

## FLUORESCENT COMPOUNDS

### Technical Field

This invention relates generally to novel fluorescent compounds and to compounds which can function as fluorescent dyes, methods of isolating  
5 such compounds from microorganisms and uses of such compounds in scientific applications.

### Background Art

Compounds that fluoresce have many uses and are known to be particularly suitable for biological applications where fluorescence is  
10 required for the detection of whole cells, cellular components, and cellular functions. For example, many diagnostic and analytical techniques require the samples to be fluorescently tagged so that they can be detected. This is achieved by using fluorescent dyes or probes which interact with a wide variety of materials such as cells, tissues, proteins, antibodies, enzymes,  
15 drugs, hormones, lipids, nucleotides, nucleic acids, carbohydrates, or natural or synthetic polymers to make fluorescent conjugates.

With synthetic fluorescent probes, ligands are frequently used to confer a specificity for a biochemical reaction that is to be observed and the fluorescent dye provides the means of detection or quantification of the  
20 interaction. These applications include, among others, the detection of proteins (for example in gels or aqueous solution), cell tracking, the detection of proteins via fluorescently labelled antibodies, the assessment of enzymatic activity, the staining of nucleic acids.

Long wavelength absorbance usually increases the utility of a  
25 fluorescent probe since it reduces the interference from cellular autofluorescence and reduces the cytotoxic effect of the fluorophore in combination with light. Although lasers are particularly useful as a concentrated light source for the excitation of fluorescence, at present the output of lasers is restricted to particular wavelengths of light. Compounds  
30 whose excitation spectra coincide with laser output are therefore of high utility. The argon laser is the most common light source for excitation of fluorescence, and has principal outputs, light at 488 nm and 514 nm. Fluorescent compounds that are excited by either of these wavelengths are therefore of particular utility. Alternatively, excitation of fluorescence can  
35 be achieved using solid state light sources such as Light emitting diodes.

Fluorescent compounds excited by the light emitted from these alternative sources are also of particular utility.

Red fluorescent compounds are used extensively in many fields of biological study. Many of these, including Texas red, Tetramethyl  
5 rhodamine-isothiocyanate or red emitting BODIPY dyes require excitation at green wavelengths such as 542 nm. This limits their use in many applications, especially those where the argon-ion laser is used for excitation. Compounds such as ethidium bromide, can be excited with light from the argon-ion laser (520 nm band), but are not generally suitable for tagging of  
10 organic molecules other than nucleic acids. Other compounds such as phycoerythrin, can be excited using the argon-ion laser (488 nm) and does emit in the orange/red wavelengths. Phycoerythrin, however, has poor stability and a high molecular weight making it unsuitable for many applications such as cell tracking, labelling of nucleic acids or staining  
15 proteins.

For staining of proteins, there are a number of methods available. These methods can utilise non-fluorescent compounds, or fluorescent compounds. The most commonly used method utilises Coomassie blue which is non-fluorescent, can require the use of large amounts of organic  
20 solvents and is time consuming. Other fluorescence-based protein-detection methods are available which are potentially more sensitive than non-fluorescent methods. However, these methods are in general much more expensive than non-fluorescent methods which limits their widespread use. Therefore, compounds that combine useful spectral characteristics, and  
25 relatively high sensitivity will be of particular utility.

There are several methods for the quantification of protein in solution. These methods are based on a range of techniques, and include methods where dyes bind to soluble proteins. These dyes can be either non-fluorescent or fluorescent compounds. Fluorescent dye-based methods are  
30 often more sensitive than the non-fluorescent dyes, and allow for the determination of protein concentration over a wide range of concentrations. Compounds that combine useful spectral characteristics with an ability to bind proteins will be of particular utility.

In enzymatic studies, there is widespread use of fluorescent  
35 compounds for the detection of particular enzymatic activities. For example, fluorescein di- $\beta$ -D-galactopyranoside (FDG) is a non-fluorescent compound

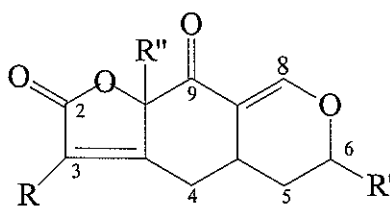
that is sequentially hydrolysed by the enzyme  $\beta$ -galactosidase first to generate fluorescein monogalactoside and then to fluorescein which is highly fluorescent. The cleavage of the FDG compound can be monitored by the increase in fluorescence in the solution, and thus allows sensitive quantification of enzymatic activity. At present, only  
5 a limited number of fluorophores are suitable for this procedure. Therefore, novel fluorescent compounds that can be conjugated to a variety of substrates will be of utility.

