UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF ILLINOIS

BELOTECA, INC., Plaintiff, v. APICORE US LLC and MYLAN INSTITUTIONAL LLC, Defendants.	Civil Action No.

COMPLAINT FOR DECLARATORY JUDGMENT

Plaintiff Beloteca, Inc. ("Plaintiff" or "Beloteca"), for its Complaint against Apicore US LLC ("Apicore") and Mylan Institutional LLC ("Mylan") (together, "Defendants") alleges as follows:

NATURE OF THE ACTION

1. This is an action for declaratory judgment of non-infringement and invalidity under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and under the patent laws of the United States, Title 35, U.S.C. § 100, *et seq*., regarding Beloteca's Abbreviated New Drug Application ("ANDA") with the U.S. Food and Drug Administration ("FDA"), to manufacture and sell an isosulfan blue for injection product prior to the expiration of U.S. Patent Nos. 7,662,992 ("the '992 patent"), 8,969,616 ("the '616 patent") and 9,353,050 ("the '050 patent").

2. The isosulfan blue for injection product is used as an aid in lymphography (a medical imaging procedure) to test how well the lymphatic system is working in certain parts of the body. It is a blue dye that works by staining the lymph nodes and lymph vessels.

THE PARTIES

3. Beloteca, Inc. is a California corporation having a place of business at 10525 Vista Sorrento Parkway, Suite 100, San Diego, California 92121.

4. On information and belief, Apicore is a limited liability company organized and existing under the laws of the State of Delaware, having a place of business at 49 Napoleon Court, Somerset, New Jersey 08873.

5. On information and belief, Apicore is a pharmaceutical company in the field of active pharmaceutical ingredient manufacturing.

6. On information and belief, Mylan is a limited liability company organized and existing under the laws of the State of Delaware, having a place of business at 1718 Northrock Court, Rockford, Illinois 61103.

7. On information and belief, Mylan is a pharmaceutical company that develops and commercializes injectable pharmaceutical products, including isosulfan blue.

8. On information and belief, Apicore is the assignee of the ’992 patent, entitled “Process for Preparation of Isosulfan Blue,” a copy of which is attached hereto as Exhibit A.

9. On information and belief, Apicore is the assignee of the ’616 patent, entitled “Process for Preparation of Isosulfan Blue,” a copy of which is attached hereto as Exhibit B.

10. On information and belief, Apicore is the assignee of the ’050 patent, entitled “Process for Preparation of Isosulfan Blue,” a copy of which is attached hereto as Exhibit C.

11. On information and belief, Mylan is Apicore’s exclusive licensee of the ’992, ’616 and ’050 patents and holds all substantial rights in the’992, ’616 and ’050 patents.

JURISDICTION AND VENUE

12. These claims arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and under the patent laws of the United States, Title 35 of the United States Code.

13. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1337 and 1338(a), in that it involves substantial claims arising under the United States Patent Act, 35 U.S.C. § 1 et seq.

14. This Court may declare the rights and other legal relations of the parties pursuant to 28 U.S.C. §§ 2201 and 2202 because this is a case of actual controversy within the Court's jurisdiction which seeks a declaratory judgment that the '992, '616 and '050 patents are invalid and/or are not infringed.

15. This Court has personal jurisdiction over Mylan because Mylan has a principal place of business within this District; because Mylan is currently registered with the Illinois Secretary of State, file number 02052377, and has an Illinois registered agent; and/or because, on information and belief, Mylan has affiliations with Illinois and this District that are pervasive, continuous and systematic, including but not limited to its conducting of substantial and regular business therein through the direct or indirect manufacturing, marketing, distribution, offering for sale, and/or sale of its pharmaceutical drug products, including isosulfan blue, within Illinois and this District.

16. This Court has personal jurisdiction over Apicore because, on information and belief, Apicore has affiliations with Illinois and this District that are pervasive, continuous and systematic, including but not limited to its conducting of substantial and regular business therein through the direct or indirect manufacturing, marketing, distribution, offering for sale, and/or sale of its pharmaceutical drug products, including isosulfan blue, within Illinois and this

District; its licensing the ’992, ’616 and ’050 patents to Mylan, a company located in Illinois and this District; and/or because Apicore is registered as a licensed drug distributor with the Illinois Division of Financial & Professional Regulation, license no. 004004580.

17. Venue is proper in this judicial district based on 28 U.S.C. §1391(b), (c), and (d).

PATENTS-IN-SUIT

18. On its face, the ’992 patent issued from Application No. 12/180,057 on February 16, 2010, with assignee “Apicore, LLC,” naming Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial as inventors. The ’992 patent claims processes for preparing compounds purporting to include isosulfan blue.

19. On its face, the ’616 patent issued from Application No. 13/951,034 on March 3, 2015, with assignee “Apicore US LLC,” naming Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial as inventors. The ’616 patent claims processes for preparing compounds purporting to include isosulfan blue.

20. On its face, the ’050 patent issued from Application No. 13/310,019 on May 31, 2016, with assignee “Apicore US LLC,” naming Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial as inventors. The ’050 patent claims compounds purporting to include isosulfan blue with purity of at least 99.0% by HPLC.

CONTROVERSY

21. On information and belief, Apicore has entered into an exclusive licensing arrangement with Mylan permitting Mylan to commercialize isosulfan blue products purportedly manufactured according to the claims of the ’992, ’616 and ’050 patents.

22. On information and belief, Mylan, by itself or through affiliated entities, markets a 1% isosulfan blue for injection product in the United States, purportedly pursuant to ANDA No. 90,874. ANDA No. 90,874 was approved July 20, 2010.

23. ANDA No. 90,874 was originally held by Synerx.

24. On information and belief, Mylan acquired Synerx on or around January, 2011.

25. On information and belief, Mylan, through its exclusive license with Apicore, markets, offers to sell, manufactures, distributes, and/or sells isosulfan blue products, in the United States, including in Illinois and in this District.

26. On approximately July 26, 2017, Beloteca submitted to FDA Abbreviated New Drug Application ("ANDA") No. 210714 for 1% strength isosulfan blue for injection. This ANDA sought approval to market Beloteca's isosulfan blue for injection product in the United States.

27. On January 16, 2019, FDA approved Beloteca's ANDA No. 210714.

28. Based on the approval of its ANDA No. 210714, Plaintiff Beloteca intends to manufacture, market, distribute and/or sell in the United States and this District the isosulfan blue drug product that is the subject of ANDA No. 210714.

29. On information and belief, upon learning of FDA's approval of ANDA No. 210714, Defendants will assert that Beloteca is infringing the '992, '616 and '050 patents by manufacturing, marketing, distributing and/or selling in the United States the isosulfan blue drug product, and will bring legal action against Beloteca for patent infringement.

30. In 2016, Defendants asserted patent infringement and filed a legal action against another drug company (Aurobindo Pharma) when that company obtained approval of its ANDA seeking to engage in the commercial manufacture, use, marketing, offer for sale and/or sale of

isosulfan blue—the same drug at issue in Plaintiff's ANDA No. 210714. That legal action, titled *Mylan Institutional LLC et al. v. Aurobindo Pharma Ltd*. et al., Civil Action No. 2:16-cv-491-RWS-RSP, was filed in the United States District Court for the Eastern District of Texas. *See* Exhibit D attached (copy of the *Aurobindo* Complaint, without exhibits).

31. There is a case or controversy as to Beloteca's non-infringement of the '992, '616 and/or '050 patents through its preparation, marketing, and/or sale of isosulfan blue for injection products made pursuant to ANDA No. 210714, and as to the invalidity of the '992, '616 and/or '050 patents.

32. The facts alleged herein, under all the circumstances, show that there is an actual, substantial, continuing and justiciable controversy between the parties having adverse legal interests, of sufficient immediacy and reality to warrant the issuance of a declaratory judgment as to the infringement, validity, and/or enforceability of the '992, '616, and '050 patents.

COUNT I

DECLARATORY JUDGMENT OF NONINFRINGEMENT OF ANY VALID CLAIM OF THE '992 PATENT

33. Beloteca repeats and incorporates by reference the foregoing paragraphs of its Complaint as if fully set forth herein.

34. This declaratory judgment claim arises under the patent laws of the United States, 35 U.S.C. § 1 et seq. and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and seeks a declaration that no valid claim of the '992 patent will be infringed by the manufacture, use, sale, offer for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714.

35. There is an actual and justiciable controversy between the parties concerning whether the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for

injection product that is the subject of ANDA No. 210714 will infringe any valid and enforceable claim of the ’992 patent.

36. Beloteca is entitled to a judicial declaration that the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714 will not infringe, directly or indirectly, any valid claim of the ’992 patent.

COUNT II

DECLARATORY JUDGMENT OF NONINFRINGEMENT OF ANY VALID CLAIM OF THE ’616 PATENT

37. Beloteca repeats and incorporates by reference the foregoing paragraphs of its Complaint as if fully set forth herein.

38. This declaratory judgment claim arises under the patent laws of the United States, 35 U.S.C. § 1 et seq. and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and seeks a declaration that no valid claim of the ’616 patent will be infringed by the manufacture, use, sale, offer for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714.

39. There is an actual and justiciable controversy between the parties concerning whether the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714 will infringe any valid and enforceable claim of the ’616 patent.

40. Beloteca is entitled to a judicial declaration that the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714 will not infringe, directly or indirectly, any valid claim of the ’616 patent.

COUNT III

DECLARATORY JUDGMENT OF NONINFRINGEMENT OF ANY VALID CLAIM OF THE '050 PATENT

41. Beloteca repeats and incorporates by reference the foregoing paragraphs of its Complaint as if fully set forth herein.

42. This declaratory judgment claim arises under the patent laws of the United States, 35 U.S.C. § 1 et seq. and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and seeks a declaration that no valid claim of the '050 patent will be infringed by the manufacture, use, sale, offer for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714.

43. Among other things, the '050 patent is invalid for the reasons stated in the Petition for *Inter Partes* Review of U.S. Patent No. 9,353,050, in the matter captioned *Luitpold Pharmaceuticals, Inc. v. Apicore US LLC*, Case No. IPR2018-01640, currently pending before the United States Patent and Trademark Office, Patent Trial and Appeal Board.

44. There is an actual and justiciable controversy between the parties concerning whether the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714 will infringe any valid and enforceable claim of the '050 patent.

45. Beloteca is entitled to a judicial declaration that the manufacture, use, sale, offering for sale, or importation of the isosulfan blue for injection product that is the subject of ANDA No. 210714 will not infringe, directly or indirectly, any valid claim of the '050 patent.

PRAYER FOR RELIEF

WHEREFORE, Beloteca respectfully requests that the Court enter a Judgment and Order in its favor and against Defendants as follows:

(a) For a declaration that the manufacture, use, offer to sell, sale, and/or importation into the United States of the isosulfan blue for injection product that is the subject of ANDA No. 210714 does not, and will not, infringe any valid and enforceable claim of the '992, '616 and '050 patents;

(b) For a declaration that the claims of the '992, '616 and '050 patents are invalid;

(c) For a declaration that this case is exceptional in favor of Beloteca and awarding attorneys' fees pursuant to 35 U.S.C. § 285, other statutes or rules, or the general power of the Court;

(d) For an award of costs and expenses; and

(e) For such other relief as the Court determines to be just and proper.

By: s/ Don Mizerk

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Attorneys for Plaintiff BELOTECA, INC.

Dated: January 17, 2019

Exhibit A

US007662992B2

(12) **United States Patent**
Kovi et al.

(10) **Patent No.: US 7,662,992 B2**
(45) **Date of Patent: Feb. 16, 2010**

(54) **PROCESS FOR PREPARATION OF ISOSULFAN BLUE**

(75) Inventors: **Ravishanker Kovi**, Monroe, NJ (US); **Satyam Nampalli**, Belle Mead, NJ (US); **Peter Xavier Tharial**, Piscataway, NJ (US)

(73) Assignee: **Apicore, LLC**, Somerset, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/180,057**

(22) Filed: **Jul. 25, 2008**

(65) **Prior Publication Data**

US 2008/0293963 A1 Nov. 27, 2008

Related U.S. Application Data

(63) Continuation of application No. 11/747,291, filed on May 11, 2007, now abandoned.

(51) **Int. Cl.**
C07C 309/00 (2006.01)

(52) **U.S. Cl.** **562/46**; 562/59

(58) **Field of Classification Search** 568/30
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

1,531,507	A	3/1925	Rosenbaum	
1,805,925	A	5/1931	Schmidt	
1,878,530	A	9/1932	Kyrides	
2,422,445	A	6/1947	Stryker	
2,726,252	A	12/1955	Balon	
4,330,476	A	5/1982	Hermann	
4,710,322	A	12/1987	Metz	
5,659,053	A	8/1997	Gessner et al.	
2006/0224003	A1 *	10/2006	Kulkarni et al.	552/111

OTHER PUBLICATIONS

Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition vol. III Part F 110-113.
International Search and Written Opinion of Apr. 23, 2003 of International Application No. PCT/US07/84051.

* cited by examiner

Primary Examiner—Jafar Parsa
Assistant Examiner—Chukwuma O Nwaonicha
(74) *Attorney, Agent, or Firm*—Timothy X. Gibson; Gibson & Dernier LLP

(57) **ABSTRACT**

A process for the preparation of isosulfan blue (Active Pharmaceutical Ingredient) is provided. A process is also provided for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), used in the preparation thereof and a procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3). Also provided is a process for the preparation of an isoleuco acid of formula (4), which upon mild oxidation gives rise to isosulfan blue of pharmaceutical grade which can be used for preparation of pharmaceutical formulations. The isolation and purification procedures provided in the process provide substantially pure isosulfan blue with HPLC purity 99.5% or greater.

