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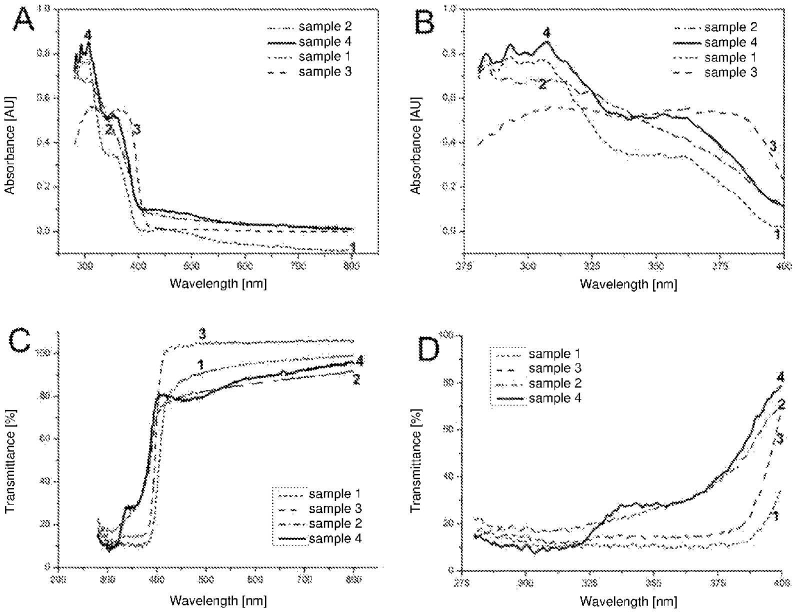

Fig. 1

(57) **Abstract:** The object of the invention is a process for the isolation and culture of strains, the strains, use thereof, media for culturing thereof and a form of scytonemin.

[Continued on next page]

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A process for the isolation and culture of strains, the strains, use thereof, media for culturing thereof and a form of scytonemin

The object of the invention is a process for the isolation and culture of strains, the strains, use thereof, media for culturing thereof and a form of scytonemin. The invention is applicable to biotechnological and cosmetic industry.

Standard isolation and culture methods are known (Rippka et al., 1979; Anahas and Muralitharan, 2015; Singh et al., 2014) which involve collecting a biological material from the endolytic microenvironment, for example pores in stone, either directly into a culture medium (BG11) (Singh et al., 2014) or else placing an isolated biological material on plates with 2% agar and the BG11 growth medium for the selection of monoclonal *Cyanobacterium* (according to Wolk, 1998), and subsequently transferring the colonies directly into the BG11 liquid medium (Anahas and Muralitharan, 2015). Direct collection of the biological material into the BG11 liquid medium may not be applicable in many cases, because this would require additional purification and isolation of monoclonal bacterial strains. The culture and purification method reported by Anahas and Muralitharan (2015) on plates with agar and BG11 (according to Wolk, 1998) does not achieve the expected results, because *Cyanobacteria* colonies collected from extremely dry environments proliferate extremely slowly or not at all. It is desirable to provide a culture method that would enable easy proliferation of various *Cyanobacteria* strains isolated from the environment whose culture in laboratory conditions using methods known in the art is impossible or at least difficult to accomplish. It is expected that application of the method would as a result enable the isolation of new *Cyanobacteria* strains having uniquely favorable properties, in particular high productivity of pigments, being also natural ultraviolet (UV) radiation filters. The present invention unexpectedly solved the aforementioned problems.

The fist object of the invention is a process for the isolation and culture of *Cyanobacteria* strains, in particular that deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0068B or BEA_IDA_0075B, characterized in that it comprises:

a) preparation of a growth medium by enriching it in micro- and macronutrients found in natural sandstone originating from Nubian formations with the following contents in mass percentages: 97.6% quartz, 0.4% muscovite-biotite 1.2% apatite and 0.8 % other minerals in trace quantities in the amount of 200 g per 1000 mL of an aqueous medium solution having the following composition per 1000 mL of the medium: 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \times 7H_2O$, 0.036 g $CaCl_2 \times 2H_2O$, 6.0 mg citric acid, 6.0 mg ammonium ferric citrate, 1 mg EDTA, 0.02 g Na_2CO_3, 1 mL of the A5 blend of trace metals with the following composition per 1000 mL of the aqueous A5 blend solution: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \times 4H_2O$, 0.222 g $ZnSO_4 \times 7H_2O$, 0.39 g $Na_2MoO_4 \times 2H_2O$, 0.079 g $CuSO_4 \times 5H_2O$, 49.4 mg $Co(NO_3)_2 \times 6H_2O$, subsequently stirring the resulting suspension for 24 hours at 25°C and subsequent 5-hour sedimentation at 25°C and filtration thereof; whenever the Applicant uses the phrase pure BG11 medium or agar-free BG11 medium or BG11 medium or medium according to Rippka et al. (1979) or medium with the composition of Table 1 or medium whose composition is disclosed in stage a), but without addition of the stone, this is meant to be the medium with the composition listed below in Table 1.

Ingredient no.	Chemical name	Ingredient quantity per 1000 mL of the medium being an aqueous solution
1	$NaNO_3$	1.5 g
2	K_2HPO_4	0.04 g
3	$MgSO_4 \times 7H_2O$	0.075 g
4	$CaCl_2 \times 2H_2O$	0.036 g
5	Citric acid	6.0 mg
6	Ammonium ferric citrate	6.0 mg
7	EDTA	1 mg
8	Na_2CO_3	0.02 g
9	A5 blend of trace metals with the following composition per 1000 mL of the aqueous A5 blend solution: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \times 4H_2O$, 0.222 g $ZnSO_4 \times 7H_2O$, 0.39 g $Na_2MoO_4 \times 2H_2O$, 0.079 g $CuSO_4 \times 5H_2O$, 49.4 mg $Co(NO_3)_2 \times 6H_2O$	1 mL

Table 1

b) collection of bacteria from the environment;

c) passaging the biological material collected in stage b) in the liquid medium obtained in stage a), i.e., according to Table 1, enriched with stone, with additional agar with ultimate contents between 2% in the beginning and 0.5% by weight in the end, preferably in three intermediate stages of 4 weeks each of the five stages, i.e. two ultimate stages (initial, final) and three intermediate stages, wherein the growth media in the intermediate stages contain the following quantities of additional agar: 1.75%, 1.5%, 1% by weight, respectively, with respect to the medium obtained in stage a);

d) dissolving the culture solution from final stage c), i.e. containing 0.5% agar, in the aqueous medium solution whose composition is disclosed in stage a) but without addition of the stone and incubation at 25°C for 2 weeks with stirring.

The second object of the invention is a bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0068B.

The third object of the invention is a bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0075B.

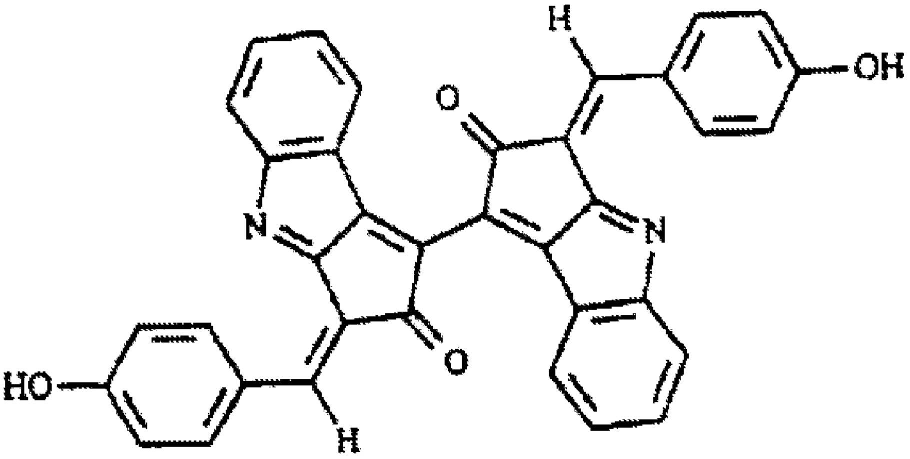
The fourth object of the invention is the use of the strain of the invention defined as the second object of the invention for the preparation of a pigment with UV absorption properties, in particular scytonemin or derivatives thereof. Equally preferably the use of the invention comprises application of the resulting pigment, in particular scytonemin or derivatives thereof, for the manufacture of cosmetic products, in particular for sunscreens.

The fifth object of the invention is a medium for culturing *Cyanobacteria*, containing in 1000 mL of the aqueous medium solution 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \times 7H_2O$, 0.036 g $CaCl_2 \times 2H_2O$, 6.0 mg citric acid, 6.0 mg ammonium ferric citrate, 1 mg EDTA, 0.02 g Na_2CO_3, 1 mL of the A5 blend of trace metals with the following composition per 1000 mL of the aqueous A5 blend solution: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \times 4H_2O$, 0.222 g $ZnSO_4 \times 7H_2O$, 0.39 g $Na_2MoO_4 \times 2H_2O$, 0.079 g $CuSO_4 \times 5H_2O$, 49.4 mg $Co(NO_3)_2 \times 6H_2O$, characterized in that it contains natural Nubian sandstone with the following contents in mass percentages: 97.6% quartz, 0.4% muscovite-biotite 1.2% apatite and 0.8 % other minerals in trace quantities in the amount of 200 g ground stone / 1000 mL of the medium. The disclosed medium is suitable for culturing *Cyanobacteria* which produce pigments, in particular scytonemin.

The sixth object of the invention is scytonemin crystals having at least one property selected from the following:

- X-ray powder diffraction spectrum with characteristic peaks at 2 theta angle values of 2.500°, 4.589°, 5.062°, 8.630° and 9.197°,
- specific infrared absorption bands at 3345, 3065, 2961, 2926, 1713, 1591, 1516, 1449, 1296, 1175, 1145, 957, 932, 930, 833 [cm^{-1}] in the IR spectrum (KBr),
- decomposition temperature in a range between 365°C and 380.3°C with a peak at about 380.3°C in thermogravimetric/differential thermal analysis (heating/cooling rate: 15/20°C/min),
- 1H NMR spectrum recorded in pyridine-d5 containing signals at δ 8.98 ppm, 7.99 ppm, 7.86 ppm, 7.75 ppm, 7.48 ppm, 7.33 ppm and 7.22 ppm.

Owing to the invention, it was possible to effectively provide a culture method that enabled easy proliferation of a desirable *Cyanobacteria* strain isolated from the natural environment whose culture in laboratory conditions using methods known in the art was impossible or at least difficult to accomplish. Owing to the use of the process of the invention, a specific *Cyanobacterium* strain could be isolated having extremely favorable properties, in particular high productivity for scytonemin of Formula 1, in particular its oxidized form, i.e., at least 1.5% per dry weight of *Cyanobacterium*.



Formula 1

The taxonomic characteristics of the *Cyanobacterium* strain were determined based on optical microscopy analysis and on the latest guidelines published in Komerek et al. (2014) and the literature reports found in that paper. Micromorphological characteristics of the test strain show it belongs to the family *Chroococcidiopsidaceae* (Komerek et al., 2014) and the genus *Chroococcidiopsis* (Geitler, 1932).

Micromorphological description of the resulting strains:

Cyanobacterium cells obtained in a culture of the invention had the following features:

a) color: blue-green, yellow to light brown;

b) form: single and spherical cells with a diameter of between 1.5 and 5 µm, clustered in colonies - from several to less than twenty cells or else forming aggregates, typically surrounded by a distinct sheath;

c) division: cells divide along two or more planes. After division cell coats typically extend and include daughter cells, which is seen as layering of a colony sheath;

d) thylakoid arrangement: arranged circularly near the cell wall.

Embodiments of the invention are shown in the drawings, wherein Fig. 1 shows comparative results of absorbance (A and B) and transmittance (C and D) tests for selected commercially available creams with SPF 30 and 50 (samples 1-3) and samples (no. 4) with scytonemin added, wherein the tests were performed using a thin-layer material to simulate artificial skin (3M® surgical tape), and Fig. 1a shows absorbance curves for the samples in a range between UVB (280–320 nm), UVA (320-400 nm) and up to 800 nm and 1B in a range between UVB (280-320 nm) and UVA (320-400 nm), and Fig. 1C shows transmittance curves for the samples in a range between UVB (280–320 nm), UVA (320-400 nm) and up to 800 nm and 1D in a range between UVB (280-320 nm) and UVA (320-400 nm), wherein curve symbols: continuous line "bolt" – formulation 4 with scytonemin added; sample 1 ----- ; sample 2 -.-.-.- and sample 3 - - - -, Fig. 2 illustrates the FTIR spectrum of the SCY sample, Fig. 3 shows the weight loss curve depending on sample heating temperature (black curve) and the heat flow curve (gray curve) in a temperature range of 350-520 °C with maximum decomposition temperature at 380.3 °C, Fig. 4 shows an X-ray diffractogram obtained using PXRD (polycrystalline X-ray diffraction method) of the scytonemin form of the invention with the major peaks marked with "*",Fig. 5 and 5a present a proton nuclear magnetic resonance (^{1}H NMR) spectrum of the scytonemin sample recorded in pyridine-d_5, in the δ scale [ppm], wherein Fig. 5 contains a complete spectrum (range: -0.5 to 10.5 ppm), and Fig. 5a shows an extended range of 7.1 - 9.1 ppm, while Fig. 6 presents results of investigation into the degree of scytonemin dispersion in selected solutions used in cosmetics disclosed in Example 2.2, Fig. 7a and 7b show the crystal described in Example 8, Fig. 8a-8d show graphic models of the SCY structure obtained using MERCURY software (Macrae et al., 2020): asymmetric unit (Fig. 8a), general view of unit cell packing (Fig. 8b), and unit cell packing, view along the [001] direction (Figure 8c) and along the [100] direction (Figure 8d)

Example 1

1.1 Preparation of growth medium

Preparation of the growth medium involved enrichment in micro- and macronutrients found in sandstone originating from the Nubian formation from which *Cyanobacteria* are sourced. Therefore, 200 g of sterilized stone was ground in an agate mortar and added per each 1000 mL of a pure BG11 medium according to Table 1. The resulting mixture was subsequently stirred for 24 hours at 25°C, subjected to final 5-hour sedimentation and filtered through a filter with a diameter of 25 mm and pore size of 0.2 µm (Cyclopore Track-Etch Membranes, Whatman). The resulting BG11 medium enriched in micro- and macronutrients from sandstone was heated to 60°C. Subsequently, dry agar in the final amount of 2% by weight was added to the resulting solution. Subsequently the entire contents were stirred until the agar dissolved and poured on Petri dishes (R), cooled to 25°C and kept covered in sterile conditions for *Cyanobacteria* collection from the environment and seeding on plates with the medium.

1.1.1.

Characteristics of the stone

Stones with endolytic microorganisms originate from the Nubian Sandstone formation. X-day diffraction (XRD) was used for the quantitative analysis of the mineralogical composition of five samples of the stones with a total weight of 52 g. The sandstone had mean contents of: quartz 97.6%, muscovite-biotite 0.4%, apatite 1.2% and other minerals in trace quantities of 0.8%.

1.2 collection of *Cyanobacteria* from the environment

Two small stone fragments with endolytic colonization by two *Cyanobacteria* strains being the object of the present invention were scraped mechanically using a sterile lancet onto the Petri dishes of item 1.1 above so that two separate cultures were set up for two strains, enriched in micro- and macronutrients from Nubian sandstone. The *Cyanobacteria* were seeded at 25°C in the light/dark regimen (12/12 hrs.) at 25°C, with light intensity in the PAR (photosynthetic active radiation, 400-700 nm) range of approx. 30–50 µmol photons m^{-2} s^{-1}, provided by 18 W cool fluorescent lamps (PhilipsTLD18W / 33). After 5–10 weeks, the agar dishes were tested for the presence of *Cyanobacateria* colonies using a Euromex Oxion Inverso OX.2053-PL light microscope + Cmex 3 camera.

1.3 passaging

Gradual passaging of the biological material from stage 1.2 was performed on solid media obtained in stage 1.1 and was conducted from the additional agar content of initially 2% by weight of the medium to 0.5% finally, preferably in three intermediate stages with agar contents of 1.75%, 1.5%, 1%. The passaging time was 4 weeks for each intermediate and final stages at 25°C with continuous PAR (400 – 700 nm) irradiation at 35 µmol photons m^{-2} s^{-1}.

1.4 culture

The resulting colonies from stage 1.3 for two strains from the medium with an agar content of 0.5% by weight were dissolved in an aqueous solution of the medium from stage 1.1 whose composition is disclosed in Table 1, which had not been modified using the addition of micro- and macronutrients from the stone and were incubated at 25°C for 2 weeks with simultaneous continuous orbital shaking (20 rpm) using an IKA KS 501 Orbital Shaker and with continuous PAR irradiation (400–700 nm) at 35 µ mol photons m^{-2} s^{-1} and two separate cultures for the two strains being the object of the invention were further maintained.

Preparation of a monoclonal stable culture of two strains of the invention in the medium of stage 1.1, not modified using the addition of micro- and macronutrients from the stone.

Cyanobacteria colonies isolated under the microscope were placed in an aqueous solution of the medium of stage 1.1 whose composition is listed in Table 1 without the addition of the stone at pH 8.2; temp. 25°C and PAR light intensity of 20 μmol photons m^{-2} s^{-1} and were shaken at certain intervals for resuspension. Part (2 mL) of the culture was added every two weeks to 100 mL fresh standard medium of stage 1.1 without the addition of the stone to maintain a fresh culture. The photoperiod during cyanobacterial culture in the liquid medium was 10-12 hours of light and 12-14 hours of dark in a continuous or mixed mode.

