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(54) **Short-lived γ-emitting metal isotopes phthalocyanine tetrasulfonic acid**

(57) New phthalocyanine tetrasulfonic acid metal complexes selected from technetium-99m, gallium-67, gallium-68, copper-64, chromium-51, cobalt-57, indium-111, mercury-197 and zinc-62 have been found to have affinity for malignant growth and thus are useful in detecting the presence, size and location thereof with radiation imaging device.

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SPECIFICATION

Short-lived γ-emitting metal isotopes phthalocyanine tetrasulfonic acid

The present invention relates to a novel organometallic complex containing a short-lived γ-emitting metal isotope. More specifically, the invention relates to the production of novel complexes between phthalocyanine tetrasulfonic acid and of short-lived γ-emitting metal isotopes. The novel products of the present invention are suitable for injection into the blood stream of a mammal when dissolved or dispersed in a biologically sterile aqueous medium substantially isotonic with mammalian body fluids so as to permit the detection of malignant growths by the usual scanning procedures.

The art of radiochemistry has found many applications in the fields of medicine and biology. It has long been known that the introduction into an organism of compounds containing (or "labeled" with) a radioisotope can provide insight into the anatomy, physiology and metabolic processes of the organism. These compounds, generally referred to as radiopharmaceuticals are particularly useful in diagnostic techniques which involve studying the structure or function of various internal organs, e.g. the brain, kidney, or liver with radiation detection means. For diagnostic work, isotopes with a short half life and an emission spectrum rich in gamma rays (as opposed to alpha or beta particles) are preferred.

The metastable isotope Tc-99m has a 6 hour half-life and an emission spectrum, 99% gamma radiation at 140 KeV, which is extremely well suited for techniques of diagnostic nuclear medicine. Thus, Tc-99m has a high specific activity, 5.28×10^2 millicuries per gram (mc/g), and a conveniently rapid rate of decay; whereas its daughter product, Tc-99, has a specific activity which is almost nine orders of magnitude lower and a half life which is roughly nine orders of magnitude longer. For the organism being studied or diagnosed, the slow rate of decay from the relatively stable low specific activity Tc-99 to its degradation product (ruthenium) would not normally produce any hazardous amounts of radiation, regardless of the biological means or route of elimination of a Tc-99m radiopharmaceutical. For the researcher or clinician, the emission spectrum of Tc-99m can provide high levels of accuracy in radio-diagnostic measurements and calculations. In recent years, Tc-99m has become readily available in hospitals through the use of selective elution from a so-called molybdenum-99 (Mo-99) generator. The isotope Mo-99 produces Tc-99m as a radioactive decay product.

Although Tc-99m compounds would appear to be ideal radiopharmaceuticals for diagnostic use, providing or selecting Tc compounds or complexes with a view toward organ specificity and tolerable levels of toxicity is a complex task. Obviously, compounds with a very low LD 50 are undesirable for human or veterinary use, even in the small amounts called for by diagnostic work. Compounds with insufficient 'in vivo' stability may be poor diagnostic tools, since radioactive ions or other chemical species with insufficient or undesired organ specificity may be liberated. Stable compounds which become distributed generally throughout the organism, despite their stability, or which do not reach a desired destination in the organism are also poorly suited for many studies or organ function or structure, e.g. liver and gallbladder studies. For these studies of organ function, compounds which are specific to an organ, but which are not excreted by it (or if excreted, are easily reabsorbed) are also poor candidates.

Some Tc compounds or complexes have been developed for specific investigations. For example a liver specific 99m-Tc 6,8 - dihydrothioctic acid complex has been developed to provide a meaningful picture of liver function by measuring the radioactivity emitted from the liver, gallbladder, intestines, and feces of the organism or patient being studied (U.S.P. 3.873.680). Technetium-99m joined with calcium organo chelates in the presence of ferrous sulfate has been found useful as agents for kidney visualization, renal function studies and other vascular studies (U.S.P. 3.446.361). A preparation of ethane - 1 - hydroxy - 1, 1 - diphosphosphorate in acidic solution of stannous chloride and mixed with 99 m TcO_4 has been proposed for use in radiographic skeletal bone-scawning procedures (U.S.P. 3.735.001). Brain and kidneys can be examined with an iron complex labelled with 99m Tc (U.S.P. 3.787.565). It is also known that macro-aggregates of serum albumin labelled with 99mTc are particularly useful in lung function investigations (U.S.P. 3.803.299 and 3.862.299). Other derivatives of 99m Technetium has been proposed for various other uses in U.S.P. 3.812.264, 3.852.413, 3.863.004 and 3.683.066.