23 Claims, No Drawings

PROCESS FOR PREPARATION OF ISOSULFAN BLUE

CROSS-REFERENCE TO RELATED APPLICATIONS

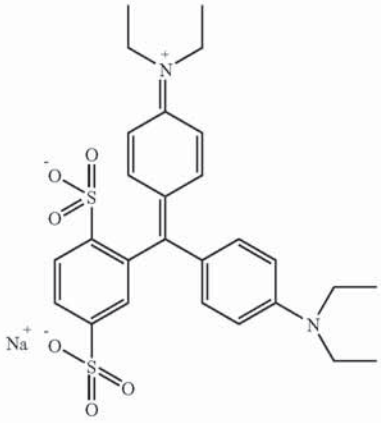
This is a continuation application, and claims the benefit, of U.S. patent application Ser. No. 11/747,291 filed May 11, 2007, the entirety of which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a process for the production of isosulfan blue, and in particular, to a process for the production of isosulfan blue in a substantially pure form.

BACKGROUND OF THE INVENTION

Isosulfan blue, having a chemical name, N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt and the formula



(5)

is a triarylmethane dye used as a contrast agent for the delineation of lymphatic vessels and is particularly useful as a cancer diagnostic agent. Also known chemically as sulfan blue or patent blue, isosulfan blue is an active pharmaceutical ingredient used in the Lymphazurin™ blue dye pharmaceutical dosage form, available as 1% (10 mg/ml) 5 ml solution in phosphate buffer for injection. It is commonly used in a procedure called "mapping of the sentinel lymph nodes". It is an adjunct to lymphography for visualization of the lymphatic system draining the region of injection. It has been used with increasing frequency in localizing sentinel lymph nodes in breast cancer patients. Isosulfan blue-guided surgical removal of cancerous tissue has been on the rise as it is cost effective and safer to use than technetium 99M radioisotope-labeled sulfur colloid. Isosulfan blue is a structural isomer of sulphan blue; both belong to the family of triarylmethane dyestuffs. Generally, preparation of triarylmethane dyes involves condensation of suitably substituted aryl aldehydes with 2 equivalents of alkyl-aryl amines giving rise to leuco-bases or leuco-acids followed by oxidation. Although the literature is replete with methods of preparing triarylmethane dyes, most of the methods involve strong acids for condensation resulting in leuco-bases or leuco-acids, hazardous oxidizing agents (lead oxide, chloranil, iron phthalocyanine/oxone) for converting to triarylmethane dyes, and crude methods (precipitation with sodium sulfate) of purification. See for example U.S. Pat. Nos. 4,330,476, 4,710,322, 1,531,507, 5,659,053, 1,805,925, 2,422,445, 1,878,530 and 2,726,252. Prior art methods of isolation of the crude leuco-acids or leuco-bases involve tedious neutralization/basification with strong bases and typically using the reaction mixtures in the oxidation step, giving rise to crude triarylmethane dyes. The triarylmethane dyestuffs thus prepared are used mainly for dyeing fabric, coloring paper, and printing inks. The literature cites utilization of the same aforementioned synthetic and isolation methods for the preparation of diagnostically important dyes, such as isosulfan blue, sulphan blue and patent blue V. See, Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition, Volume III Part F, 110-133.

Therefore there is a need in the art for an improved method in the process chemistry of isosulfan blue to be prepared in the purest form which is suitable for large scale cGMP production for its pharmaceutical formulation manufacturing.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention is to provide a simple, safe, cost-effective, time saving and reliable process for the preparation of isosulfan blue in bulk scale and in substantially pure form. "Substantially pure" is defined herein as 99.0% or greater.

Another object of the invention is to provide a simple, cost-effective and reliable process for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), required in the preparation of isosulfan blue. This embodiment provides a process step that does not require tedious neutralization with very large quantities of sodium carbonate and effervescence, as is the case in prior art processes.

Another object of the invention is to provide a simplified procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3) that does not include acidifying the reaction mixture with concentrated sulfuric acid and boiling until excess sulfurous acid is expelled, as is taught in the prior art.

Yet another object of the invention is to provide a procedure for obtaining the benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3) free of inorganic salts, which essentially simplifies the isolation procedures to be implemented during isolation of isoleuco acid.

Yet another, object of the invention is to provide a process for the preparation of an isoleuco acid of formula (4), through the urea derivative as an in-situ intermediate. The isoleuco acid of formula (4) on further oxidation gives rise to the target compound, isosulfan blue (5). Still another object of the invention is to use very mild oxidation agent to avoid any over oxidized products and also to improve the stability of the isosulfan blue under reaction conditions.

According to this invention, there is provided a simple procedure for the isolation of benzaldehyde-2,5-disulfonic acid, isoleuco acid and isosulfan blue at acid stage and also at sodium salt formation stage by incorporating crystallization techniques, thereby avoiding distillation and other techniques using high temperatures which jeopardize the compound stability during the manufacturing process.

These and other aspects of the invention will be apparent to those skilled in the art.

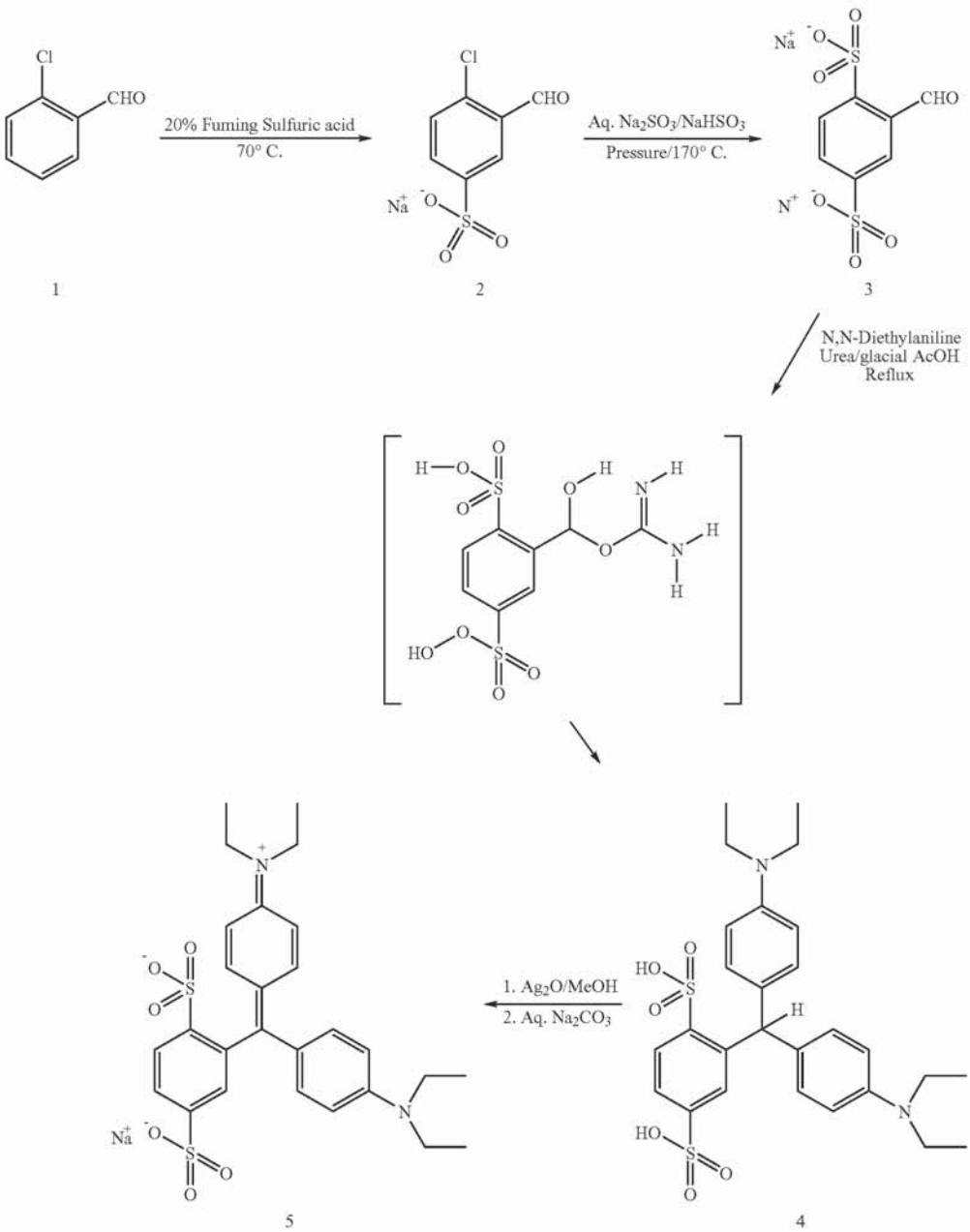
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following description, for purposes of explanation, specific numbers, materials and configurations are set forth in order to provide a thorough understanding of the invention. It will be apparent, however, to one having ordinary skill in the art that the invention may be practiced without these specific details. In some instances, well-known features may be omitted or simplified so as not to obscure the present invention.

Furthermore, reference in the specification to phrases such as "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. The appearances of phrases such as "in one embodiment" in various places in the specification are not necessarily all referring to the same embodiment. In accordance with one embodiment the present invention relates to a process for the preparation of isosulfan blue.

Scheme

The following provides a process for the production of isosulfan blue of formula (5):

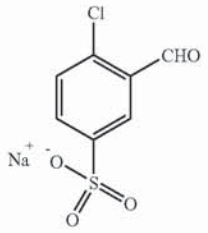


1 2 3 4 5

Experimental Procedures

In accordance with one embodiment of the present invention a first step involves sulfonation of the commercially available starting material of the formula (1) to 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2).

(2)



In one example, the sulfonation process involved reacting one equivalent of the 2-chlorobenzaldehyde of formula (1) with 2.0 equivalents of 20% fuming sulfuric acid at 15° C. to 70° C. for 16 hrs. The reaction mixture was poured into ice-water carefully followed by stirring with solid sodium chloride resulting in a cream colored precipitate, which upon filtration, washing with ether and drying afforded 2-chlorobenzaldehyde-5-sulfonic acid of the formula (2) in 86% yield.

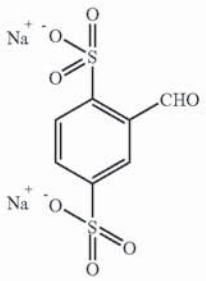
In accordance with one embodiment of the present invention, a second step of the process involves nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2) with an alkali metal sulfite/bisulfite such as sodium sulfite/sodium bisulfite at elevated temperatures under closed conditions.

In one example, this reaction was carried out in a Parr pressure vessel equipped with overhead magnetic stirring. 2-Chlorobenzaldehyde-5-sulfonic acid (2), sodium sulfite (2.29 equivalents), sodium bisulfite (10% of sodium sulfite), and water (3.45 mL/g) were charged into the Parr pressure vessel. The reaction mixture in the vessel was stirred and heated at 170-180° C. for 5-7 hours generating 140-150 psi pressure.

The reaction mixture, after cooling, was poured into methanol while stirring, so as to make 20% aqueous content of the whole volume. This process ensured total precipitation of the inorganic salts, which could be removed by filtration. The solvent from the filtrate was removed under reduced pressure to obtain a solid residue, which was triturated with methanol and filtered to afford light yellow colored compound, benzaldehyde-2,5-disulfonic acid, di sodium salt of the formula (3) in 93.9% yield.

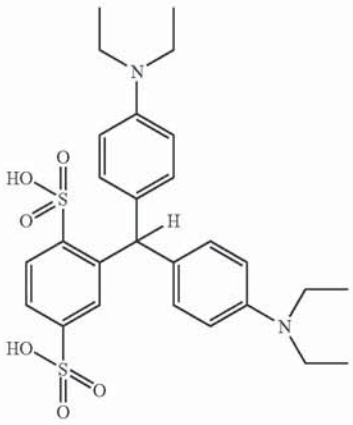
In accordance with one embodiment a purification procedure for removing the inorganic salts essentially involves dissolving the crude solid in N, N dimethylformamide and stirring the contents for 1-2 hours at ambient temperature followed by filtration. The filtrate is precipitated by dichloromethane to afford the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% and with HPLC assay greater than 90% w/w.

(3)



In accordance with one embodiment of the present invention, a third step of the process involved condensing benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N,N-diethylaniline to provide isoleuco acid of the formula (4).

(4)



In one example, pure isoleuco-acid of the formula (4) with chromatographic purity greater than 98.0% was obtained in the solid form out of the reaction mixture. A mixture of benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3), N,N-diethylaniline (2.2 equivalents), and urea (0.75 equivalents) in glacial acetic acid was stirred and refluxed for 20-25 hrs. The reaction progressed through the intermediate formation in-situ which is a urea derivative of benzaldehyde-2,5-disulfonic acid disodium salt. To the above cooled reaction mixture after 20-25 hrs reflux, methanol was added to form a precipitate, which was collected by vacuum filtration and washed with diethyl ether to afford the isoleuco acid of the formula (4) in 56.8% yield.

The purification of isoleuco acid was carried out by dissolving the crude solid in 5 volumes of water and stirred for 1-2 hours at ambient temperature and filtering the solid. The above process was repeated twice before the final solid was washed with acetone to generate isoleuco acid of the formula (4) with chromatographic purity greater than 99.5%.

