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Datasheet for the decision of 11 May 2021

Case Number: T 0539/18 - 3.3.06

Application Number: 12733657.6

Publication Number: 2726592

IPC: C11D3/386, C07K5/06, C07K5/08

Language of the proceedings: EN

Title of invention:
STABILISED SUBTILISIN COMPOSITION

Patent Proprietor:
Novozymes A/S

Opponent:
BASF SE

Headword:
Stabilised Subtilisin/Novozymes

Relevant legal provisions:
EPC Art. 52(1), 56, 100(a), 100(b)
RPBA Art. 12(2), 12(4)

Keyword:
Late-filed ground/objection of lack of novelty - admitted in first-instance proceedings (no) - reasons to overrule the discretionary decision taken in first-instance proceedings (no)
Late submitted material - justification for late filing (no)
Sufficiency of disclosure - main request (yes)
Industrial application - main request (yes)
Inventive step - main request (yes)

Decisions cited:
T 2221/10, T 0197/10

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0539/18 - 3.3.06

D E C I S I O N
of Technical Board of Appeal 3.3.06
of 11 May 2021

Appellant: (Opponent)	BASF SE Carl-Bosch-Strasse 38 67056 Ludwigshafen (DE)
Representative:	BASF IP Association BASF SE G-FLP-C006 67056 Ludwigshafen (DE)
Respondent: (Patent Proprietor)	Novozymes A/S Krogshoejvej 36 2880 Bagsvaerd (DK)
Representative:	Potter Clarkson The Belgrave Centre Talbot Street Nottingham NG1 5GG (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 3 January 2018 rejecting the opposition filed against European patent No. 2726592 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	J.-M. Schwaller
Members:	G. Santavicca
	E. Mille

Summary of Facts and Submissions

I. The appeal of the opponent lies from the decision of the Opposition Division to reject the opposition filed against European patent No. 2 726 592, independent claims 1, 11 and 12 of which read as follows:

"*1. A composition, which comprises a subtilisin and a peptide aldehyde hydrosulfite adduct having the formula X-B^1-NH-CHR-CHOH-SO_3M, wherein:*
a) M is H (hydrogen) or an alkali metal;
b) R is a group such that NH-CHR-CO is an L or D-amino acid residue;
c) B^1 is one amino acid residue; and
d) X consists of one or more amino acid residues, optionally comprising an N-terminal protection group."

"*11. A method of preparing the composition of any preceding claim, which method comprises mixing a subtilisin, an aqueous solution comprising a peptide aldehyde hydrosulfite adduct having the formula X-B^1-NH-CHR-CHOH-SO_3M, wherein M, R, B1 and X are defined as in claim 1, and optionally a surfactant.*"

"*12. A compound for use in the composition of any of claims 1-10, which compound is a peptide aldehyde hydrosulfite adduct having the formula X-B^1-NH-CHR-CHOH-SO_3M, wherein M and X are defined as in claim 1, B^1 is an amino acid residue which is different from proline (Pro), and R is a group such that NH-CHR-CO is an L or D-amino acid residue of Tyr, m-tyrosine, 3,4-dihydroxyphenylalanine, Phe, Val, Met, Nva or Nle.*"

II. With its grounds of appeal, the appellant filed

D20: Siezen et al., Protein Engineering, 1991, vol. 4, no 7, p 719-737

D21: Siezen et al., Protein Science, 1997, 6:501-523

D22: Crutzen and Douglass, in Zoller's Handbook of Detergents, part. A by Guy Broze; Properties,

and maintained its grounds for opposition (Articles 100 (a) - in conjunction with Articles 54, 56 and 57 EPC - and (b) EPC). As regards inventive step, it held that each of D13 (EP 0 670 310 A), D14 (US 4,691,007 A), D5 (WO 2011/036153 Al) and D7 (WO 2009/118375 A2) was a suitable starting point for assessing inventive step, as claim 1 was broad and not restricted to detergent compositions, and that "*subtilisin*" encompassed, in view of [0026] and [0028] of the patent, serine proteases in general or at least a larger part thereof, e.g. trypsin and thrombin.

III. With letter of 19 July 2018, the appellant filed further items of evidence D23 to D28 and argued that, since the claims at issue also encompassed e.g. medical compositions, the skilled person would consider as closest prior art also documents disclosing medical/ pharmaceutical enzymes, such as thrombin, trypsin, and chymotrypsin, which were closely related to subtilisin, all being serine proteases. This was also apparent from the fact that the first protease used in detergents was trypsin, a protease of mammals, a target for medical applications. Thus, the skilled person would consider D13, the disclosure of which was not merely restricted to medical applications. Indeed, also the patent in suit ([0022]) mentioned as preferred embodiments hydrosulfites of therapeutic peptide aldehydes for the treatment of human diseases, such as the hydrosulfite adducts of peptide aldehydes “Antipain”, “Chymostatin A, B and C”.

