wash 3x 100 ml of demin water. Evaporate the solvent. The analysis of FFA and menthol esters fractions are shown in table below:

	Fame of CLA FFA	Fame of CLA menthol esters
C16:0	2.4	2.3
C16:1	0.1	0.1
C18:0	2.5	1.7
CLA,t,t	2.8	0.6
CLAc9,c11	1.1	0.4
CLAc10,c12	1.3	0.4
CLAc11,c13	0.6	0.9
C18:1,t	1.1	0.0
C18:1,c	10.8	13.7
CLA-OX	0.0	0.2
C18:2,t	0.4	0.6
C18:2c	2.1	3.6
C20:0	0.7	0.1
C20:1	0.3	0.0
C22:0	0.1	0.1
CLA,c9,t11	28.1	62.1
CLA,t10,c12	45.5	13.2
Ratio c9,t11: t10,c12	38:62	83:17
Conversion	32 %	
FFA, %	99.3	1.85

2) CLA geraniol ester:

20 g CLA 50:50

5 3.64 g geraniol

0.24 g demin water

0.2 g *Candida rugosa* lipase

T 38 °C

Reaction time 1 hour

10

The CLA, geraniol and water were mixed in a vial and incubated in a shaking water bath at 38 °C. After 30 minutes the lipase was added, mixed by shaking and the vial

replaced in the water bath. After 1 hour the reaction mixture was diluted with an equal volume of cold hexane and filtered through a 0.45 μm PTFE membrane to remove the enzyme. After evaporation of the solvent 17 g of the
5 remaining oil was dissolved in 100 ml of hexane and mixed with 100 ml of demin water in which 2.84 g of sodium hydroxide had been dissolved. After vigorous shaking, 10 ml of ethyl alcohol was added to ease the separation of the layers.

- 10 The aqueous phase was removed and added to 100 ml of a 20% hydrochloric acid solution and 100 ml of hexane and shaken vigorously. The organic layer was removed and washed with 3x 100 ml of demin water, 100 ml of brine and the solvent evaporated to leave 14 g of oil comprising 85 % FFA.
- 15 The organic phase was washed with 2x 100 ml of hot alkaline demin water, 100 ml of demin water and 100 ml of brine. The dry organic phase was passed through a column of basic alumina to remove any traces of remaining FFA and the solvent evaporated to yield 3 g of oil.

	ester	ffa
C16:0	4.91	4.33
C16:1	0.22	0
C18:0	0.69	1.98
CLA tt	0.82	4.31
CLA c9,c11	0.39	1.38
CLA c10,c12		1.3
CLA c11,c13	0.93	0.52
C18:1 t	0.12	0.16
C18:1 c	36.12	23.13
CLA - ox	0	0
C18:2 t	1.15	0.95
C18:2 c	2.64	1.52
C20:0	0	0
C20:1	0	0.2
C22:0	0	0.24
CLA c9,t11	44.7	20.62
CLA t10,c12	7.31	39.36
Conversion	30 %	
RATIO	85.9	34.4

The two product fractions were examined by TLC and the respective bands for FFA and ester removed and worked up 5 FAME analysis.

3) CLA citronellol ester:

5 g CLA
 1.1 g citronellol
 10 0.06 g demin water
 0.1 g *Candida rugosa* lipase
 T 30 °C
 Reaction time 30 minutes

The CLA, citronellol and water were mixed in a vial and incubated in a shaking water bath at 30 °C. After 30 minutes the lipase was added, mixed by shaking and the vial replaced in the water bath. After 30 minutes the reaction mixture was filtered through a 0.45 µm PTFE membrane to remove the enzyme. The reaction had reached 20 % conversion.

The reaction mixture was analysed by TLC. The ester bands 10 was worked up for FAME analysis.