Induction of a *Cyanobacteria* culture for scytonemin synthesis and determination of its productivity

The methodology was taken from Fleming and Castenholtz (2007). Briefly, *Cyanobacteria* solutions from the above medium, i.e. from stage 1.1, without the addition of the stone were filtered and the filters were placed on a BG-11 solid agar medium with an agar content of 2%. The dishes with the filters were subjected to PAR (65 μmol photons m^{-2} s^{-1}, or 40 W/m^2) and UV irradiation (1.8 W/m^2). Some filters with irradiated *Cyanobacteria* were analyzed for scytonemin content every three days. Therefore, a spectrophotometry technique was used as presented below: absorption spectra of extracted (methanol/ethyl acetate (v/v 1:1)) scytonemin were obtained using an HP 8452A Diode Array single-beam spectrophotometer (Hewlett-Packard, Tokyo, Japan).

Absorbance values for the specific wavelength (maximum peaks for respective pigments) were selected for the semi-quantitative assay of scytonemin [mg/g dry weight (DW)] using trichromatic equations and extinction coefficients (Lichtenthaler, 1987).

1.5 method for the evaluation of scytonemin productivity of the invention

The culture solutions containing *Cyanobacteria* from stage 1.4 after the end of culture were filtered using a 0.2 µm filter. The filters were placed on a BG-11 solid agar medium (2%). The dishes with the filters were subjected to PAR irradiation at 65 µmol photons m^{-2} s^{-1} (or 40 W/m^2) and UVA irradiation at 1.8 W/m^2. Some filters with irradiated *Cyanobacteria* were analyzed for scytonemin content every three days. Therefore, a spectrophotometry technique was used as shown below:
Absorption spectra of extracted (methanol/ethyl acetate (v/v 1:1)) scytonemin (with other pigments) were obtained using an HP 8452A Diode Array single-beam spectrophotometer (Hewlett-Packard, Tokyo, Japan). Absorbance values for the specific wavelength (maximum peaks for respective pigments) were selected for the semi-quantitative assay of scytonemin [mg/g dry weight (DW)] using trichromatic equations and extinction coefficients (Lichtenthaler, 1987).
The resulting scytonemin productivity was at least 1.75% for the bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0075B and for the bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under numberBEA_IDA_0068B as per dry weight of *Cyanobacterium,* that is, much higher than in the art in which it was between 0.03 - 0.09% scytonemin per dry weight of *Cyanobacteria* (DW) (Balskus et al., 2011); therefore, productivity was between 19 and 58 times as high.

1.6 extraction and purification of scytonemin

The biomass obtained according to the description in the items above suspended in the culture liquid is separated by centrifugation (or filtration). The resulting biomass is subjected to preliminary purification in a chloroform:hexane mixture (v/v 1:1). In this stage, the biomass with the mixture of solvents is shaken for 10 minutes and sonicated, also for 10 minutes. This is subsequently centrifuged (6000 rpm for 10 min) and the supernatant is collected from above the sediment. Another fresh portion of the mixture of solvents is added to the sediment and the procedure is repeated. After another centrifugation, the supernatant from both centrifugations is merged and may be purified using a vacuum evaporator for reuse. The biomass after the first stage of purification is subsequently subjected to primary extraction in an ethyl acetate : methanol mixture (v/v 1:1) or in acetone. Centrifugation and sonication in 10-minute cycles is also used at this stage. Centrifugation follows every cycle and the supernatant is collected. Extraction is repeated with further fresh portions of the solvent until the supernatant starts to lose color (typically 3 to 5 times). The collected supernatant is subsequently evaporated using a vacuum evaporator (40°C) for reuse. The dried residue after evaporation is subjected to the final purification procedure. The chloroform:hexane (v/v 1:1) is also used at this stage, with shaking, sonication and centrifugation. The number of purification stages depends on the degree of sample contamination and it is repeated until a clear colorless supernatant is obtained after centrifugation. When this effect is achieved, the sediment (scytonemin) is additionally washed with hexane twice. After the last centrifugation and collection of the supernatant from above the sediment, it is dried in a vacuum dryer (40°C) and then weighed. The dried sediment is assayed by HPLC to asses the purity of the resulting product.

Example 2 Use of scytonemin

2.1. Efficiency of sun protection

To demonstrate the efficiency of sun protection of various brands of sunscreens and the sunscreen product proposed by the applicant (sample 4) with scytonemin based on *Cyanobacterium* extracts as the active ingredient, a spectrophotometry technique was used. Therefore, commercially available sunscreen products and the tested sample 4 were analyzed for absorbance and transmittance in an experiment using simulation of human skin (3M surgical tape). The commercially available 3M (R) tape was applied on a 2x2 cm quartz glass tape onto which a thin layer of the products being evaluated was applied. The plates were tested for absorbance and transmittance after 20 minutes using a FLAME-S (Ocean Optics, Florida, USA) system and spectrometer.

The efficiency of sun protection of the scytonemin product of the invention is shown in Figure 1 which presents comparative results of testing absorbance (A and B) and transmittance (C and D) for selected commercially available products with SPF (Sun Protection Factor) (Greiter, 1974) of 30 and 50 (samples 1-3) and sample 4 with scytonemin added. The tests were conducted using a thin-layer material that simulated artificial skin (3M® surgical tape). Fig. 1A shows absorbance curves for the samples in a range between UVB (280-320 nm), UVA (320-400 nm) and up to 800 nm and 1B in a range between UVB (280-320 nm) and UVA (320-400 nm), and Fig. 1C shows transmittance curves for the samples in a range between UVB (280-320 nm), UVA (320-400 nm) and up to 800 nm and 1D in a range between UVB (280-320 nm) and UVA (320-400 nm), wherein curve symbols: continuous line "bolt" -

formulation 4 with scytonemin added; sample 1 ----- ; sample 2 -.-.-.- and sample 3 - - - - -.

Commercially available products whose specific compositions are listed below were selected for comparative analysis:

Sample 1 2-Ethylhexyl 4-methoxycinnamate / Octinoxate + 2-Hydroxy-4-methoxybenzophenone / Oxybenzone + titanium dioxide (TiO_2) [percentage contents in the product: 7.5%, 4% and 10%, respectively] + q.s. (quantum satis): glycerin + glycerol stearate + water + silica + alcohol.

Sample 2 titanium dioxide (TiO_2) + zinc oxide (ZnO) [percentage contents in the product: 10% and 17%, respectively] + q.s.: glycerin + glycerol stearate + water + silica + alcohol.

Sample 3 zinc oxide (ZnO) + (2-Ethylhexyl 4-methoxycinnamate) / Octinoxate [percentage contents in the product: 15.5% and 7.5%, respectively] + q.s.: glycerin + glycerol stearate + water + silica + alcohol.

Sample 4 0.8% SCYTONEMIN + Diprobase q.s. with the composition: white petrolatum, liquid paraffin, macrogol cetostearyl ether, cetostearyl alcohol, sodium dihydrogen phosphate dihydrate, chlorocresol, sodium hydroxide, concentrated phosphoric acid, purified water.

Observation: the formulation with scytonemin added showed high absorbance values and low transmittance values in the UVB and UVA range at a level similar to commercially available creams with SPF 30 and 50.

2.2 Testing the degree of scytonemin dispersion in selected solutions used in cosmetics

2.2.1

0.5 mg scytonemin was weighed out on an analytical balance and suspended in 1 g of the solution:

1) Propylene glycol (INCI: Propylene Glycol)

2) Refined apricot oil (INCI: Prunus Armeniaca (Apricot) Kernel Oil)

3) Glycerin (INCI: Glycerin)

4) Isohexadecane (INCI: Isohexadecane)

5) 2-octyldodecan-1-ol ODD (INCI: Octyldodecanol)

6) SLP Emulsifier (INCI: Sorbitan Laurate / Polyglyceryl-4 Laurate / Dilauryl Citrate)

Subsequently, the sample was mixed using a shaker for approx. 1 min and maintained for 10 min in an ultrasonic bath to achieve a higher dispersion level.

Results:

1. High dispersion level of the active ingredient; the glycol solution immediately turns brown-green; small particles of the suspension are seen (see Fig. 6a).

2. Very low dispersion level of the active ingredient in the solution; undissolved suspension is clearly seen which sediments over time (see Fig. 6b)

3. Low dispersion level of the active ingredient; suspension is clearly seen which gradually dissolves over time and slightly tints the solution (see Fig. 6c)

4. High dispersion level of the active ingredient. After suspending, an evenly dispersed grayish suspension is obtained; particles of the substance are seen (very fine) which sediment over time (see Fig. 6d)

5. Relatively high dispersion level of the active ingredient. A pale green solution was obtained; the suspension settling on the bottom is seen (see Fig. 6e)

6. Low dispersion level of the active ingredient in the emulsifier solution. Fine particles settling on the bottom are seen; the solution gradually became colored over time (see Fig. 6f).

Summary:

The best dispersion level of the active ingredient was obtained in sample 1 at a concentration of 0.5 mg/g glycol. Decreasing dispersion levels were seen in successive samples in the following order (the samples are arranged from the highest to the lowest dispersion level of scytonemin in the matrix):

1 > 4 > 5 > 6 > 3 > 2.

2.2.2

Preparation:

0.5 mg of scytonemin was weighed out on an analytical balance and suspended in 5 g of the composition:

7) glycerin + glycerol stearate + water + silica + alcohol

The sample was stirred for 5 min using a laboratory stirrer

Result:

After mixing the raw material base with the active ingredient, a homogenous off-white base was obtained with visible scytonemin particles. The substance did not dissolve but disintegrated into smaller particles. The effect was similar as with sample 4 with isohexadecane (suspension) - see Fig. 6g.

1 > 4, 7 > 5 > 6 > 3 > 2.

Example 3. Comparative example - state of the art - standard isolation and culture method

Using a standard method by Rippka et al. (1979) in which culture in the BG11 medium was used; Anahas and Muralitharan (2015) in which culture in the BG-11N_0 medium was used; Singh et al. (2014) in which culture in the BG11 medium modified with 10 mM $NaHCO_3$ was used, bacteria of the strain being the object of the patent application could not be isolated, much less cultured. Bacterial colonies died early and biomass necessary to produce scytonemin could not be obtained.

Example 4

Fourier transform infrared spectroscopy (FTIR) with Thermogravimetric/Differential Thermal Analysis TG/DTA)

SAMPLE IDENTIFICATION: SCY
Dark brown solid in a powder form
weight: 8.3 mg

INTRODUCTION

A sample, hereinafter referred to as SCY, obtained from the strain being the object of the invention with deposit number BEA_IDA_0075B was prepared for further analysis using techniques described below: differential thermal analysis/thermogravimetry (TG/DTA) and Fourier transform infrared spectroscopy (FTIR) according to the specific procedures described below. No prior sample preparation was necessary for the analysis. Subsequently repeated experiments for a sample of strain BEA_IDA_0068B provided similar results. All results presented in the examples refer to the same substance obtained from two strains being the object of the invention.

A sample of SCY was stored until analysis in a closed container at room temperature.

4.1

FTIR

209.9 mg KBr previously dried at 110°C for five hours was weighed out for FTIR analysis and cooled in a vacuum desiccator to room temperature. 0.4 mg of the sample was added to KBr and the mixture was ground in an agate mortar under an infrared lamp to avoid absorption of moisture.

The spectrum was obtained in the following conditions:

Equipment

Shimadzu FTIR 8400 spectrophotometer (Shimadzu, Kyoto, Japan).
PIKE press (Pike Technologies, Madison, USA).

Measurement parameters:

Method: % Transmittance
Range: 400 - 4000 cm-1
Apodization: Happ-Genzel
Scan number: 45
Resolution: 4.0

Results:

The data were processed using Shimadzu software to obtain peak values. Peak values and bands were assigned according to the available literature (Pretsch, E. et al.: "Tablas para la elucidación estructural de compuestos orgánicos por métodos espectroscópicos" Ed. Alhambra. Madrid, 1980 and Flett, M. "Characteristic Frequencies of Chemical Groups in the Infra-red" Elsevier Monographs. Elsevier, 1963. Figure 2 shows the FTIR spectrum of a sample of SCY (obtained in Example 1) with major bands marked. Table 2 shows wavenumber values assigned to the most probable identified functional groups/bonds.

Table 2

3345	*O-H stretching*
3065	*=C-H stretching (alkenes, aromatics)*
1713	*C=O stretching*
1591	*C=C stretching (aromatics)*
1516	*C=C stretching (aromatics)*
1449	*C=C stretching (aromatics)*
1296	*O-H deformation*
1175	*C-O stretching*
957	*C-H in-plane deformation (aromatics)*
833	*C-H out-of-plane deformation (aromatics)*

Identical analysis was performed for the compound prepared in Example 1 from the strain deposited under number BEA_IDA_0068B. The resulting spectrum was identical as that in Figure 2.

4.2 TG/DTA

3.7 mg of the sample was weighed out into an alumina crucible and 34.6 mg of pure gold wire (99.999%) was added to balance the rod weight with the microbalance counterweight.

SAMPLE PREPARATION

No special preparation was needed for the analysis.

APPARATUS

SETARAM SETSYS 6000 analyzer

EXPERIMENTAL CONDITIONS

Measurement parameters:

Argon flow at 2 atm

Sample weight: 3.7 mg
Crucible: 100 µL Al_2O_3
Reference crucible: Al_2O_3
Temperature ramp:

Temperature (start – end) in [°C]	Time [s]	Rate [°C/minute]
25-25	300	-

25-900	5250	15
900-40	2580	20

Results

Weight losses in successive stages were calculated based on the thermogravimetric curve, and the presence of exothermic and endothermic processes during sample heating were determined from the heat flow curve.

Figure 3 shows the weight loss curve depending on sample heating temperature (black curve) and the heat flow curve (gray curve) in a temperature range of 350°C to 520°C. A distinct weight loss of SCY (black curve) was seen in this temperature range. Thermal decomposition of a substance is an exothermic process (heat flow value increment on the gray curve), starts at 365.4°C (vertical dashed line in Fig. 3) and achieves its maximum at 380.3°C (vertical solid line in Fig. 3). It was found that a weight loss of 4.32% of SCY occurred in a temperature range of 365.4°C to 380.3°C related to an exothermic process, which confirmed decomposition temperature of SCY in this temperature range with a distinct maximum at 380.3°C. Weight loss of SCY was still seen above this temperature, associated with an endothermic process (decreasing values on the gray curve), which confirmed a process of gas release and restructuring of SCY decomposition products except for the temperature range of 405-412°C, in which an exothermic process was seen, associated with secondary decomposition of SCY decomposition products.

T range of SCY decomposition = 365.4 - 380.3°C
Maximum of weight loss = 380.3°C
Δ weight = 0.16 mg (4.32%)

Similar spectra were obtained for compounds obtained from the two strains being the object of the invention, cultured and isolated according to Example 1.

Example 5

Polycrystalline X-ray diffraction (PXRD)

Measurement methodology

After extracting SCY from the two strains being the object of the invention, the solvent was evaporated and 8.3 mg of brown powder was obtained (crystalline form, form of the invention) with single needle-like crystals with a size of 2 to 20 micrometers or as their aggregates with radial arrangement of needle-like crystals. The characteristics of the crystalline material were observed using optical microscopy (AXIO Imager DM2 microscope, Zeiss, Carl Zeiss, Germany, Apochrome 63x lens, n=1.4 Zeiss).

PXRD powder analysis was performed in a crystalline material (form of the invention) composed of SCY, obtained from the two strains being the object of the invention, for which two similar PXRD diffractograms were recorded. The PXRD measurement was performed using a Bruker D8-Discover polycrystalline diffractometer. Powder diffractograms were obtained at room temp. with an X-ray tube as the X-ray source (Cu anode, Kα at 50 kV, 30 mA and collimator with a slit of 2 mm). Measurements were recorded in a continuous operating mode; 2 Theta angle scanning range between 2 and 60 degrees, measurement step of 0.02 degree, scanning rate of 0.7 sec/measurement step.

Diffrac.EVA v5.1 software was used for the analysis of the resulting diffractogram data.

The results of the analysis of powder X-ray diffraction spectra (form of the invention) presented in Fig. 4 are as follows:

1. The diffraction pattern for SCY does not show any amorphous phases.

2. The PXRD diffraction pattern contains approx. 35 significant diffraction peaks in the 2 Theta angle range of 2 to 43 degrees. Because the quality of the diffraction pattern was poorer and it was not possible to unambiguously determine (identify) peak parameters above this value, analysis was not performed.

3. Considering their characteristics (low full width at half maximum (FWHM), which confirms a high degree of crystallinity), at least five low-angle diffraction angles at the following 2 Theta angle values determined based on the available software: 2.500°, 4.589°, 5.062°, 8.630° and 9.197°, can be used to identify the material.

The peaks are marked in Fig. 4 with "*".

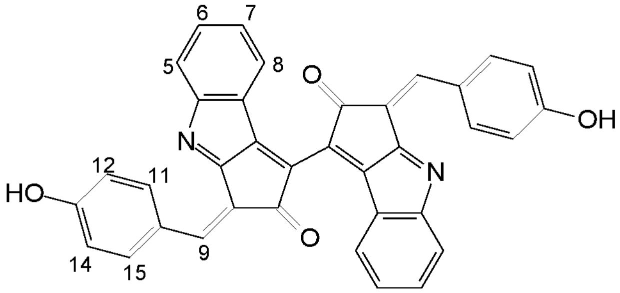
4. The presence of other lower and broader peaks with higher FWHM values shows that a crystalline material with a lower degree of crystallinity (reduced crystallite size) occurs in the sample.

5. The recorded diffraction peaks are specific for SCY and may be used to identify the substance. Based on the available crystallographic databases of polycrystalline data, no other known substance with this diffraction pattern was found.

