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DECISION
of 25 February 2004

Case Number: T 1003/99 - 3.3.1

Application Number: 82306557.8

Publication Number: 0082636

IPC: C07D401/12

Language of the proceedings: EN

Title of invention:

Luminescent labelling materials and procedures

Patentee:

The Welsh National School of Medicine

Opponent:

ECKART H. Otto, Dr.

Headword:

Acridinium compounds/THE WELSH NATIONAL SCHOOL OF MEDICINE

Relevant legal provisions:

EPC Art. 54, 56

Keyword:

"Main request: novelty (no) - broad interpretation of functional feature"

"Auxiliary request 1: inventive step (no) - obvious solution of the technical problem"

"Auxiliary request 2: inventive step (yes) - non obvious solution of the technical problem"

Decisions cited:

-

Catchword:

-



Case Number: T 1003/99 - 3.3.1

D E C I S I O N
of the Technical Board of Appeal 3.3.1
of 25 February 2004

Appellant: The Welsh National School of Medicine
(Proprietor of the patent) Heath Park
Glamorgan
Cardiff CF4 4XN (GB)

Representative: Newell, William Joseph
Wynne-Jones, Lainé & James
Morgan Arcade Chambers
33 St. Mary Street
Cardiff CF10 1AF (GB)

Respondent: ECKART H.Otto, Dr.
(Opponent) 119, rue Berckmanns
1060 Brussels (BE)

Representative: Strehl, Peter, Dipl.-Ing.
Patentanwälte
Strehl Schübel-Hopf Groening u. Partner
Postfach 22 14 55
D-80504 München (DE)

Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 26 August 1999
revoking European patent No. 0082636 pursuant
to Article 102(1) EPC.

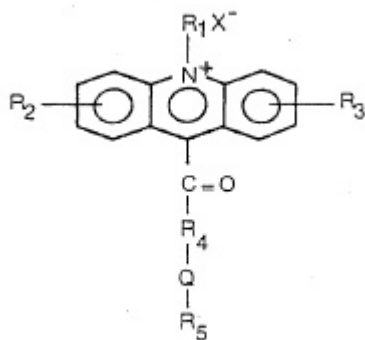
Composition of the Board:

Chairman: A. J. Nuss
Members: J. M. Jonk
J. H. Van Moer
P. F. Ranguis
S. C. Perryman

Summary of Facts and Submissions

- I. The Appellant (Proprietor of the patent) lodged an appeal against the decision of the Opposition Division revoking the European patent No. 0 082 636 (European patent application No. 82 306 557.8), the independent Claim 1 as granted reading as follows:

A luminescent labelling compound for use as a 'label' with a substance of biological interest, of the following formula:



- where X⁻ is any anion, R₁ represents H, C₁-C₁₀ alkyl, alkenyl, alkynyl, or aryl substituents, and R₂ and R₃ are hydrogen, amino, carboxyl, hydroxyl, alkoxy, nitro-, or halide substituents, and R₄ is a phenoxy-moiety, or derivative of said moiety, R₅ is a group capable of reacting with said substance of biological interest and Q is either a direct link or a carbon, nitrogen or oxygen containing group optionally substituted by a hydrophilic substituent.
- II. The opposition was filed against the patent as a whole, and based on the grounds of lack of novelty and

inventive step as indicated in Article 100(a) EPC. It was supported by several documents including:

- (1) Woodhead et al in Monoclonal antibodies and developments in immunoassay , Elsevier (1981), pages 135 to 145,
- (32) Corrie et al, Methods in Enzymology, Vol. 73 (1981), pages 79 to 126, and
- (54) The Peptides, Academic Press, Vol. 1 (1979), pages 79 and 80.

The decision of the Opposition Division was based on Claim 1 filed with letter dated 11 February 1999 and Claims 2 to 12 as granted. Said Claim 1 corresponded to Claim 1 as granted, except that it was additionally indicated that the claimed compound substantially preserved its quantum yield on reaction with protein.

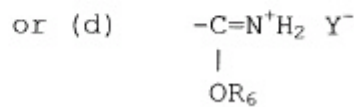
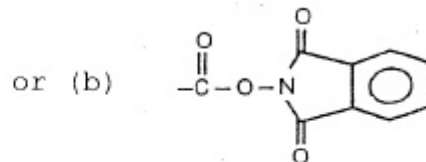
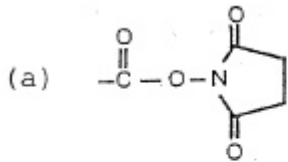
III. The Opposition Division held that the subject-matter of Claim 1 then on file met the requirements of Articles 84 and 123 EPC, but lacked novelty in view of document (1), since the carboxyl group fulfilled the definition of R₅ in that it was a reactive group allowing retention of the quantum yield as e.g. exemplified in

- Dr Ramakrishnan's experimental tests filed by the Respondent (Opponent) on 1 August 1991.