For dual colour staining, there is a very limited choice of low molecular weight fluorophores. The predominant green fluorophore is fluorescein, which strongly absorbs light from the 488 nm band of the argon ion laser, and re-emits at 518 nm. At present  
10 there are few compatible red or orange fluorophores that are of low molecular weight and are excited by the 488 nm or 514 nm bands of the argon ion laser. Therefore, low molecular weight compounds that are excited by argon ion lasers and emit at wavelengths greater than 600 nm will be of utility, particularly if there is minimal spectral overlap with fluorescein.

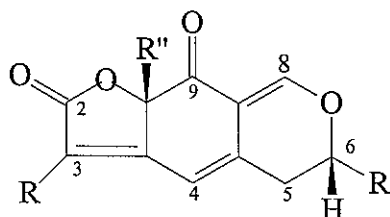
15 The present inventors have isolated new compounds derived from a fungus that are capable of combining readily with a range of organic molecules to produce fluorescent complexes.

### Disclosure of Invention

In a first aspect, there is provided a compound according to formulae:

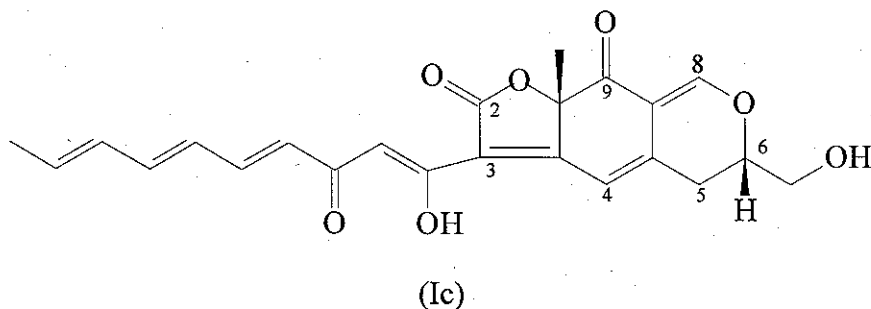


(Ia) or



(Ib)

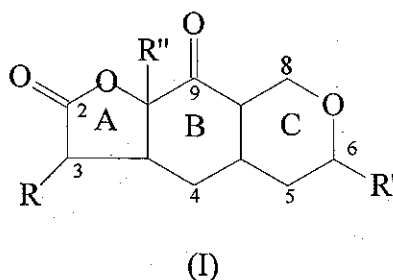
wherein R is  $-\text{C}(\text{OH})\text{CHC}(\text{O})(\text{CH})_6\text{CH}_3$ , R' is  $-\text{CH}_2\text{OH}$  and R'' is Me, being the compound 5,6-dihydro-3-[(1Z, 4E, 6E, 8E)-1-hydroxy-3-oxo-1,4,6,8-decatetraenyl]-6-hydroxymethyl-9a-methyl-2H-furo [3,2-g][2]benzopyran-2-9(9aH)-dione according to formula (Ic), including tautomers thereof:



and the compound is capable of interacting with an organic compound so as to fluoresce after excitation.

Preferably, the compound is excited at wavelengths in the range of 300-560 nm, more preferably, 380-530 nm and even more preferably, UV wavelengths and/or blue wavelengths. Preferably the compound emits in the wavelengths of 460 to 700 nm. More preferably its emission peak is centered around 530 nm when interacting with an organic compound such as sodium dodecyl sulphate and centered around 605 nm when interacting with a biomolecule such as a protein or cell.

In a second aspect, there is provided a compound according to formula (I):



wherein each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched

chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo groups;

rings A, B and C are optionally substituted with one or more halogen atoms; and

rings A, B and C optionally include one or more double bonds;

5 with the proviso that:

(i)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = -CH_2OH$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a;

10 (ii)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a;

(iii)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

15 (iv)  $R \neq -C(O)(CH_2)_4Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

20 (v)  $R \neq -C(O)(CH_2)_6Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(vi)  $R \neq -C(O)(CH_2)_5Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

25 (vii)  $R \neq -C(O)(CH_2)_6Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(viii)  $R \neq -Ac$  when  $R' = -(CH)_2C(Me)CHCH(Me)(Et)$ ,  $R'' = Me$ ,  $C4=C1$ , ring A

includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

5 (ix)  $R \neq \text{Ac}$  when  $R' = (\text{CH})_2\text{C}(\text{Me})\text{CHCH}(\text{Me})(\text{Et})$ ,  $R'' = \text{Me}$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(x)  $R \neq \text{Ac}$  when  $R' = (\text{CH})_2\text{C}(\text{Me})\text{CH-}i\text{-propyl}$ ,  $R'' = \text{Me}$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