It has been reported in J. Nucl. Med *5*, 462 (1964) that Co-57 labelled derivatives of tetraphenyl porphynesulfate can be useful in detecting cerebral tumors but not other tumors. Approximately 40% of the Co-57 was dissociated which in part explained the failure of the radioisotope to localize in the tumors.

Notwithstanding these developments there remains a need for radiopharmaceuticals which rapidly and selectively accumulate in specific tissues. It would appear highly desirable to provide radiopharmaceuticals which exhibit an affinity for malignant growths or tumor cells so as to provide an early diagnosis of tumors and tumor metastases.

It is also known that phthalocyanines and particularly the tetrasulfonic acid derivatives thereof have a tendency to behave like the naturally occurring porphyrins.Phthalocyanine is a heterocyclic ring compound consisting of four benzisoindole nuclei fused via nitrogen bridges. They are known to form stable chelates with metal ions and certain metal oxides. Metal phthalocyanines may be prepared by exchange of the desired metal ion with the central ion of lithium phthalocyanine. One such class of metal phthalocyanines which have been prepared are the actinide and lanthanide rare earth phthalocyanines and more particularly uranyl sulfonated phthalcocyanine which are disclosed in U.S.P. 3.027.391 as useful in the treatment of a locatable

tumor by direct injection of the uranyl phthalocyanine into the tumor of the animal.

As can be appreciated these heavy metal sulfonated phthalocyanines are useful only when a tumor has already been located by other means and they are only useful for the therapeutic treatment of said located treatment either when the starting heavy metal is a radioactive nuclide where the radiation will destroy the tumor or where the heavy metal is a fissionable or a neutron-activatable nuclide. Unfortunately, this method provides not for the location of a tumor but only for its radioactive treatment and thus leaves no place for detecting the presence of a tumor and its treatment by other means, such as surgery.

Since, it is well known that the main problem of malignant growths is their early detection so that proper treatment can be initiated as soon as possible, it would therefore appear highly desirable to provide a safe radioactive method for this type of detection.

In accordance with the present invention, it has now been found that malignant tumors can be readily detected by the use of novel complexes of short-lived γ-emitting metal isotopes and phthalocyanine tetrasulfonic acid. It has been found that these novel complexes because of the affinity for malignant growths can be particularly useful in detecting, the presence, the location and size of said malignant growth by the usual radioactivity scanning method.

The short-lived γ-emitting metal isotopes which can be combined with phthalocyanine tetrasulfonic acid to yield the novel radiopharmaceutical complexes of the present invention are technetium-99m, gallium-67, gallium-68, mercury-197, copper-64, chromium-51, colbalt-57, indium-111 and zinc-62. Preferred complexes for studies imaging within 12 hours are the technetium-99m phthalocyanine tetrasulfonic acid and gallium-68 phthalocyanine tetrasulfonic acid, because technetium-99m and gallium-68 are readily obtained when desired from a generator and because of the short half life of 6 hours and 68 minutes respectively. Although technetium-99 is the principal isotope presently used in clinical practice, gallium-68 thus becomes equally important because of recent developments in positron tomographic instrumentation. For application requiring imaging at longer time intervals up to 120 hours post injection complexes of metal isotopes with a half life of about 3 days are preferred including gallium-67 phthalocyanine tetrasulfonic acid and indium-111 phthalocyanine tetrasulfonic acid.

The novel compounds of the present invention may be prepared either by the condensation procedure disclosed in Inor. Chem. 4, 469 (1965) Weber et al or the direct labelling method.