IV. With letter of 11 September 2018, the respondent/patent proprietor enclosed auxiliary requests 1 (already pending in opposition proceedings) to 4 and requested not to admit the opponent's submissions of 19 July 2018 and late-filed documents D23 to D28. The Opponent had not submitted any justification whatsoever as to why the late-filed documents were not cited earlier. Moreover, these were no more relevant that those already on file. Hence, their late-filed submission was a clear abuse of procedure.

It also stressed that the feature "*subtilisin*" was clear per se without recourse to the description and could not be relativised to include e.g. the trypsin erroneously mentioned in paragraph [0028] of the patent. In this respect, the appellant had not disproved the finding in the decision under appeal that subtilisin and trypsin were classified differently according to International Union of Biochemistry, which meant that they were different enzymes.

The fresh ground of lack of novelty had correctly not be admitted, because trypsin and thrombin were not subtilisin. D13 did not concern compositions as defined in claim 1, thus was not the closest prior art. As to D14, it concerned elastase enzymes, i.e. not a subtilisin enzyme, and also pertained to the medical field rather than to household products field.

The closest prior art was disclosed by D7 or D5, and the composition of claim 1 differed therefrom in the use of a bisulphite adduct of a peptide aldehyde. This difference produced a technical effect, as the examples of the patent showed that it permitted the use of concentrated solutions ([0089]) in which subtilisin was effectively inhibited as with known peptide aldehyde.

The technical problem to be solved was easier handling of the inhibitor while maintaining its inhibitory effect on subtilisin. This problem was effectively solved by the composition of claim 1 across its whole breadth and was not obvious over the cited prior art. D7 and D5 did not address the same problem nor provided any incentive to replace the peptide aldehyde, let alone with bisulphite adducts. Without hindsight, there was no reason to consider prior art on organic synthesis of aldehydes which were not peptide aldehydes, such as
D15 (Khosropour et al., "*A New, Efficient and Chemoselective One-Pot Protocol for Synthesis of 4-Arylidene-2-phenyl-5(4H)-oxazolones from Aryl Aldehyde bisulphite Adducts Promoted by $POCl_3$*", J. Heterocyclic Chem.,45, 683 (2008),
D16 (Pandit et al., "*Expedient Reductive Amination of Aldehyde Bisulphite Adducts*", Synthesis, 2009, 23, p. 4032-4036,
D17 (Ragan et al., "*Safe Execution of a Large-Scale Ozonolysis: Preparation of the Bisulphite Adduct of 2-Hydroxyindane-e-carboxaldehyde and Its Utility in a Reductive Amination*", Organic Process Research & Development, 2003, 7, p. 155-160), or
D18 (Matthew M. et al.,"*A Practical, Efficient Synthesis of 1,1-Dioxohexahydro- 1A6-thiopyran-4-carbaldehyde*", Organic Process Research & Development, 2003, 12, p. 892-895).
Late-filed documents D23 to D28 were not relevant either.

V. With its response of 18 December 2019, the appellant enclosed still further items of evidence D29 and D30, and argued that there was a strong link between peptide aldehydes used in detergents and/or in medical fields.

VI. In its provisional opinion, the board held that there was no reason for overruling the discretionary decision of the Opposition Division not to admit the late-filed ground for opposition of lack of novelty, that late-filed documents D23 to D30 were not admissible and that the grounds of insufficiency of disclosure and lack of industrial application did not prejudice the maintenance of the patent as granted. As regards inventive step, D7 was the most promising closest prior art document and the crucial question was whether the skilled person starting from this document would have considered the use of aldehyde bisulphite adducts taught e.g. in examples 79 and 80 of D13 for making stabilised subtilisin compositions.

VII. At the oral proceedings held on 11 May 2021 the appellant referred to its written submissions in respect of admittance issues, insufficiency of disclosure and lack of industrial application. The final requests of the parties were as follows:

The **appellant**/opponent requested that the decision under appeal be set aside, that the patent be revoked and that the new ground of appeal under Article 100(a) EPC (lack of novelty over D13) and new items of evidence D20 to D30 be admitted in the appeal proceedings.

The **respondent**/patent proprietor requested that the appeal be dismissed or, auxiliarly, that the patent be maintained on the basis of the amended claims according to one of auxiliary requests 1 to 4, all filed with letter dated 11 September 2018, and that the new ground for opposition, the appellant's submission of 19 July 2018 and new items of evidence D23 to D30 not be admitted in the appeal proceedings.

Reasons for the Decision

1. Admittance of the new ground for opposition

1.1 According to an established principle (Case Law of the Boards of Appeal of the EPO, 2019, IV.C.4.5.2), a Board will only overrule a first instance discretionary decision if it was taken according to wrong principles, or without taking into account the right principles, or in unreasonable way, and thus exceeding the proper limits of the discretion.

1.2 In the present case, the Opposition Division has considered that the new ground of opposition was late filed and *prima facie* not substantiated nor relevant, insofar as Trypsin disclosed in D13 (classified as EC 3.4.21.4) was not a subtilisin (classified as 3.4.21.62) and because there was no evidence that the Thrombin disclosed in D13 was identical to "Pancreatic Trypsin" mentioned in paragraph [0028] of the patent.