10 (xi)  $R \neq \text{C}(\text{O})(\text{CH})_4\text{Me}$  when  $R' = (\text{CH})_2\text{Me}$ ,  $R'' = \text{Me}$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(xii)  $R \neq \text{C}(\text{O})(\text{CH})_4\text{Me}$  when  $R' = n\text{-propyl}$ ,  $R'' = \text{Me}$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

15 (xiii)  $R \neq \text{C}(\text{O})(\text{CH})_4\text{Me}$  when  $R' = n\text{-propyl}$ ,  $R'' = \text{Me}$ , rings A, B and C do not include double bonds.

(xiv)  $R \neq \text{C}(\text{O})(\text{CH})_6\text{Me}$  when  $R' = (\text{CH})_2\text{Me}$ ,  $R'' = \text{Me}$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

20 (xv)  $R \neq \text{C}(\text{O})(\text{CH}_2)_4\text{Me}$  when  $R' = \text{C}(\text{CH}_2)(\text{Me})$ ,  $R'' = \text{Me}$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

25 (xvi)  $R \neq \text{C}(\text{O})(\text{CH}_2)_4\text{Me}$  when  $R' = (\text{CH})_2\text{Me}$ ,  $R'' = \text{Me}$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6, including tautomers thereof.

Preferably, the compound according to the second aspect of the invention is in accordance with formula (Ia) and more preferably, has the stereochemistry as depicted in formula (Ib).

Even more preferably,  $R = -C(OH)CHC(O)(CH)_6CH_3$ ,  $R^I = -CH_2OH$  and  $R^{II} = Me$  such that the present invention according to the second, third and fourth aspect consists in the compound 5,6-dihydro-3-[(1Z, 4E, 6E, 8E)-1-hydroxy-3-oxo-1,4,6,8-decatetraenyl]-6-hydroxymethyl-9a-methyl-2H-furo[3,2-g][2]benzopyran-2-9(9aH)-dione according to  
5 formula (Ic).

It will be appreciated that the compound according to formula (I)-(Ic) includes all corresponding tautomeric structures.

Chemically, the compounds of formula (I)-(Ic), such as 5,6-dihydro-3-[(1Z, 4E, 6E, 8E)-1-hydroxy-3-oxo-1,4,6,8-decatetraenyl]-6-hydroxymethyl-9a-methyl-2H-  
10 furo[3,2-g][2]benzopyran-2-9(9aH)-dione, are members of the azaphilone family. The azaphilone compounds are produced by a variety of fungi, and have been investigated for their antibiotic functions, their ability to inhibit enzymatic function, and their role as colouring agents in food.

The azaphilone nucleus has also been found in the pigments produced by  
15 *Monascus* spp. The pigments containing the common azaphilone core include dihydro-monascins and monoscorubin. There is no record of their utility as fluorescent dyes for the staining of biomolecules or organic compounds.

None of the prior art known to the applicants teaches or suggests that a compound of the structure described in formula (I)-(Ic) would be fluorescent, nor suggests that it  
20 would be suitable for use in the fluorescent staining of bio-molecules or organic compounds. There are accordingly unexpected and advantageous properties associated with this compound including sensitive detection of proteins in gels, cell tracking and dual colour staining.

The present inventors have unexpectedly found that compounds according to  
25 formula (I)-(Ic) are not fluorescent in aqueous solution, but are capable of interacting with organic compounds to produce an intensely fluorescent stain. In a preferred embodiment, the compounds according to formula (I)-(Ic) of the invention are used in the detection of tagging of organic molecules.

Preferably, the compound according to formula (I)-(Ic), when bound to an organic  
30 molecule emits fluorescence after excitation at blue wavelengths. More preferably, the compounds according to formula (I)-(Ic) are excited by light in the absorbance range 300-560 nm.

In a preferred embodiment, a compound according to formula (I)-(Ic) interacts with proteins to produce a fluorescent complex that can be excited by light generated by standard UV transilluminators (307 nm). Upon excitation, the fluorescent complex emits light over a wide range of wavelengths allowing the protein complexes to be  
5 detected. The excitation wavelength of the protein/dye complex includes that of ethidium bromide complexed with DNA (Absorption maximum 518 nm, Emission maxima 605 nm). This is of particular utility because it allows the same equipment to be used to detect both DNA and protein in gels. The broad range of excitation wavelengths allows the compound to be excited strongly by 488 nm and 514 nm bands of the argon  
10 ion laser, as well as absorb light emitted from diode light sources such as those that emit at around 400 nm.