In accordance with one embodiment of the present invention a fourth step of the process involves conversion of the isoleuco acid (4) to isosulfan blue of the formula (5) under conditions that employ milder oxidizing agents with no strong acidic reagents and are less hazardous than the prior art.

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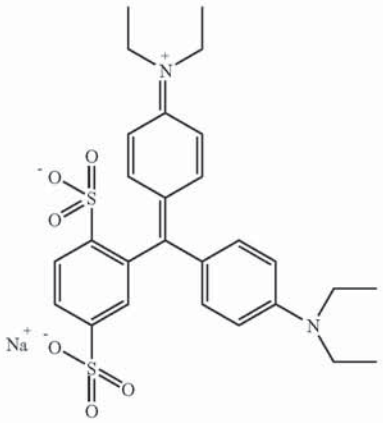

In an example of the present inventive process, a suspension of isoleuco acid of the formula (4) in methanol was stirred at room temperature for 12-14 hrs with silver oxide (2.5 equivalents). The blue colored reaction mixture was filtered through a pad of silica gel and Celite followed by filtration through an acidic zeolite bed and further through a 0.2 micron membrane filtration unit. The filtrate was then precipitated with isopropyl ether at room temperature to obtain crude isosulfan blue acid.

The isosulfan blue acid thus obtained was then purified by recrystallization from aqueous isopropyl alcohol/acetone to afford isosulfan blue acid of chromatographic purity NLT 99.5% performed by High Performance Liquid Chromatography.

The final product of isosulfan blue sodium (formula 5) was obtained when isosulfan blue acid was adjusted to a pH greater than 6.0 in aqueous acetone medium using sodium bicarbonate solution for pH adjustment. The reaction mass was filtered to give isosulfan blue sodium of formula (5) having purity greater than 99.5% by HPLC and also free of silver with silver content estimated by Atomic absorption spectrometer less than 20 ppm.

EXAMPLES

2-Chlorobenzaldehyde-5-sulfonic Acid, Sodium Salt of the Formula (2)

113.82 g (based on SO_3 molecular weight, 569 mL) of 20% fuming sulfuric acid (FSA) was charged into a 1 L three-neck flask fitted with a dropping funnel, overhead stirrer, and thermometer. The reaction mass was cooled to 15 to 20° C. 100 g of 2-chlorobenzaldehyde of the formula (1) was added dropwise to the stirred and cooled FSA over a period of 40 minutes, so that the temperature didn't rise above 20° C. The reaction mixture was stirred and heated at 70° C. for 16 hours to obtain a dark-brown colored reaction solution. The HPLC results indicated the absence of the starting material. The dark-brown colored reaction solution was carefully poured into a beaker containing 1200 g of crushed ice and stirred. 500 g of solid sodium chloride was added portion wise to the stirred colored acidic solution to precipitate a light-yellow colored solid. The light-yellow colored solid was collected by vacuum filtration and washed with diethyl ether to afford 150.0 g (86.92%) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2).

Benzaldehyde-2,5-disulfonic Acid, Sodium Salt of the Formula (3)

50 g (0.206 mol) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2), 59.75 g (0.474 mol, 2.3 eq.) of Na_2SO_3 and 5.97 g (10% of Na_2SO_3) of $NaHSO_3$ were dissolved in 400 mL of water. The solution was charged into a 600 mL capacity Parr pressure cylinder equipped with stirring and heating. The reaction mixture was stirred (300-310 RPM) and heated at 180° C. (generates ~150 psi pressure) for 5-7 hours. HPLC results indicate the absence of the starting material. After cooling and releasing the pressure, the reaction mixture was poured into 1600 mL of stirred methanol and stirred for 15-30 minutes to precipitate the unwanted inorganic salts. The inorganic salts were filtered off using a pad of Celite and the filtrate evaporated under reduced pressure to obtain a solid residue. The solid residue obtained was triturated with 200 mL methanol, collected by filtration and washed with ether to give 60 g (93.9%) of benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3).

Purification of Benzaldehyde-2,5-disulfonic Acid, Sodium Salt Formula (3)

60 g of crude benzaldehyde-2,5-disulfonic acid, disodium salt prepared as per the procedure above was dissolved in 500 mL of N,N-dimethylformamide and stirred for 2 hours at 20-25° C. The mixture was filtered through a buchner funnel and the filtrate was precipitated using 1500 mL of dichloromethane to afford 20 g of the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% w/w.

Isoleuco Acid of the Formula (4)

60 g of benzaldehyde-2,5-disulfonic acid sodium salt of formula (3), 8.76 g of urea (0.75 eq), and 1000 mL of glacial acetic acid were charged into a 3 L 3-neck flask fitted with a mechanical stirrer and reflux condenser. 65.61 mL (2.2 eq) of N,N-diethyl aniline was added to the stirred mixture and refluxed for 20-25 hrs. When the HPLC results indicated the content of starting material was less than 5%, the reaction mass was cooled to room temperature. After cooling to room temperature, 600 mL of methanol was added and the separated solid collected on a sintered funnel by vacuum filtration. The collected solid was washed with methanol to obtain 55-60 g (56.8%) of crude isoleuco acid of the formula (4).

Purification of Isoleuco Acid of Formula (4)

50 g of crude isoleuco acid along with 250 ml of water was charged into a 1 L 3-neck round bottom flask fitted with a mechanical stirrer. The reaction mixture was stirred for 1 hour at 20-25° C. The solid was filtered through a buchner funnel.

The above process was repeated twice. The final product thus obtained was then washed with 25 ml of acetone and then dried to obtain 40-45 g of the desired isoleuco acid of formula (4).

Isosulfan Blue of the Formula (5)

15 g (0.027 mol) of isoleuco acid of the formula (4) and 225 mL of Methanol were charged into a 1 L round bottomed flask and the suspension was stirred. To the stirred suspension,

15.91 g (0.068 mol, 2.5 eq.) of silver oxide was added in one portion at room temperature and stirred at room temperature for 12-14 hours. The reaction mixture turned blue in color as the oxidation to the desired product progressed. The HPLC results indicated the absence of starting material. The blue colored reaction mixture was filtered through a buchner funnel and the solid silver oxide collected was taken into the reaction flask and the filtrate was kept aside. 225 ml of methanol was added to the silver oxide taken in the reaction flask and stirred at 20-25° C. for 30 minutes and filtered through the buchner funnel. This silver oxide washing procedure with methanol was carried out twice more.

The combined filtrates along with the initial filtrate were then filtered through a bed of silica gel/celite (2 inch silica gel/1 inch of celite) and finally the bed was washed with 50 mL of methanol.

The filtrate was then subjected to a filtration through an acidic zeolite bed of 2 inch height (pH of the zeolite bed was adjusted to acidic pH by using 0.1N hydrochloric acid aqueous solution) followed by filtration through a 0.2 micron filtration unit.

Isopropyl ether was added three times the volume of the filtrate and the isosulfan blue acid was precipitated as a solid at about 10 gram (68.8%) yield.

In order to prepare the Isosulfan blue sodium salt of the formula (5), 10.0 g of the solid obtained above was dissolved in 30 mL deionized water. Saturated sodium bicarbonate solution was added drop wise to adjust the pH to 8.0. To this 300 mL of acetone was added and stirred at 20-25° C. for 30 minutes. The crystallized product was then filtered through a buchner funnel and the solid thus obtained was dried at 40° C. under vacuum to obtain the isosulfan blue sodium salt of formula (5).

While the preferred embodiments have been described and illustrated it will be understood that changes in details and obvious undisclosed variations might be made without departing from the spirit and principle of the invention and therefore the scope of the invention is not to be construed as limited to the preferred embodiment.

What is claimed is:

1. A process of preparing N-[4-[[4-(diethyl amino) phenyl] (2, 5-disulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt comprising combining a suspension of isoleuco acid of the formula

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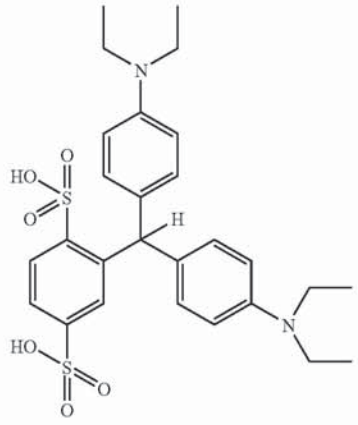
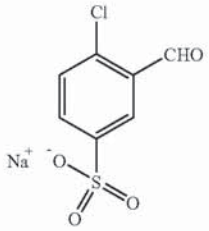


in a polar solvent with 2.0 to 3.0 equivalents of silver oxide, recovering isosulfan blue acid, and treating the isosulfan blue acid with a sodium solution.

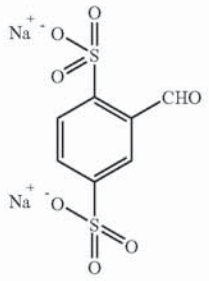
2. The process according to claim **1** comprising sulfonation of 2-chlorobenzaldehyde to obtain 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula

(2)



followed by nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt with an alkali metal sulfite and bisulfite to obtain benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



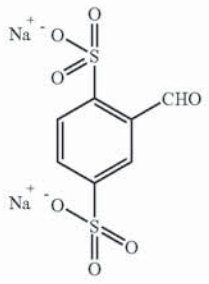
and condensing the benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N, N-diethylaniline using urea and glacial acetic acid to provide isoleuco acid of the formula (4).

3. The process of preparing 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2) according to claim **2** comprising reacting one equivalent of 2-chlorobenzaldehyde with 2 equivalents, based on SO_3 content, of 20% fuming sulfuric acid.

4. The process according to claim **1** wherein the polar solvent is methanol.

5. The process according to claim **2** of preparing free benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula

(3)



wherein the alkali metal sulfite and bisulfite comprise sodium sulfite and sodium bisulfite salts.

6. The process according to claim **5** wherein the reaction is carried out in a pressure vessel at 170-180° C. for 5 to 7 hours.

7. The process according to claim **6** wherein the reaction is carried out under a pressure of 140 to 150 psi.

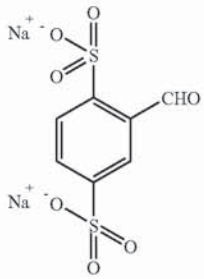
8. The process according to claim **2** comprising precipitating inorganic salts which will hinder the rate of reaction using methanol or one or more C_{1-4} lower alcohols.

9. The process according to claim **2** in which the benzaldehyde-2,5-disulfonic acid disodium salt is purified by extracting with a non-aqueous polar solvent followed by its precipitation in a halogenated or non-halogenated non-polar solvent which is miscible with the non-aqueous polar solvent.

10. The process according to claim **9** wherein the nonaqueous polar solvent is N,N dimethylformamide and the nonpolar solvent is dichloromethane.

11. The process according to claim **1** wherein the isoleuco acid of the formula (4)
is prepared by combining a benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



with N,N-diethylaniline, and urea and glacial acetic acid.

12. The process according to claim **11** performed at reflux conditions for 20-25 hours at 115 to 120° C.

13. The process according to claim **11** comprising precipitating a crude solid using methanol or a C_{1-4} lower alcohol.

14. The process according to claim **11** in which the crude solid is further purified using water.

15. The process according to claim **1** comprising oxidation of isoleuco acid of the formula (4) with 2.5 equivalents of silver oxide in methanol, resulting in a reaction mass, stirring the reaction mass at 20 to 25° C. for 12-14 hours, and filtering the silver oxide to provide a filtrate.

16. The process according to claim **15** comprising passing the filtrate through a bed of silica gel and celite and passing the filtrate through a zeolite bed optionally treated with an acid or base.

17. The process according to claim **16** further comprising passing the filtrate through a 0.2 micron filtration unit.

18. The process according to claim **15** comprising precipitating the filtrate using a non-polar solvent miscible with the filtrate.

19. The process according to claim **18** wherein the non-polar solvent is isopropyl ether.

20. The process according to claim **1** comprising adjusting the N-[4-[[4-(diethylamino) phenyl] (2,5-disulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-N -ethylethanaminium to a pH greater than 6.0 using an aqueous inorganic or organic derivative of sodium or a combination thereof.

21. The process according to claim **20** wherein the pH is adjusted using sodium bicarbonate solution.

22. The process according to claim **1** comprising recrystallization of N-[4-[[4-(diethylamino) phenyl] (2,5-disulfophenyl) methylene]-2,5-cyclohexadien-1 -ylidene]-N -ethylethanaminium using a solvent selected from the group consisting of a polar solvent, a non-polar solvent and a combination thereof to afford HPLC purity greater than 99.5%

23. The process according to claim **22** wherein the solvent is selected from an aqueous acetone medium and 80%_aqueous isopropanol/acetone.

* * * * *

Exhibit B

US008969616B2

(12) **United States Patent**
Kovi et al.

(10) **Patent No.: US 8,969,616 B2**
(45) **Date of Patent: *Mar. 3, 2015**

(54) **PROCESS FOR PREPARATION OF ISOSULFAN BLUE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/951,034**

(22) Filed: **Jul. 25, 2013**

(65) **Prior Publication Data**

US 2013/0310600 A1 Nov. 21, 2013

Related U.S. Application Data

(63) Continuation of application No. 13/310,019, filed on Dec. 2, 2011, which is a continuation of application No. 12/643,056, filed on Dec. 21, 2009, now abandoned, which is a continuation of application No. 12/180,057, filed on Jul. 25, 2008, now Pat. No. 7,662,992, which is a continuation of application No. 11/747,291, filed on May 11, 2007, now abandoned.