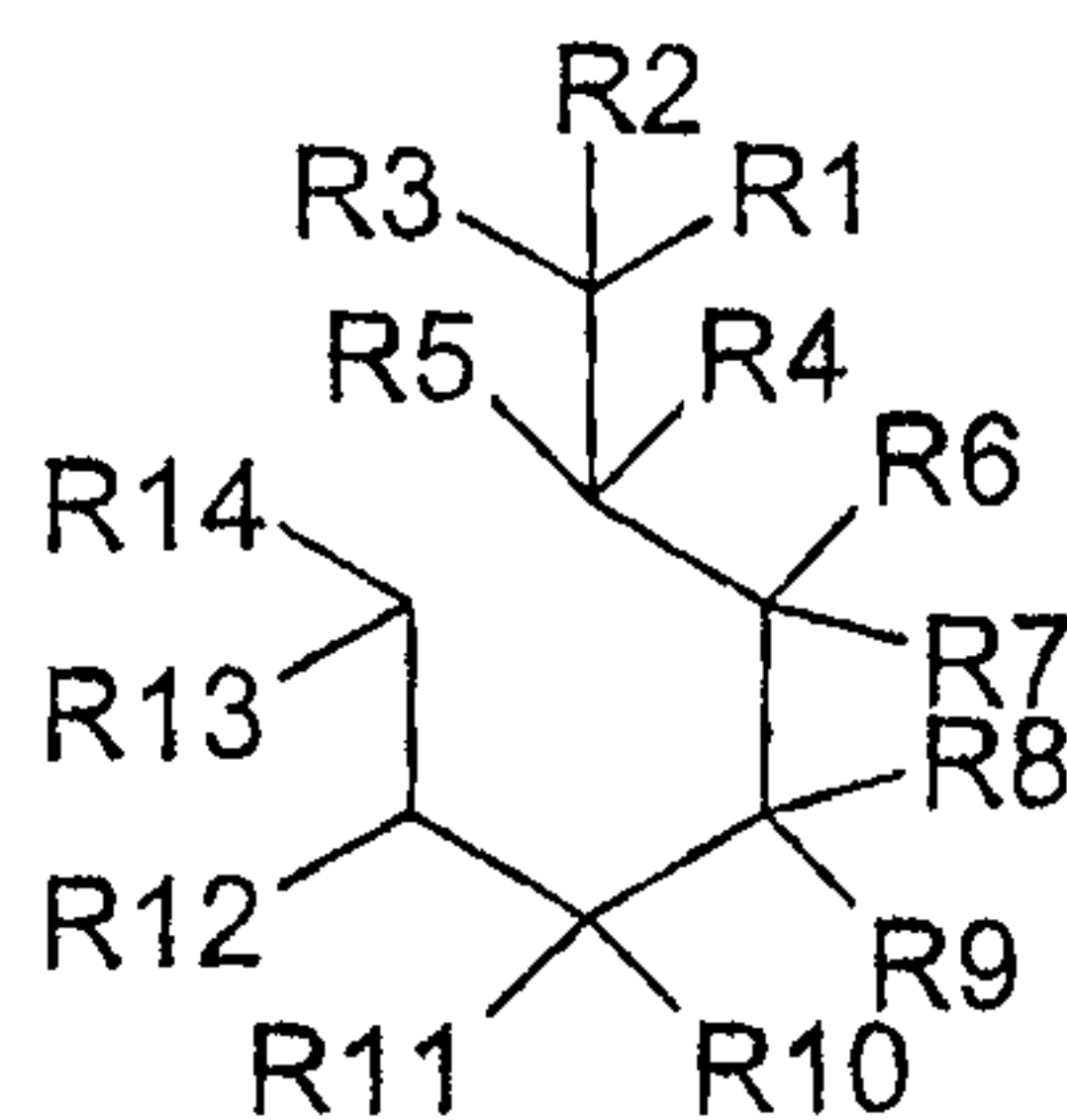
C16:0	2.89
C16:1	0.21
C18:0	0.71
C18:1	9.98
C18:2	1.47
C20:0	0.47
C20:1	0.28
C22:1	0.41
CLA c9,t11	39.65
CLA t10,c12	7.65
CLA c,c	1.78
CLA t,t	6.99
Conversion	20 %
Ratio	84:16

CLAIMS:

1. An ester of conjugated lioleic acid (CLA) with a food allowable alcohol, wherein the food allowable alcohol is a
5 terpene alcohol or a sesquiterpene alcohol.

2. An ester of conjugated linoleic acid (=CLA) and a food allowable alcohol according to Claim 1 wherein the terpene alcohol or sequiterpene alcohol is of the general formula 1,

10



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wherein:

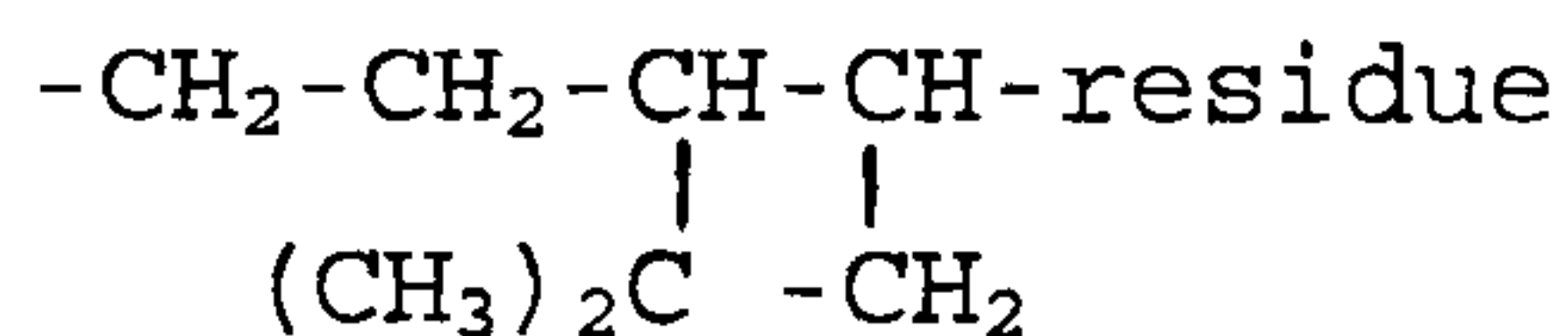
15 R1 = H, or forms together with R4 a CH₂-CH₂ bond,

R2 = H or CH₃

R3 = H, CH₃ or OH

R4 = H or forms together with R1, or with R6 or with R14 a CH₂-CH₂ bond, or forms together with R11 a -C(CH₃)₂-bridge or forms

20 together with R9 a -CH₂- bridge or forms together with R9 a



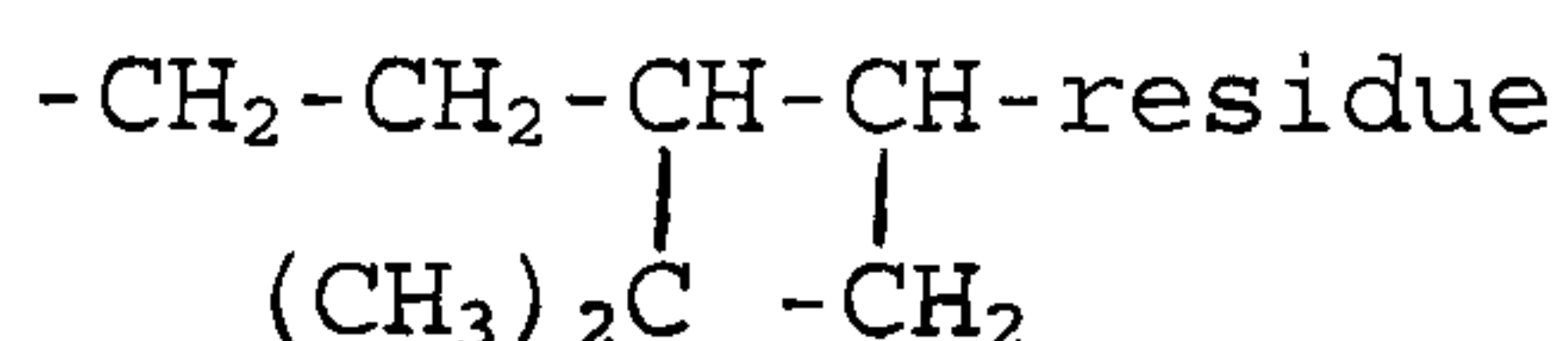
R5 = H or forms together with R13 or with R14 a CH₂-CH₂ bond

R6 = H or forms together with R4 a CH₂-CH₂ bond

25 R7 = H, or OH

R8 = H or OH or forms together with R10 a CH₂-CH₂ bond

R9 = H, or forms together with R4 a -CH₂- bridge or a



30 R10 = H or forms together with R8 or with R12 a CH₂-CH₂ bond

R11 = H, i-propyl, i-propenyl, CH₃ or forms together with R4 a -C(CH₃)₂- bridge

R12 = H or forms together with R10 a CH₂-CH₂ bond

5 R13 = H, OH, or -C(CH₃)=CH-CH(OH)- or forms together with R5 a CH₂-CH₂ bond

R14 = H, or form together with R4 or R5 a CH₂-CH₂ bond.

10 3. An ester according to Claim 1 or 2, wherein the alcohol is menthol, isopulegol, menthenol, carveol, carvomenthenol, carvomenthol, isobornylalcohol, caryophyllenealcohol, geraniol, farnesol or citronellol.