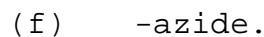
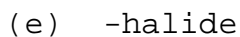
IV. Oral proceedings before the Board were held on 25 February 2004.

V. The Appellant defended the patentability of the subject-matter of the patent in suit on the basis of the claims as granted as his main request and 2 sets of claims filed during the oral proceedings before the Board as Auxiliary requests 1 and 2, respectively.

Claim 1 of the Auxiliary request 1 corresponded to Claim 1 as granted, except that the meaning of R_4 : "or derivative of said moiety," was deleted, and the meaning of R_5 was restricted to one of the following:

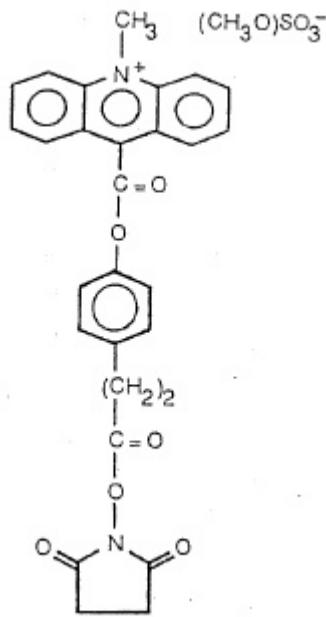


where R_6 represented any one of the R_1 groups and Y represented a halide



Claim 1 of the Auxiliary request 2 read as follows:

"A luminescent labelling compound for use as a label with a substance of biological interest, of the following formula:



VI. The Appellant argued with respect to Claim 1 of his main request that its subject matter was novel since the compound of document (1), i.e. a compound having a formula as indicated in Claim 1 of the patent in suit in which R₅ is a carboxyl group, did not fall under the scope of Claim 1, since a carboxyl group as such was not capable of reacting with a substance of biological interest. Furthermore, he argued concerning inventive step that the provision of a compound as claimed being capable of directly coupling with a compound of biological interest under mild reaction conditions, having an adequate stability on storage and being able to provide an improved luminescence activity was not obvious in the light of the cited prior art, since it was commonly known to the skilled person that in preparing a peptide bond the use of a carbodiimide was deemed necessary. In this context he referred to document (32) and document

(14) Ian Weeks et al, Clin. Chem. 29/8 (1983),
pages 1474 to 1479.

With respect to Claim 1 of the Auxiliary request 1 he also argued that its subject-matter was not obvious to the skilled person, since the skilled person would immediately understand that the achievement of the advantages of an indirect labelling carbodiimide coupling of a carboxyl compound with an amino compound (e.g. antibody or antigen) via a reactive succinimide ester as indicated in document (32) would depend on the circumstances of each particular case, and that it was in fact known from document

(10) Hartmut R. Schroeder et al, Clin. Chem. 27/8
(1981), pages 1378 to 1384,

that the coupling of a reactive luminescent succinimide ester with an antibody lead to an unsatisfying light production.

VII. The Respondent (Opponent) argued that the definition of R₅ in the claims as any reactive group capable of (directly) reacting with said substance of biological interest did not meet the requirements of Articles 84 and 123 EPC.

Moreover, he argued that Claim 1 of the present main request lacked novelty in view of document (1). In this context, he submitted that the compound as disclosed therein would at least be capable of coupling with amino group containing substances of biological interest to a certain extent even without the use of the carbodiimide coupling method. Anyhow, in using the

carbodiimide coupling method as indicated in document (1), an intermediate product falling under the scope of Claim 1 would be obtained.

Concerning Claim 1 of the Auxiliary request 1 he did not raise a novelty objection, but considered that its subject-matter lacked inventive step in view of documents (1), (32) and (54). The documents (32) and (54) clearly showed that it was common general knowledge that succinimide esters were particularly advantageous as intermediate or starting compounds for peptide bond formation. It was therefore obvious to the skilled person to modify the compound of document (1) accordingly. In this context, he disputed by referring to the Experimental Report from Dr Ramakrishnan considered by the Opposition Division that by using a compound of the patent in suit an improved luminescence would be obtained.