Example 6

^{1}H NMR (proton nuclear resonance) measurement of scytonemin

The ^{1}H NMR spectrum of scytonemin (1.6 mg) was recorded in pyridine-d_5 (0.75 ml) on a Bruker Avance II 300 spectrometer at the basic frequency of 300.13 MHz at room temperature. δ chemical shifts are given in ppm, and values of J coupling constants in Hz. The spectrum was standardized with respect to the residual signal of H-2 protons of pyridine-d_5 at 8.727 ppm. The phase and baseline were corrected manually. Integration regions were selected in a similar fashion. Signals were assigned based on earlier literature data (Proteau et al., 1993). Experimental δ chemical shift data are consistent with the cited data. Due to rapid exchange of labile protons of -OH phenol groups, their signals were not included in the description. Signals from trace impurities (water and *n*-hexane) are found at δ 4.93 and about δ 1.0, respectively.



^{1}H NMR (pyridine-d_5) δ [ppm]: 8.98 (d, 2H, *J* 8.7; H-11,15); 7.99 (s, 1H; H-9); 7.86 (d, 1H, *J* 7.5; H-8); 7.75 (d, 1H, *J* 7.6; H-5); 7.48 (td, 1H, *J* 7.6, 1.2; H-6); 7.33 (d, 2H, *J* 8.8; H-12,14); 7.22 (m, 1H, H-7 /overlaps with the residual signal of pyridine-d_5 H-3 protons/).

Figures 5 and 5a show the proton spectrum (^{1}H NMR) of a scytonemin sample recorded in pyridine-d_5 in the δ scale [ppm], wherein Figure 5 contains a complete spectrum (range of -0.5 to 10.5 ppm), and Figure 5a contains an extended range of 7.1 - 9.1 ppm.

Example 7

The compound prepared in Example 1 was stored in room conditions (temp. 25°C) for 10 months. Absorbance spectra before and after the storage test are identical, which confirms stability of the compound. In addition, the high stability of scytonemin was confirmed in papers (Fleming and Castenholz 2007)and (Rastogi and Incharoensakdi 2014) in which it was shown that scytonemin still had practically unchanged characteristic absorbance spectra after 2 months of continuous UVA irradiation (5 W/m2) or heating to 60°C for 2 months. The crystalline form of scytonemin of the invention is stable.

Example 8

Determination of the scytonemin structure model (SCY) using X-ray diffraction (XRD)

Preparation of a monocrystalline sample of SCY

A monocrystalline sample of scytonemin for analysis using X-ray diffraction (XRD) was prepared by crystallization in the tetrahydrofuran (THF)-ethanol (EtOH) system in a 2:1 volumetric ratio. Approx. 30 mg of the compound and 12 mL of the THF-EtOH mixture was used in the process. The sample was initially dissolved in 8 mL THF, and subsequently, after 4 mL EtOH was added, the resulting solution was slowly (approx. 7 days) concentrated by free evaporation at room temperature.

Data collection and reduction

One dark brown parallelepiped crystal with dimensions of 0.011 x 0.035 x 0.131 mm was selected from the test sample (Figure 7a and 7b).

Diffraction data for the selected SCY crystal were collected at 100 K using a Rigaku Oxford Diffraction Synergy-S four-cycle diffractometer equipped with a CuKα radiation source (1.54184 Å), graphite monochromator and an Oxford CryoStream 800 sample cooling system for low-temperature measurements. Refinement of cell parameters and data reduction were performed using software from the diffractometer manufacturer (Rigaku Oxford Diffraction, 2018).

Structure solving and refining

The phase problem was solved by intrinsic phasing and atom positions in the structure model were determined using SHELXT (Sheldrick, 2015- Section A). Considering the quality of diffraction data, full-matrix refinement of positions and isotropic atomic displacement parameters of non-hydrogen atoms based on structure factor squares ($F^2(hkl)$) was only performed. To improve structure refinement and correct molecular geometry parameters, geometric constraints for benzene rings (AFIX 66) and terminal five-members rings having carbonyl groups (AFIX 56) were used.

Structure model refinement and additional calculations were performed using SHELXL2014 (Sheldrick, 2015-Section C)

Parameters of the diffraction measurement, crystal lattice and structure model refinement for SCY are listed in Table 3. Parameters of the geometrically determined structure model of SCY are listed in Tables 4-7. These are, respectively: atomic coordinates expressed as fractions of unit cell parameters ($\times 10^4$) and equivalent isotropic atomic displacement parameters U_{eq} ($Å^2 \times 10^3$) for SCY, wherein U_{eq} values are defined as 1/3 of the trace of the orthogonalized U_{IJ} tensor (Table 4), bond lengths (Table 5) as well as valence (Table 6) and torsion angles (Table 7).

Graphic representations of the SCY structure model (Figures 8a-d) were obtained using MERCURY software (Macrae et al., 2020): asymmetric unit (Figure 8a),

general view of unit cell packing (Figure 8b) and unit cell packing, view along the [001] direction (Figure 8c) and along the [100] direction (Figure 8d).

Table 3

Name	SCY
Molecular formula	$C_{36}H_{20}N_2O_4$
Molecular weight/g mol^{-1}	544.57
Measurement temperature/K	100.00(15)
Crystal system	monoclinic
Space group	Pc
a/Å	35.5024(13)
b/Å	3.78158(10)
c/Å	20.4422(6)
α/°	90
β/°	95.338(3)
γ/°	90
Unit cell volume/Å^3	2732.57(15)
Z	4
Density$_{calc}$/g cm^{-3}	1.304
μ/mm^{-1}	0.716
Crystal dimensions/mm^3	0.131 × 0.035 × 0.011
Radiation source	CuKα (λ = 1.54184)
2Θ angle range for recorded data/°	5 to 149.748.
Ranges of determined *hkl* parameters	$-41 \leq h \leq 44$, $-4 \leq k \leq 4$, $-24 \leq l \leq 24$
Number of reflections recorded	6816
Number of symmetrically independent reflections recorded	6816 [R_{sigma} = 0.0239]

Number of reflections/number of constraints/number of refined parameters	6816/16/215
Structure model goodness of fit parameter based on $F(hkl)^2$	3.675
R factors [$I \geq 2\sigma$ (I)]	R_1 = 0.2424, wR_2 = 0.5534
Highest/lowest value in the difference electron density map/e Å^{-3}	2.38/-1.65

Table 4

Atom	x	y	z	U_{eq}
C1	7477(4)	-3830(50)	6963(8)	30(4)
C2	7274(5)	-2300(50)	6402(7)	43(6)
C3	6889(4)	-2040(60)	6526(10)	37(5)
C4	6853(4)	-3410(70)	7164(10)	68(9)
C12	7217(5)	-4520(60)	7434(7)	39(5)
N5	6580(6)	-2680(60)	7543(9)	39(5)
C11	7088(5)	-5090(60)	8061(6)	55(8)
C6	6716(5)	-4110(70)	8129(8)	47(7)
C7	6568(4)	-4510(70)	8731(10)	62(8)
C8	6792(5)	-5880(70)	9265(7)	56(8)
C9	7164(5)	-6860(50)	9197(6)	37(5)
C10	7312(4)	-6460(50)	8595(7)	21(4)
C13	6634(14)	-210(150)	6110(30)	80(12)
C14	6238(6)	790(70)	5931(12)	41(6)
C15	6071(10)	1590(120)	5306(12)	95(16)
C16	5694(11)	2630(150)	5220(20)	110(20)
C17	5484(8)	2870(170)	5760(30)	140(30)

C18	5650(10)	2070(170)	6380(20)	180(50)
C19	6027(10)	1030(110)	6470(13)	72(10)
O20	5077(9)	3230(90)	5546(18)	72(8)
O21	7389(10)	-1730(100)	5910(20)	83(9)
C22	7872(4)	-4610(60)	7096(9)	43(6)
C23	8059(5)	-4180(60)	7736(7)	38(6)
C24	8437(5)	-5380(50)	7725(8)	32(5)
C25	8484(4)	-6570(60)	7079(9)	40(6)
C33	8135(6)	-6090(60)	6690(6)	49(7)
N26	8740(8)	-7660(120)	6664(12)	79(10)
C32	8180(4)	-7000(50)	6027(6)	29(5)
C27	8550(4)	-8240(50)	6046(7)	34(5)
C28	8693(4)	-9390(50)	5473(9)	36(5)
C29	8467(6)	-9310(60)	4881(7)	48(7)
C30	8097(5)	-8070(70)	4861(6)	65(10)
C31	7954(4)	-6920(60)	5434(8)	48(7)
C34	8684(5)	-5060(50)	8250(9)	17(3)
C35	9093(5)	-6120(70)	8481(9)	51(7)
C36	9236(4)	-5740(50)	9133(8)	34(5)
C37	9598(4)	-6940(50)	9338(8)	25(4)
C38	9817(4)	-8530(60)	8890(10)	82(12)
C39	9673(6)	-8910(70)	8238(10)	44(6)
C40	9311(7)	-7710(80)	8033(8)	130(30)
O41	10201(6)	-9340(60)	9131(12)	49(5)
O42	7964(6)	-2120(50)	8222(10)	41(4)
C51	2566(3)	13380(50)	2948(7)	44(6)

C52	2750(4)	11940(40)	3534(5)	26(4)
C53	3136(3)	11400(40)	3433(6)	17(3)
C54	3190(3)	12500(40)	2785(6)	16(3)
C62	2838(4)	13730(50)	2485(5)	30(4)
N55	3453(6)	12750(70)	2335(10)	47(6)
C61	2864(4)	14900(50)	1841(6)	26(4)
C56	3247(4)	14290(60)	1805(7)	34(5)
C57	3412(4)	15100(70)	1232(10)	68(10)
C58	3193(6)	16510(70)	696(8)	51(7)
C59	2810(6)	17130(70)	733(8)	71(10)
C60	2645(4)	16320(70)	1305(9)	58(8)
C63	3389(5)	10270(50)	4038(9)	19(3)
C64	3783(3)	9350(60)	4039(8)	81(12)
C65	3921(4)	8090(50)	4654(7)	36(5)
C66	4301(4)	7240(40)	4779(6)	21(4)
C67	4545(3)	7660(50)	4290(8)	38(5)
C68	4407(4)	8920(50)	3675(7)	36(5)
C69	4026(4)	9770(50)	3549(7)	29(4)
O70	4886(11)	5840(110)	4330(20)	89(10)
O71	2577(4)	11830(40)	4085(7)	18(3)
C72	2128(3)	14310(50)	2856(7)	41(5)
C73	1915(4)	13840(40)	2239(6)	20(4)
C74	1539(4)	14990(60)	2303(8)	58(8)
C75	1520(4)	16160(50)	2959(9)	37(5)
C83	1884(4)	15740(50)	3301(6)	23(4)
N76	1276(4)	17990(40)	3265(7)	21(3)

C82	1841(4)	17100(40)	3931(5)	35(5)
C77	1470(4)	18330(50)	3868(5)	26(4)
C78	1309(3)	19640(50)	4413(8)	49(7)
C79	1519(4)	19700(40)	5023(6)	23(4)
C80	1889(4)	18470(40)	5086(5)	20(4)
C81	2050(3)	17170(40)	4540(6)	21(4)
C84	1242(6)	15190(60)	1567(12)	29(4)
C85	896(4)	16160(50)	1462(8)	38(6)
C86	755(5)	15490(60)	817(8)	64(9)
C87	390(5)	16520(60)	595(7)	49(7)
C88	165(4)	18230(50)	1019(9)	27(4)
C89	305(4)	18890(50)	1665(8)	63(9)
C90	671(4)	17860(50)	1886(7)	21(4)
O91	-169(7)	18650(70)	817(13)	53(5)
O92	2030(5)	12180(50)	1792(9)	33(4)
C101	10609(7)	-12320(60)	8307(12)	29(5)
C102	-592(11)	22390(110)	1630(20)	59(8)

Table 5

Atom	Atom	Length/Å		Atom	Atom	Length/Å
C1	C2	1.4200		C51	C52	1.4200
C1	C12	1.4200		C51	C62	1.4200
C1	C22	1.434(18)		C51	C72	1.586(15)
C2	C3	1.4200		C52	C53	1.4200
C2	O21	1.13(4)		C52	O71	1.333(17)
C3	C4	1.4200		C53	C54	1.4200

C3	C13	1.36(6)		C53	C63	1.52(2)
C4	C12	1.4200		C54	C62	1.4200
C4	N5	1.325(18)		C54	N55	1.373(18)
C12	C11	1.418(15)		C62	C61	1.400(14)
N5	C6	1.362(18)		N55	C56	1.380(19)
C11	C6	1.3900		C61	C56	1.3900
C11	C10	1.3900		C61	C60	1.3900
C6	C7	1.3900		C56	C57	1.3900
C7	C8	1.3900		C57	C58	1.3900
C8	C9	1.3900		C58	C59	1.3900
C9	C10	1.3900		C59	C60	1.3900
C13	C14	1.47(5)		C63	C64	1.44(2)
C14	C15	1.3900		C64	C65	1.3900
C14	C19	1.3900		C64	C69	1.3900
C15	C16	1.3900		C65	C66	1.3900
C16	C17	1.3900		C66	C67	1.3900
C17	C18	1.3900		C67	C68	1.3900
C17	O20	1.48(4)		C67	O70	1.39(4)
C18	C19	1.3900		C68	C69	1.3900
C22	C23	1.4200		C72	C73	1.4200
C22	C33	1.4200		C72	C83	1.4200
C23	C24	1.4200		C73	C74	1.4200
C23	O42	1.33(2)		C73	O92	1.21(2)
C24	C25	1.4200		C74	C75	1.4200
C24	C34	1.33(2)		C74	C84	1.76(3)
C25	C33	1.4200		C75	C83	1.4200

C25	N26	1.36(2)		C75	N76	1.314(15)
C33	C32	1.422(15)		C83	C82	1.409(13)
N26	C27	1.39(2)		N76	C77	1.360(15)
C32	C27	1.3900		C82	C77	1.3900
C32	C31	1.3900		C82	C81	1.3900
C27	C28	1.3900		C77	C78	1.3900
C28	C29	1.3900		C78	C79	1.3900
C29	C30	1.3900		C79	C80	1.3900
C30	C31	1.3900		C80	C81	1.3900
C34	C35	1.54(2)		C84	C85	1.28(2)
C35	C36	1.3900		C85	C86	1.3900
C35	C40	1.3900		C85	C90	1.3900
C36	C37	1.3900		C86	C87	1.3900
C37	C38	1.3900		C87	C88	1.3900
C38	C39	1.3900		C88	C89	1.3900
C38	O41	1.44(3)		C88	O91	1.23(3)
C39	C40	1.3900		C89	C90	1.3900

Table 6

Atom	Atom	Atom	Angle/°		Atom	Atom	Atom	Angle/°
C2	C1	C22	130.7(14)		C52	C51	C62	108.0
C12	C1	C2	108.0		C52	C51	C72	123.7(12)
C12	C1	C22	121.3(14)		C62	C51	C72	128.3(11)
C1	C2	C3	108.0		C53	C52	C51	108.0
O21	C2	C1	126(2)		O71	C52	C51	120.8(12)
O21	C2	C3	125(2)		O71	C52	C53	130.2(11)
C2	C3	C13	121(3)		C52	C53	C54	108.0

C4	C3	C2	108.0		C52	C53	C63	115.3(11)
C4	C3	C13	130(3)		C54	C53	C63	136.1(11)
C12	C4	C3	108.0		C62	C54	C53	108.0
N5	C4	C3	126.4(17)		N55	C54	C53	143.5(12)
N5	C4	C12	121.5(17)		N55	C54	C62	108.4(12)
C1	C12	C4	108.0		C54	C62	C51	108.0
C11	C12	C1	157.9(15)		C61	C62	C51	139.9(12)
C11	C12	C4	92.2(14)		C61	C62	C54	112.1(12)
C4	N5	C6	102.3(17)		C54	N55	C56	102.1(15)
C6	C11	C12	116.0(14)		C56	C61	C62	98.5(12)
C6	C11	C10	120.0		C56	C61	C60	120.0
C10	C11	C12	124.0(14)		C60	C61	C62	141.5(12)
N5	C6	C11	106.3(14)		N55	C56	C61	118.6(14)
N5	C6	C7	133.5(14)		N55	C56	C57	121.3(14)
C11	C6	C7	120.0		C57	C56	C61	120.0
C8	C7	C6	120.0		C56	C57	C58	120.0
C9	C8	C7	120.0		C57	C58	C59	120.0
C8	C9	C10	120.0		C58	C59	C60	120.0
C9	C10	C11	120.0		C59	C60	C61	120.0
C14	C13	C3	148(5)		C64	C63	C53	124.7(15)
C15	C14	C13	127(3)		C65	C64	C63	110.1(12)
C15	C14	C19	120.0		C65	C64	C69	120.0
C19	C14	C13	113(3)		C69	C64	C63	129.8(12)
C16	C15	C14	120.0		C66	C65	C64	120.0
C15	C16	C17	120.0		C65	C66	C67	120.0
C16	C17	C18	120.0		C68	C67	C66	120.0

C16	C17	O20	111(4)		O70	C67	C66	120(2)
C18	C17	O20	128(4)		O70	C67	C68	117(2)
C19	C18	C17	120.0		C67	C68	C69	120.0
C18	C19	C14	120.0		C68	C69	C64	120.0
C23	C22	C1	121.1(14)		C73	C72	C51	120.8(11)
C23	C22	C33	108.0		C83	C72	C51	131.1(12)
C33	C22	C1	130.8(14)		C83	C72	C73	108.0
C22	C23	C24	108.0		C74	C73	C72	108.0
O42	C23	C22	128.9(16)		O92	C73	C72	123.1(14)
O42	C23	C24	120.3(16)		O92	C73	C74	127.7(14)
C25	C24	C23	108.0		C73	C74	C75	108.0
C34	C24	C23	120.8(15)		C73	C74	C84	115.5(12)
C34	C24	C25	131.0(15)		C75	C74	C84	135.8(12)
C24	C25	C33	108.0		C83	C75	C74	108.0
N26	C25	C24	144.7(17)		N76	C75	C74	135.0(13)
N26	C25	C33	106.8(17)		N76	C75	C83	115.8(13)
C22	C33	C32	142.3(15)		C75	C83	C72	108.0
C25	C33	C22	108.0		C82	C83	C72	148.2(12)
C25	C33	C32	109.4(15)		C82	C83	C75	103.7(12)
C25	N26	C27	108(2)		C75	N76	C77	100.4(13)
C27	C32	C33	104.1(14)		C77	C82	C83	102.8(11)
C27	C32	C31	120.0		C77	C82	C81	120.0
C31	C32	C33	135.9(14)		C81	C82	C83	136.9(11)
C32	C27	N26	110.5(15)		N76	C77	C82	116.6(12)
C28	C27	N26	128.9(15)		N76	C77	C78	123.3(12)
C28	C27	C32	120.0		C82	C77	C78	120.0