1.3 The appellant merely argued that the patent was its own dictionary.

1.4 The board in this respect notes that claim 1 specifically defines a "*subtilisin*" (not a "subtilase" or a "subtilisin-like", nor generically a "subtilisin-like serine protease"). As the feature "*subtilisin*" is clear per se and since, according to the jurisprudence of the Boards of Appeal (e.g. T 2221/10 and T 197/10), the description cannot be used to interpret a term in a different way when the term used in a claim has a clear technical meaning, the appellant's argument cannot be retained here.

1.5 It follows from the above that the board has no reason to doubt that the Opposition Division exercised its discretion in an improper way, and so has no reason to overrule its decision not to admit the new ground for opposition under Article 100(a) EPC (in combination with Article 54 EPC) into the proceedings.

2. Admittance of late-filed items of evidence

2.1 D20, D21 and D22 having been filed with the grounds of appeal and concerning common general knowledge in dispute, they are admitted into the appeal proceedings, as they shed light on the breadth of the claimed "*subtilisin*".

2.2 The submission of 19 July 2018 and D23 to D28 have all been filed well after the expiry of the period for opposition or for reacting to the decision under appeal, without any justification whatsoever as to why they could not be submitted earlier. The admittance of submissions and documents filed after the grounds and the reply thereto being at the Board's discretion under Article 12(4) RPBA 2007, in the present case, since the amended case and the late documents could and should have been submitted during the opposition proceedings, they are not admitted into the appeal proceedings.

2.3 The same conclusion applies to D29 and D30 filed still later with letter of 18 December 2019.

3. Sufficiency of disclosure of the invention

3.1 An objection based on Article 100(b) EPC presupposes that there are serious doubts, substantiated by verifiable facts, that the patent does not disclose the invention in a manner sufficiently clear and complete

for it to be carried out by a person skilled in the art. The burden of proof is upon the opponent to establish that a skilled person, using its common general knowledge, would be unable to carry out the invention (Case Law, *supra*, II.C.9).

3.2 In the present case, the invention concerns a composition comprising subtilisin (an enzyme) and an inhibitor as defined by its chemical formula. As no effect or result to be achieved is defined in claim 1, the only ability required to the skilled person is the preparation of the composition.

3.3 As the appellant has not proven that the skilled person cannot make the claimed composition, this ground for opposition does not succeed.

4. Main Request - Industrial application

In line with case law (*supra*, I.E.3), the boards finds that the appellant has not proven that the invention has no industrial application. The attack against the alleged impossibility of preparing stable commercial compositions comprising "capless" peptide aldehydes, in view of rapid disintegration, has not been proven. As the application of the composition relies on the known inhibition of subtilisin by peptide aldehydes (D7), and as the composition finds commercial application in household products, as for D7, the claimed subject-matter has manifestly an industrial application.

5. Main request - Inventive step

5.1 The patent ([0001]) relates to liquid compositions comprising the enzyme subtilisin stabilised by a

peptide aldehyde derivative, to a method of making the composition and to a compound for use therein.

5.1.1 According to the patent (e.g. paragraph [0007]), the invention is based on the findings that peptide aldehydes are sparingly soluble; the bisulphite adduct is itself effective as a subtilisin inhibitor and stabiliser, and can further stabilise a second (non-subtilisin) enzyme if present. Further it maintains its inhibitory and stabilising effect in a liquid detergent during storage, so that the use of the bisulphite adduct can avoid the cost and time of converting it back to the peptide aldehyde and subsequent drying of the peptide aldehyde can be saved, and this can avoid the inconvenience of handling the peptide aldehyde in powder form or as highly diluted aqueous solution in the large-scale production of these compositions. Furthermore, the addition of a peptide aldehyde bisulphite adduct may also improve the wash performance of a subtilisin-containing detergent.

5.2 Closest prior art

5.2.1 It is not in dispute that D5 or D7 can be taken as the closest prior art for assessing inventive step. It is in dispute that also D13 or D14 might be taken. Hence, it has to be decided which of these documents more closely addresses the objectives of the patent and proposes similar solutions.

5.2.2 For the Board, D13 cannot be retained as its primary object is to provide configurational inversion stabilised bisulphite adducts of L-arginine aldehyde derivatives that are potent thrombin inhibitors useful as anticoagulants (page 2, lines 45-47), thus D13 does **not** aim to provide stabilised liquid compositions of

thrombin enzymes, and neither belongs to the technical field of household products nor concerns subtilisin enzymes therefor.

5.2.3 Similar considerations apply to D14, which concerns potent elastase inhibitors for use in the treatment and/or prevention of diseases such as pulmonary emphysema, or as research tools in pharmacological and related studies. D14 thus neither belongs to the technical field of household products nor concerns subtilisin enzymes.

5.2.4 D5 (see summary of the invention; examples) concerns the addition of a protease inhibitor to a protease-containing detergent composition in order to improve its detergency, i.e. the use of a reversible protease inhibitor suitable to address the known poor long term storage stability of proteases in some liquid detergents. In the examples, *inter alia*, a particular Savinase™ (subtilisin) is used, in particular with a Z-GAY-H (a capped glycinyl-alaninyl-tyrosinal peptide aldehyde), in relation to detergency. Thus, D5 shares same elements of the problems addressed in the patent in suit and proposes a similar solution.