(51) **Int. Cl.**

C07C 309/00	(2006.01)
C07C 303/02	(2006.01)
C07C 303/22	(2006.01)

(52) **U.S. Cl.**

CPC ***C07C 303/02*** (2013.01); ***C07C 303/22*** (2013.01)

USPC **562/43**; 562/41

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

1,531,507	A	3/1925	Rosenbaum	
1,805,925	A	5/1931	Schmidt	
1,878,530	A	9/1932	Kyrides	
2,422,445	A	6/1947	Stryker	
2,726,252	A	12/1955	Balon	
4,330,476	A	5/1982	Hermann	
4,710,322	A	12/1987	Metz	
5,659,053	A	8/1997	Gessner et al.	
2006/0224003	A1 *	10/2006	Kulkarni et al.	552/111

OTHER PUBLICATIONS

Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition vol. III Part F 110-113.

International Search and Written Opinion of Apr. 23, 2003 of International Application No. PCT/US2007/084051.

Office Action for corresponding U.S. Appl. No. 12/180,057, dated Feb. 23, 2009.

Office Action for corresponding U.S. Appl. No. 11/747,291, dated Feb. 7, 2008.

Office Action for corresponding U.S. Appl. No. 12/643,056, dated Jul. 19, 2011.

Coleman et al., "Unexplained Decrease in Measured Oxygen Saturation by Pulse Oximetry Following Injection of Lymphazurin 1% (isosulfan Blue) During a Lymphatic Mapping Procedure", Journal of Surgical Oncology 1999, 70: 126-129.

Office Action for corresponding U.S. Appl. No. 13/310,019, dated Jul. 10, 2012.

* cited by examiner

Primary Examiner — Karl J Puttlitz

(74) *Attorney, Agent, or Firm* — Timothy X. Gibson, Esq.; Gibson & Dernier LLP

(57) **ABSTRACT**

A process for the preparation of isosulfan blue (Active Pharmaceutical Ingredient) is provided. A process is also provided for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), used in the preparation thereof and a procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3). Also provided is a process for the preparation of an isoleuco acid of formula (4), which upon mild oxidation gives rise to isosulfan blue of pharmaceutical grade which can be used for preparation of pharmaceutical formulations. The isolation and purification procedures provided in the process provide substantially pure isosulfan blue with HPLC purity 99.5% or greater.

23 Claims, No Drawings

PROCESS FOR PREPARATION OF ISOSULFAN BLUE

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation application, and claims the benefit, of U.S. patent application Ser. No. 13/310,019, filed Dec. 2, 2011, which is a continuation of U.S. patent application Ser. No. 12/643,056, filed Dec. 21, 2009, now abandoned, which is a continuation of U.S. Ser. No. 12/180,057 filed Jul. 25, 2008, now U.S. Pat. No. 7,662,992, which is a continuation of U.S. Ser. No. 11/747,291 filed May 11, 2007, now abandoned, the entireties of which are incorporated herein by reference.

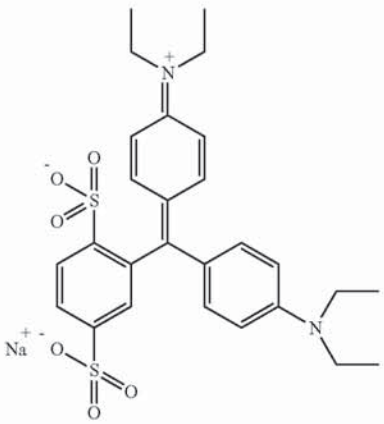
FIELD OF THE INVENTION

The present invention relates to a process for the production of isosulfan blue, and in particular, to a process for the production of isosulfan blue in a substantially pure form.

BACKGROUND OF THE INVENTION

Isosulfan blue, having a chemical name, N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt and the formula

(5)



is a triarylmethane dye used as a contrast agent for the delineation of lymphatic vessels and is particularly useful as a cancer diagnostic agent. Also known chemically as sulfan blue or patent blue, isosulfan blue is an active pharmaceutical ingredient used in the Lymphazurin™ blue dye pharmaceutical dosage form, available as 1% (10 mg/ml) 5 ml solution in phosphate buffer for injection. It is commonly used in a procedure called "mapping of the sentinel lymph nodes". It is an adjunct to lymphography for visualization of the lymphatic system draining the region of injection. It has been used with increasing frequency in localizing sentinel lymph nodes in breast cancer patients. Isosulfan blue-guided surgical removal of cancerous tissue has been on the rise as it is cost effective and safer to use than technetium 99M radioisotope-labeled sulfur colloid. Isosulfan blue is a structural isomer of sulphan blue; both belong to the family of triarylmethane dyestuffs. Generally, preparation of triarylmethane dyes involves condensation of suitably substituted aryl aldehydes with 2 equivalents of alkyl-aryl amines giving rise to

leuco-bases or leuco-acids followed by oxidation. Although the literature is replete with methods of preparing triarylmethane dyes, most of the methods involve strong acids for condensation resulting in leuco-bases or leuco-acids, hazardous oxidizing agents (lead oxide, chloranil, iron phthalocyanine/oxone) for converting to triarylmethane dyes, and crude methods (precipitation with sodium sulfate) of purification. See for example U.S. Pat. Nos. 4,330,476, 4,710,322, 1,531, 507, 5,659,053, 1,805,925, 2,422,445, 1,878,530 and 2,726, 252. Prior art methods of isolation of the crude leuco-acids or leuco-bases involve tedious neutralization/basification with strong bases and typically using the reaction mixtures in the oxidation step, giving rise to crude triarylmethane dyes. The triarylmethane dyestuffs thus prepared are used mainly for dyeing fabric, coloring paper, and printing inks The literature cites utilization of the same aforementioned synthetic and isolation methods for the preparation of diagnostically important dyes, such as isosulfan blue, sulphan blue and patent blue V. See, Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition, Volume III Part F, 110-133.

Therefore there is a need in the art for an improved method in the process chemistry of isosulfan blue to be prepared in the purest form which is suitable for large scale cGMP production for its pharmaceutical formulation manufacturing.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention is to provide a simple, safe, cost-effective, time saving and reliable process for the preparation of isosulfan blue in bulk scale and in substantially pure form. "Substantially pure" is defined herein as 99.0% or greater.

Another object of the invention is to provide a simple, cost-effective and reliable process for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), required in the preparation of isosulfan blue. This embodiment provides a process step that does not require tedious neutralization with very large quantities of sodium carbonate and effervescence, as is the case in prior art processes.

Another object of the invention is to provide a simplified procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3) that does not include acidifying the reaction mixture with concentrated sulfuric acid and boiling until excess sulfurous acid is expelled, as is taught in the prior art.

Yet another object of the invention is to provide a procedure for obtaining the benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3) free of inorganic salts, which essentially simplifies the isolation procedures to be implemented during isolation of isoleuco acid.

Yet another, object of the invention is to provide a process for the preparation of an isoleuco acid of formula (4), through the urea derivative as an in-situ intermediate. The isoleuco acid of formula (4) on further oxidation gives rise to the target compound, isosulfan blue (5). Still another object of the invention is to use very mild oxidation agent to avoid any over oxidized products and also to improve the stability of the isosulfan blue under reaction conditions.

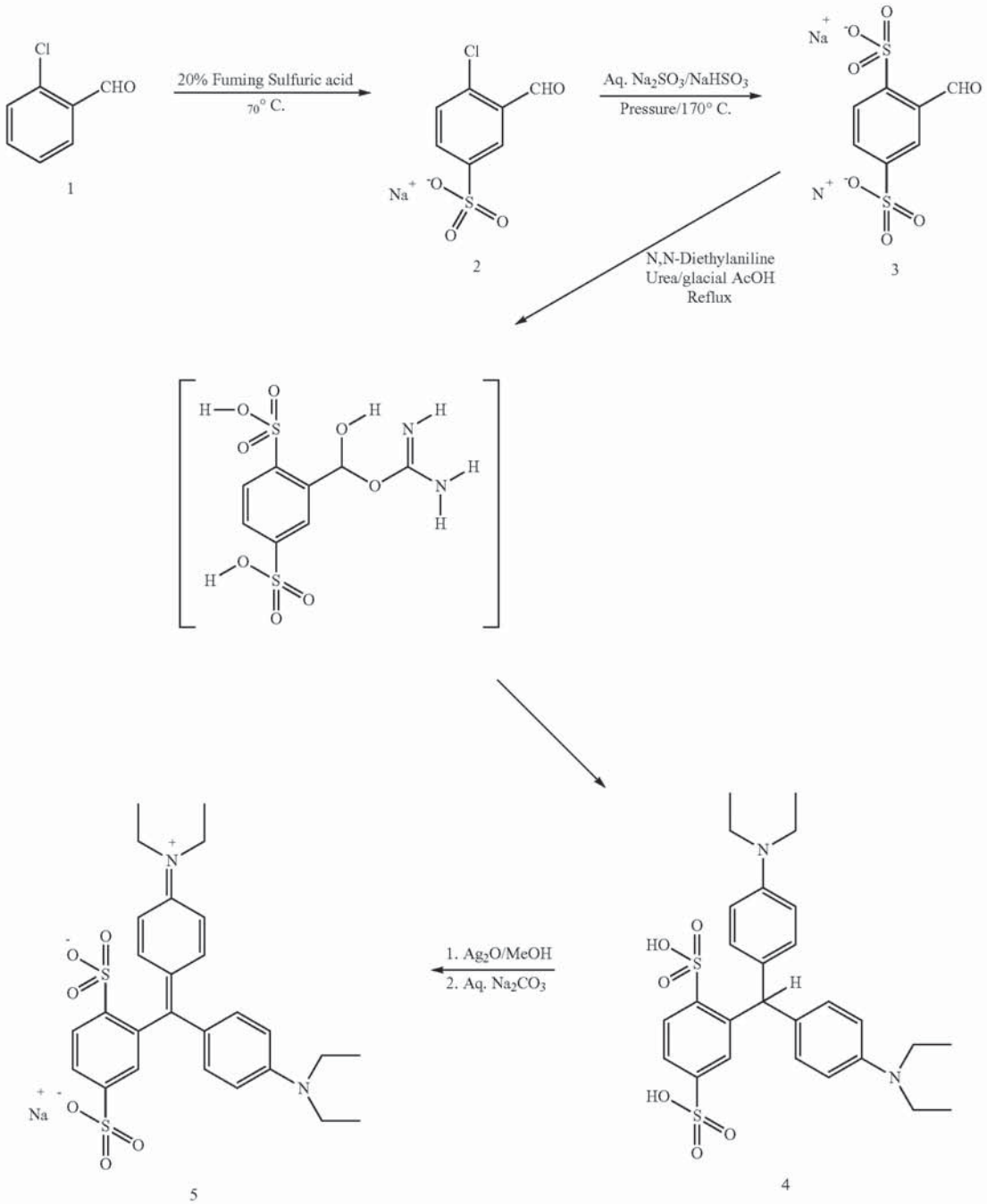
According to this invention, there is provided a simple procedure for the isolation of benzaldehyde-2,5-disulfonic acid, isoleuco acid and isosulfan blue at acid stage and also at sodium salt formation stage by incorporating crystallization techniques, thereby avoiding distillation and other techniques using high temperatures which jeopardize the compound stability during the manufacturing process.

These and other aspects of the invention will be apparent to those skilled in the art.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following description, for purposes of explanation, specific numbers, materials and configurations are set forth in order to provide a thorough understanding of the invention. It will be apparent, however, to one having ordinary skill in the art that the invention may be practiced without these specific details. In some instances, well-known features may be omitted or simplified so as not to obscure the present invention. Furthermore, reference in the specification to phrases such as "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. The appearances of phrases such as "in one embodiment" in various places in the specification are not necessarily all referring to the same embodiment. In accordance with one embodiment the present invention relates to a process for the preparation of isosulfan blue.

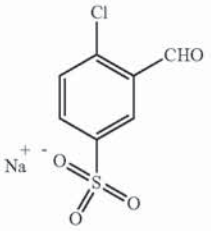
Scheme The following provides a process for the production of isosulfan blue of formula (5):



Experimental Procedures

In accordance with one embodiment of the present invention a first step involves sulfonation of the commercially available starting material of the formula (1) to 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2).

(2)



In one example, the sulfonation process involved reacting one equivalent of the 2-chlorobenzaldehyde of formula (1) with 2.0 equivalents of 20% fuming sulfuric acid at 15° C. to 70° C. for 16 hrs. The reaction mixture was poured into ice-water carefully followed by stirring with solid sodium chloride resulting in a cream colored precipitate, which upon filtration, washing with ether and drying afforded 2-chlorobenzaldehyde-5-sulfonic acid of the formula (2) in 86% yield.

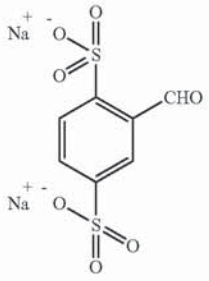
In accordance with one embodiment of the present invention, a second step of the process involves nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2) with an alkali metal sulfite/bisulfite such as sodium sulfite/sodium bisulfite at elevated temperatures under closed conditions.