C27	C28	C29	120.0		C77	C78	C79	120.0
C30	C29	C28	120.0		C80	C79	C78	120.0
C29	C30	C31	120.0		C81	C80	C79	120.0
C30	C31	C32	120.0		C80	C81	C82	120.0
C24	C34	C35	139.2(18)		C85	C84	C74	130.3(19)
C36	C35	C34	121.2(14)		C84	C85	C86	111.1(15)
C36	C35	C40	120.0		C84	C85	C90	128.8(15)
C40	C35	C34	118.7(14)		C86	C85	C90	120.0
C35	C36	C37	120.0		C85	C86	C87	120.0
C38	C37	C36	120.0		C86	C87	C88	120.0
C37	C38	O41	115.8(15)		C89	C88	C87	120.0
C39	C38	C37	120.0		O91	C88	C87	116.3(18)
C39	C38	O41	123.8(15)		O91	C88	C89	123.1(18)
C38	C39	C40	120.0		C90	C89	C88	120.0
C39	C40	C35	120.0		C89	C90	C85	120.0

Table 7

A	B	C	D	Angle/°		A	B	C	D	Angle/°
C1	C2	C3	C4	0.0		C51	C52	C53	C54	0.0
C1	C2	C3	C13	171(3)		C51	C52	C53	C63	172.9(16)
C1	C12	C11	C6	152(4)		C51	C62	C61	C56	179.0(17)
C1	C12	C11	C10	-27(6)		C51	C62	C61	C60	-1(3)
C1	C22	C23	C24	176(2)		C51	C72	C73	C74	-178(2)
C1	C22	C23	O42	-23(3)		C51	C72	C73	O92	14(2)
C1	C22	C33	C25	-175(3)		C51	C72	C83	C75	178(2)
C1	C22	C33	C32	11(4)		C51	C72	C83	C82	2(3)
C2	C1	C12	C4	0.0		C52	C51	C62	C54	0.0

C2	C1	C12	C11	-155(5)		C52	C51	C62	C61	-180(2)
C2	C1	C22	C23	142.9(16)		C52	C51	C72	C73	-144.8(14)
C2	C1	C22	C33	-42(3)		C52	C51	C72	C83	38(2)
C2	C3	C4	C12	0.0		C52	C53	C54	C62	0.0
C2	C3	C4	N5	157(3)		C52	C53	C54	N55	-178(3)
C2	C3	C13	C14	173(7)		C52	C53	C63	C64	175.7(18)
C3	C4	C12	C1	0.0		C53	C54	C62	C51	0.0
C3	C4	C12	C11	171(2)		C53	C54	C62	C61	179.7(16)
C3	C4	N5	C6	-169.2(19)		C53	C54	N55	C56	-179.1(18)
C3	C13	C14	C15	-150(7)		C53	C63	C64	C65	-173.7(14)
C3	C13	C14	C19	32(10)		C53	C63	C64	C69	11(3)
C4	C3	C13	C14	-19(11)		C54	C53	C63	C64	-14(3)
C4	C12	C11	C6	-5.0(15)		C54	C62	C61	C56	-0.5(16)
C4	C12	C11	C10	176.5(16)		C54	C62	C61	C60	179.8(17)
C4	N5	C6	C11	9(2)		C54	N55	C56	C61	-4(3)
C4	N5	C6	C7	-175(2)		C54	N55	C56	C57	178.7(14)
C12	C1	C2	C3	0.0		C62	C51	C52	C53	0.0
C12	C1	C2	O21	-172(3)		C62	C51	C52	O71	169.7(16)
C12	C1	C22	C23	-36(3)		C62	C51	C72	C73	34(2)
C12	C1	C22	C33	139.2(18)		C62	C51	C72	C83	-143.8(14)
C12	C4	N5	C6	-15(3)		C62	C54	N55	C56	3(2)
C12	C11	C6	N5	-2(2)		C62	C61	C56	N55	3(2)
C12	C11	C6	C7	-179(2)		C62	C61	C56	C57	-179.8(15)

C12	C11	C10	C9	178(2)		C62	C61	C60	C59	180(2)
N5	C4	C12	C1	-158(3)		N55	C54	C62	C51	178.5(18)
N5	C4	C12	C11	12(2)		N55	C54	C62	C61	-2(2)
N5	C6	C7	C8	-175(3)		N55	C56	C57	C58	177(2)
C11	C6	C7	C8	0.0		C61	C56	C57	C58	0.0
C6	C11	C10	C9	0.0		C56	C61	C60	C59	0.0
C6	C7	C8	C9	0.0		C56	C57	C58	C59	0.0
C7	C8	C9	C10	0.0		C57	C58	C59	C60	0.0
C8	C9	C10	C11	0.0		C58	C59	C60	C61	0.0
C10	C11	C6	N5	176(2)		C60	C61	C56	N55	-177(2)
C10	C11	C6	C7	0.0		C60	C61	C56	C57	0.0
C13	C3	C4	C12	-170(4)		C63	C53	C54	C62	-171(2)
C13	C3	C4	N5	-12(4)		C63	C53	C54	N55	12(3)
C13	C14	C15	C16	-177(4)		C63	C64	C65	C66	-176(2)
C13	C14	C19	C18	178(4)		C63	C64	C69	C68	175(2)
C14	C15	C16	C17	0.0		C64	C65	C66	C67	0.0
C15	C14	C19	C18	0.0		C65	C64	C69	C68	0.0
C15	C16	C17	C18	0.0		C65	C66	C67	C68	0.0
C15	C16	C17	O20	-167(5)		C65	C66	C67	O70	-159(3)
C16	C17	C18	C19	0.0		C66	C67	C68	C69	0.0
C17	C18	C19	C14	0.0		C67	C68	C69	C64	0.0
C19	C14	C15	C16	0.0		C69	C64	C65	C66	0.0
O20	C17	C18	C19	165(5)		O70	C67	C68	C69	160(3)
O21	C2	C3	C4	172(3)		O71	C52	C53	C54	-168.4(18)
O21	C2	C3	C13	-17(4)		O71	C52	C53	C63	5(2)
C22	C1	C2	C3	-179(2)		C72	C51	C52	C53	178.8(19)

C22	C1	C2	O21	9(4)		C72	C51	C52	O71	-11(2)
C22	C1	C12	C4	179(2)		C72	C51	C62	C54	-179(2)
C22	C1	C12	C11	24(6)		C72	C51	C62	C61	2(3)
C22	C23	C24	C25	0.0		C72	C73	C74	C75	0.0
C22	C23	C24	C34	175(2)		C72	C73	C74	C84	172.0(19)
C22	C33	C32	C27	177(2)		C72	C83	C82	C77	172(2)
C22	C33	C32	C31	-3(4)		C72	C83	C82	C81	-15(3)
C23	C22	C33	C25	0.0		C73	C72	C83	C75	0.0
C23	C22	C33	C32	-173(3)		C73	C72	C83	C82	-175(3)
C23	C24	C25	C33	0.0		C73	C74	C75	C83	0.0
C23	C24	C25	N26	170(5)		C73	C74	C75	N76	166(2)
C23	C24	C34	C35	175(2)		C73	C74	C84	C85	-179(2)
C24	C25	C33	C22	0.0		C74	C75	C83	C72	0.0
C24	C25	C33	C32	176(2)		C74	C75	C83	C82	177.5(15)
C24	C25	N26	C27	-177(2)		C74	C75	N76	C77	-174.2(15)
C24	C34	C35	C36	-169(2)		C74	C84	C85	C86	-172.5(19)
C24	C34	C35	C40	6(3)		C74	C84	C85	C90	11(4)
C25	C24	C34	C35	-11(4)		C75	C74	C84	C85	-10(4)
C25	C33	C32	C27	4.0(17)		C75	C83	C82	C77	-3.6(14)
C25	C33	C32	C31	-176.2(16)		C75	C83	C82	C81	169.7(15)
C25	N26	C27	C32	9(4)		C75	N76	C77	C82	6.2(18)
C25	N26	C27	C28	-179.5(19)		C75	N76	C77	C78	-169.9(13)
C33	C22	C23	C24	0.0		C83	C72	C73	C74	0.0

C33	C22	C23	O42	161(3)		C83	C72	C73	O92	-168.4(19)
C33	C25	N26	C27	-6(4)		C83	C75	N76	C77	-9(2)
C33	C32	C27	N26	-8(3)		C83	C82	C77	N76	-1.5(15)
C33	C32	C27	C28	179.8(18)		C83	C82	C77	C78	174.7(14)
C33	C32	C31	C30	-180(3)		C83	C82	C81	C80	-172(2)
N26	C25	C33	C22	-174(3)		N76	C75	C83	C72	-169.2(19)
N26	C25	C33	C32	1(3)		N76	C75	C83	C82	8(2)
N26	C27	C28	C29	-171(3)		N76	C77	C78	C79	176.0(17)
C32	C27	C28	C29	0.0		C82	C77	C78	C79	0.0
C27	C32	C31	C30	0.0		C77	C82	C81	C80	0.0
C27	C28	C29	C30	0.0		C77	C78	C79	C80	0.0
C28	C29	C30	C31	0.0		C78	C79	C80	C81	0.0
C29	C30	C31	C32	0.0		C79	C80	C81	C82	0.0
C31	C32	C27	N26	172(3)		C81	C82	C77	N76	-176.2(16)
C31	C32	C27	C28	0.0		C81	C82	C77	C78	0.0
C34	C24	C25	C33	-175(2)		C84	C74	C75	C83	-170(2)
C34	C24	C25	N26	-4(5)		C84	C74	C75	N76	-3(3)
C34	C35	C36	C37	176(2)		C84	C85	C86	C87	-177(2)
C34	C35	C40	C39	-176(2)		C84	C85	C90	C89	176(2)
C35	C36	C37	C38	0.0		C85	C86	C87	C88	0.0
C36	C35	C40	C39	0.0		C86	C85	C90	C89	0.0
C36	C37	C38	C39	0.0		C86	C87	C88	C89	0.0
C36	C37	C38	O41	173(2)		C86	C87	C88	O91	-171(2)
C37	C38	C39	C40	0.0		C87	C88	C89	C90	0.0

C38	C39	C40	C35	0.0		C88	C89	C90	C85	0.0
C40	C35	C36	C37	0.0		C90	C85	C86	C87	0.0
O41	C38	C39	C40	-173(2)		O91	C88	C89	C90	170(2)
O42	C23	C24	C25	-163(2)		O92	C73	C74	C75	168(2)
O42	C23	C24	C34	13(2)		O92	C73	C74	C84	-20(2)

Notes

It is noted that the crystal was found to be multiply twinned within the processing of collected diffraction data, and adequate procedures implemented by the software manufacturer had to be used. Structure model refinement parameters (R_1, wR_2, GoF) (Table 4) are far from satisfactory, but the fact that the original model obtained during phase problem solving agreed with expectations and was chemically consistent strongly suggested that the assumed structure model was correct. In addition, the model had relatively stable behavior during refinement, which means that no model disintegration occurred, even though this frequently occurs whenever a structure model does not correspond to reality.

The proposed structure model assumes lattice symmetry consistent with the Pc space group. The asymmetric unit contains two molecules of the analyzed compound (Figure 8a).

Each of the molecules consists of two largely coplanar fragments. Therefore, torsion angles to a significant degree determine the conformation of the analyzed molecules: C2-C1-C22-C23 -142.9(16)° and C52-C51-C72-C73 -144.8(14)°, respectively.

The distance of 2.71 Å between the O20 and O70 terminal hydroxyl oxygen atoms suggests a hydrogen bond between the atoms.

Two C101 and C102 atoms not bound covalently are noted in the model, preliminarily classified as carbon atoms. These could be artifacts resulting from the quality of obtained data, but their distances from hydroxyl oxygen atoms (O41-C101 2.679 Å and O91-C102 2.734 Å) suggest that hydrogen bonds could be present in this location. It could therefore be supposed that these would be oxygen atoms found in residual solvent molecules.

Example 9

A scytonemin sample obtained according to Example 1 was dissolved in DMSO (dimethylsulfoxide) to a concentration of 1% by weight and subsequently, with a spectrophotometer used according to the standard procedures (manufacturer: Varian, model: CARY 100 Scan) absorbance was measured for two wavelengths (305 and 393 nm) in a cuvette with 1 cm thickness. The following results were obtained:

UV absorption and extinction coefficients:

- Specific extinction / 1% at 305 nm: 330
- Specific extinction / 1% to 393 nm: 730
-

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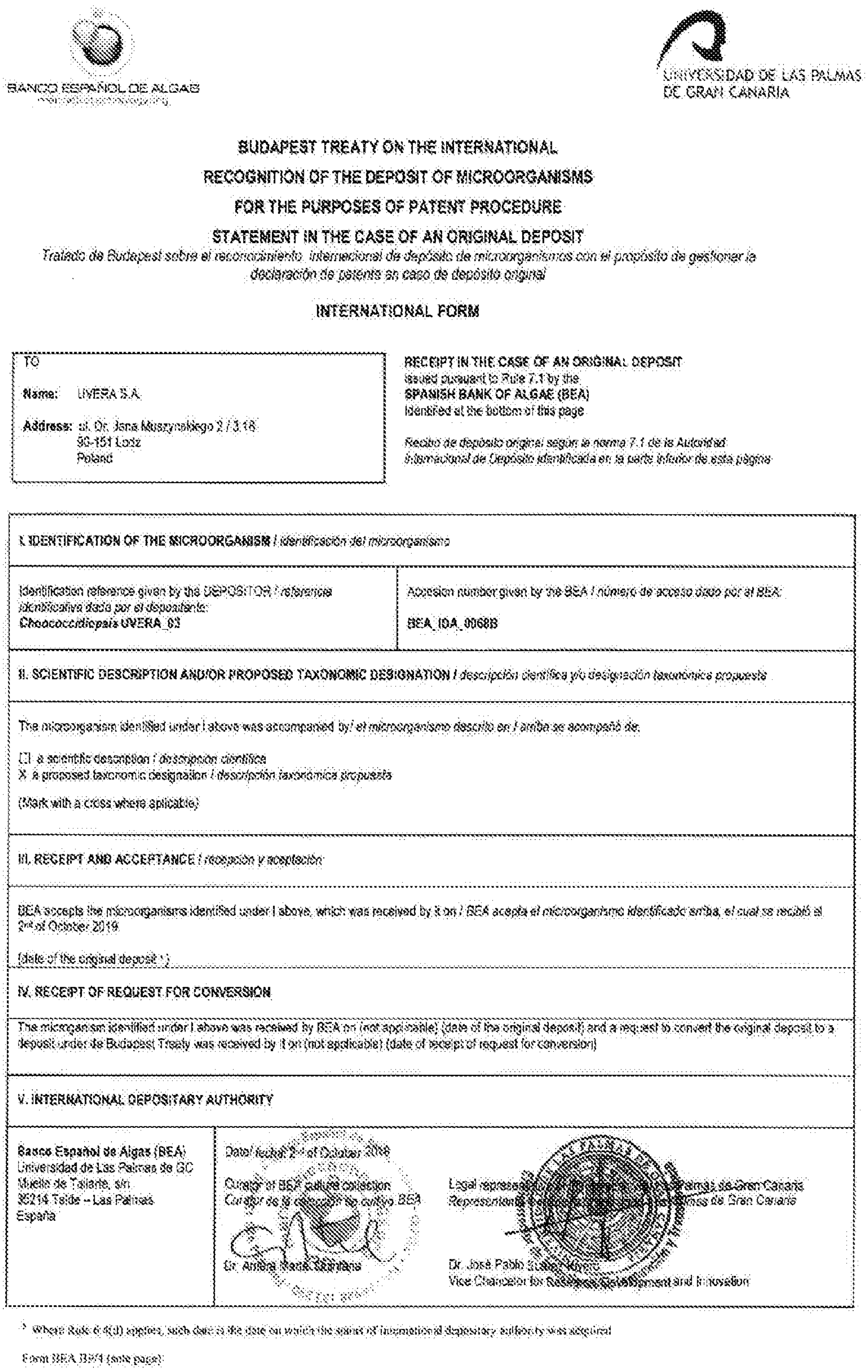


BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE
STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT
Tratado de Budapest sobre el reconocimiento internacional de depósito de microorganismos con el propósito de gestionar la declaración de patente en caso de depósito original

INTERNATIONAL FORM

TO

Name: UVERA S.A.