5.2.5 D7 (see field of the invention) relates to a liquid composition comprising a subtilisin and a peptide compound as a stabiliser for the subtilisin, as well as to a peptide compound which is useful as a stabiliser for subtilisin enzymes. It also addresses (see background art) the problems arising from the degradation of subtilisin and other enzymes in liquid detergent composition, which reduce the wash performance thereof. D7 proposes the use of peptide aldehydes derivatives, including peptide compounds with OH-substituted phenylalanine as the C-term residue. As

apparent from page 5, lines 26-28, of D7, the liquid composition needs not to contain surfactants. From the examples of D7, it is apparent that tyrosinal peptide aldehyde Z-GAY-H is the most efficient, but the peptide aldehydes of D7 were dissolved in DMSO before use.

5.2.6 Consequently, D7 is more specific than D5, in particular about liquid compositions as defined in claim 1 at issue and about the problems arising from using the disclosed peptide aldehydes in combination with DMSO as solvent, and so D7 represents the closest state of the art to the claimed subject-matter.

5.3 Technical problem

5.3.1 In its reply to the grounds of appeal the respondent has formulated the technical problem over D7 as "to provide inhibitors whose handling in liquid form is easier while maintaining the inhibitory effect on the subtilisin".

5.3.2 As the application as filed on which the patent in suit was granted acknowledged D7 as prior art, and since the now formulated technical problem is fairly derivable from that formulated in the penultimate paragraph of page 1 and in the paragraph bridging pages 1 and 2 of the application as filed ("*In the large-scale production of stabilized subtilisin compositions [...] this can avoid the inconvenience of handling the peptide aldehyde in powder form or as highly diluted aqueous solution*"), the Board has no reason to take another stance on the formulation of the technical problem to be solved.

5.4 As a solution to this technical problem, the patent in suit proposes the composition according to claim 1 and

the compound of claim 12, both being characterised by a **particular** peptide aldehyde **hydrosulfite adduct.**

5.5 Problem effectively solved

5.5.1 On the respondent's argument that the bisulphite adduct as such is the inhibitor, the Board considers that according to the patent itself ([0041],[0042]) it was known that the "conversion into a hydrosulphite adduct is reversible [...] thus the adduct may partly or fully revert to release the peptide aldehyde [...] in a liquid subtilisin formulation ...". Also Experimental Report D6 (Results, points 3 and 4), comparing the performance of a peptide aldehyde according to D7 (page 5, line 2) with the corresponding bisulphite adduct used in example 1 of the patent, shows that the protease inhibitory activity of both inhibitors does not differ significantly, unless a particular buffer is used, such that the peptide aldehyde is the inhibitor, not its bisulphite adduct.

5.5.2 However, as the known peptide aldehydes were used in highly diluted state (page 1, 6th paragraph), and significant differences were not always attained, the effects of "easier handling in liquid form while maintaining the inhibitory effect on the subtilisin" as formulated originally are not disproved by D6.

5.5.3 Moreover, the patent in suit contains a number of examples showing the successful use of adducts to prepare concentrated compositions ([0089]) and their inhibition activity as a function of the concentration thereof and on a second enzyme (lipase).

5.5.4 Hence, the technical problem formulated by the respondent can be seen as being effectively solved.

5.6 (Non)Obviousness of the proposed solution

5.6.1 The question which arises is whether the skilled person starting from D7 and faced with the technical problem posed would have found motivation, without the benefit of hindsight, for replacing the peptide aldehyde of D7 with a known peptide aldehyde **bisulphite adduct** in the expectation of easier handling (according to [0007] of the patent) while maintaining the effectiveness as subtilisin enzymes inhibitor and whether in doing so he would have arrived at a composition falling within the ambit of claim 1 at issue or of a compound as defined in claim 12.

5.6.2 The board notes that D7 (e.g. page 2, last full paragraph; page 5, lines 26-28; page 13, last paragraph; examples) only concerns peptide aldehydes for use as subtilisin stabiliser or inhibitor in liquid compositions, in particular tyrosinal peptide aldehydes such as Z-GAY, and discloses the use of DMSO for dissolving the peptide aldehydes before use in a liquid detergent.

5.6.3 The appellant argued that in view of the disclosed use of DMSO for dissolving the peptide aldehydes in D7, it was immediately apparent to the skilled person that it could not result in easier handling, as DMSO was known to be expensive and a not-easy-to-handle solvent.

5.6.4 However, D7, although disclosing the use of DMSO, does not mention any problem with the handling of this solvent for the disclosed peptide aldehydes when preparing the stabilised subtilisin liquid compositions, let alone in large scale, nor does D7 contain any suggestion whatsoever towards the use of other solvents or of other forms of the peptides, let

alone peptide aldehyde in form of **bisulphite adducts**. Thus, the recognition of the problem faced by the skilled person (Patent, [0007]) is not immediately apparent from D7.