The condensation method essentially involves condensing monosodium sulfophthallic acid with the short-lived γ-emitting metal isotope in nitrobenzene at 200°C or higher in an inert gas atmosphere in the presence of a reducing agent made up of hydroxylamine, urea and ammonium chloride and in the presence of a catalyst such as ammonium molybdate. The reaction mixture is heated to about 90°C and concentrated in a stream of nitrogen. Condensation will occur upon heating the residue to 235°C or higher depending on the selected metal isotope for about one half hour.

Nevertheless, this method has drawbacks in that a mixture of labelled isotopes of tetrasulfophthalocyanine complexes are obtained and these must be purified in order to eliminate unreacted starting materials such as the reactants and the free metal isotope so that a certain amount of time is thus consumed which becomes a disadvantage when a metal isotope having a very short half-life of about three hours is used, thus creating a race against time to obtain the desired procedure for injection in the host to be tested. Nevertheless, this method is the only one available when using a metal isotope such as technetium which is not particularly suited for use in the direct labelling method because of its chemical properties.

Alternatively, the desired products of the present invention can also be obtained by the direct labelling method. In this method, the tetrasulfophthalocyanine which is obtained by the sulfonation of phthalocyanine is separated so as to recover the major constituent which is then labelled directly with the desired metal isotope in accordance with procedures known in the art. This method is preferably used with metal isotopes other than technetium. In the direct labelling procedure the metal isotope is added to the aqueous solution of tetrasulfophthalocyanine and heated for 10 to 30 minutes at 100°C after adjusting the pH to neutral or slightly basic.

The novel phthalocyanine tetrasulfonic acid complexes of short-lived γ-emitting metal isotopes are useful upon injection in the blood stream for diagnosing the presence of tumors in the animal body.

Phthalocyanines and their sulfonated analogues are non-toxic, and even their complexes with toxic metals result in non-toxic metal complexes. Furthermore, since the actual amount of short-lived metal isotopes required for scanning purposes in animals are negligible, the corresponding phthalocyanine tetrasulfonic acid complexes have been found to exhibit no noticeable possibility of adverse pharmacological effect when administered for detection purposes in animals.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention only.

Example 1.

Production of Technetium-99m phthalocyanine tetrasulfonic acid (Tc-99m-PcTs).

To a freshly eluted pertechnetate ($^{99m}TcO_4^-$) solution (2 ml containing 50-150 μCi ^{99m}Tc, from a $^{99}Mo/^{99m}Tc$ generator) was added monosodium sulfophthalic acid (8.04 mg, 3×10^{-5} mol), urea (6 mg, 10^{-4} mol) and 0.1 ml of a solution containing 6×10^{-4} M ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and 10^{-1} M ammonium chloride. After the addition of hydroxylamine (8.34 mg, 12×10^{-5} mol) which reduces the pertechnetate, probably to technetium oxide ($^{99m}TcO_2$), the mixture was heated to 90°C and concentrated in a stream of nitrogen. The residue was heated up within 25 min. to 235°C to allow condensation to occur. After the mixture was cooled to

room temperature, the residue was taken up in 1 ml of water and applied to a weak anion exchange column (1 ml of Amberlite® 1R-45-(OH), packed in a 1 ml plastic syringe, column A). The column was washed with distilled water (9 ml) and eluted in the reversed direction with 0.1 N sodium hydroxide (10 ml). The eluate was directly passed over a cation exchange column (0.5 ml of Amberlite® 12-120 (H), column B) to yield 5 fractions of 2 ml each. The purified Tc-99mPcTs (10% yield based on radioactivity of the original pertechnetate sample), was collected in the third fraction (Fraction III, column B). After adjusting the pH to 7.0 with 0.1 N hydrochloric acid (about 0.1 ml), the preparation was ready for injection into laboratory animals.

By proceeding in the same manner and starting with the nitrate or chloride salt of gallium-67, copper-64, chromium-51, cobalt-57, indium-111 and zinc-62 there is obtained the corresponding gallium-67 phthalocyanine tetrasulfonic acid, copper-64 phthalocyanine tetrasulfonic acid, chromium-51 phthalocyanine tetrasulfonic acid, cobalt-57 phthalocyanine tetrasulfonic acid. indium-111 phthalocyanine tetrasulfonic acid and zinc-62 phthalocyanine tetrasulfonic acid.