15 4. An ester according to Claim 1, 2 or 3 wherein the CLA residue of the esters comprise more than 80 wt% of c9t11 plus t10c12 CLA isomers.

20 5. An ester according to Claim 1, 2 or 3 wherein the CLA residue of the esters comprise more than 90 wt% of c9t11 plus t10c12 CLA isomers.

6. An ester according to Claims 1-3 wherein the CLA residue of the esters comprise more than 95 wt% of c9t11 plus t10c12 CLA isomers.

25 7. An ester according to Claim 4, 5 or 6 wherein the c9t11 and t10c12 CLA-isomers are present in a weight ratio of 50:50.

30 8. An ester according to Claim 4, 5 or 6 wherein the c9t11 : t10c12 isomer weight ratio in the CLA-esters is more than 70 : 30.

9. An ester according to Claim 4, 5 or 6 wherein the c9t11 : t10c12 isomer weight ratio in the CLA-esters is more than 80 : 20.

10. Composition comprising an ester of conjugated linoleic acid (CLA) and a food allowable alcohol according to Claim 1, wherein the food allowable alcohol is menthol and wherein more than 80 wt % of the CLA residue of the ester consists of c9t11-CLA, while at least 98 wt % of the total CLA-residue consists of c9t11 and t10c12 CLA-isomers and the composition contains less than 5 wt % of free fatty acids.
11. Composition according to Claim 10, wherein more than 90 wt % of the CLA residue of the esters consists of c9t11-CLA.
12. Composition according to Claim 10, containing less than 3 wt % of free fatty acids.
13. Process for the preparation of an ester of conjugated linoleic acid (=CLA) and a food allowable alcohol as defined in Claim 1, wherein free conjugated linoleic acid is reacted with a food allowable alcohol as defined in Claim 1 in the presence of less than 1.8 wt % water and a lipase.
14. Process for the preparation of a composition comprising esters of conjugated linoleic acid (CLA) and a food allowable alcohol as defined in Claim 1 wherein the esters obtained are enriched in c9t11-CLA and wherein the reaction is performed by partially converting a mixture of c9t11 and t10c12 CLA in a weight ratio of about 50:50 and a food allowable alcohol from the group as defined in Claim 1 in the presence of an enzyme than can discriminate between c9t11 and t10c12-CLA isomers, whereupon CLA-esters are isolated enriched in c9t11 CLA-isomer and free fatty CLA is isolated as unconverted reactant enriched in t10c12 CLA isomer.
15. Process according to Claim 14 wherein the enzyme is *Candida rugosa* lipase.

16. Process according to Claim 14 or 15 wherein the CLA-esters enriched in c9t11 CLA-isomer are isolated from the mixture having a water phase and an organic phase by:

(a) first adding a diluted base to the mixture;

5 (b) separating the water phase and the organic phase of the mixture obtained in (a);

(c) washing the organic phase and adjusting the pH of this phase to pH 5-7; and

(d) removing the water from the phase obtained in (c).

10

17. Process according to Claim 16 wherein said step (d) of removing the water comprises distillation.

18. Process according to Claim 14 or 15 wherein the free fatty CLA is isolated as unconverted reactant enriched in t10c12-CLA isomer by:

15

- acidifying to a pH < 3.0 the water phase obtained according to the process of Claim 16,

- whereupon the mixture obtained is separated in an upper layer and a residue and

20

- the upper layer is washed with water and the water is removed therefrom.

19. Process according to Claim 18 wherein said water phase is acidified to a pH < 1.5.

25

20. Food products comprising a fat and a health component wherein the health component is one or more esters according to Claims 1 to 9 or the composition according to any one of Claims 10 to 12.

30

21. Food products according to Claim 20 wherein the food product is a spread having a fat content of from 10-90% by weight, a dressing, mayonnaise, cheese, ice cream, ice cream

coating, confectionery coating or filling, a sauce or a culinary product.

22. Food products according to Claims 20 and 21 wherein the
5 food product contains on total product 0.5 to 20 wt % of an ester of CLA according to Claims 1-9.

23. Process for the preparation of triglycerides wherein at least one of the fatty acid residues is a conjugated linoleic
10 acid (CLA) residue wherein an ester according to any one of Claims 1 to 9 or a composition according to any one of Claims 10 to 12 is converted with glycerol or with a vegetable oil in the presence of a base or an enzyme while in the instance that glycerol is converted the liberated alcohol residue from the
15 esters of the CLA-esters is removed from the reaction mix during the conversion.