5.6.5 To further back up its **obviousness** objections in respect of the use of the peptide aldehyde bisulphite adduct in a composition as defined in claim 1, the appellant essentially argued that:

(a) the need for stabilising against the autolysis of proteases was a well-known issue for the skilled person. Subtilisin enzymes were stabilised long before the patent in suit to prevent their autolysis by use of boric/boronic acid derivatives as stabilisers;

(b) the equilibrium between protease and peptide aldehyde was well-known to the skilled person, as peptide aldehydes were used since long time for inhibition of subtilisin enzymes, as e.g. in D7;

(c) the equilibrium between peptide aldehyde and bisulphite adduct was known to the skilled person, at least from the cited prior art using bisulphite adducts as a surrogate for the aldehyde, as e.g. in D15 to D18;

(d) it was generally known that introducing a negative charge into the peptide aldehyde molecule by adding the sulphite group increased the solubility compared to the aldehyde and that the equilibrium in solution - under basic pH conditions usually used in detergents - was in favor of the aldehyde. The skilled person had a sound background in organic chemistry as in D15 to D18;

(e) also epimerisation of peptide aldehydes was a known problem, which was solved by N-terminal protection;

(f) the skilled person was aware of D13 and D14 in view of the common catalytic mechanism of proteases such as chymotrypsin, trypsin and subtilisin. This was also mentioned in D7 (fourth paragraph on page 2), *inter alia* disclosing that peptide aldehydes are suitable for stabilising not only subtilisin but also e.g. chymotrypsin-type proteases. Thrombin and trypsin as disclosed in D13 might be different from subtilisin but are remarkably similar in their activity. D13 and D14 disclose the use of bisulphite adducts of peptide stabilisers to effectively stabilise serine proteases. Thus the skilled person knew therefrom that the equilibrium in water provided sufficient peptide aldehyde to effectively inhibit serine proteases, and inferred, e.g. from D13, that a bisulphite adduct of a peptide aldehyde would react also on a subtilisin. The fact that D13 showed successful inhibition of proteases indicated to the skilled person that at least for thrombin and trypsin the dissociation kinetics were not an issue at all. There was no reason to believe that the skilled person, in view of the effective inhibition in D13 and the high similarity of trypsin and thrombin to subtilisin, would have had doubts at using bisulphite adducts for subtilisin inhibition.

(g) The skilled person thus had a reasonable expectation of success and a strong motivation to try to apply the positive finding of D13 on bisulphite adducts to stabilisation of subtilisin.

(h) Hence, the skilled person starting from D7 and considering, on the one hand, the breadth of claim 1 (encompassing also medical compositions) and, on the other hand, a passage on page 2, lines 18 and 19, of D7, mentioning the use of Leu-Leu-TyrH for stabilising

chymotrypsin-types proteases, would have taken D13 as additional prior art to try to solve the faced problem.

5.6.6 These arguments do not convince the board, firstly because it is not in dispute that the need for stabilising against the auto-proteolysis of proteases was a well-known issue for the skilled person, nor that subtilisin enzymes were stabilised long before the patent in suit; nor that the equilibrium between protease and peptide aldehyde was well known to the skilled person, as apparent from *inter alia* D7; or that the equilibrium between the peptide aldehyde and its bisulphite adduct was known to the skilled person.

In these respects, attention is drawn to the patent in suit ([0004], [0008], first sentence thereof, and [0041] to [0044]), which acknowledges the sparing solubility of known peptide aldehydes and also that their known conversion into water-soluble hydrosulfite adducts was known from textbooks. This is why the board, during the oral proceedings, warned not to include the "water-soluble" aspect in the formulation of the technical problem, as otherwise, this pointer would immediately point to, thus render obvious the claimed solution.

In fact, as apparent from the invoked prior art for stabilised subtilisin compositions for household products (D7 (Background art) or D5 (Background art, page 1, lines 8-12), despite the said knowledge from a textbook published in 1992, these prior art items, and the prior art to which they refer in 2008 or 2009, still concerns peptide aldehydes as such, not their bisulphite adducts.

Indeed, as apparent from the patent in suit ([0008]), the invention is based on the findings that this adduct stabilises and inhibits the subtilisin during storage, not only the subtilisin but also other enzymes, so that liquid enzymatic compositions for detergent products can be made thereof.

5.6.7 The alleged general knowledge of introducing a negative charge into the peptide aldehyde molecule by adding the sulphite group to increase the solubility compared to the aldehyde is acknowledged in the patent ([0008]).

5.6.8 The argument that it was known that the equilibrium of the bisulphite in solution - e.g. under basic pH conditions usually used in detergents was in favor of the aldehyde has been backed up by prior art D4 (Kokesh et al., J. Org. Chem. 1975, Vol. 40, n° 11, pp. 1632-36) (see Figure 1) but only for benzaldehyde.