In another preferred embodiment, a fluorescent form of the compound according to formula (I)-(Ic) is capable of strongly absorbing light from the 488 nm output of the argon-ion laser, and to re-emit the light at wavelengths longer than 600 nm.

15 In another preferred embodiment, the compound according to formula (I) is included in a composition, along with an analytically acceptable carrier, and may be used in combination with a fluorochrome such as fluorescein in dual staining applications. Such a fluorochrome may be included in the composition or separately used.

The compound according to formula (I)-(Ic) may be produced by a fungal  
20 species. Preferably, the fungal species is the strain deposited at the Australian Government Analytical Laboratories (AGAL) on 15 January 1998 identified by Accession Number NM98/00507.

The methods of purification, isolation, as well as the structure of the novel fluorophore 5,6-dihydro-3-[(1Z, 4E, 6E, 8E)-1-hydroxy-3-oxo-1,4,6,8-decatetraenyl]-6-  
25 hydroxymethyl-9a-methyl-2H-furo[3,2-g][2] benzopyran-2-9(9aH)-dione and related compounds have not previously been described.

The binding of the compound according to formula (I)-(Ic) to organic molecules may involve direct chemical or physical binding or may be achieved by the use of "linking molecules" known to the art. Organic molecules which may be bound by the

compound of the invention when used as a fluorescent dye include, for example, proteins, peptides, sugars, nucleic acids, antibodies, cell surface biomolecules, detergents and cells. Due to the compound's fluorescent and organic compound-binding characteristics, it will be appreciated that the compound will have use in any application  
5 where detection of a fluorescent dye attached to an organic compound is required.

In a third aspect, the present invention consists in a process of producing a compound according to the first or second aspects of the invention, the process comprising culturing a fungal species under conditions such that the fungal species  
10 produces the compound; and separating the compound from the culture.

The compound according to formula (I)-(Ic) can be used as a fluorescent dye when in a crude culture extract or when purified by extraction and separation techniques.

15 It has been found by the present inventors that after inoculation and incubation of the strain identified by AGAL Accession Number NM98/00507 on the growth medium, a compound which is suitable as a fluorescent dye is produced by the fungus.

It will be appreciated that it should be possible to produce compounds according to  
20 formula (I)-(Ic) of the first, second, third and fourth aspect of the present invention by using techniques other than from the supernatant of a suitable fungal culture. For example, if the fungus produces a "pre-cursor", then it will be possible to modify that pre-cursor to its fluorescent form by chemical, physical or enzymatic means. The knowledge that such compounds can be obtained from microorganisms should allow the  
25 discovery and production of other compounds suitable for use as fluorescent dyes belonging to the same family or quite distinct compounds with useful characteristics. The compound according to formula (I) may also be produced synthetically by direct chemical synthesis, or by modification of intermediate(s) in the biosynthetic pathway used by the fungi.

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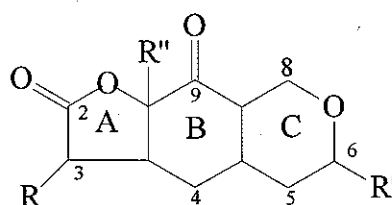
The compound according to formula (I)-(Ic) of the present invention may also be produced synthetically by chemical means. The knowledge that a new fluorescent

compound is produced by fungi may lead to other means of producing the compound apart from culturing the fungi under the required conditions.

The compound according to formula (I)-(Ic) of the present invention has the distinct advantage that it binds to cells and other organic molecules in its fluorescent form so can be used as a means to track the cells or the other organic molecules when  
5 labelled with the compound.

In a fourth aspect, the present invention consists in use of a compound according to formula (I):

10



(I)

15

wherein:

each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;

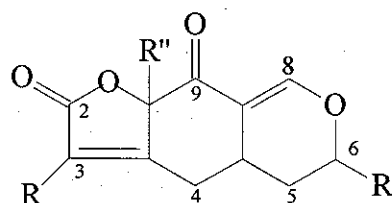
rings A, B and C optionally include one or more double bonds;

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rings B and C are optionally substituted with one or more halogen atoms, including tautomers of formula (I),

or a compound according to the formula (1a), including tautomers thereof:

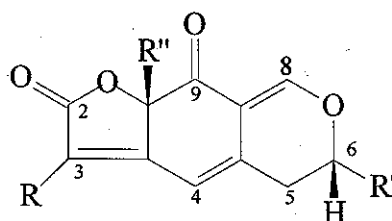
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(Ia) ,

or a compound having the stereochemistry as depicted in formula (Ib), including tautomers thereof:

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(Ib)