In one example, this reaction was carried out in a Parr pressure vessel equipped with overhead magnetic stirring. 2-Chlorobenzaldehyde-5-sulfonic acid (2), sodium sulfite (2.29 equivalents), sodium bisulfite (10% of sodium sulfite), and water (3.45 mL/g) were charged into the Parr pressure vessel. The reaction mixture in the vessel was stirred and heated at 170-180° C. for 5-7 hours generating 140-150 psi pressure.

The reaction mixture, after cooling, was poured into methanol while stirring, so as to make 20% aqueous content of the whole volume. This process ensured total precipitation of the inorganic salts, which could be removed by filtration. The solvent from the filtrate was removed under reduced pressure to obtain a solid residue, which was triturated with methanol and filtered to afford light yellow colored compound, benzaldehyde-2,5-disulfonic acid, di sodium salt of the formula (3) in 93.9% yield.

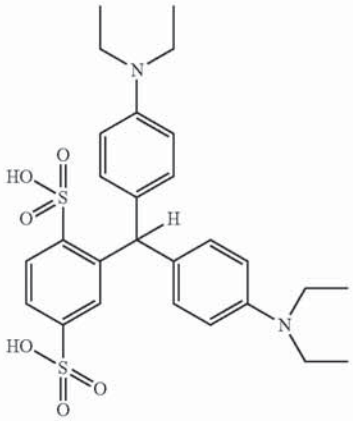
In accordance with one embodiment a purification procedure for removing the inorganic salts essentially involves dissolving the crude solid in N,N dimethylformamide and stirring the contents for 1-2 hours at ambient temperature followed by filtration. The filtrate is precipitated by dichloromethane to afford the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% and with HPLC assay greater than 90% w/w.

(3)



In accordance with one embodiment of the present invention, a third step of the process involved condensing benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N, N-diethylaniline to provide isoleuco acid of the formula (4).

(4)

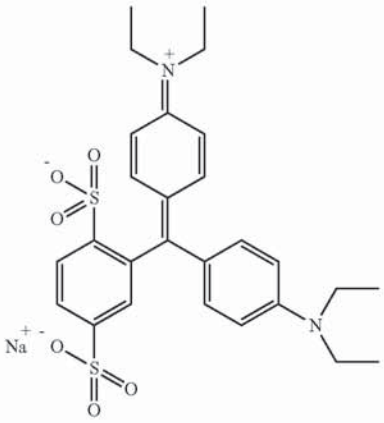


In one example, pure isoleuco-acid of the formula (4) with chromatographic purity greater than 98.0% was obtained in the solid form out of the reaction mixture. A mixture of benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3), N,N-diethylaniline (2.2 equivalents), and urea (0.75 equivalents) in glacial acetic acid was stirred and refluxed for 20-25 hrs. The reaction progressed through the intermediate formation in-situ which is a urea derivative of benzaldehyde-2,5-disulfonic acid disodium salt. To the above cooled reaction mixture after 20-25 hrs reflux, methanol was added to form a precipitate, which was collected by vacuum filtration and washed with diethyl ether to afford the isoleuco acid of the formula (4) in 56.8% yield.

The purification of isoleuco acid was carried out by dissolving the crude solid in 5 volumes of water and stirred for 1-2 hours at ambient temperature and filtering the solid. The above process was repeated twice before the final solid was washed with acetone to generate isoleuco acid of the formula (4) with chromatographic purity greater than 99.5%.

In accordance with one embodiment of the present invention a fourth step of the process involves conversion of the isoleuco acid (4) to isosulfan blue of the formula (5) under conditions that employ milder oxidizing agents with no strong acidic reagents and are less hazardous than the prior art.

(5)



In an example of the present inventive process, a suspension of isoleuco acid of the formula (4) in methanol was stirred at room temperature for 12-14 hrs with silver oxide (2.5 equivalents). The blue colored reaction mixture was filtered through a pad of silica gel and Celite followed by filtration through an acidic zeolite bed and further through a 0.2 micron membrane filtration unit. The filtrate was then precipitated with isopropyl ether at room temperature to obtain crude isosulfan blue acid.

The isosulfan blue acid thus obtained was then purified by recrystallization from aqueous isopropyl alcohol/acetone to afford isosulfan blue acid of chromatographic purity NLT 99.5% performed by High Performance Liquid Chromatography.

The final product of isosulfan blue sodium (formula 5) was obtained when isosulfan blue acid was adjusted to a pH greater than 6.0 in aqueous acetone medium using sodium bicarbonate solution for pH adjustment. The reaction mass was filtered to give isosulfan blue sodium of formula (5) having purity greater than 99.5% by HPLC and also free of silver with silver content estimated by Atomic absorption spectrometer less than 20 ppm.

EXAMPLES

2-Chlorobenzaldehyde-5-sulfonic acid, Sodium Salt of the Formula (2)

113.82 g (based on SO_3 molecular weight, 569 mL) of 20% fuming sulfuric acid (FSA) was charged into a 1 L three-neck flask fitted with a dropping funnel, overhead stirrer, and thermometer. The reaction mass was cooled to 15 to 20° C. 100 g of 2-chlorobenzaldehyde of the formula (1) was added dropwise to the stirred and cooled FSA over a period of 40 minutes, so that the temperature didn't rise above 20° C. The reaction mixture was stirred and heated at 70° C. for 16 hours to obtain a dark-brown colored reaction solution. The HPLC results indicated the absence of the starting material. The dark-brown colored reaction solution was carefully poured into a beaker containing 1200 g of crushed ice and stirred. 500 g of solid sodium chloride was added portion wise to the stirred colored acidic solution to precipitate a light-yellow colored solid. The light-yellow colored solid was collected by vacuum filtration and washed with diethyl ether to afford 150.0 g (86.92%) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2).

Benzaldehyde-2,5-disulfonic acid, Sodium Salt of the Formula (3)

50 g (0.206 mol) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2), 59.75 g (0.474 mol, 2.3 eq.) of Na_2SO_3 and 5.97 g (10% of Na_2SO_3) of $NaHSO_3$ were dissolved in 400 mL of water. The solution was charged into a 600 mL capacity Parr pressure cylinder equipped with stirring and heating. The reaction mixture was stirred (300-310 RPM) and heated at 180° C. (generates ~150 psi pressure) for 5-7 hours. HPLC results indicate the absence of the starting material. After cooling and releasing the pressure, the reaction mixture was poured into 1600 mL of stirred methanol and stirred for 15-30 minutes to precipitate the unwanted inorganic salts. The inorganic salts were filtered off using a pad of Celite and the filtrate evaporated under reduced pressure to obtain a solid residue. The solid residue obtained was triturated with 200 mL methanol, collected by filtration and washed with ether to give 60 g (93.9%) of benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3).

Purification of Benzaldehyde-2,5-disulfonic acid, Sodium Salt Formula (3)

60 g of crude benzaldehyde-2,5-disulfonic acid, disodium salt prepared as per the procedure above was dissolved in 500 mL of N,N-dimethylformamide and stirred for 2 hours at 20-25° C. The mixture was filtered through a buchner funnel and the filtrate was precipitated using 1500 mL of dichloromethane to afford 20 g of the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% w/w.

Isoleuco Acid of the Formula (4)

60 g of benzaldehyde-2,5-disulfonic acid sodium salt of formula (3), 8.76 g of urea (0.75 eq), and 1000 mL of glacial acetic acid were charged into a 3 L 3-neck flask fitted with a mechanical stirrer and reflux condenser. 65.61 mL (2.2 eq) of N,N-diethyl aniline was added to the stirred mixture and refluxed for 20-25 hrs. When the HPLC results indicated the content of starting material was less than 5%, the reaction mass was cooled to room temperature. After cooling to room temperature, 600 mL of methanol was added and the separated solid collected on a sintered funnel by vacuum filtration. The collected solid was washed with methanol to obtain 55-60 g (56.8%) of crude isoleuco acid of the formula (4).

Purification of Isoleuco Acid of Formula (4)

50 g of crude isoleuco acid along with 250 ml of water was charged into a 1 L 3-neck round bottom flask fitted with a mechanical stirrer. The reaction mixture was stirred for 1 hour at 20-25° C. The solid was filtered through a buchner funnel.

The above process was repeated twice. The final product thus obtained was then washed with 25 ml of acetone and then dried to obtain 40-45 g of the desired isoleuco acid of formula (4).

Isosulfan Blue of the Formula (5)

15 g (0.027 mol) of isoleuco acid of the formula (4) and 225 mL of Methanol were charged into a 1 L round bottomed flask and the suspension was stirred. To the stirred suspension, 15.91 g (0.068 mol, 2.5 eq.) of silver oxide was added in one portion at room temperature and stirred at room temperature for 12-14 hours. The reaction mixture turned blue in color as

the oxidation to the desired product progressed. The HPLC results indicated the absence of starting material. The blue colored reaction mixture was filtered through a buchner funnel and the solid silver oxide collected was taken into the reaction flask and the filtrate was kept aside. 225 ml of methanol was added to the silver oxide taken in the reaction flask and stirred at 20-25° C. for 30 minutes and filtered through the buchner funnel. This silver oxide washing procedure with methanol was carried out twice more.

The combined filtrates along with the initial filtrate were then filtered through a bed of silica gel/celite (2 inch silica gel/1 inch of celite) and finally the bed was washed with 50 mL of methanol.

The filtrate was then subjected to a filtration through an acidic zeolite bed of 2 inch height (pH of the zeolite bed was adjusted to acidic pH by using 0.1N hydrochloric acid aqueous solution) followed by filtration through a 0.2 micron filtration unit.

Isopropyl ether was added three times the volume of the filtrate and the isosulfan blue acid was precipitated as a solid at about 10 gram (68.8%) yield.

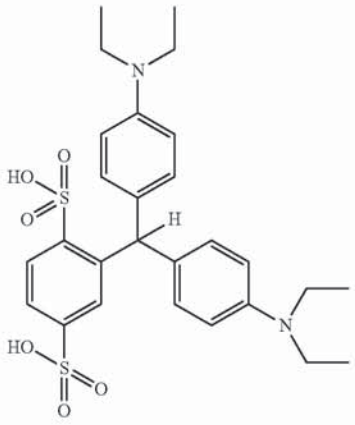
In order to prepare the Isosulfan blue sodium salt of the formula (5), 10.0 g of the solid obtained above was dissolved in 30 mL deionized water. Saturated sodium bicarbonate solution was added drop wise to adjust the pH to 8.0. To this 300 mL of acetone was added and stirred at 20-25° C. for 30 minutes. The crystallized product was then filtered through a buchner funnel and the solid thus obtained was dried at 40° C. under vacuum to obtain the isosulfan blue sodium salt of formula (5).

While the preferred embodiments have been described and illustrated it will be understood that changes in details and obvious undisclosed variations might be made without departing from the spirit and principle of the invention and therefore the scope of the invention is not to be construed as limited to the preferred embodiment.

The invention claimed is:

1. A process of preparing N-[4-[[4-(diethyl amino) phenyl] (2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt comprising combining a suspension of isoleuco acid of the formula

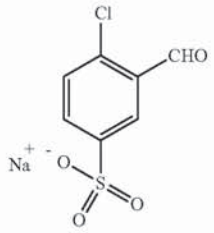
(4)



in a polar solvent with silver oxide, recovering isosulfan blue acid, and treating the isosulfan blue acid with a sodium solution.

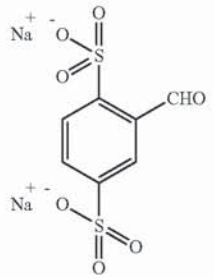
2. The process according to claim **1** comprising sulfonation of 2-chlorobenzaldehyde to obtain 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula

(2)



followed by nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt with an alkali metal sulfite and bisulfite to obtain benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



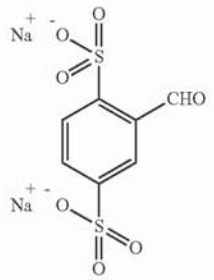
and condensing the benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N, N-diethylaniline using urea and glacial acetic acid to provide isoleuco acid of the formula (4).

3. The process of preparing 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2) according to claim **2** comprising reacting 2-chlorobenzaldehyde with sulfuric acid.

4. The process according to claim **1** wherein the polar solvent is methanol.

5. The process according to claim **2** of preparing free benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula

(3)



wherein the alkali metal sulfite and bisulfite comprise sodium sulfite and sodium bisulfite salts.

6. The process according to claim **5** wherein the reaction is carried out in a pressure vessel at 170-180° C. for 5 to 7 hours.

7. The process according to claim **6** wherein the reaction is carried out under a pressure of 140 to 150 psi.

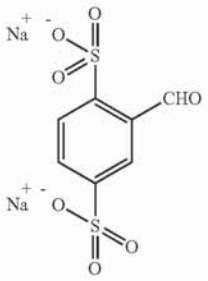
8. The process according to claim **2** comprising precipitating inorganic salts which will hinder the rate of reaction using methanol or one or more C_{1-4} lower alcohols.

9. The process according to claim **2** in which the benzaldehyde-2,5-disulfonic acid disodium salt is purified by extracting with a non-aqueous polar solvent followed by its precipitation in a halogenated or non-halogenated non-polar solvent which is miscible with the non-aqueous polar solvent.

10. The process according to claim **9** wherein the nonaqueous polar solvent is N,N dimethylformamide and the nonpolar solvent is dichloromethane.

11. The process according to claim **1** wherein the isoleuco acid of the formula (4) is prepared by combining a benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



with N,N-diethylaniline, and urea and glacial acetic acid.