Example 2.

In order to establish the chemical nature of the Tc-99m entity in the purified Tc-99m-PcTs fraction (example 1, fraction III, from column B), a condensation experiment with Tc-99, the long-lived decay product of Tc-99m, was conducted. Technetium-99 metal (49.5 mg, 0.5×10^{-3} mol) was dissolved in a few drops of concentrated nitric acid whereafter a small amount of urea was added to destroy any nitrite (HNO_2) formed. After the addition of a small amount of hydroxylamine, the mixture was dried in a vacuum to yield a light green solid. The material was taken up in an aqueous solution of hydroxylamine (2 ml) containing 3 - sulfophthalic acid (432 mg, 1.61×10^{-3} mol), urea (600 mg, 10^{-2} mol), ammonium molybdate (7.4 mg, 6×10^{-6} mol) and ammonium chloride (53 mg, 10^{-3} mol). The reaction mixture was covered with nitrobenzene (b.p. 210.9°C) and heated on an oil bath under a nitrogen atmosphere. After the water was evaporated, the hydroxylamine decomposed as evinced by the sudden gas formation. The color of the reaction mixture changed from light violet to black. After addition of a further 5 ml of nitrobenzene, the mixture was refluxed for 20 min. The black precipitate was collected, suspended in absolute methanol, filtered, washed thoroughly with absolute methanol and dried to give 235 mg of a black powder. The specific activity (β-decay of the Tc-99) indicated the presence of 15.3% of Tc (W/W), or a 5.6% excess of Tc calculated for a Tc:PcTs ratio of 1:1. Accordingly, the preparation was either contaminated by Tc salts other than the Tc-99-PcTs complex, or the latter complex contains more than 1 mol of Tc per mol of PcTs.

Cochromatography of Tc-99-PcTs (1 mg) and a Tc-99m-PcTs preparation in the ion exchange system (see example 1) followed by UV analysis of the purified Tc-99m-PcTs (fraction III from column B), was also performed. The UV-spectrum showed the characteristic absorption maxima at 685 and 595 nM together with a strong absorption below 300 nM and a shoulder at 305 nM, which confirms the sulfophthalocyanine nature of the eluted material.

Example 3.

In vivo distribution of Tc-99m-PcTs (fraction III from column B, see example 1).

Female rabbits (2 kg) were anaesthetized by i.p. injection of sodium Nembutal® (pentobarbital, 30 mg/kg). The purified Tc-99m-PcTs fraction (2 ml, 30 μCi) was administered i.v. via the marginal vein of the ear. Distribution of the radioactivity was visualized via scintillation scanning with a Dyna®-IV camera (Picker Nuclear). This camera is equipped with a high resolution, parallel collimator and contains a matrix of 37 phototubes to yield an intrinsic resolution of ⅛".

Five min. after injection, activity accumulates in the heart and in the hepatic region. The kidneys have become visible whereas the bones, and in particular the bone joints of the hind legs, can also be recognized. Six hours post injection, a strong fixation is observed in the liver, the cardiac region, the kidneys and the spleen. The stability of the Tc-99m-PcTs complex is evident from the absence of activity in the stomach, thyroid and salivary glands. For comparison, a scintillation scan of a rabbit injected with a similar dose of activity in the form of the free pertechnetate ion shows that in contrast with the Tc-99m-PcTs experiment, the stomach, salivary glands and thyroid are now strongly labeled.

Example 4.

In order to study the organ distribution of Tc-99m-PcTs as a function of time, seven female Fisher 344 CRBL rats were injected via the caudal vein with the purified radiopharmaceutical (45 μCi in 0.2 ml saline per rat). Animals were sacrificed at different time intervals and dissected. Samples of the liver, kidneys, lungs, muscle, spleen and blood were collected, weighed and counted for the content of Tc-99m in a Spectron®-100 multichannel analyser, calibrated for 140 KeV photons (Picker Nuclear). Values were adjusted for Tc-99m decay and for weight differences between the animals, and the changes in specific activities were plotted. Some obvious distribution patterns can be inferred from this graph. A high specific activity in the kidneys is maintained throughout the study, indicating irreversible binding of Tc-99m-PcTs in these organs. Hepatic fixation, with a maximum uptake after 12 hours, is also significant. The spleen and lungs are less active although their specific activities remain constant during the experiment. The activity of the blood pool decreases exponentially with a half time of elimination of 12 hours. The uptake of Tc-99m-PcTs by the muscle is insignificant and parallels the activity of the blood.