In view of the fact that the prior art did not provide any pointer to the skilled person to modify the compound of document (1) by introducing an ethylene group as structural feature Q of the formula indicated in Claim 1 as granted, the Respondent did not object to the patentability of the Auxiliary request 2.

VIII. The Appellant requested that the decision under appeal be set aside and that the patent be maintained as granted or in the alternative on the basis of the Auxiliary request 1 or 2 filed at the oral proceedings.

The Respondent requested that the appeal be dismissed.

IX. At the conclusion of the oral proceedings the Board's decision was pronounced.

Reasons for the Decision

1. The appeal is admissible.

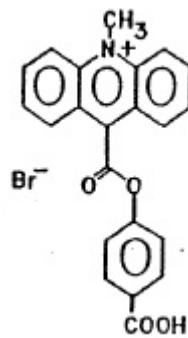
2. *Main request*

2.1 Formal matters

2.1.1 The Respondent raised formal objections with respect to the subject-matter of Claim 1 as granted under Articles 84 and 123 EPC. However, the Board does not concur with his submissions in this respect, since the objections under Article 84 did not relate to the amendments of the claim and because the Board has come to the conclusion that its subject-matter does not extend beyond the content of the application as filed. Having regard to the Board's findings indicated below concerning the question of novelty of the claimed subject-matter, the Board sees no need to consider this matter in more detail.

2.2 Novelty

2.2.1 Document (1) discloses the labelling of an antibody with an acridinium ester having the formula:



in which the coupling of said ester with the antibody is carried out by using the carbodiimide coupling procedure (see page 36 and page 139, penultimate paragraph).

Therefore, this document discloses the use of a chemiluminescent compound of the general structural formula given in Claim 1 of the patent in suit as granted, wherein R₅ is COOH.

2.2.2 The question to be decided is thus whether the carboxyl group on the compound of document (1) is a group falling under the scope of Claim 1 of the patent in suit as granted.

2.2.3 Claim 1 as granted relates to a luminescent labelling compound as defined by the specified general formula for use as a label with a substance of biological interest. In this general formula R₅ represents a group capable of reacting with said substance of biological interest. In the Board's judgment, this functionally defined feature comprises in fact any group capable of coupling with any amino group containing substance, such as an antibody, without any restriction with respect to the reaction conditions.

2.2.4 Thus, in view of the fact that the broad definition of R_5 in Claim 1 as granted does not exclude a group capable of being coupled with a substance of biological interest under particular reaction conditions, such as the use of a carbodiimide as activating agent, the Board comes to the conclusion that the carboxyl group on the compound of document (1) is a group falling under the scope of Claim 1 of the patent in suit as granted and that consequently the subject-matter of Claim 1 of this request lacks novelty.

3. *Auxiliary request 1*

3.1 Amendments

3.1.1 The subject-matter of Claim 1 of this request concerns a group of luminescent labelling compounds which is restricted with respect to Claim 1 as granted, in that R_5 is one of the specified groups (a) to (f) and in that as a meaning of R_4 the feature "or derivative of said moiety" was deleted. Contrary to the Respondent's point of view, the Board has come to the conclusion that the subject-matter of this claim meets the formal requirements of the EPC. Having regard to the Board's findings indicated below concerning the question of inventive step of the claimed subject-matter, the Board sees no need to consider this matter in detail.

3.2 Novelty

3.2.1 In view of the fact that none of the cited documents discloses a compound falling under the scope of Claim 1 of this request, the Board finds that the claimed subject-matter is novel over the state of the art. The

Respondent did not raise an objection with respect to the novelty either.

3.3 Inventive step

3.3.1 Article 56 EPC states that an invention is held to involve an inventive step if, having regard to the state of the art (in the sense of Article 54(2) EPC), it is not obvious to a person skilled in the art.

3.3.2 For deciding whether or not a claimed invention meets this criterion, the Boards of Appeal consistently apply the problem and solution approach, which involves essentially identifying the closest prior art, determining in the light thereof the technical problem which the claimed invention addresses and successfully solves, and examining whether or not the claimed solution to this problem is obvious for the skilled person in view of the state of the art.

3.3.3 The Board considers, in agreement with the parties to the proceedings, that the closest state of the art with respect to the claimed subject-matter of the patent in suit is the disclosure of document (1).