5.6.9 In view of these considerations the items of prior art invoked by the appellant, namely D15 to D18, relating to organic synthesis of particular aldehydes, which are not peptide aldehydes as claimed, cannot be more relevant to show that it was immediately evident for the skilled person to replace peptide aldehydes with hydrosulphite adducts in view of their solubility.

5.6.10 The argument that the skilled person had a sound background in organic chemistry and was therefore aware of D15 to D18 (page 894, left column, 2nd paragraph) does not convince the Board as they deal with different situations than that of the patent in suit, namely:
- in D15 (page 683, left column, last four sentences; last paragraph on page 684, right column), the use of the bisulphite adduct serves the purpose of having a more stable functional group, to overcome the problem

that the aldehyde is difficult to isolate, because of hydrolysation or polymerisation;
- in D16 (Abstract; first page, left column, first sentences), the bisulphite adduct is used for isolation and purification of carbonyl compounds e.g. aldehydes, because these adducts are generally crystalline solids, thus easy to handle and stable for prolonged time;
- in D17 (second paragraph of the introduction), which concerns an organic synthesis and addresses the need for effecting a reductive amination of aldehyde 1 (see the schemes on the first page), not a peptide aldehyde, it was found that isolation of aldehyde 1 as its bisulfite adduct 2 (Scheme 2) offered significant advantages in terms of handling ease and stability (e.g. aldehyde 1 readily forms dimers and higher oligomers). D17 thus describes an efficient preparation of bisulfite adduct 2 and its utility in a representative reductive amination.
- in D18 (Abstract), the hydrosulphite adduct is used for isolation and purification of aldehydes, because the produced aldehyde as such is highly water-soluble.
The situations and problems dealt with in D15 to D18 are thus different from those dealt with in the patent, where the peptide aldehyde is sparingly water-soluble.

5.6.11 It follows from the foregoing analysis that, since D15 to D18 deal with purification/isolation of aldehydes from organic synthesis, their combination with D7 can only be made with hindsight, and thus cannot succeed.

5.6.12 As regards the combination of D7 with documents such as D13 and D14, the Board remarks the following:

(a) The argument that claim 1 at issue is not limited to detergents does not inevitably imply that it also covers medical/pharmaceutical compositions. Indeed, in

this respect, it has not been argued, nor shown, that the claimed subtilisin composition also has any medical indication. The argument is thus not convincing.

(b) The fourth paragraph on page 1 of D7 discloses that peptide aldehydes are suitable for stabilising not only subtilisin but also e.g. chymotrypsin-type proteases, and concerns "liquid detergents". Hence, this part of D7 cannot point to a technical field other than that of household products as detergents, let alone to the medical/pharmaceutical technical fields of D13 or D14.

(c) Also the disclosure in D19 (Nielsen et al, "*Design of liquid enzyme products with built-in liquid detergent stabilization system*", 2007, Computer-aided chemical engineering, 23, page 150, second paragraph (i.e. "the catalytic mechanism of the subtilisin is the same as that of the digestive enzymes trypsin and chymotrypsin as well as that of enzymes in the blood clotting cascade, reproduction and other mammalian enzymes. These enzymes are known as serine proteases due to the serine residue which is crucial for catalysis") happens within the context of household products or detergents.

(d) Thus, if any, the skilled person understands from D7 and D19 (this document being equivalent to common general knowledge), that the use of peptide aldehyde as inhibitor also for chymotrypsin-type proteases is due to the common catalytic mechanism of these serine proteases, used to hydrolyse the peptide bond via tetrahedral intermediates, thus to the formation of a covalent bond with the inhibitor for the stabilisation of the enzyme. Therefrom, nothing can be implied on whether chymotrypsin, trypsin and subtilisin also have a common specificity. This was particularly stressed by

the patent proprietor at the oral proceedings, arguing that the binding pockets of these serine proteases are indeed different, i.e. do not have a common specificity for the same amino acid residues of the substrate.

This is plausible for the board, as in general an enzyme operates on only one type of substrate (substrate specificity) (IUPAC Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"), by A. D. McNaught and A. Wilkinson, Blackwell Scientific Publications, Oxford (1997). Or https://doi.org/10.1351/goldbook.). Serine proteases preferentially hydrolyse the peptide bonds of polypeptide substrates depending on the amino acids preceding and/or following the cleavage site. This specificity for the substrate is due to the favorable binding interaction of the substrate amino acid side chain with residues that form the binding site of the serine protease. Thus, these interactions are a major determinant of the substrate/inhibitor specificity for subtilisin, trypsin, chymotrypsin and elastase. As this has not been disproved by the appellant, the invoked disclosure in D7 and D19 does not point to technical fields other than household products, nor to the use therein of peptide aldehydes from the pharmaceutical field as subtilisin inhibitors.

(e) In stereochemistry, "epimerisation" is the interconversion of one epimer to the other epimer, i.e. the formation of diastereoisomers having the opposite configuration at only one of two or more tetrahedral stereogenic centres present in the molecular entities (D13 or PAC, 1996, 68, 2193 (Basic terminology of stereochemistry (IUPAC Recommendations 1996)) p. 2208).