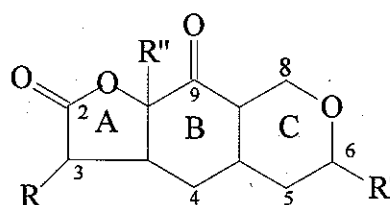
or a compound wherein R is a straight or branched chain C<sub>1-20</sub> conjugated alkenyl group optionally substituted with hydroxy and oxo groups; R' is a straight or branched chain C<sub>1-20</sub> alkyl group optionally substituted with a hydroxy group and R'' is a straight or branched chain C<sub>1-20</sub> alkyl group, including tautomers thereof, or a compound according to any one of claims 1 to 13, as a fluorescent dye or marker after interaction with an organic compound.

15

Examples of the use of the compound according to the first, second, third and fourth aspect of the present invention include but are not restricted to cell tracking dyes for microscopy, membrane fluidity dyes, conjugation with antibodies, conjugation to nucleic acids, cell surface ligand imaging dyes, conjugation to sugars, cytometric analysis, and confocal microscopy. It will be appreciated, however, that the compound would be suitable for any use where fluorescence in the red wavelengths is required, including when excited at 488 nm.

In a fifth aspect, the present invention consists in a method for fluorescently labelling an organic compound, comprising causing a compound according to formula (I):

25



(I)

wherein:

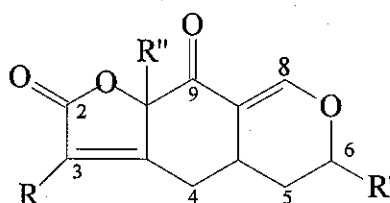
- 5 each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;

rings A, B and C optionally include one or more double bonds;

rings B and C are optionally substituted with one or more halogen atoms,

- 10 including tautomers of formula (I),

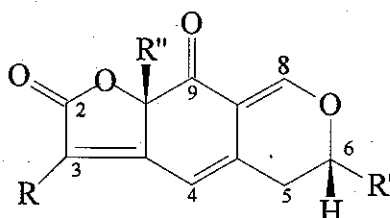
or a compound according to the formula (1a), including tautomers thereof:



(Ia) ,

15

or a compound having the stereochemistry as depicted in formula (Ib), including tautomers thereof:



(Ib)

- 20 or a compound wherein R is a straight or branched chain C<sub>1-20</sub> conjugated alkenyl group optionally substituted with hydroxy and oxo groups; R' is a straight or branched chain C<sub>1-20</sub> alkyl group optionally substituted with a hydroxy group and R'' is a straight or

branched chain C<sub>1-20</sub> alkyl group, including tautomers thereof, or a compound according to any one of claims 1 to 13, to bind to an organic compound in a manner such that the organic compound is fluorescently labelled with the compound.

5        Preferably, the fluorescently labelled organic compound is detected when exposed to a wide range of wavelengths, preferably wavelengths in the range of 300-560 nm.

      The organic compound may include, but is not limited to, proteins, peptides, sugars, nucleic acids, antibodies, cell surface biomolecules, detergents and cells. The  
10    compound may bind directly to the organic compound due to a chemical or physical association or may bind to the organic compound via a linking molecule. If the compound is attached to a ligand specific for the organic compound, for example an antibody or lectin, then the binding of that ligand to the organic compound will cause the organic compound to be fluorescently labelled.

15

      In a sixth aspect, the present invention consists in a method of detecting an organic compound in a sample including the organic compound, the method comprising  
labelling the organic compound according to method of the fifth aspect; and detecting  
the organic compound in the sample by monitoring or detecting its fluorescence.

20

      Preferably, the labelled organic compound is detected when the sample is exposed to light from a wide range of wavelengths, preferably wavelengths in the range of 300-560 nm, more preferably blue wavelengths.

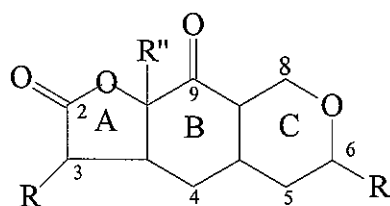
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      The monitoring or detecting of the fluorescence of the labelled-organic compound may be by any means known to the art. Such means include, but are not limited to, transillumination, spectroscopy microscopy and cytometry.

      In a seventh aspect, there is provided a composition including:

30

(i)        a compound according to formula (I)



(I)

wherein:

- each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;
- rings A, B and C optionally include one or more double bonds;
- rings B and C are optionally substituted with one or more halogen atoms, including tautomers of formula (I), and
- (ii) an organic compound

wherein the compound is capable of interacting with an organic compound so as to fluoresce after excitation.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the inclusion of any other element, integer or step, or group of elements, integers or steps.