12. The process according to claim **11** performed at reflux conditions for 20-25 hours at 115 to 120° C.

13. The process according to claim **11** comprising precipitating a crude solid using methanol or a C_{1-4} lower alcohol.

14. The process according to claim **11** in which the crude solid is further purified using water.

15. The process according to claim **1** comprising oxidation of isoleuco acid of the formula (4) with silver oxide in methanol to obtain a reaction mass.

16. The process according to claim **15** comprising stirring the reaction mass for 12-14 hours, and filtering the silver oxide to provide a filtrate.

17. The process according to claim **16** comprising passing the filtrate through a bed of silica gel and celite and passing the filtrate through a zeolite bed optionally treated with an acid or base.

18. The process according to claim **17** further comprising passing the filtrate through a 0.2 micron filtration unit.

19. The process according to claim **16** comprising precipitating the filtrate using a non-polar solvent miscible with the filtrate.

20. The process according to claim **19** wherein the non-polar solvent is isopropyl ether.

21. The process according to claim **1** comprising adjusting the N-[4-[[4-(diethylamino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium to a pH greater than 6.0 using an aqueous inorganic or organic derivative of sodium or a combination thereof.

22. The process according to claim **21** wherein the pH is adjusted using sodium bicarbonate solution.

23. The process according to claim **1** comprising recrystallization of N-[4-[[4-(diethylamino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium using a solvent selected from the group consisting of a polar solvent, a non-polar solvent and a combination thereof to afford HPLC purity greater than 99.5%.

* * * * *

Exhibit C

US009353050B2

(12) **United States Patent**
Kovi et al.

(10) **Patent No.: US 9,353,050 B2**
(45) **Date of Patent: May 31, 2016**

(54) **PROCESS FOR PREPARATION OF ISOSULFAN BLUE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 584 days.

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(22) Filed: **Dec. 2, 2011**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. 12/643,056, filed on Dec. 21, 2009, now abandoned, which is a continuation of application No. 12/180,057, filed on Jul. 25, 2008, now Pat. No. 7,662,992, which is a continuation of application No. 11/747,291, filed on May 11, 2007, now abandoned.

(51) **Int. Cl.**
C07C 303/02 (2006.01)
C07C 303/22 (2006.01)

(52) **U.S. Cl.**
CPC ***C07C 303/02*** (2013.01); ***C07C 303/22*** (2013.01)

(58) **Field of Classification Search**
CPC C09B 11/10; C07C 309/22; C07C 309/52
USPC 564/80; 562/58, 59
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

1,531,507	A	3/1925	Rosenbaum
1,805,925	A	5/1931	Schmidt
1,878,530	A	9/1932	Kyrides
2,422,445	A	6/1947	Stryker
2,726,252	A	12/1955	Balon
4,330,476	A	5/1982	Hermann
4,710,322	A	12/1987	Metz
5,659,053	A	8/1997	Gessner et al.
2006/0224003	A1	10/2006	Kulkarni

OTHER PUBLICATIONS

Dan et al., "1% Lymphazurin vs. 10% Fluorescein for Sentinel Node Mapping in Colorectal Tumors," Arch. Surg., 139, 1180-1184, 2004.*

Argentine et al., "Strategies for the investigation and control of process-related impurities in drug substances," Advanced Drug Delivery Reviews, 59, 12-28, 2007.*

Hiranaka et al., "Chemical Structure and Purity of Dyes Used in Lymphangiograms," Investigative Radiology, 10(1), 79, 1975.*

Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition vol. III Part F 110-113.

International Search and Written Opinion of Apr. 23, 2003 of International Application No. PCT/US07/84051.

Office Action for corresponding U.S. Appl. No. 12/180,057, dated Feb. 23, 2009.

Office Action for corresponding U.S. Appl. No. 11/747,291, dated Feb. 7, 2008.

Office Action for corresponding U.S. Appl. No. 12/643,056, dated Jul. 19, 2011.

Coleman et al., "Unexplained Decrease in Measured Oxygen Saturation by Pulse Oximetry Following Injection of Lymphazurin 1% (isosulfan Blue) During a Lymphatic Mapping Procedure", Journal of Surgical Oncology 1999, 70: 126-129.

* cited by examiner

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(57) **ABSTRACT**

A process for the preparation of isosulfan blue (Active Pharmaceutical Ingredient) is provided. A process is also provided for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), used in the preparation thereof and a procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3). Also provided is a process for the preparation of an isoleuco acid of formula (4), which upon mild oxidation gives rise to isosulfan blue of pharmaceutical grade which can be used for preparation of pharmaceutical formulations. The isolation and purification procedures provided in the process provide substantially pure isosulfan blue with HPLC purity 99.5% or greater.

18 Claims, No Drawings

PROCESS FOR PREPARATION OF ISOSULFAN BLUE

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation application, and claims the benefit, of U.S. patent application Ser. No. 12/643,056, filed Dec. 21, 2009, which is a continuation of U.S. Ser. No. 12/180,057 filed Jul. 25, 2008, now U.S. Pat. No. 7,662,992, which is a continuation of U.S. Ser. No. 11/747,291 filed May 11, 2007, now abandoned, the entireties of which are incorporated herein by reference.

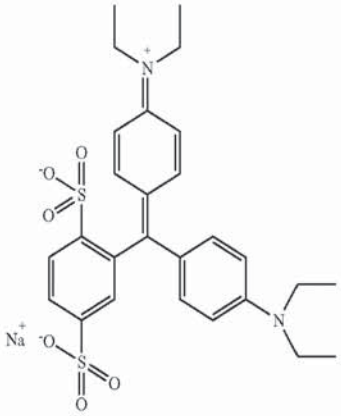
FIELD OF THE INVENTION

The present invention relates to a process for the production of isosulfan blue, and in particular, to a process for the production of isosulfan blue in a substantially pure form.

BACKGROUND OF THE INVENTION

Isosulfan blue, having a chemical name, N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt and the formula

(5)



is a triarylmethane dye used as a contrast agent for the delineation of lymphatic vessels and is particularly useful as a cancer diagnostic agent. Also known chemically as sulfan blue or patent blue, isosulfan blue is an active pharmaceutical ingredient used in the Lymphazurin™ blue dye pharmaceutical dosage form, available as 1% (10 mg/ml) 5 ml solution in phosphate buffer for injection. It is commonly used in a procedure called "mapping of the sentinel lymph nodes". It is an adjunct to lymphography for visualization of the lymphatic system draining the region of injection. It has been used with increasing frequency in localizing sentinel lymph nodes in breast cancer patients. Isosulfan blue-guided surgical removal of cancerous tissue has been on the rise as it is cost effective and safer to use than technetium 99M radioisotope-labeled sulfur colloid. Isosulfan blue is a structural isomer of sulphan blue; both belong to the family of triarylmethane dyestuffs. Generally, preparation of triarylmethane dyes involves condensation of suitably substituted aryl aldehydes with 2 equivalents of alkyl-aryl amines giving rise to leuco-bases or leuco-acids followed by oxidation. Although the literature is replete with methods of preparing triarylmethane dyes, most of the methods involve strong acids for condensation resulting in leuco-bases or leuco-acids, hazardous oxidizing agents (lead oxide, chloranil, iron phthalocyanine/oxone) for converting to triarylmethane dyes, and crude methods (precipitation with sodium sulfate) of purification. See for example U.S. Pat. Nos. 4,330,476, 4,710,322, 1,531,507, 5,659,053, 1,805,925, 2,422,445, 1,878,530 and 2,726,252. Prior art methods of isolation of the crude leuco-acids or leuco-bases involve tedious neutralization/basification with strong bases and typically using the reaction mixtures in the oxidation step, giving rise to crude triarylmethane dyes. The triarylmethane dyestuffs thus prepared are used mainly for dyeing fabric, coloring paper, and printing inks. The literature cites utilization of the same aforementioned synthetic and isolation methods for the preparation of diagnostically important dyes, such as isosulfan blue, sulphan blue and patent blue V. See, Rodd's Chemistry of Carbon Compounds by S. Coffey, 1974 2nd Edition, Volume III Part F, 110-133.

Therefore there is a need in the art for an improved method in the process chemistry of isosulfan blue to be prepared in the purest form which is suitable for large scale cGMP production for its pharmaceutical formulation manufacturing.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention is to provide a simple, safe, cost-effective, time saving and reliable process for the preparation of isosulfan blue in bulk scale and in substantially pure form. "Substantially pure" is defined herein as 99.0% or greater.

Another object of the invention is to provide a simple, cost-effective and reliable process for preparation of the intermediate, 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2), required in the preparation of isosulfan blue. This embodiment provides a process step that does not require tedious neutralization with very large quantities of sodium carbonate and effervescence, as is the case in prior art processes.

Another object of the invention is to provide a simplified procedure for the isolation of benzaldehyde-2,5-disulfonic acid, di-sodium salt of the formula (3) that does not include acidifying the reaction mixture with concentrated sulfuric acid and boiling until excess sulfurous acid is expelled, as is taught in the prior art.

Yet another object of the invention is to provide a procedure for obtaining the benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3) free of inorganic salts, which essentially simplifies the isolation procedures to be implemented during isolation of isoleuco acid.

Yet another, object of the invention is to provide a process for the preparation of an isoleuco acid of formula (4), through the urea derivative as an in-situ intermediate. The isoleuco acid of formula (4) on further oxidation gives rise to the target compound, isosulfan blue (5). Still another object of the invention is to use very mild oxidation agent to avoid any over oxidized products and also to improve the stability of the isosulfan blue under reaction conditions.

According to this invention, there is provided a simple procedure for the isolation of benzaldehyde-2,5-disulfonic acid, isoleuco acid and isosulfan blue at acid stage and also at sodium salt formation stage by incorporating crystallization techniques, thereby avoiding distillation and other techniques using high temperatures which jeopardize the compound stability during the manufacturing process.

These and other aspects of the invention will be apparent to those skilled in the art.

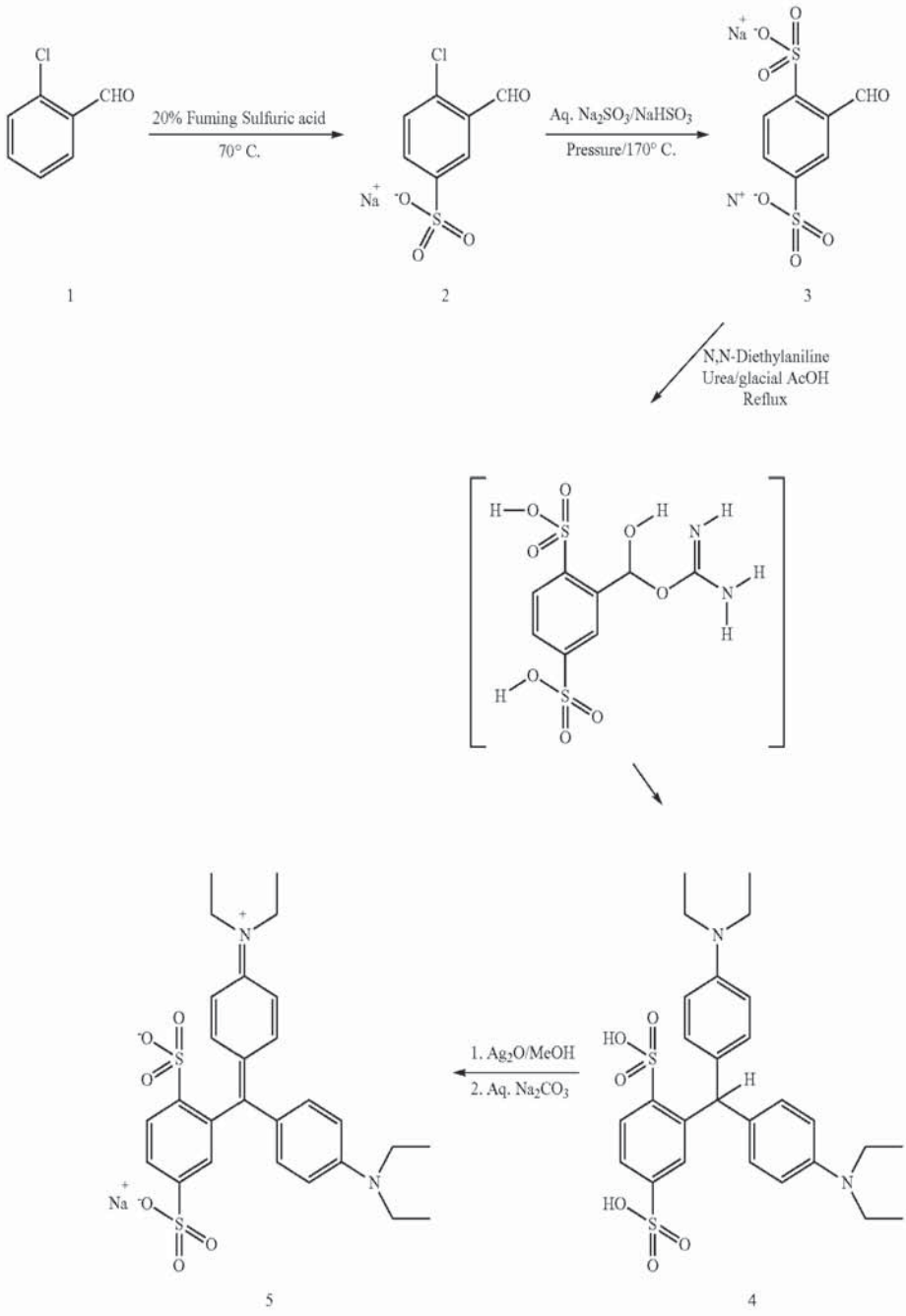
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following description, for purposes of explanation, specific numbers, materials and configurations are set forth in order to provide a thorough understanding of the invention. It will be apparent, however, to one having ordinary skill in the art that the invention may be practiced without these specific details. In some instances, well-known features may be omitted or simplified so as not to obscure the present invention.