In the present case, that epimerisation of peptide aldehydes was a known problem, which was solved by N-terminal protection, is not in dispute. Moreover, this issue cannot be decisive, since claim 1 at issue (see also [0016]) does not appear to require any particular stereo specificity (IUPAC, *supra*), e.g. only the L-form of the amino acid residue, nor does it mandatorily require the presence of a N-terminal protection group. Thus the skilled person would not look at D13 therefor.

(f) Still as regards the catalytic similarity between serine proteases, as found in the decision under appeal, chymotrypsin (3.4.21.1) and trypsin (3.4.21.4) are classified differently from subtilisin (3.4.21.62), i.e. they carry the common classification 3.4.21 of the serine proteases but are not the same serine protease. The fact acknowledged on page 640 of D22, point B, second paragraph, that the first commercial enzymatic laundry product was made with pancreatin (a protease mixture with mainly trypsin and chymotrypsin obtained from pancreatic glands animals), which were not efficient actually because instable, cannot backup the argument that the skilled person would look at the pharmaceutical fields of inhibition of natural enzymes. The modern enzymes used in household products such as the subtilisin are engineered for use at the harsh conditions of the detergent's use.

(g) Hence, from the long known similarity of catalytic mechanism of the serine proteases, the skilled person starting from D7 does not get any motivation to look in technical fields such as the medical/pharmaceutical fields, where inhibition of serine protease is used for purposes (in animals) that are very different from those in household products. This becomes more evident from the following analysis of two documents.

5.6.13 D13, concerns the inhibition of thrombin (page 2, lines 42-47) and addresses the problem of providing configurational inversion stabilised bisulphite adducts of L-arginine aldehyde derivatives, that are potent thrombin inhibitors useful as anticoagulants in mammals The object of D13 is not (even) to produce a stabilised liquid composition of inhibitor and thrombin.

More particularly, D13 discloses as thrombin inhibitor a bisulphite adduct of an arginine aldehyde compound having the formula I on the top of its page 3. Page 104 thereof, table 1, discloses two specific bisulphite adducts of arginine aldehyde peptides, in examples 79 and 80, synthesised as disclosed on page 91 of D13. The peptide aldehyde bisulphite adducts of D13 do not fulfil the conditions stipulated in claim 12 for B^1 and R, which respectively exclude proline and arginine as amino acid residues of the peptide.

D13 departs from:
- the anticoagulation currently achieved by the (parenteral) administration of heparin and coumarins, which can be ineffective, are not selective and require monitoring by assays,
- the interest in small synthetic peptides that are recognised by proteolytic enzymes in a manner similar to that of natural substrates, i.e. tripeptide aldehydes such as D-Phe-Pro-Arg-H, Boc-D-Phe-Pro-Arg-H,and D-MePhe-Pro-Arg-H, shown to produce potent direct inhibition of thrombin,
- the search for analogs, to develop pharmaceutical agents, from the clinical studies demonstrating that D-MePhe-Pro-Arg-H sulf**a**te is an anticoagulant in man, whereby the small synthetic peptide derivatives contain an aldehyde group bonded to an arginine residue,

- the fact that no drug had then emerged from the known tripeptide aldehydes, despite the continuing promise for this class of compounds, and the need for anticoagulants that act selectively on thrombin, and independent of antithrombin III, exert inhibitory action shortly after administration, preferably by an oral route, and do not interfere with lysis of blood clots, as required to maintain hemostasis, as well as
- that protection of the arginine aldehyde group from configurational inversion would greatly enhance the prophylatic and therapeutic efficacy of small synthetic peptides containing said group as thrombin inhibitors, as epimerisation to the D diastereomer inactivates the molecule as a thrombin inhibitor.

Thus, D13 seeks to provide a drug and a method of inhibiting coagulation in mammals comprising administering to a mammal in need of treatment, a coagulation inhibiting dose of a bisulfite adduct of the disclosed peptide aldehyde adducts. It follows from the foregoing analysis that background and objects of D13 having nothing in common with the production of stabilised liquid enzymatic compositions suitable for household products such as detergents. The citation of D13 by the appellant implies hindsight.

5.6.14 Indeed, the retrospective approach adopted is confirmed by:

(a) D13 (page 9, lines 13-27) also discloses the use of the pharmaceutically acceptable salts of the adducts defined by formula I, as its particular adduct possesses one or more sufficiently basic functional groups (this is not surprising for the board, as the mandatory arginine of its peptide aldehyde is the most basic amino acid), which can react with (non-toxic)

inorganic/organic acids to form pharmaceutically acceptable salts thereof. The preferred pharmaceutically acceptable acid addition salts of D13 are formed from mineral acids such as sulfuric acid, i.e. the L-arginine aldehyde sulfate.

(b) The bisulphite adducts of D13 are prepared by commonly used procedures, wherein the corresponding arginine aldehyde compound (page 9, lines 32-38), or its sulphate salt (Examples 79 - page 91, lines 18-24 - and 80 - page 92, lines 37-53), is combined with about a stoichiometric amount of a pharmaceutically acceptable alkali metal or alkaline earth metal bisulphite in a polar solvent such as water or mixture of solvents to afford the desired bisulphite adduct. The adduct is then isolated and lyophilised by conventional methods or formulated as described below.