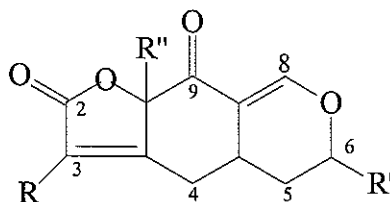
Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

A micro-organism is cultured under conditions to promote sporulation and Extract B is used to facilitate the identification of sporulated cells within a predominantly vegetative cell culture. For example, *Saccharomyces cerevisiae* was grown in a water based broth containing 10 g/L Glucose, 5 g/L yeast  
5 extract, and 10 g/L peptone. After a 16 hour incubation at 30°C, the cells were harvested by centrifugation and resuspended in 1mL of water. The 5 µL aliquots of the culture were spotted onto a semi-solid medium composed of 5% Potassium acetate, 2% Agar and water. The cultures were incubated at 22°C for 4 days to allow sporulation of the yeast cells. The sporulated culture was  
10 resuspended in water to a density of  $1 \times 10^6$  cells per mL. 5 µL of Extract B was added to 1 mL of the sporulated culture. To counterstain, 5 µL of a 10 mM solution (in DMSO) of 5 (6)-carboxyfluorecein diacetate (CFDA), was added to the sporulated culture. After 5 minutes, the sporulated culture was harvested by centrifugation and resuspended in 1mL of water. Examination under an  
15 epifluorescence microscope (excitation 488 nm) allowed for rapid differentiation between sporulated and vegetative cells.

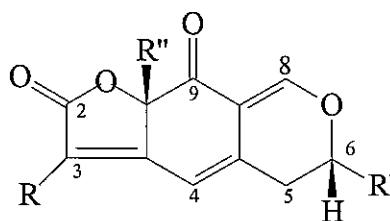
It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the  
20 invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A compound according to formulae:

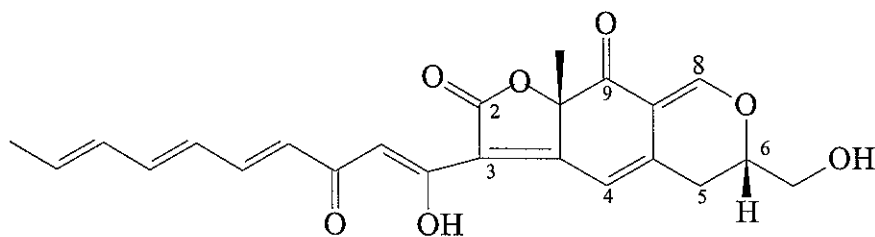


(Ia) or



(Ib)

- wherein R is  $-\text{C}(\text{OH})\text{CHC}(\text{O})(\text{CH})_6\text{CH}_3$ , R' is  $-\text{CH}_2\text{OH}$  and R'' is Me, being the compound 5,6-dihydro-3-[(1Z, 4E, 6E, 8E)-1-hydroxy-3-oxo-1,4,6,8-decatetraenyl]-6-hydroxymethyl-9a-methyl-2H-furo [3,2-g][2]benzopyran-2-9(9aH)-dione according to formula (Ic), including tautomers thereof:

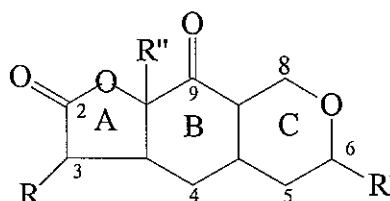


(Ic)

and the compound is capable of interacting with an organic compound so as to fluoresce after excitation.

2. A compound as claimed in claim 1, wherein the organic compound is a nitrogen containing compound.

3. A compound according to claim 2, wherein the nitrogen containing compound is selected from the group consisting of proteins, amino acids, peptides, nucleic acids and aliphatic or aromatic amines.
4. A compound as claimed in any one of claims 1 to 3, wherein the compound  
5 interacts with the organic compound in the presence of a surfactant so as to fluoresce after excitation.
5. A compound as claimed in claim 4, wherein the surfactant is selected from the group consisting of salts of long chain fatty acids or sulfonic acids.
6. A compound according to formula (I):



(I)

wherein each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo groups;

- 15 rings A, B and C are optionally substituted with one or more halogen atoms; and rings A, B and C optionally include one or more double bonds; with the proviso that:
  - (i)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = -CH_2OH$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond  
20 between C4 and C4a; and ring C includes a double bond between C8 and C8a;
  - (ii)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a;
  - (iii)  $R \neq -C(OH)CHC(O)(CH)_6CH(Et)(Me)$  when  $R' = Me$ ,  $R'' = Me$ , ring A  
25 includes a double bond between C3 and C3a; ring B includes a double bond

between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

5 (iv)  $R \neq -C(O)(CH_2)_4Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(v)  $R \neq -C(O)(CH_2)_6Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