Address: ul. Dr. Jana Muszynskiego 2 / 3.18
90-151 Lodz
Poland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
SPANISH BANK OF ALGAE (BEA)
identified at the bottom of this page

Recibo de depósito original según la norma 7.1 de la Autoridad Internacional de Depósito identificada en la parte inferior de esta página

I. IDENTIFICATION OF THE MICROORGANISM / *identificación del microorganismo*

Identification reference given by the DEPOSITOR / *referencia identificativa dada por el depositante:* ***Chroococcidiopsis* UVERA_03**	Accesion number given by the BEA / *número de acceso dado por el BEA:* **BEA_IDA_0068B**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION / *descripción científica y/o designación taxonómica propuesta*

The microorganism identified under I above was accompanied by / *el microorganismo descrito en I arriba se acompañó de:*

[ ] a scientific description / *descripción científica*
X a proposed taxonomic designation / *descripción taxonómica propuesta*

(Mark with a cross where aplicable)

III. RECEIPT AND ACCEPTANCE / *recepción y aceptación*

BEA accepts the microorganisms identified under I above, which was received by it on / *BEA acepta el microorganismo identificado arriba, el cual se recibió el* 2nd of October 2019

(date of the original deposit ¹)

IV. RECEIPT OF REQUEST FOR CONVERSION

The microrganism identified under I above was received by BEA on (not applicable) (date of the original deposit) and a request to convert the original deposit to a deposit under de Budapest Treaty was received by it on (not applicable) (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Banco Español de Algas (BEA)
Universidad de Las Palmas de GC
Muelle de Taliarte, s/n
35214 Telde – Las Palmas
España

Date/ *fecha*: 2nd of October 2019

Curator of BEA culture collection
Curador de la colección de cultivo BEA

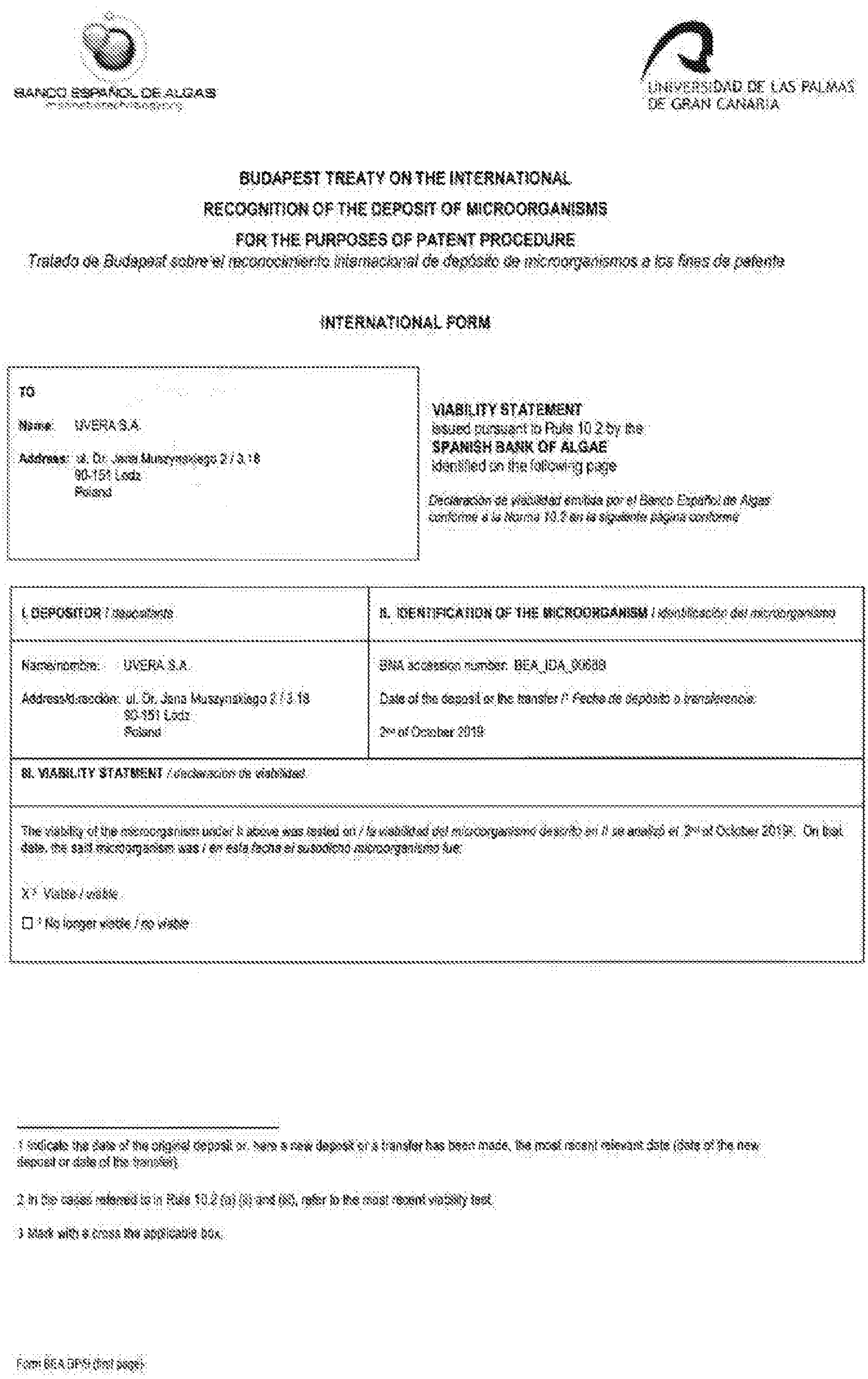
Dr. Antera Martel Quintana

Legal representative [illegible] Las Palmas de Gran Canaria
Representante [illegible] *Las Palmas de Gran Canaria*

Dr. José Pablo Suárez Rivero
Vice Chancellor for Research, Development and Innovation

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired

Form BEA BP/4 (sole page)



BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

BUDAPEST TREATY ON THE INTERNATIONAL
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Tratado de Budapest sobre el reconocimiento internacional de depósito de microorganismos a los fines de patente

INTERNATIONAL FORM

TO

Name: UVERA S.A.

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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
SPANISH BANK OF ALGAE
identified on the following page

Declaración de viabilidad emitida por el Banco Español de Algas conforme a la Norma 10.2 en la siguiente página conforme

I. DEPOSITOR / *depositante*	**II. IDENTIFICATION OF THE MICROORGANISM** / *identificación del microorganismo*
Name/nombre: UVERA S.A. Address/dirección: ul. Dr. Jana Muszynskiego 2 / 3.18 90-151 Lodz Poland	BEA accession number: BEA_IDA_0068B Date of the deposit or the transfer[1] / *Fecha de depósito o transferencia*: 2nd of October 2019

III. VIABILITY STATMENT / *declaración de viabilidad*

The viability of the microorganism under II above was tested on / *la viabilidad del microorganismo descrito en II se analizó el* 3rd of October 2019[2]. On that date, the said microorganism was / *en esta fecha el susodicho microorganismo fue*:

X[3] Viable / *viable*

☐[3] No longer viable / *no viable*

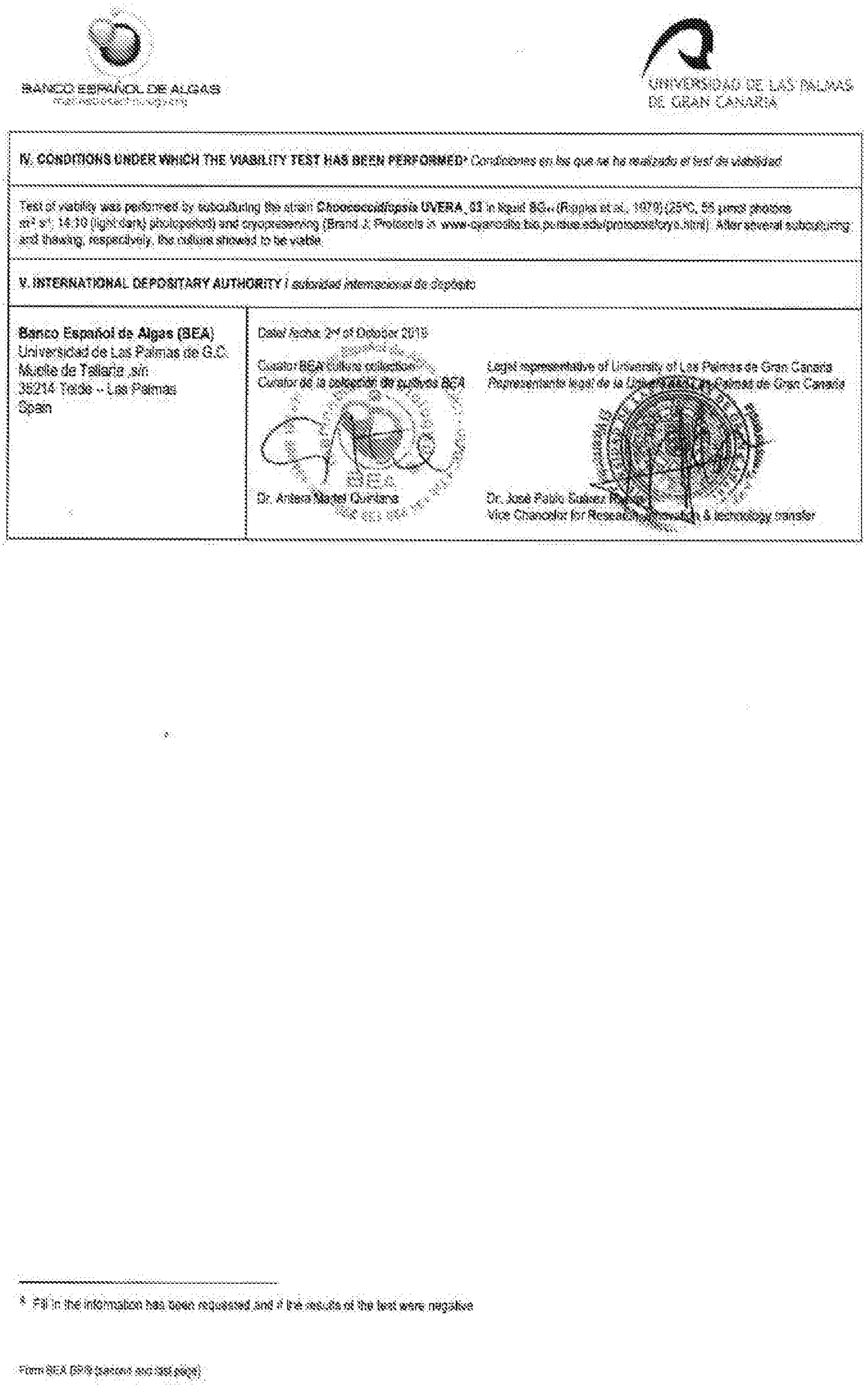
---

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2 (a) (ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BEA BPS (first page)



BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

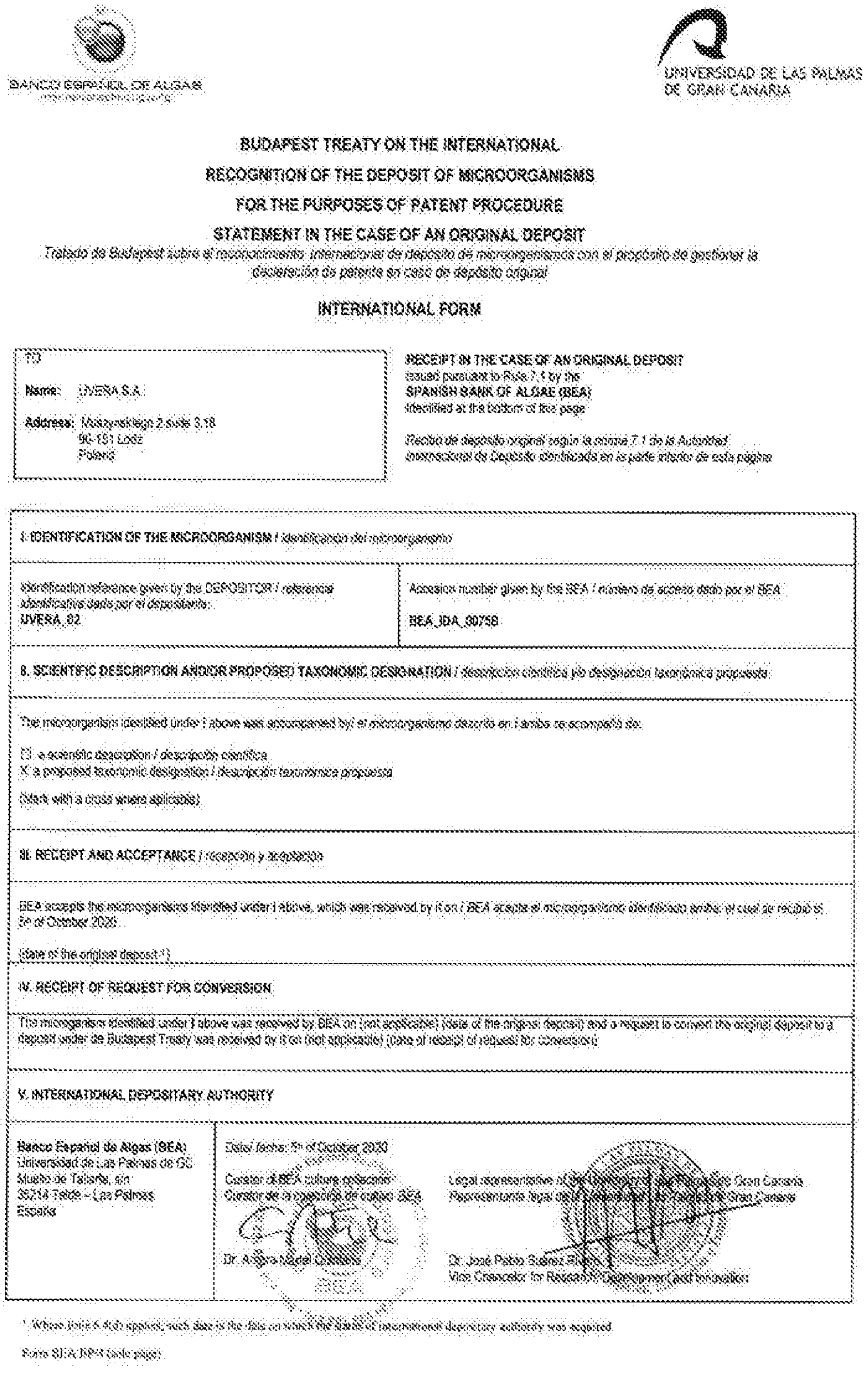
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[8] *Condiciones en las que se ha realizado el test de viabilidad*
Test of viability was performed by subculturing the strain ***Chroococcidiopsis* UVERA_03** in liquid BG_{11} (Rippka et al., 1979) (25ºC, 55 µmol photons m^{-2} s^{-1}, 14:10 (light:dark) photoperiod) and cryopreserving (Brand J. Protocols in www-cyanosite.bio.purdue.edu/protocols/cryo.html). After several subculturing and thawing, respectively, the culture showed to be viable.
V. INTERNATIONAL DEPOSITARY AUTHORITY / *autoridad internacional de depósito*

Banco Español de Algas (BEA) Universidad de Las Palmas de G.C. Muelle de Taliarte ,s/n 35214 Telde – Las Palmas Spain	Date/ *fecha*: 2nd of October 2019 Curator BEA culture collection *Curador de la colección de cultivos BEA* BEA Dr. Antera Martel Quintana	Legal representative of University of Las Palmas de Gran Canaria *Representante legal de la Universidad de Las Palmas de Gran Canaria* Dr. José Pablo Suárez Rivero Vice Chancellor for Research, Innovation & technology transfer

---

[8] Fill in the information has been requested and if the results of the test were negative

Form BEA BP/9 (second and last page)



BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT

Tratado de Budapest sobre el reconocimiento internacional de depósito de microorganismos con el propósito de gestionar la declaración de patente en caso de depósito original

INTERNATIONAL FORM

TO

Name: UVERA S.A.

Address: Moszynskiego 2 suite 3.18
90-151 Lodz
Poland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
SPANISH BANK OF ALGAE (BEA)
identified at the bottom of this page

Recibo de depósito original según la norma 7.1 de la Autoridad Internacional de Depósito identificada en la parte inferior de esta página

I. IDENTIFICATION OF THE MICROORGANISM / *identificación del microorganismo*

Identification reference given by the DEPOSITOR / *referencia identificativa dada por el depositante:* **UVERA_82**	Accession number given by the BEA / *número de acceso dado por el BEA* **BEA_IDA_0075B**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION / *descripción científica y/o designación taxonómica propuesta*

The microorganism identified under I above was accompanied by/ *el microorganismo descrito en I arriba se acompaña de:*

☐ a scientific description / *descripción científica*
X a proposed taxonomic designation / *descripción taxonómica propuesta*

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE / *recepción y aceptación*

BEA accepts the microorganism identified under I above, which was received by it on / *BEA acepta el microorganismo identificado arriba, el cual se recibió el:* 5th of October 2020

(date of the original deposit [1])

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by BEA on (not applicable) (date of the original deposit) and a request to convert the original deposit to a deposit under de Budapest Treaty was received by it on (not applicable) (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Banco Español de Algas (BEA)
Universidad de Las Palmas de GC
Muelle de Taliarte, s/n
35214 Telde – Las Palmas
España

Date/ *fecha:* 5th of October 2020

Curator of BEA culture collection
Curador de la colección de cultivo BEA

Dr. Antera Martel Quintana

BEA

Legal representative of the University of Las Palmas de Gran Canaria
Representante legal de la Universidad de Las Palmas de Gran Canaria

Dr. José Pablo Suárez Rivero
Vice Chancellor for Research, Development and Innovation

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[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BEA/BP/4 (sole page)

BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

Tratado de Budapest sobre el reconocimiento internacional de depósito de microorganismos a los fines de patente

INTERNATIONAL FORM

TO

Name: UVERA S.A.