(c) From the preparation illustrated in examples 79 and 80 of D13, it is apparent that the peptide aldehyde salt (sulfate) used is already water-soluble, and that the further step for preparing the bisulphite adduct thereof only serves the purpose of preventing epimerisation, not for easier handling. This is even more apparent from the procedure disclosed on page 114, lines 3-49, showing epimerisation as a function of time in absence or presence of increasing amounts of sodium bisulphite, wherefrom it becomes apparent that the hydrosulphite adduct strongly reduces epimerisation (to a value of 6.72% of isomer after 8 hours). Hence, the solution disclosed in D13 does not correspond to the problem/solution dealt with in the patent in suit, where the peptide aldehyde used to stabilise subtilisin is sparingly soluble, poses a handling problem if large quantities of the composition have to prepared. Without hindsight, the skilled person was not motivated to

consider D13 to find a solution to the problems dealt with in the patent in suit.

(d) Finally, Table 1 and page 101, lines 3-7, of D13 were invoked by the appellant to show that peptide aldehyde bisulphite adducts were disclosed in connection with different serine proteases such as Thrombin, Trypsin, Plasmin, hence were suitable to inhibit all serine proteases. However, this disclosure of D13, starting from page 100, line 31, relates to an assay for demonstrating *in vitro* that the adducts of D13 selectively inhibit the action of thrombin in mammals. The hypothetical equilibrium constant (Kass) for the reaction between thrombin and the test compound of D13 is determined by this assay. The liquid composition disclosed by D13 for the assay on page 100, lines 37-47, comprises a buffer, a chromogenic substrate, a solution of human thrombin, a test compound of D13 in a 50% v/v aqueous solution with methanol. It is immediately evident that this liquid composition for the assay is not comparable to a liquid composition with subtilisin for use in household products, pertaining to industrial scale production.

5.6.15 The same conclusion can be drawn from D14, as:

(a) D14 (column 1, lines 10-17) does not pertain to the technical field of the composition according to claim 1 at issue but to a medical/pharmaceutical technical field, to provide certain proline derivatives which are useful as human leukocyte elastase inhibitors, e.g. in the treatment of tissue degenerative diseases, and pharmaceutical compositions prepared with such derivatives. Already these aspects show that also the choice of D14 arises from hindsight.

(b) D14 departs from the background art of peptide aldehyde inhibitors of porcine pancreatic elastase (PPE) and human leukocyte elastase (HLE), none of which however had then been found to be clinically useful in the treatment of any tissue degenerative disease. This is a completely different situation to that arising from D7.

(c) the tri- and tetra-peptide aldehyde derivatives of D14 include the bisulphite as adducts (column 3, lines 63-65), which are prepared (column 4, lines 3-10) by dissolving the aldehyde in a water-methanol solvent and by adding an excess of sodium hydrogen sulphite, and by then removing the solvent. According to D14 (column 4, lines 20-30), also the pharmaceutically-acceptable base- or acid-addition salts of the peptide aldehyde of D14 may be used.

(d) The examples of D14 invoked by the appellant, respectively on columns 50 - line 10 - and 51 - lines 60-65, merely show how to produce the specific bisulphite adducts illustrated therein. These specific adducts are N-protected L-valyl- or L-lysyl-L-prolyl-L-valinal bisulphite, which thus comprise as amino acid residue B^1 (as defined in claim 12) always proline, i.e. which do not fulfil at least the condition set for B^1 defined in claim 12 at issue.

(e) the assessment of the potency of inhibition human leukocyte **elastase** (HLE) on a low molecular weight peptide substrate, in D14 (column 14, lines 3-59), is carried out by obtaining a kinetic determination of the dissociation constant, Ki, of the complex formed from the interaction of an inhibitor with HLE, by using an appropriate substrate (anilide methoxysuccinyl-alanyl-alanylprolyl-valine-p-nitroanilide). Thus, the liquid

composition in which this was determined comprised, in addition to the substrate in DMSO, a buffer, an **HLE** enzyme and an inhibitor to be tested in solution with DMSO. Hence, also the liquid composition of D14, like that of D13, is merely disclosed for carrying out the assay.

5.6.16 It follows from the foregoing analysis that the skilled person starting from D7 and facing the problem posed had no motivation to consider the disclosure of D13 or D14 for combination with that of D7. The skilled person would thus have not tried without hindsight to use the peptide aldehyde bisulphite adducts of e.g. example 79 or 80 of D13, or those of D14, instead of the peptide aldehyde of D7, and he would thus have not arrived in an obvious way to the composition of claim 1, or to the compound of claim 12 at issue.

The composition or compound at issue was thus not obvious over D7, even if D13, D14, D15 to D19 were considered.

5.7 As none of the grounds of opposition under Article 100(a) or (b) EPC prejudices the maintenance of the patent, the appeal of the opponent does not succeed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Pinna J.-M. Schwaller

Decision electronically authenticated