10 (vi)  $R \neq -C(O)(CH_2)_5Me$  when  $R' = -(CH_2)Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(vii)  $R \neq -C(O)(CH_2)_6Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

15 (viii)  $R \neq -Ac$  when  $R' = -(CH)_2C(Me)CHCH(Me)(Et)$ ,  $R'' = Me$ ,  $C4=C1$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

20 (ix)  $R \neq -Ac$  when  $R' = -(CH)_2C(Me)CHCH(Me)(Et)$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(x)  $R \neq -Ac$  when  $R' = -(CH)_2C(Me)CH\text{-}i\text{-propyl}$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

25 (xi)  $R \neq -C(O)(CH)_4Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A includes a double bond between C3 and C3a; ring B includes a double bond between C4 and C4a; and ring C includes a double bond between C8 and C8a and C5 and C6;

(xii)  $R \neq -C(O)(CH)_4Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

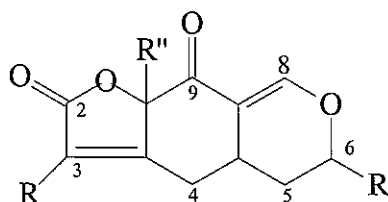
30 (xiii)  $R \neq -C(O)(CH)_4Me$  when  $R' = n\text{-propyl}$ ,  $R'' = Me$ , rings A, B and C do not include double bonds.

(xiv)  $R \neq -C(O)(CH)_6Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

5 (xv)  $R \neq -C(O)(CH_2)_4Me$  when  $R' = -C(CH_2)(Me)$ ,  $R'' = Me$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6;

(xvi)  $R \neq -C(O)(CH_2)_4Me$  when  $R' = -(CH)_2Me$ ,  $R'' = Me$ , ring A does not include a double bond; and rings B and C include double bonds between C8a and C4a and C5 and C6, including tautomers thereof.

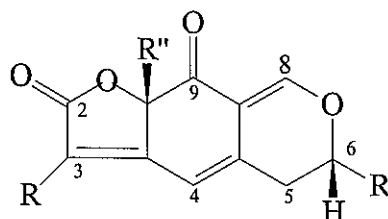
10 7. A compound as claimed in claim 6 according to the formula (Ia):



(Ia)

wherein R, R' and R'' are defined as in claim 6.

15 8. A compound as claimed in claim 7 having the stereochemistry as depicted in formula (Ib):

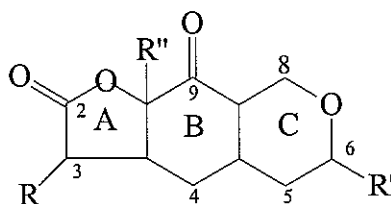


(Ib)

wherein R, R' and R'' are defined as in claim 6.

20 9. A compound as claimed in any one of claims 1 to 8, wherein the compound when interacting with an organic compound, emits fluorescence after excitation at ultraviolet to blue wavelengths.

10. A compound as claimed in any one of claims 1 to 8, wherein the compound emits fluorescence when excited by light in the range 300-560 nm.
11. A compound as claimed in claim 9 or claim 10, wherein the organic compound is a protein and the compound when interacting with the protein emits fluorescence after excitation at 307 nm.
12. A compound as claimed in claim 11, wherein compound emits fluorescence when excited by light at about 400 nm, 488 nm and 514 nm.
13. A compound as claimed in claim 12, wherein compound emits fluorescence at about 600 nm after excitation at 488 nm.
14. A composition including a compound as claimed in any one of claims 1 to 13, an analytically acceptable carrier and optionally, a flurochrome.
15. A process for the preparation of a compound as claimed in any one of claims 1 to 13 comprising culturing a fungal species under conditions such that the fungal species produces the compound, and separating the compound from the culture.
16. Use of a compound according to formula (I):



(I)

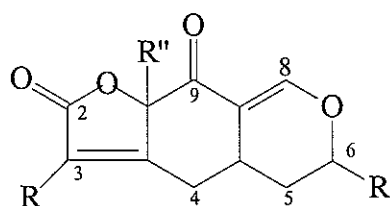
wherein:

- each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;

rings A, B and C optionally include one or more double bonds;