Address: Muszynskiego 2 suite 3.18
90-151 Lodz
Poland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
SPANISH BANK OF ALGAE
identified on the following page

Declaración de viabilidad emitida por el Banco Español de Algas conforme a la Norma 10.2 en la siguiente página conforme

I. DEPOSITOR / *depositante*	**II. IDENTIFICATION OF THE MICROORGANISM** / *identificación del microorganismo*
Name/nombre: **UVERA S.A.** Address/dirección: Muszynskiego 2 / 3.18 90-151 Lodz Poland	BNA accession number: **BEA_IDA_0075B** Date of the deposit or the transfer¹/ *Fecha de depósito o transferencia*: 5th of October 2020

III. VIABILITY STATMENT / *declaración de viabilidad*

The viability of the microorganism under II above was tested on / *la viabilidad del microorganismo descrito en II se analizó el* 2nd of October 2019². On that date, the said microorganism was / *en esta fecha el susodicho microorganismo fue*:

X ³ Viable / *viable*

☐ ³ No longer viable / *no viable*

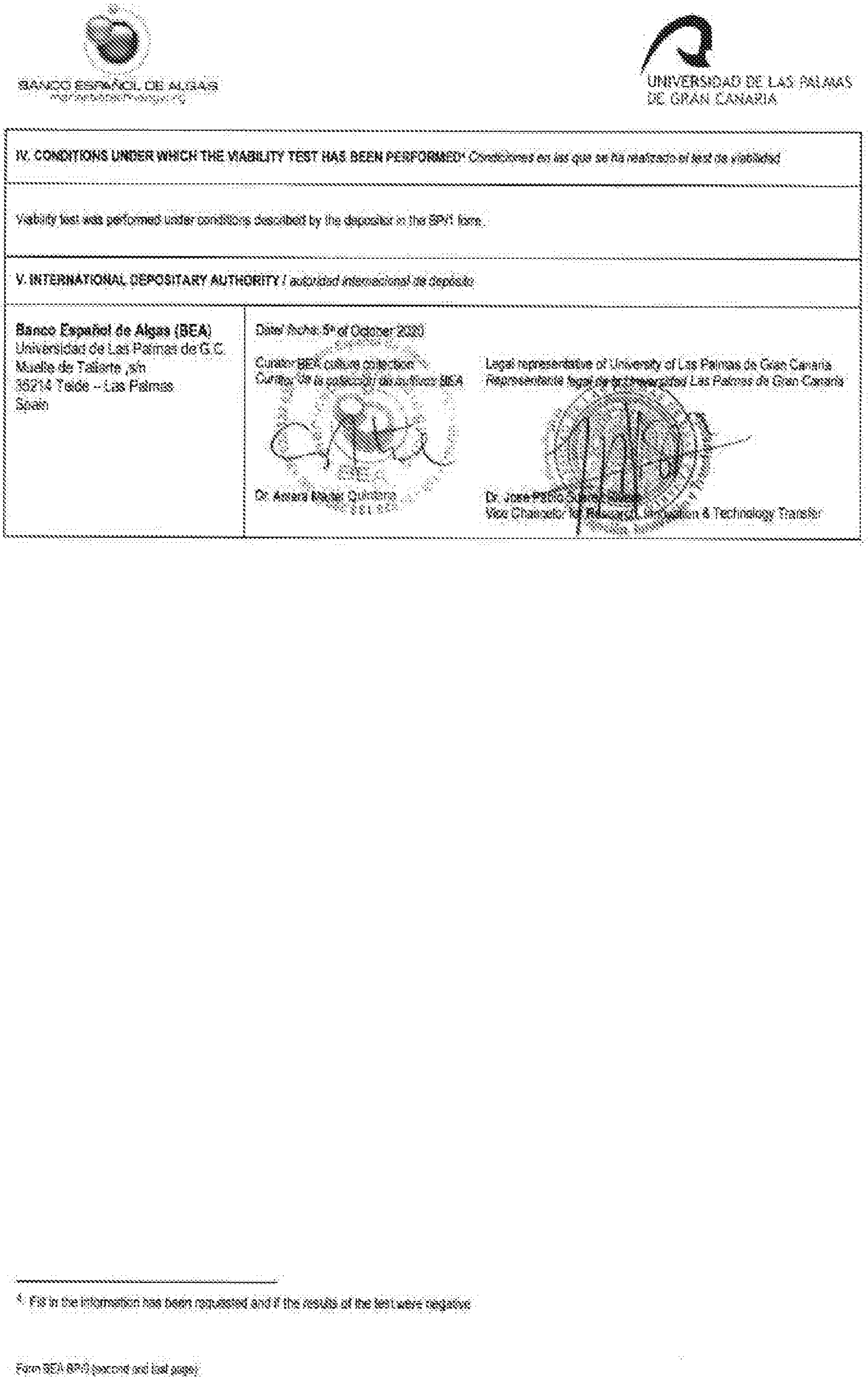
---

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2 (a) (ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

Form BEA SP/3 (first page)



BANCO ESPAÑOL DE ALGAS

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4] *Condiciones en las que se ha realizado el test de viabilidad*

Viability test was performed under conditions described by the depositor in the BP/1 form.

V. INTERNATIONAL DEPOSITARY AUTHORITY / *autoridad internacional de depósito*

Banco Español de Algas (BEA) Universidad de Las Palmas de G.C. Muelle de Taliarte ,s/n 35214 Telde – Las Palmas Spain	Date/ fecha: 5th of October 2020 Curator BEA culture collection *Curator de la colección de cultivos BEA* Dr. Antera Martel Quintana	Legal representative of University of Las Palmas de Gran Canaria *Representante legal de la Universidad Las Palmas de Gran Canaria* Dr. Jose Pablo Suárez Rivero Vice Chancellor for Research, Innovation & Technology Transfer

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[4] Fill in the information has been requested and if the results of the test were negative

Form BEA BP/9 (second and last page)

Claims

1. A process for the isolation and culture of Cyanobacteria strains, in particular that deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0068B or BEA_IDA_0075B, characterized in that it comprises:

a) preparation of a growth medium by enriching it in micro- and macronutrients found in natural sandstone originating from Nubian formations with the following contents in mass percentages: 97.6% quartz, 0.4% muscovite-biotite 1.2% apatite and 0.8% other minerals in trace quantities in the amount of 200 g per 1000 mL of an aqueous medium solution having the following composition per 1000 mL of the medium: 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \times 7H_2O$, 0.036 g $CaCl_2 \times 2H_2O$, 6.0 mg citric acid, 6.0 mg ammonium ferric citrate, 1 mg EDTA, 0.02 g Na_2CO_3, 1 mL of the A5 blend of trace metals with the following composition per 1000 mL of the aqueous A5 blend solution: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \times 4H_2O$, 0.222 g $ZnSO_4 \times 7H_2O$, 0.39 g $Na_2MoO_4 \times 2H_2O$, 0.079 g $CuSO_4 \times 5H_2O$, 49.4 mg $Co(NO_3)_2 \times 6H_2O$, subsequently stirring the resulting suspension for 24 hours at 25°C and subsequent 5-hour sedimentation at 25°C and filtration thereof;

b) collection of bacteria from the environment;

c) passaging the biological material collected in stage b) in the liquid medium obtained in stage a), i.e., according to Table 1, enriched with stone, with additional agar with end contents between 2% in the beginning and 0.5% by weight in the end, preferably in three intermediate stages of 4 weeks each of the five stages, i.e. two end stages (initial, final) and three intermediate stages, wherein the growth media in the intermediate stages contain the following quantities of additional agar: 1.75%, 1.5%, 1% by weight, respectively, with respect to the medium obtained in stage a);

d) dissolving the culture solution from final stage c), i.e. containing 0.5% agar, in the aqueous medium solution whose composition is disclosed in stage A but without addition of the stone and incubation at 25°C for 2 weeks with stirring.

2. A new bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0068B.

3. A new bacterial strain deposited in Banco Espanol de Algas Universidad de Las Palmas de GC under number BEA_IDA_0075B.

4. Use of the strain as defined in claim 2 or 3 for the manufacture of a pigment having UV absorption properties, in particular scytonemin or derivatives thereof.

5. The use of claim 4 comprises application of the resulting pigment, in particular scytonemin or derivatives thereof, for the manufacture of cosmetic products, in particular for sunscreens.

6. A medium for culturing *Cyanobacteria*, containing in 1000 mL of the aqueous medium solution 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4$ x $7H_2O$, 0.036 g $CaCl_2$ x $2H_2O$, 6.0 mg citric acid, 6.0 mg ammonium ferric citrate, 1 mg EDTA, 0.02 g Na_2CO_3, 1 mL of the A5 blend of trace metals with the following composition per 1000 mL of the aqueous A5 blend solution: 2.86 g H_3BO_3, 1.81 g $MnCl_2$ x $4H_2O$, 0,222 g $ZnSO_4$ x $7H_2O$, 0.39 g Na_2MoO_4 x $2H_2O$, 0.079 g $CuSO_4$ x $5H_2O$, 49.4 mg $Co(NO_3)_2$ x $6H_2O$, characterized in that it contains natural Nubian sandstone with the following contents in mass

percentages: 97.6% quartz, 0.4% muscovite-biotite 1.2% apatite and 0.8% other minerals in trace quantities in the amount of 200 g ground stone/ 1000 mL of the medium.

7. Scytonemin crystals having at least one property selected from the following:

- X-ray powder diffraction spectrum with characteristic peaks at 2 theta angle values of 2.500°, 4.589°, 5.062°, 8.630° and 9.197°,
- specific infrared absorption bands at 3345, 3065, 2961, 2926, 1713, 1591, 1516, 1449, 1296, 1175, 1145, 957, 932, 930, 833 [cm^{-1}] in the IR spectrum (KBr),
- decomposition temperature in a range between 365°C and 380.3°C with a peak at about 380.3°C in thermogravimetric/differential thermal analysis (heating/cooling rate: 15/20°C/min).

- ^{1}H NMR spectrum recorded in pyridine-d_5 containing signals at δ 8.98 ppm; 7.99 ppm; 7.86 ppm; 7.75 ppm; 7.48 ppm; 7.33 ppm; 7.22 ppm.

- structure model based on structural X-ray analysis (XRD) described by the geometric parameters listed in Tables 4 to 7 and presented in Figures 8a to d.

- specific extinction 1% in DMSO at 393 nm: 730 and specific extinction 1% in DMSO at 305 nm: 330

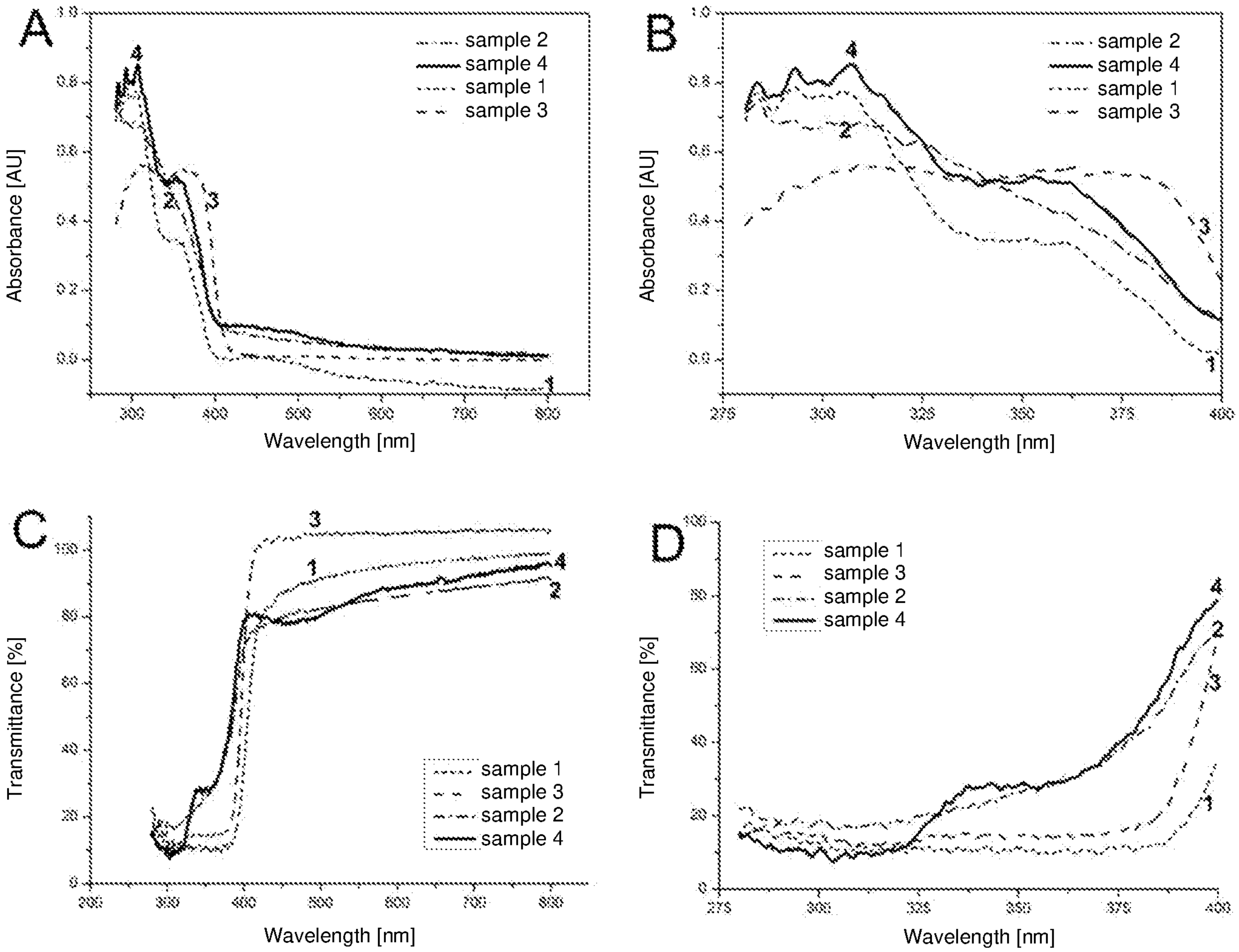

A
Absorbance [AU]
Wavelength [nm]
sample 2
sample 4
sample 1
sample 3
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800
4
2
3
1
B
Absorbance [AU]
Wavelength [nm]
sample 2
sample 4
sample 1
sample 3
1.0
0.8
0.6
0.4
0.2
0.0
275
300
325
350
375
400
4
2
3
1
C
Transmittance [%]
Wavelength [nm]
sample 1
sample 3
sample 2
sample 4
100
80
60
40
20
0
200
300
400
500
600
700
800
3
1
4
2
D
Transmittance [%]
Wavelength [nm]
sample 1
sample 3
sample 2
sample 4
100
80
60
40
20
0
275
300
325
350
375
400
4
2
3
1

Fig. 1

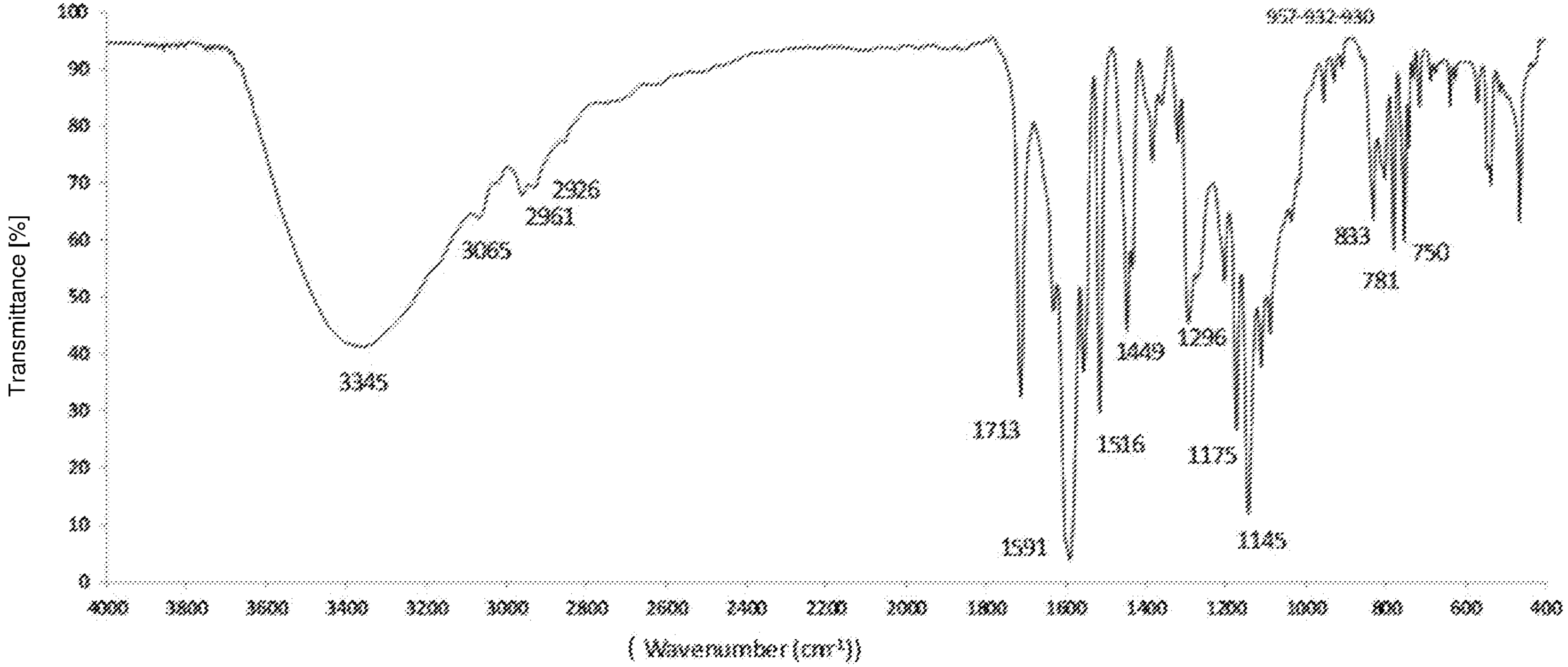

Transmittance [%]
100
90
80
70
60
50
40
30
20
10
0
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
(Wavenumber (cm⁻¹))
3345
3065
2961
2926
1713
1591
1516
1449
1296
1175
1145
957-932-930
833
781
750