This document is - as indicated above under point 2.2.1 - concerned with labelling of an antibody with a chemiluminescent compound of the general structural formula given in Claim 1 of the patent in suit as granted, wherein R₅ is COOH. Moreover, it discloses that the coupling of said compound with the antibody is carried out by using the carbodiimide coupling procedure.

3.3.4 Having regard to this closest prior art the Appellant contended that the compounds of present Claim 1 had the advantages that they were capable of directly coupling with a compound of biological interest under mild reaction conditions, that they provided an improved luminescence activity and that they were stable under normal storage conditions.

3.3.5 The Respondent did not deny that the claimed compounds had an improved capability for a direct coupling with a compound of biological interest, but he disputed the presence of an improvement of the luminescence activity by referring to the Experimental Report from Dr Ramakrishnan.

On the other hand, the Appellant did not deny the validity of the test results of this Experimental Report, but he submitted that Dr Ramakrishnan had carried out the teaching of Document (1) with substantially more than average skill and with full awareness of the compound of the invention and its luminescence properties, so that his experiments did not reflect a true interpretation of how the notional person skilled in the art would have attempted to reproduce the teachings of document (1).

However, in the Board's judgement, the reproduction of the teaching of document (1) has been performed using common general knowledge represented by, for example, the documents (54) and (32) discussed below and applying the generally preferred method of peptide bond formation involving the indirect coupling of the acridinium compound with the amine via the reactive N-hydroxysuccinimide ester intermediate.

Moreover, the Board also cannot accept the Appellant's submissions in support of the contended luminescence improvement based on documents (10) and (14) for the following reasons:

It is true, that document (10) discloses that using 6-[carboxy-methoxyacetyl-N-(6-aminohexyl)-N-ethylamino]-2,4-dihydrophtalazine-1,4-dione (or CMAHEI) in the form of its active N-hydroxy-succinimide ester (NHS-CMAHEI) for preparing labelled protein gives a lower incorporation as judged from chemiluminescence yield than that from absorbance estimates due to a possible quenching of the label by individual amino acids and - as demonstrated - by self-coupling of the NHS-CMAHEI with the phthalhydrazide ring nitrogens, which abolishes light production (see page 1381, the paragraph bridging the columns and Table 1). Such a detrimental self-coupling cannot, however, occur in applying a labelling compound of the patent in suit. In fact, this document rather teaches that in applying labelling conditions producing conjugates that allows greatest sensitivity a highly convenient immunoassay has been achieved (see page 1381, right column, penultimate paragraph, and page 1378, first paragraph).

Furthermore, document (14) indeed teaches that the compound of document (1), used as a control, should not spontaneously couple covalently to a protein (see page 1477, left column, lines 1 to 4), but this teaching is not relevant, since it does not relate to an active ester coupling procedure as applied in the Experimental Report from Dr Ramakrishnan.

Finally, the Board observes that labelled compounds achieved by coupling a compound of biological interest with the compound of document (1) or with a compound of the patent in suit are identical and that already for this reason the presence of an improvement of the luminescence activity would be unlikely. The possibility, that by using a compound of the patent in suit as claimed as starting material for the coupling procedure a more efficient reaction may occur or less detrimental by-products may be obtained is not relevant in this context, since present Claim 1 of the patent in suit relates to compounds as such and not to a particular coupling procedure.

3.3.6 With respect to the stability of the compounds as claimed compared to the compound of document (1) the Board observes that the Appellant did not provide any evidence of an improvement in this respect. Therefore, and in view of the broad scope of the claimed compounds, the Board does not consider it plausible that an improved stability within the whole scope of Claim 1 has been achieved.

3.3.7 Thus, in view of the established jurisprudence of the Boards of Appeal that alleged advantages to which a patent proprietor refers, without offering sufficient evidence to support the comparison with the closest prior art, cannot be taken into consideration in determining the problem underlying the invention, the technical problem underlying the patent in suit in the light of document (1), which has credibly been solved by the claimed invention, can be seen in the provision of labelling compounds having an adequate luminescence activity, a sufficient stability under normal storage

conditions and, as such, being capable of more efficiently reacting with amino group containing substances of biological interest (see also page 2, lines 44 to 49, and page 5, lines 42 to 45, of the patent in suit).