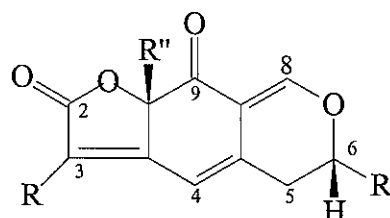
rings B and C are optionally substituted with one or more halogen atoms, including tautomers of formula (I),

- or a compound according to the formula (1a), including tautomers thereof:



(Ia) ,

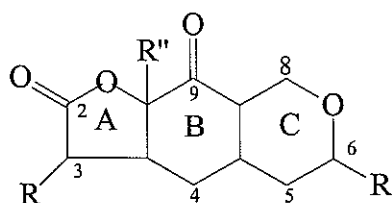
or a compound having the stereochemistry as depicted in formula (Ib), including tautomers thereof:



(Ib)

or a compound wherein R is a straight or branched chain C<sub>1-20</sub> conjugated alkenyl group optionally substituted with hydroxy and oxo groups; R' is a straight or branched chain C<sub>1-20</sub> alkyl group optionally substituted with a hydroxy group and R'' is a straight or branched chain C<sub>1-20</sub> alkyl group, including tautomers thereof, or a compound according to any one of claims 1 to 13, as a fluorescent dye or marker after interaction with an organic compound.

17. Use as in claim 16 in a technique for staining, labelling and/or detecting organic molecules.
- 15 18. A method for fluorescently labelling an organic compound, comprising causing a compound according to formula (I):



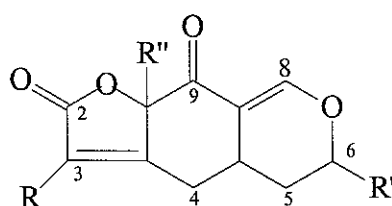
(I)

wherein:

each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;

- 5 rings A, B and C optionally include one or more double bonds;  
rings B and C are optionally substituted with one or more halogen atoms, including tautomers of formula (I),

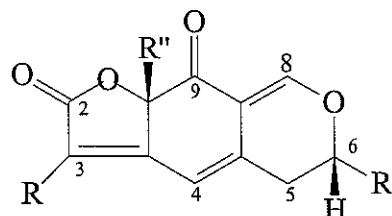
or a compound according to the formula (1a), including tautomers thereof:



10

(Ia) ,

or a compound having the stereochemistry as depicted in formula (Ib), including tautomers thereof:

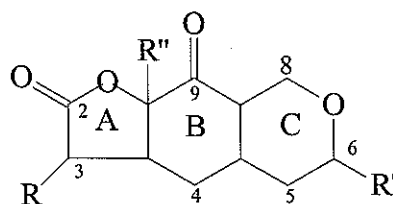


(Ib)

- 15 or a compound wherein R is a straight or branched chain C<sub>1-20</sub> conjugated alkenyl group optionally substituted with hydroxy and oxo groups; R' is a straight or branched chain C<sub>1-20</sub> alkyl group optionally substituted with a hydroxy group and R'' is a straight or branched chain C<sub>1-20</sub> alkyl group, including tautomers thereof, or a compound according to any one of claims 1 to 13, to bind to an organic compound in a manner such that the  
20 organic compound is fluorescently labelled with the compound.

19. A compound, method, composition or use as claimed in any one of claims 1 to 18, wherein the organic compound is a protein, peptide, sugar, nucleic acid, cell surface molecule or detergent, or is included in an antibody or a cell.

20. A compound, method, composition or use as claimed in claim 19, wherein the compound is bound to the organic compound through a linking molecule.
21. A method of detecting a organic compound in a sample including the organic compound, the method comprising labelling the organic compound according to  
5 the method of claim 18, and detecting the organic compound in the sample by monitoring or detecting its fluorescence.
22. A method as claimed in claim 21, wherein the labelled organic compound is detected when the sample is exposed to light from a wide range of wavelengths, preferably wavelengths in the range of 300-560 nm, more preferably ultraviolet to  
10 blue wavelengths.
23. A method as claimed in claim 21 or claim 22, wherein the monitoring or detecting of the fluorescence of the labelled-organic compound is by transillumination, spectroscopy, microscopy or cytometry.
24. A composition including:  
15 (i) a compound according to formula (I)



(I)

wherein:

- each of R, R' and R'' is a hydrogen atom, halogen atom or a straight or branched  
20 chain C<sub>1-20</sub> alkyl, alkenyl or alkynyl group optionally substituted with one or more halogen, hydroxy and/or oxo group;

rings A, B and C optionally include one or more double bonds;

rings B and C are optionally substituted with one or more halogen atoms, including tautomers of formula (I), and

- 25 (ii) an organic compound  
wherein the compound is capable of interacting with an organic compound so as to fluoresce after excitation.