Fig. 2

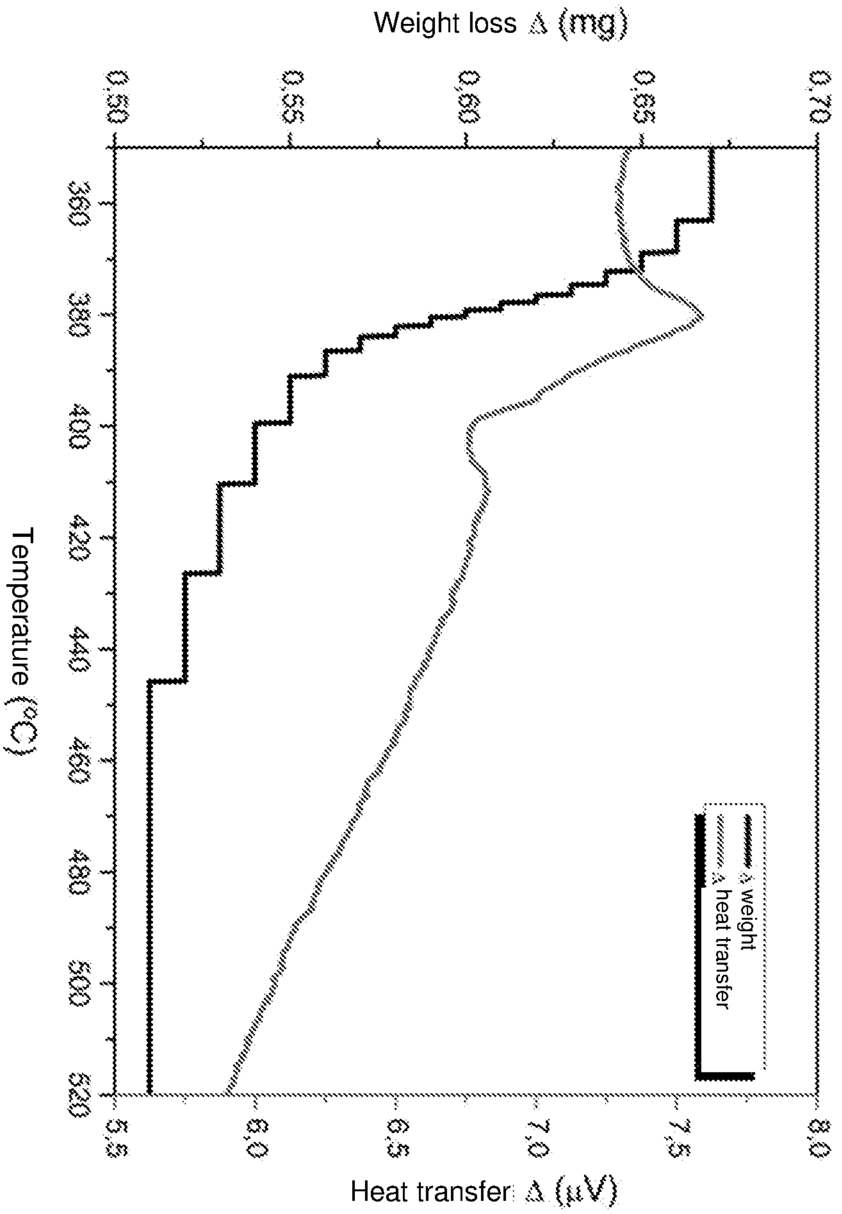
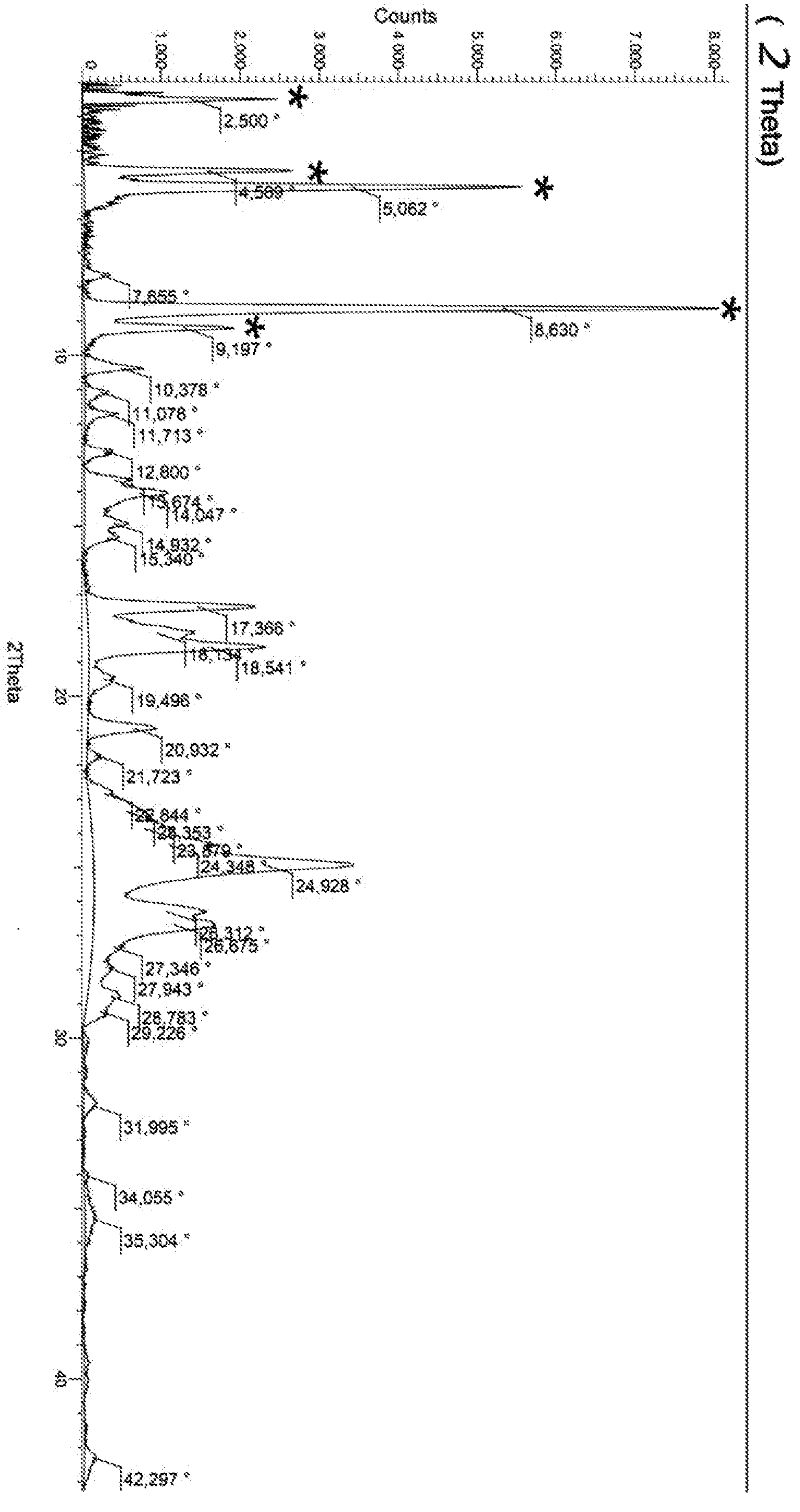

Weight loss Δ (mg)
0,50
0,55
0,60
0,65
0,70
Temperature (°C)
360
380
400
420
440
460
480
500
520
Heat transfer Δ (μV)
5,5
6,0
6,5
7,0
7,5
8,0
Δ weight
Δ heat transfer

Fig. 3


(2 Theta)
Counts
0
1.000
2.000
3.000
4.000
5.000
6.000
7.000
8.000
2Theta
10
20
30
40
*
2,500 °
*
4,569 °
*
5,062 °
7,655 °
*
8,630 °
*
9,197 °
10,378 °
11,078 °
11,713 °
12,800 °
13,674 °
14,047 °
14,932 °
15,340 °
17,366 °
18,134 °
18,541 °
19,496 °
20,932 °
21,723 °
22,844 °
23,353 °
23,879 °
24,348 °
24,928 °
26,312 °
26,675 °
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27,943 °
28,783 °
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31,995 °
34,055 °
35,304 °
42,297 °

Fig. 4

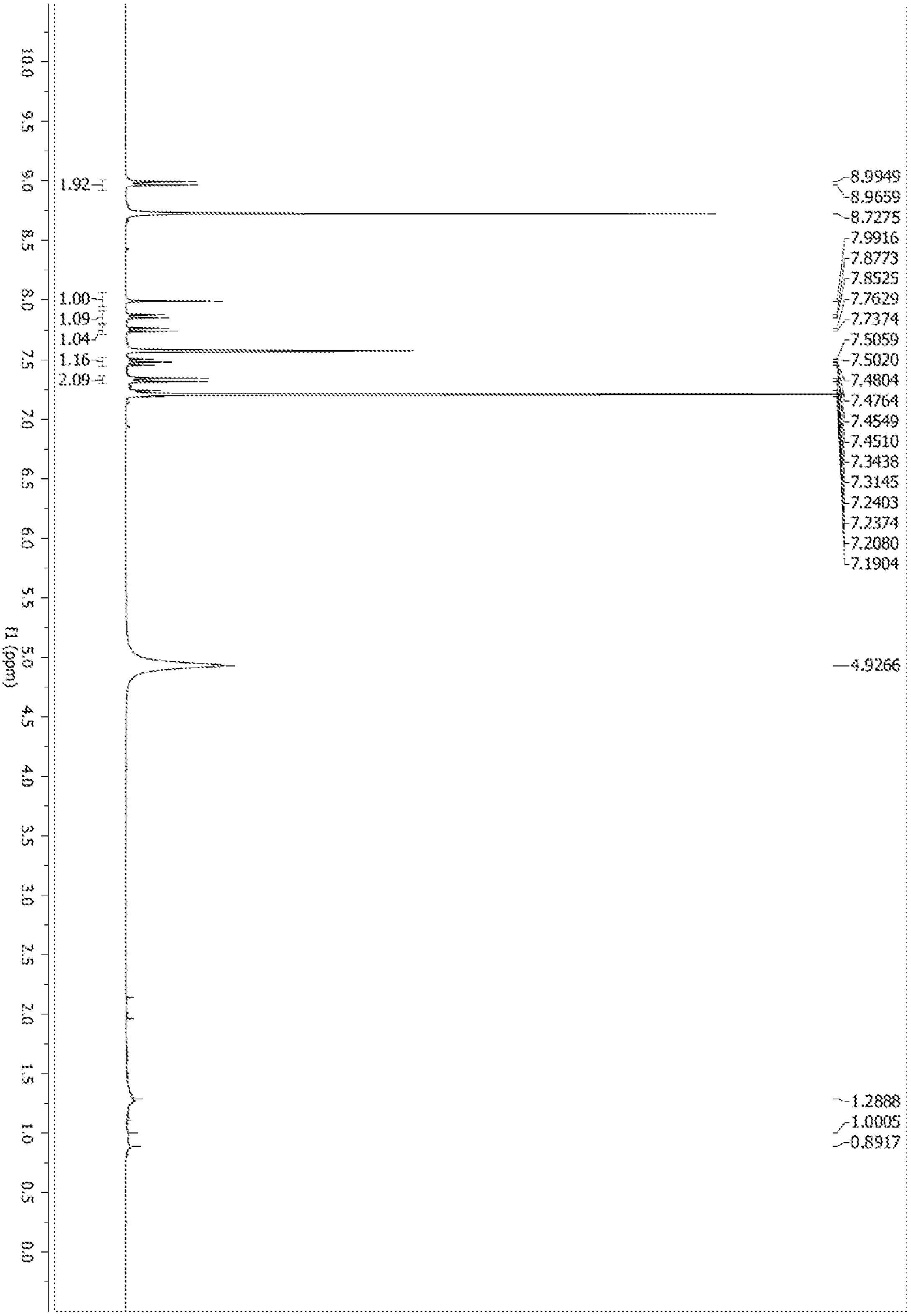
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
1.92
1.00
1.09
1.04
1.16
2.09
8.9949
8.9659
8.7275
7.9916
7.8773
7.8525
7.7629
7.7374
7.5059
7.5020
7.4804
7.4764
7.4549
7.4510
7.3438
7.3145
7.2403
7.2374
7.2080
7.1904
4.9266
1.2888
1.0005
0.8917

Fig. 5

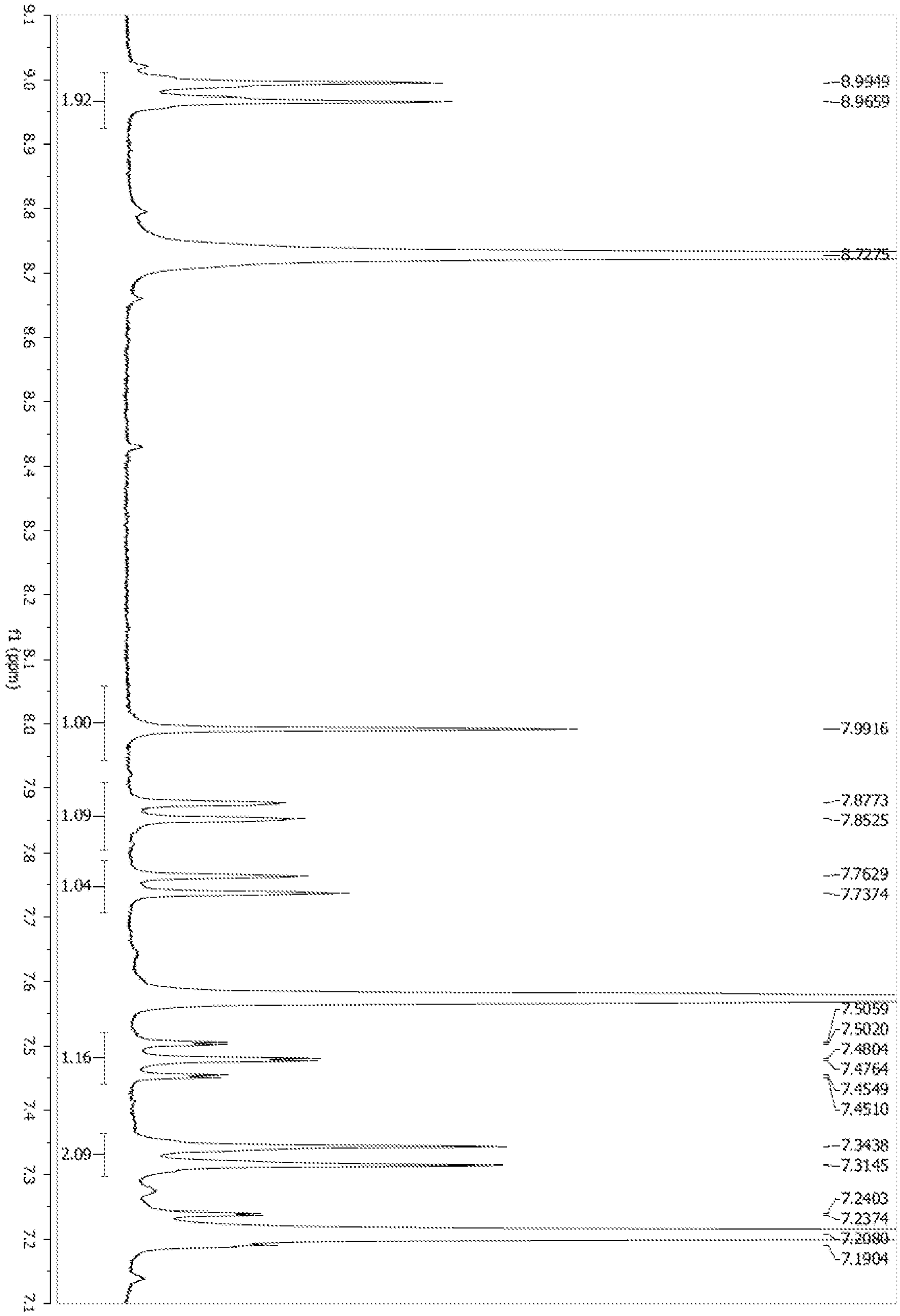
9.1
9.0
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
1.92
1.00
1.09
1.04
1.16
2.09
8.9949
8.9659
8.7275
7.9916
7.8773
7.8525
7.7629
7.7374
7.5059
7.5020
7.4804
7.4764
7.4549
7.4510
7.3438
7.3145
7.2403
7.2374
7.2080
7.1904