- 3.3.8 The question now is whether the solution of the technical problem as defined above by the compounds of present Claim 1 would have been obvious to the skilled person in view of common general knowledge and the cited prior art.
- 3.3.9 As indicated above under points 2.2.1 and 3.3.3, document (1) discloses the labelling of an antibody with a chemiluminescent compound of the general structural formula given in Claim 1 of the patent in suit as granted, in which R₅ is COOH, whereby the coupling of said compound with the antibody is carried out by using the carbodiimide coupling procedure.
- 3.3.10 Furthermore, documents (32) and (54) representing common general knowledge at the priority date of the patent in suit clearly disclose that the carbodiimide coupling procedure for the formation of peptide bonds by reacting a RCOOH compound with a carbodiimide in the presence of N-hydroxysuccinimide firstly forming the reactive ester intermediate of the acid with N-hydroxysuccinimide ester and subsequently converting said reactive ester intermediate with the amine component is an advantageous procedure in that it efficiently reduces isomerisation and the forming of by-products giving cleaner reaction products than the direct carbodiimide coupling (see document (32),

page 92, last line, to page 93, last line; and document (54), page 79 to page 80, lines 1 to 4 under Scheme 23).

3.3.11 In view of these findings, it is the Board's position that the skilled person faced with the technical problem underlying the patent in suit as defined above and, in particular, aiming to provide labelling compounds being, as such, capable of a more efficient peptide bond formation with NH_2 -compounds of biological interest, would find in the cited prior art a clear incentive to replace the COOH group of the acridinium compound of document (1) by the rest R_5 as defined under (a) in present Claim 1 of the patent in suit. Furthermore, in following this incentive, he would not expect a reduction of the luminescence activity, since the direct carbodiimide coupling indicated in document (1) and the indirect reactive ester coupling both lead to an identical labelled compound. An additional examination of whether or not the reactive ester intermediate of the acid of document (1) with N -hydroxysuccinimide ester would have a suitable stability under normal storage condition does not need any inventive skill.

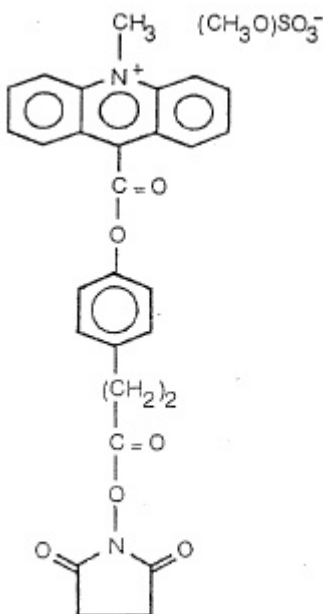
In this context, the Board observes that this point of view is in fact supported by document (10) referred to by the appellant by indicating that the active ester chemistry was chosen for labelling the antibody because this approach had been successful for coupling small molecules to proteins and avoided unwanted side reactions (see page 1381, left column, lines 1 to 4 of the second paragraph).

3.3.12 Thus, having regard to these considerations, the Board concludes that the solution of the above defined technical problem as claimed in present Claim 1 is obvious to the skilled person in the light of his common general knowledge and the cited documents, and consequently this request fails for the reason of lack of inventive step within the meaning of Article 56 EPC.

4. *Auxiliary request 2*

4.1 Amendments

4.1.1 Claim 1 of this request relates to a single luminescent labelling compound for use as a label with a substance of biological interest having the following formula:



The subject-matter of said Claim 1 of this request is based on the sole example of the application as filed and that of the patent in suit.

Furthermore, the subject-matter of Claims 2 to 4 is also supported by the example of the application as filed and that of the patent in suit, and in addition by the Claims 3 to 5 as granted.

Therefore, the claims of this request comply with the requirements of Article 123(2) and (3) EPC.

4.2 Novelty and inventive step

4.2.1 In view of the fact that the cited prior art does not provide any disclosure or incentive to the skilled person to provide a compound as defined in present Claim 1 having an ethylene group as structural feature Q of the formula indicated in Claim 1 as granted, and does not give him any pointer to the suitability of such a compound as an efficient reactive ester luminescent labelling compound either, the Board has come to the conclusion that the subject-matter of the present claim also meets the requirements of novelty and inventive step.

4.2.2 Since the Respondent did not raise objections to the patentability of the claims of this request either, the Board sees no need for a more detailed reasoning for its findings.

4.2.3 Consequently, the Board considers this request allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of Claims 1 to 4 of auxiliary request 2 filed at the oral proceedings with a description yet to be adapted.

The Registrar:

The Chairman:

N. Maslin

A. Nuss