Furthermore, reference in the specification to phrases such as "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. The appearances of phrases such as "in one embodiment" in various places in the specification are not necessarily all referring to the same embodiment. In accordance with one embodiment the present invention relates to a process for the preparation of isosulfan blue.

Scheme

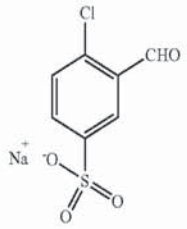
The following provides a process for the production of isosulfan blue of formula (5):



Experimental Procedures

In accordance with one embodiment of the present invention a first step involves sulfonation of the commercially available starting material of the formula (1) to 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2).

(2)



In one example, the sulfonation process involved reacting one equivalent of the 2-chlorobenzaldehyde of formula (1) with 2.0 equivalents of 20% fuming sulfuric acid at 15° C. to 70° C. for 16 hrs. The reaction mixture was poured into ice-water carefully followed by stirring with solid sodium chloride resulting in a cream colored precipitate, which upon filtration, washing with ether and drying afforded 2-chlorobenzaldehyde-5-sulfonic acid of the formula (2) in 86% yield.

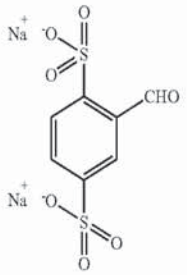
In accordance with one embodiment of the present invention, a second step of the process involves nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula (2) with an alkali metal sulfite/bisulfite such as sodium sulfite/sodium bisulfite at elevated temperatures under closed conditions.

In one example, this reaction was carried out in a Parr pressure vessel equipped with overhead magnetic stirring. 2-Chlorobenzaldehyde-5-sulfonic acid (2), sodium sulfite (2.29 equivalents), sodium bisulfite (10% of sodium sulfite), and water (3.45 mL/g) were charged into the Parr pressure vessel. The reaction mixture in the vessel was stirred and heated at 170-180° C. for 5-7 hours generating 140-150 psi pressure.

The reaction mixture, after cooling, was poured into methanol while stirring, so as to make 20% aqueous content of the whole volume. This process ensured total precipitation of the inorganic salts, which could be removed by filtration. The solvent from the filtrate was removed under reduced pressure to obtain a solid residue, which was triturated with methanol and filtered to afford light yellow colored compound, benzaldehyde-2,5-disulfonic acid, di sodium salt of the formula (3) in 93.9% yield.

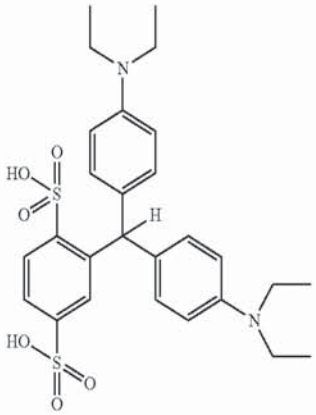
In accordance with one embodiment a purification procedure for removing the inorganic salts essentially involves dissolving the crude solid in N,N dimethylformamide and stirring the contents for 1-2 hours at ambient temperature followed by filtration. The filtrate is precipitated by dichloromethane to afford the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% and with HPLC assay greater than 90% w/w.

(3)



In accordance with one embodiment of the present invention, a third step of the process involved condensing benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N,N-diethylaniline to provide isoleuco acid of the formula (4).

(4)

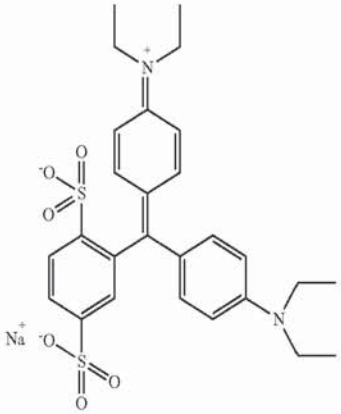


In one example, pure isoleuco-acid of the formula (4) with chromatographic purity greater than 98.0% was obtained in the solid form out of the reaction mixture. A mixture of benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3), N,N-diethylaniline (2.2 equivalents), and urea (0.75 equivalents) in glacial acetic acid was stirred and refluxed for 20-25 hrs. The reaction progressed through the intermediate formation in-situ which is a urea derivative of benzaldehyde-2,5-disulfonic acid disodium salt. To the above cooled reaction mixture after 20-25 hrs reflux, methanol was added to form a precipitate, which was collected by vacuum filtration and washed with diethyl ether to afford the isoleuco acid of the formula (4) in 56.8% yield.

The purification of isoleuco acid was carried out by dissolving the crude solid in 5 volumes of water and stirred for 1-2 hours at ambient temperature and filtering the solid. The above process was repeated twice before the final solid was washed with acetone to generate isoleuco acid of the formula (4) with chromatographic purity greater than 99.5%.

In accordance with one embodiment of the present invention a fourth step of the process involves conversion of the isoleuco acid (4) to isosulfan blue of the formula (5) under conditions that employ milder oxidizing agents with no strong acidic reagents and are less hazardous than the prior art.

(5)



In an example of the present inventive process, a suspension of isoleuco acid of the formula (4) in methanol was stirred at room temperature for 12-14 hrs with silver oxide (2.5 equivalents). The blue colored reaction mixture was filtered through a pad of silica gel and Celite followed by filtration through an acidic zeolite bed and further through a 0.2 micron membrane filtration unit. The filtrate was then precipitated with isopropyl ether at room temperature to obtain crude isosulfan blue acid.

The isosulfan blue acid thus obtained was then purified by recrystallization from aqueous isopropyl alcohol/acetone to afford isosulfan blue acid of chromatographic purity NLT 99.5% performed by High Performance Liquid Chromatography.

The final product of isosulfan blue sodium (formula 5) was obtained when isosulfan blue acid was adjusted to a pH greater than 6.0 in aqueous acetone medium using sodium bicarbonate solution for pH adjustment. The reaction mass was filtered to give isosulfan blue sodium of formula (5) having purity greater than 99.5% by HPLC and also free of silver with silver content estimated by Atomic absorption spectrometer less than 20 ppm.

EXAMPLES

2-Chlorobenzaldehyde-5-sulfonic acid, sodium salt of the Formula (2)

113.82 g (based on SO_3 molecular weight, 569 mL) of 20% fuming sulfuric acid (FSA) was charged into a 1 L three-neck flask fitted with a dropping funnel, overhead stirrer, and thermometer. The reaction mass was cooled to 15 to 20° C. 100 g of 2-chlorobenzaldehyde of the formula (1) was added dropwise to the stirred and cooled FSA over a period of 40 minutes, so that the temperature didn't rise above 20° C. The reaction mixture was stirred and heated at 70° C. for 16 hours to obtain a dark-brown colored reaction solution. The HPLC results indicated the absence of the starting material. The dark-brown colored reaction solution was carefully poured into a beaker containing 1200 g of crushed ice and stirred. 500 g of solid sodium chloride was added portion wise to the stirred colored acidic solution to precipitate a light-yellow colored solid. The light-yellow colored solid was collected by vacuum filtration and washed with diethyl ether to afford 150.0 g (86.92%) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2).

Benzaldehyde-2,5-disulfonic acid, sodium salt of the Formula (3)

50 g (0.206 mol) of 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of the formula (2), 59.75 g (0.474 mol, 2.3 eq.) of Na_2SO_3 and 5.97 g (10% of Na_2SO_3) of $NaHSO_3$ were dissolved in 400 mL of water. The solution was charged into a 600 mL capacity Parr pressure cylinder equipped with stirring and heating. The reaction mixture was stirred (300-310 RPM) and heated at 180° C. (generates ~150 psi pressure) for 5-7 hours. HPLC results indicate the absence of the starting material. After cooling and releasing the pressure, the reaction mixture was poured into 1600 mL of stirred methanol and stirred for 15-30 minutes to precipitate the unwanted inorganic salts. The inorganic salts were filtered off using a pad of Celite and the filtrate evaporated under reduced pressure to obtain a solid residue. The solid residue obtained was triturated with 200 mL methanol, collected by filtration and washed with ether to give 60 g (93.9%) of benzaldehyde-2,5-disulfonic acid, sodium salt of formula (3).

Purification of Benzaldehyde-2,5-disulfonic acid, sodium salt Formula (3)

60 g of crude benzaldehyde-2,5-disulfonic acid, disodium salt prepared as per the procedure above was dissolved in 500 mL of N,N-dimethylformamide and stirred for 2 hours at 20-25° C. The mixture was filtered through a buchner funnel and the filtrate was precipitated using 1500 mL of dichloromethane to afford 20 g of the light yellow colored compound, benzaldehyde-2,5-disulfonic acid disodium salt of formula (3) with chromatographic purity NLT 99.0% w/w.

Isoleuco Acid of the Formula (4)

60 g of benzaldehyde-2,5-disulfonic acid sodium salt of formula (3), 8.76 g of urea (0.75 eq), and 1000 mL of glacial acetic acid were charged into a 3 L 3-neck flask fitted with a mechanical stirrer and reflux condenser. 65.61 mL (2.2 eq) of N,N-diethyl aniline was added to the stirred mixture and refluxed for 20-25 hrs. When the HPLC results indicated the content of starting material was less than 5%, the reaction mass was cooled to room temperature. After cooling to room temperature, 600 mL of methanol was added and the separated solid collected on a sintered funnel by vacuum filtration. The collected solid was washed with methanol to obtain 55-60 g (56.8%) of crude isoleuco acid of the formula (4).

Purification of Isoleuco Acid of Formula (4)

50 g of crude isoleuco acid along with 250 ml of water was charged into a 1 L 3-neck round bottom flask fitted with a mechanical stirrer. The reaction mixture was stirred for 1 hour at 20-25° C. The solid was filtered through a buchner funnel.

The above process was repeated twice. The final product thus obtained was then washed with 25 ml of acetone and then dried to obtain 40-45 g of the desired isoleuco acid of formula (4).

Isosulfan Blue of the Formula (5)

15 g (0.027 mol) of isoleuco acid of the formula (4) and 225 mL of Methanol were charged into a 1 L round bottomed flask and the suspension was stirred. To the stirred suspension, 15.91 g (0.068 mol, 2.5 eq.) of silver oxide was added in one portion at room temperature and stirred at room temperature for 12-14 hours. The reaction mixture turned blue in color as the oxidation to the desired product progressed. The HPLC results indicated the absence of starting material. The blue colored reaction mixture was filtered through a buchner funnel and the solid silver oxide collected was taken into the reaction flask and the filtrate was kept aside. 225 ml of methanol was added to the silver oxide taken in the reaction flask and stirred at 20-25° C. for 30 minutes and filtered through the buchner funnel. This silver oxide washing procedure with methanol was carried out twice more.

The combined filtrates along with the initial filtrate were then filtered through a bed of silica gel/celite (2 inch silica gel/1 inch of celite) and finally the bed was washed with 50 mL of methanol.

The filtrate was then subjected to a filtration through an acidic zeolite bed of 2 inch height (pH of the zeolite bed was adjusted to acidic pH by using 0.1N hydrochloric acid aqueous solution) followed by filtration through a 0.2 micron filtration unit.

Isopropyl ether was added three times the volume of the filtrate and the isosulfan blue acid was precipitated as a solid at about 10 gram (68.8%) yield.

In order to prepare the Isosulfan blue sodium salt of the formula (5), 10.0 g of the solid obtained above was dissolved in 30 mL deionized water. Saturated sodium bicarbonate solution was added drop wise to adjust the pH to 8.0. To this 300 mL of acetone was added and stirred at 20-25° C. for 30 minutes. The crystallized product was then filtered through a buchner funnel and the solid thus obtained was dried at 40° C. under vacuum to obtain the isosulfan blue sodium salt of formula (5).

While the preferred embodiments have been described and illustrated it will be understood that changes in details and obvious undisclosed variations might be made without departing from the spirit and principle of the invention and therefore the scope of the invention is not to be construed as limited to the preferred embodiment.

The invention claimed is:

1. A compound N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt having a purity of at least 99.0% by HPLC.

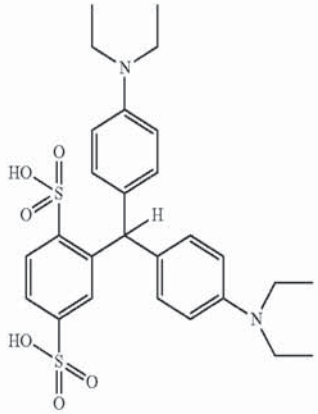
2. The compound according to claim **1** having a purity between 99.0% and 99.5% by HPLC.

3. The compound according to claim **1** having less than 20 ppm silver.

4. The compound according to claim **3** having a purity greater than 99.5% by HPLC.

5. The compound according to claim **1** prepared by a process comprising combining a suspension of isoleuco acid of the formula

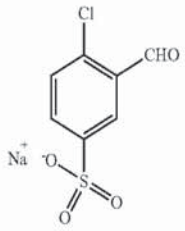
(4)



in a polar solvent with silver oxide, recovering isosulfan blue acid, and treating the isosulfan blue acid with a sodium solution.