Fig. 5a

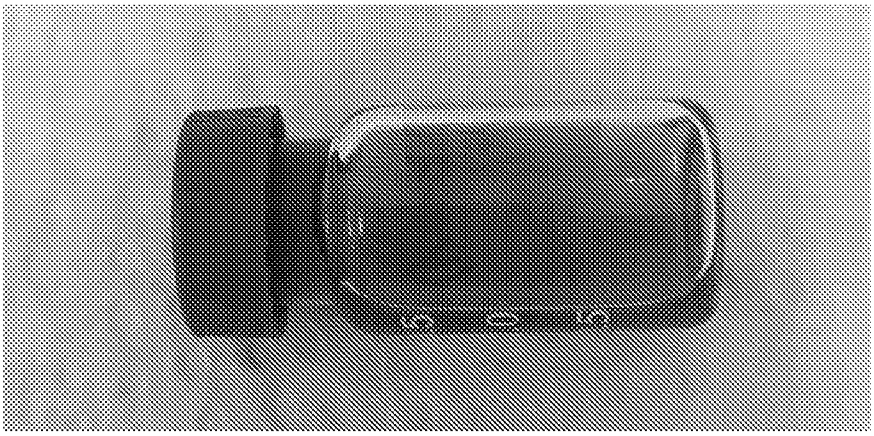

Fig. 6a

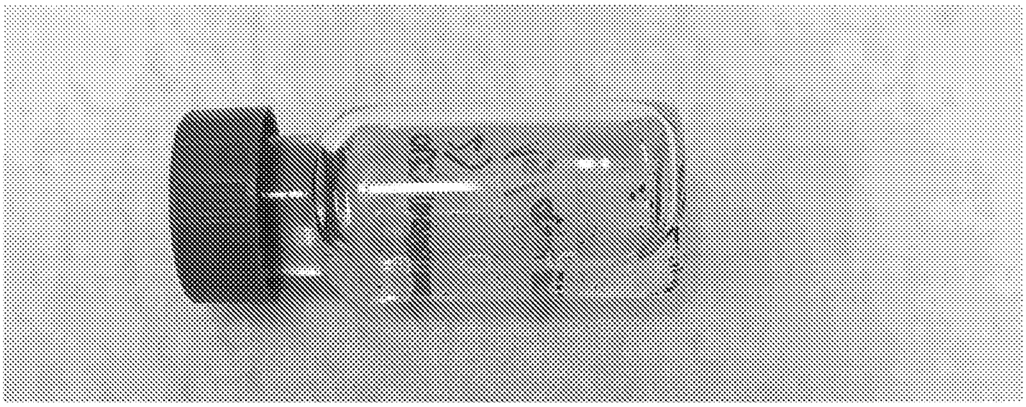

Fig. 6b

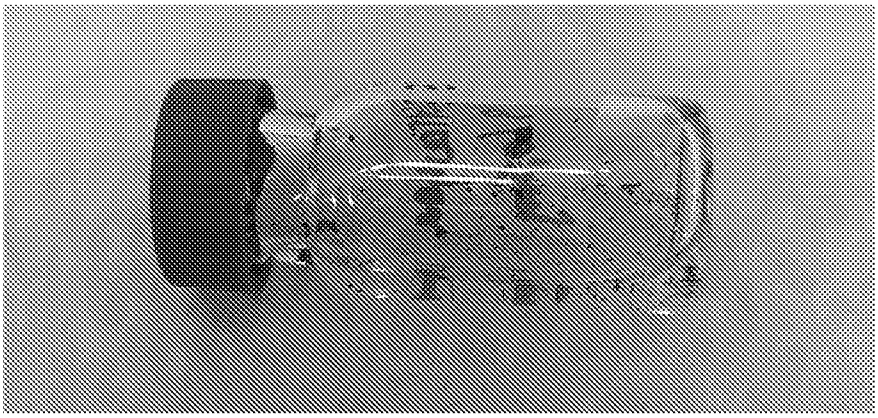

Fig. 6c

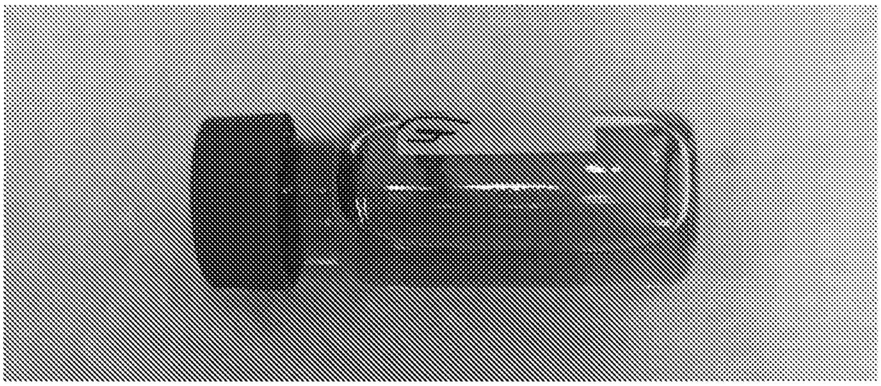

Fig. 6d

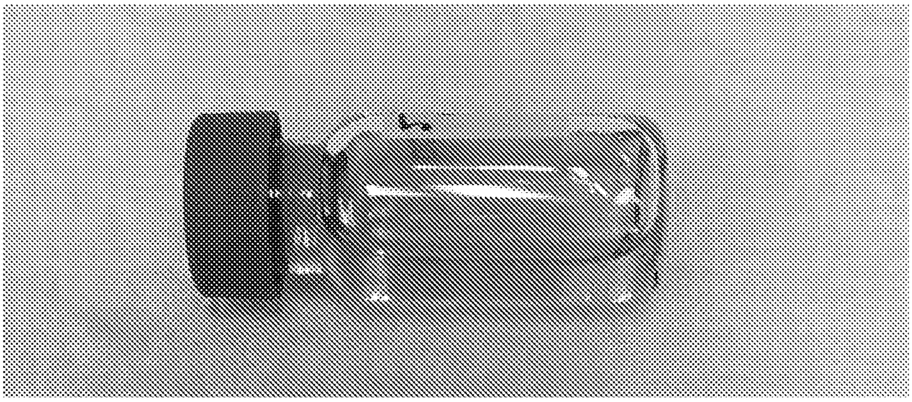

Fig. 6e

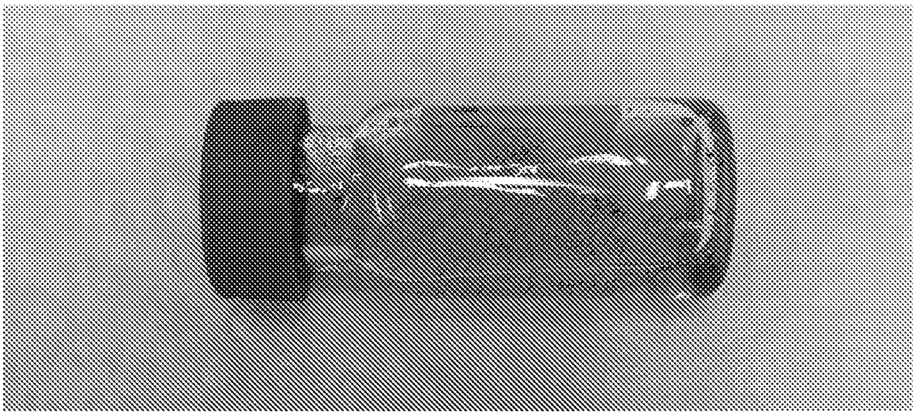

Fig. 6f

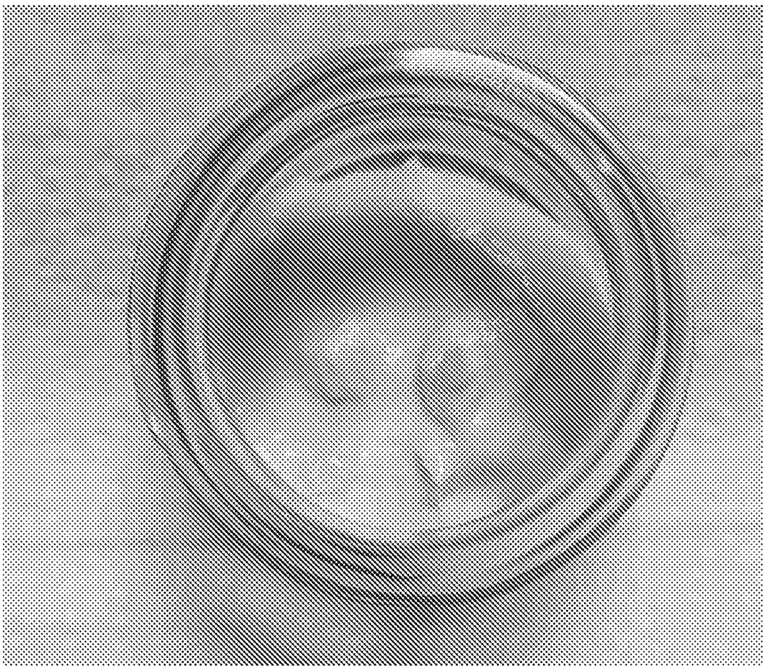

Fig. 6g

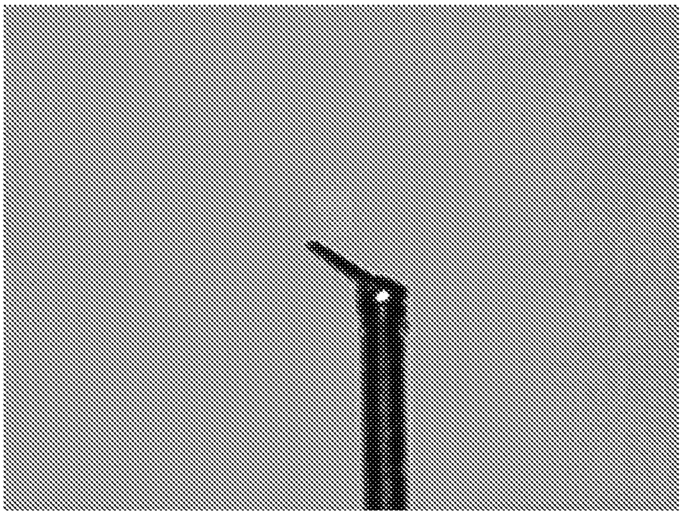

Fig. 7a

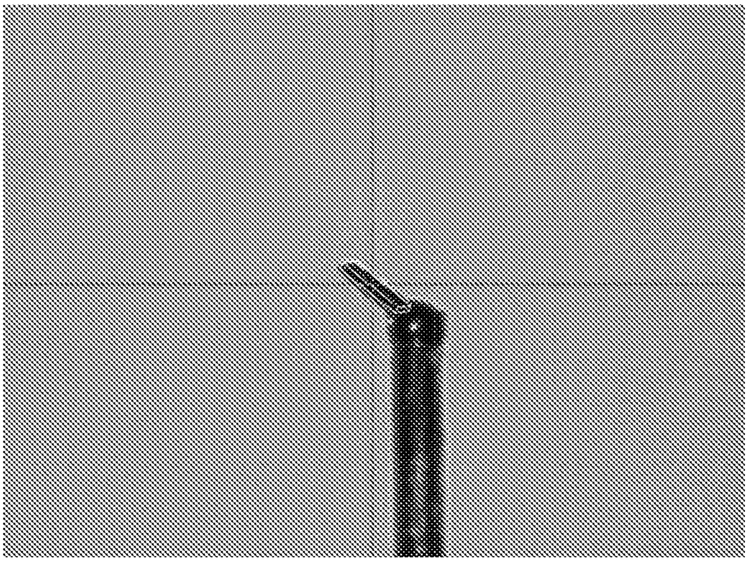

Fig. 7b

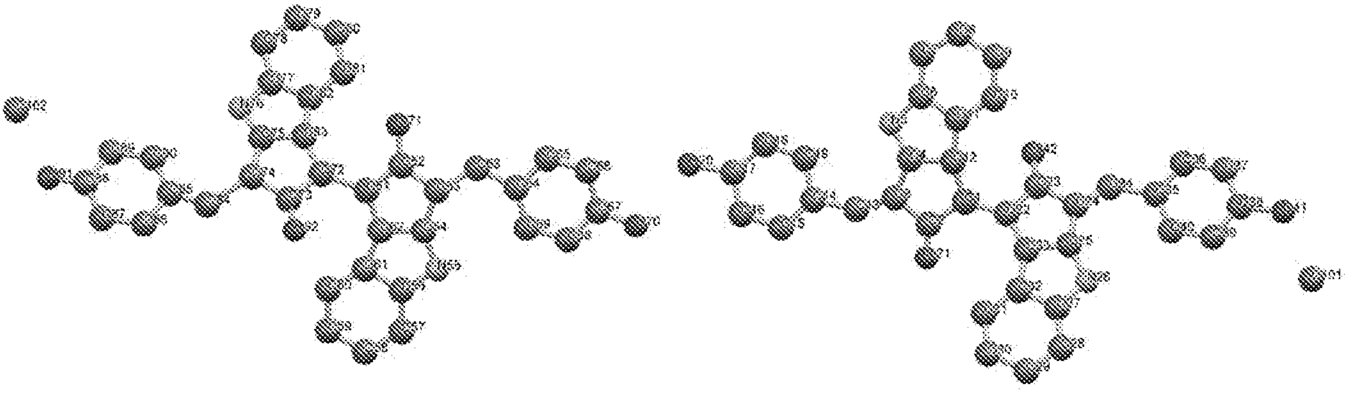

Fig. 8a

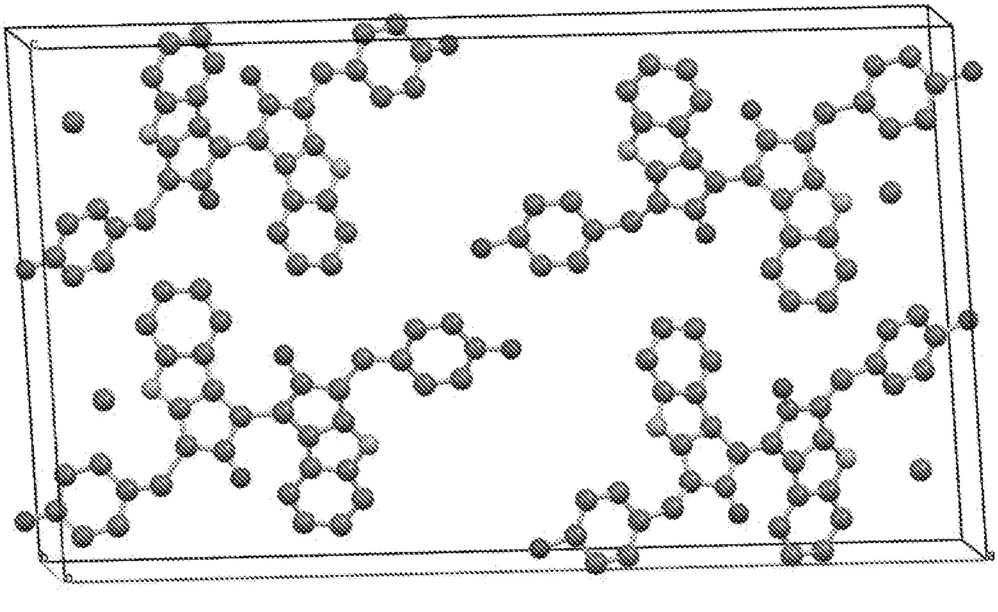

Fig. 8b

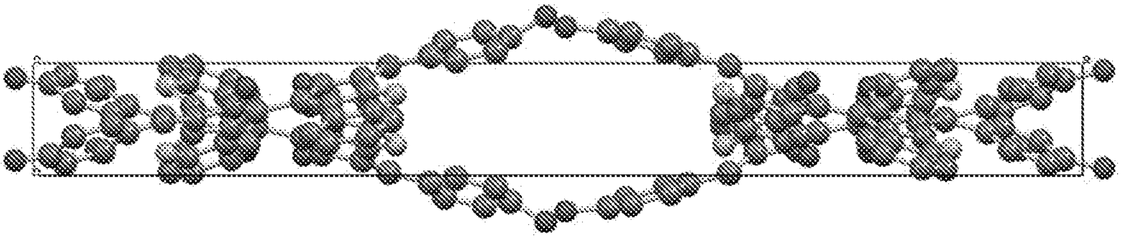

Fig. 8c

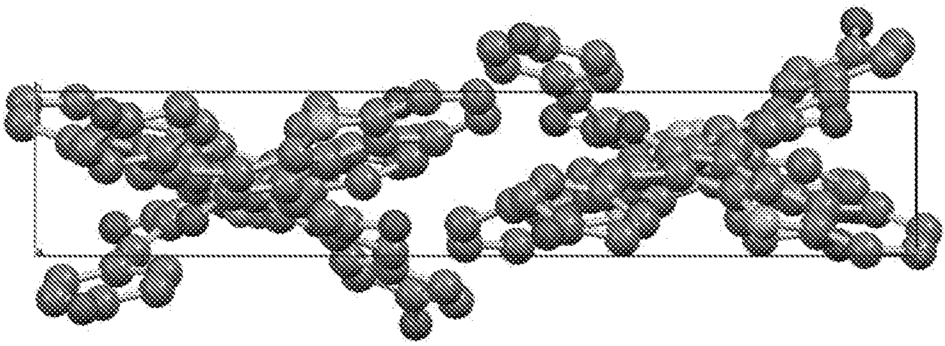

Fig. 8d

INTERNATIONAL SEARCH REPORT

International application No.
PCT/PL2022/050034

A. CLASSIFICATION OF SUBJECT MATTER

C12N1/20; C12R1/01; C12P1/04; C12P17/10; C12P17/16; A61Q17/04; C09B61/00; C07D209/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; C12R, C12P, A61Q; C09B; C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC VIA EPOQUENET, WPI VIA EPOQUENET, NPL VIA EPOQUENET, ESPACENET, INTERNET (GOOGLE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ES2668420 (A1), 2018-05-18, UNIV DE LAS PALMAS DE GRAN CANARIA [ES]; [claims, examples].	1-7
A	WO0024369 (A1), 2000-05-04, HUNER NORMAN [CA]; KROL MARIANNA [CA]; IVANOV ALEXANDER [CA]; SARHAN FATHEY [CA]; [claims, examples].	1-7
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Name and mailing address of the ISA/ Visegrad Patent Institute / Branch Office PL Al. Niepodległości 188, 00-950 Warsaw, Poland Facsimile No. +48 22 579 00 01	Authorized officer Ewa Waszkowska Telephone No. 48 22 579 02 96

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Balskus EP, Case RJ, Walsh CT. THE BIOSYNTHESIS OF CYANOBACTERIAL SUNSCREEN SCYTONEMIN IN INTERTIDAL MICROBIAL MAT COMMUNITIES. FEMS MICROBIOL ECOL. 2011 Aug;77(2):322-32; [abstract, figures].	1-7

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/PL2022/050034

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