In conclusion, these results indicate that renal tissue exhibits a strong affinity for the Tc-99m-PcTs. A number of other organs, including the liver, spleen, lungs and heart also retain this product. The distribution pattern suggests significant binding at the level of the reticulo-endothelial system.

Example 5.

The fixation of the Tc-99m-PcTs by the various organs may also be seen from the excretion pattern of this radiopharmaceutical. Excretion of Tc .99m

was determined with 3 male rats (Sprague-Sawley) of about 100 g each. In order to obtain a uniform pattern, *the animals were cystectomized (J. P. Bonjour. Helv. Acta 24, 24 (1966)*. Each rat received a dose of 0.4 μCi/g of Tc-99m-PcTs via the caudal vein. The total activity of the animals was measured at different time intervals by means of a PHO-V camera (Searle) equipped with a high resolution parallel collimator. The animals were restrained in a tight cage to control their geometry in relation to the detector. Throughout the experiment, they were able to eat and drink. The average of the values obtained with the three animals were plotted as percentage of the activity at the time of injection on a semi-logarithmic scale.

Values were corrected for decay of the Tc-99m and accordingly, represents the biological excretion of TcPcTs only. A two compartment curve is obtained indicating that a distribution equilibrium is reached within 3 hrs. This point of intersection of the two curves coincides with the maximum uptake in the various organs and in the blood pool. It is evident that during the first three hours after injection about 20% of the Tc-99m has been excreted directly from the blood pool. The remainder of the Tc-99m is released from the target tissues at an extremely low rate (0.5% per hour). These observations, together with the absence of activity in the stomach, thyroid and salivary glands (Example 3), indicate that no pertechnetate is released from the TcPcTs complex. It also suggests irreversible binding of TcPcTs at the receptor sites of the target organs.

Example 6.

In order to evaluate Tc-99m-PcTs as a tumor scanning agent, we selected the hormones sensitive 13762 mammary adenocarcinoma in Fisher 344/CRBL female rats as a model. This tumor is kept in the ascites form. However, once inoculated s.c. or in soft tissue, a solid tumor develops. In our study, 5 animals (150 g each) were inoculated in the thigh with 0.5 ml ascites liquid (10^6 cells). At time intervals of 3, 6, 8 and 11 days after inoculation, the animals were injected via the caudal vein with Tc-99m-PcTs (500 μCi in 1 ml). The activity distribution in the animals was followed by scintillation scanning with a Dyna®-IV camera coupled with a Cybernex® ordinator system (Chromemco). Selected images were registered on a magnetic disc, which allows their presentation on a matrix of 128 by 128 points. The activity of each point is presented via a scale of 8 colors which are four times repeated to cover an activity of 0-256 counts. In addition to the dramatic visualization of the scintillation scan, this technique allows for quantitative studies of the results.

The images obtained 3 and 6 days after tumor inoculation only, revealed a slight increase in activity at the inoculation site. However, scanning experiment with Tc-99m-PcTs in animals 8 days post inoculation clearly reveals selective uptake of Tc-99m in the tumor. One of the animals was sacrificed after the scintigraphic study and dissected. The tumor had developed in the thigh as a 0.5 cm solid nonvascularized, white module (25 mg). The activity of the tumor was 4 times higher as that of muscle tissue taken from the healthy thigh. Eleven days post inoculation, the tumor reached a 1 cm diameter (455 mg) and was equally well visualized with our new radiopharmaceutical (tumor/muscle ratio 5:1).

The pertinent fixation of Tc-99m-PcTs in malignant lesions at stages of tumor development, well before the vascularization phase, strongly indicates the usefulness of this novel radiopharmaceutical as a scanning agent for the early diagnosis of malignant tumors and their metastases.

Example 7.

Production of Gallium-67 phthalocyanine tetrasulfonic acid (Ga-67-PcTs) by Direct Labelling.

Phthalocyanine was converted to the tetrasulfonic derivative by the method of R. P. Linstead and F. T. Weiss, J. Chem. Soc. 2975 (1950) to a solution of phthalocyanine tetrasulfonic acid (4 mg 1.5×10^{-5} mol) in 0.6 ml of a 0.1 M phosphate buffer pH 7.3 was added 0.1 ml of the same buffer containing carrier-free gallium (67 Ga^{+++}, 30-50 microcuries) and 40 micrograms of sodium citrate. The mixture was heated for ten minutes at 100°C whereupon a sample of 10-25 microliters was applied to a silica gel thin layer chromatoplate. After developing the plate in acetone:ethylacetate:water:NH_4OH (7:3:3:0.3) and an autoradiogram revealed the presence of four radioactive zones with identical migration patterns as the major resolved blue constituents of the unlabelled phthalocyanine tetrasulfonic acid preparation. Unreacted gallium remained at the origin (84%) whereas 16% of the radioactivity was associated with the Ga-67-PcTs zones.

Purification of the Ga-67-PcTs is accomplished by applying the reaction mixture onto a silica gel column (2 × 0.5 cm) followed by elution with 0.9% saline 1 ml. The preparation was then ready for use.

By proceeding in the same manner and starting with the nitrate or chloride salt of gallium-68, copper-64, chromium-51, cobalt-57, indium-111, mercury-197 and zinc-62 there is obtained the corresponding gallium-68 phthalocyanine tetrasulfonic acid, copper-64 phthalocyanine tetrasulfonic acid, chromium-51 phthalocyanine tetrasulfonic acid, cobalt-57 phthalocyanine tetrasulfonic acid, mercury-197 phthalocyanine tetrasulfonic acid, indium-111 phthalocyanine tetrasulfonic acid and zinc-62 phthalocyanine tetrasulfonic acid.

CLAIMS

1. The phthalocyanine tetrasulfonic acid metal complexes selected from the group consisting of technetium-99m, gallium-67, gallium-68, copper-64, chromium-51, cobalt-57, indium-111, mercury-197 and zinc-62.

2. The technetium-99m phthalocyanine tetrasulfonic acid.

3. A method for detecting tumors in the body of an animal which comprises administering to the animal a diagnostic dose of a phthalocyanine tetrasulfonic acid metal complexes selected from technetium-99m, gallium-67, gallium-68, copper-64, chromium-51, cobalt-57, indium-111, mercury-197 and zinc-62, followed by examination with a radiation imaging device in order to determine any tumor to which is bound the labelled diagnostic agent.

4. A method for detecting tumors in the body of an animal which comprises administering to the

animal a diagnostic dose of the technetium-99m phthalocyanine tetrasulfonic acid followed by examination with a radiation imaging device in order to determine any tumor to which is bound the labelled diagnostic agent.

5. The gallium-67 phthalocyanine tetrasulfonic acid.

6. A metabolizable radio-active tumor seeking composition comprising a phthalocyanine tetrasulfonic acid radio-active radio-active metal complex selected from the group consisting of technetium-99m, gallium-67, gallium-68, copper-64, chromium-51, cobalt-57, indium-111, mercury-197 and zinc-62, and a suitable liquid carrier.

7. A metabolizable radio-active tumor seeking composition comprising the technetium-99m phthalocyanine tetrasulfonic acid and a suitable liquid carrier.

8. A metabolizable radio-active tumor seeking composition comprising the gallium-67 phthalocyanine tetrasulfonic acid and a suitable liquid carrier.

9. A metabolizable radio-active tumor seeking composition comprising the indium-111 phthalocyanine tetrasulfonic acid and a suitable liquid carrier.

10. A metabolizable radio-active tumor seeking composition comprising the gallium-68 phthalocyanine tetrasulfonic acid and a suitable liquid carrier.

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