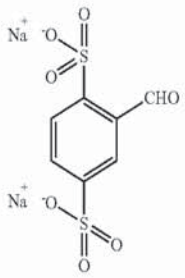
6. The compound according to claim **5** wherein the process comprises sulfonation of 2-chlorobenzaldehyde to obtain 2-chlorobenzaldehyde-5-sulfonic acid sodium salt of the formula

(2)



followed by nucleophilic displacement of the chloride in 2-chlorobenzaldehyde-5-sulfonic acid sodium salt with an alkali metal sulfite and bisulfate to obtain benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



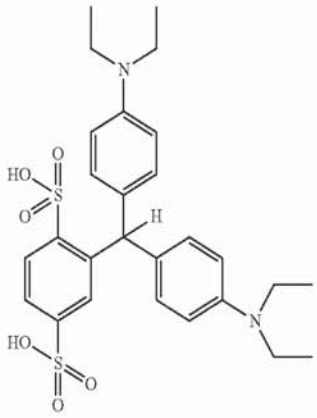
and condensing the benzaldehyde-2,5-disulfonic acid, disodium salt of the formula (3) with N, N-diethylaniline using urea and glacial acetic acid to provide isoleuco acid of the formula (4).

7. The compound according to claim **6** wherein the process of preparing 2-chlorobenzaldehyde-5-sulfonic acid, sodium salt of formula (2) comprises reacting 2-chlorobenzaldehyde with sulfuric acid.

8. The compound according to claim **5** wherein the polar solvent is methanol.

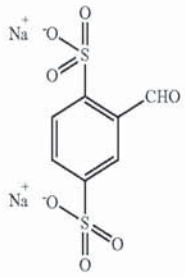
9. The compound according to claim **5** wherein the isoleuco acid of the formula

(4)



is prepared by combining a benzaldehyde-2,5-disulfonic acid, disodium salt of the formula

(3)



with N, N-diethylaniline, and urea and glacial acetic acid.

10. The compound according to claim **5** wherein the process comprises recrystallization of N-[4-[[4-(diethylamino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium using a solvent selected from the group consisting of a polar solvent, a non-polar solvent and a combination thereof to afford HPLC purity greater than 99.5%.

11. A solution containing N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt, the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt having a purity of at least 99.0% by HPLC.

12. The solution according to claim **11** wherein the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt has a purity between 99.0% and 99.5% by HPLC.

13. The solution according to claim **11** having less than 20 ppm silver.

14. The solution according to claim **13** wherein the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt has a purity greater than 99.5% by HPLC.

15. A composition consisting essentially of N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt, the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt having a purity of at least 99.0% by HPLC.

16. The composition according to claim **15** wherein the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt has a purity between 99.0% and 99.5% by HPLC.

17. The composition according to claim **15** having less than 20 ppm silver.

18. The composition according to claim **17** wherein the N-[4-[[4-(diethyl amino)phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium, sodium salt has a purity greater than 99.5% by HPLC.

* * * * *

Exhibit D

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION

MYLAN INSTITUTIONAL LLC and APICORE US LLC Plaintiffs, v. AUROBINDO PHARMA LTD., AUROBINDO PHARMA USA INC., and AUROMEDICS PHARMA LLC Defendants.	Civil Action No.: 2:16-cv-491-RWS-RSP **Jury Trial Demanded**

FIRST AMENDED COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Apicore US LLC ("Apicore") and Mylan Institutional LLC ("Mylan") (collectively, "Plaintiffs") file this First Amended Complaint for Patent Infringement ("FAC") against Aurobindo Pharma Ltd ("Aurobindo India"), Aurobindo Pharma USA Inc. ("Aurobindo USA"), and AuroMedics Pharma LLC ("AuroMedics") (collectively, "Defendants").

THE PARTIES

1. Apicore is a limited liability company organized and existing under the laws of the State of Delaware, and having a place of business at 49 Napoleon Court, Somerset, NJ 08873.

2. Apicore is a pharmaceutical company that provides innovative solutions to the pharmaceutical industry in the field of active pharmaceutical ingredient manufacturing.

3. Apicore developed a process for the manufacture of a high purity isosulfan blue product that is vastly superior to other methods of isosulfan blue synthesis and has entered into an exclusive arrangement with Mylan to commercialize this innovation.

4. Mylan is a limited liability company organized and existing under the laws of the State of Delaware, and having a place of business at 1718 Northrock Court, Rockford, IL 61103.

5. Mylan is a pharmaceutical company that develops and commercializes injectable pharmaceutical products.

6. On information and belief, Aurobindo India is an Indian corporation having a place of business at Plot No. 2, Maitri Vihar, Ameerpet, Hyderabad – 500 038, Andhra Pradesh, India.

7. On information and belief, Aurobindo India develops and manufactures certain pharmaceutical drug products for sale in the United States, including in Texas and in this district, and/or imports certain pharmaceutical drug products into the United States. In 2013, Aurobindo India commenced marketing injectable products in the United States through its subsidiary AuroMedics. In the past, Aurobindo India has designated Aurobindo USA as its U.S. agent before the FDA. In the past, Aurobindo India has designated AuroMedics as its U.S. agent before the FDA. On information and belief, AuroMedics and/or Aurobindo USA is the U.S. agent before the FDA for ANDA No. 206831.

8. On information and belief, Aurobindo USA is a Delaware corporation having a place of business at 6 Wheeling Road, Dayton, New Jersey 08810. Aurobindo USA is a wholly-owned subsidiary and agent of Aurobindo India.

9. On information and belief, Aurobindo USA markets, offers to sell, manufactures, distributes, and sells certain pharmaceutical drug products in the United States, including in Texas and in this district, and/or imports certain pharmaceutical drug products into the United States.

10. On information and belief, AuroMedics is a Delaware corporation having a place of business at 6 Wheeling Road, Dayton, New Jersey 08810. AuroMedics is a wholly-owned subsidiary of Aurobindo India.

11. On information and belief, AuroMedics markets, manufactures, offers to sell, distributes, and sells certain pharmaceutical drug products in the United States, including in Texas and in this district, and/or imports certain pharmaceutical drug products into the United States.

12. On information and belief, Aurobindo India, Aurobindo USA, and AuroMedics operate and act in concert as an integrated, unitary business for purposes of manufacturing, marketing, offering to sell, selling, and distributing generic pharmaceutical products throughout the United States, including in Texas and in this district. For example, in several of Aurobindo India's Earnings Conference Calls, including the call on February 10, 2016, Mr. Robert Cunard – CEO, Aurobindo USA and Mr. Ronald Quadrel – CEO, AuroMedics, along with individuals at Aurobindo India, were stated as representing the senior management team.

NATURE OF THE ACTION

13. This is an action for patent infringement arising under the patent laws of the United States, Title 35, United States Code, Sections 1 *et seq*., involving United States Patent No. 7,662,992 (the "'992 patent") United States Patent No. 8,969,616 (the "'616 patent") and United States Patent No. 9,353,050 (the "'050 patent) (collectively, the "Patents-in-Suit").

14. This action arises out of Defendants' offering to sell and selling in the United States and/or importing into the United States isosulfan blue, which is, or is manufactured by processes, or equivalents thereof, claimed in the Patents-in-Suit.

JURISDICTION AND VENUE

15. This Court has subject matter jurisdiction over this action pursuant to 28 U.S.C. §§ 1331 and 1338(a).

16. Venue is proper in this district pursuant to 28 U.S.C. §§ 1391 and 1400(b).

17. This Court has personal jurisdiction over Aurobindo India because, on information and belief, *inter alia*, Aurobindo India collaborated with Aurobindo USA and AuroMedics for the purpose of preparing and submitting ANDA No. 206831. Aurobindo India conducts business through and with Aurobindo USA and/or AuroMedics, its wholly-owned subsidiaries. On information and belief, Aurobindo India has affiliations with Texas and this district that are pervasive, continuous, and systematic. Aurobindo India directly or through its affiliates and agents develops, formulates, synthesizes, manufactures, markets, imports, offers to sell, and/or sells pharmaceutical drug products. On information and belief, Aurobindo India engages in direct and/or indirect marketing, offering to sell, distribution, and/or sale of pharmaceutical drug products, including isosulfan blue, within Texas and this district and to the residents of Texas and this district. On information and belief, Aurobindo India regularly conducts and/or solicits business in Texas and this district, directly or through its subsidiaries Aurobindo USA and/or AuroMedics.

18. This Court has personal jurisdiction over Aurobindo USA because Aurobindo USA has affiliations with Texas and this district that are pervasive, continuous, and systematic. Furthermore, Aurobindo USA has a Texas Taxpayer Number, which is 32038681568. In addition, Aurobindo USA is licensed with the Texas Department of State Health Services under License Number 0103142 and engages in direct and/or indirect marketing, distribution, offering for sale, and/or sale of pharmaceutical drug products, including isosulfan blue, within Texas and this district and to the residents of this district.

19. This Court has personal jurisdiction over AuroMedics because AuroMedics has affiliations with Texas and this district that are pervasive, continuous, and systematic. Furthermore, AuroMedics is licensed with the Texas Department of State Health Services under License Number 1000855 and engages in direct and/or indirect marketing, distribution, offering for sale, and/or sale of pharmaceutical drug products, including isosulfan blue, within Texas and this district and to the residents of Texas and this district.

20. On information and belief, Defendants collaborate in the manufacture, marketing, offering for sale, and sale of many pharmaceutical products (including generic drug products manufactured and sold pursuant to an approved abbreviated new drug application) within the United States generally, and in Texas and in this district specifically.

21. On information and belief, Defendants actively review pharmaceutical patents and seek opportunities to challenge those patents.

22. Moreover, because the acts of selling and/or offering for sale that give rise to infringement by Aurobindo India, Aurobindo USA, and AuroMedics have occurred within Texas and this district, personal jurisdiction is proper within this district.

PATENTS-IN-SUIT

23. Apicore was formed in 2003. For several years, the entire company was devoted to the development of isosulfan blue, a small molecule active pharmaceutical ingredient ("API"). After years of extraordinary effort and significant expense, Apicore developed a vastly superior manufacturing process that allowed for the commercial synthesis of isosulfan blue at a purity level much greater than had been previously achieved by others. That superior process is the basis for the technology claimed in the Patents-in-Suit.

24. The '992 patent, entitled "Process for Preparation of Isosulfan Blue," was duly and legally issued by the United States Patent and Trademark Office ("USPTO") on February 16,

2010. The named inventors of the ’992 patent are Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial. Apicore is the assignee of the ’992 patent. A true and correct copy of the ’992 patent is attached hereto as Exhibit A.

25. The ’616 patent, entitled “Process for Preparation of Isosulfan Blue,” was duly and legally issued by the USPTO on March 3, 2015. The named inventors of the ’616 patent are Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial. Apicore is the assignee of the ’616 patent. A true and correct copy of the ’616 patent is attached hereto as Exhibit B.

26. The ’050 patent, entitled “Process for Preparation of Isosulfan Blue,” was duly and legally issued by the USPTO on May 31, 2016. The named inventors of the ’050 patent are Ravishanker Kovi, Satyam Nampalli, and Peter Xavier Tharial. Apicore is the assignee of the ’050 patent. A true and correct copy of the ’050 patent is attached hereto as Exhibit C.

27. The ’050 patent issued from U.S. Application No. 13/310,019 (the “’019 App’n”). Pursuant to 35 U.S.C. § 122(b), the ’019 App’n was first published on March 29, 2012 as U.S. Publication No. 2012/0078007 A1 (the “’007 Pub’n”). The claims of the ’050 patent are substantially identical to the claims published in the ’007 Pub’n. A true and correct copy of the ’007 Pub’n is attached hereto as Exhibit D.

28. Apicore is the lawful owner of the Patents-in-Suit and has all right, title and interest in and to the Patents-in-Suit.

29. Mylan is an exclusive licensee of each of the Patents-in-Suit.

30. Neither Apicore nor Mylan has authorized or licensed Defendants to make, use, sell, or offer for sale any of the inventions claimed in the Patents-in-Suit.

31. On information and belief, Defendants were aware of and reviewed the ’992 patent, the ’616 patent, and the ’019 App’n prior to the commencement of this lawsuit and prior to launching their isosulfan blue product.

32. On information and belief, Aurobindo India submitted to the FDA ANDA No. 206831 seeking approval to engage in the commercial manufacture, use, marketing, offer for sale and sale of isosulfan blue. On information and belief, AuroMedics and/or Aurobindo USA served as Aurobindo India’s U.S. Agent before the FDA with respect to ANDA No. 206831.

33. On information and belief, Aurobindo India received approval of its ANDA No. 206831 on February 2, 2016. Aurobindo India thereafter announced publicly its intent to begin offering for sale and selling isosulfan blue in the United States in March 2016. The FDA-approved label for Defendants’ isosulfan blue product states that Aurobindo India is the manufacturer of the isosulfan blue product and includes directions to healthcare professionals for the proper use of the product. A true and correct copy of Defendants’ isosulfan blue label is attached as Exhibit E.

34. On information and belief, the only use for Defendants’ isosulfan blue is as an API for use in their isosulfan blue injection 1% and there are no known substantial non-infringing uses for isosulfan blue.

35. On or about March 1, 2016, Defendants began providing pricing information for Defendants’ isosulfan blue to U.S. customers, including current customers of Plaintiffs.

36. Currently, on information and belief, Defendants have contracted with major pharmaceutical wholesalers to offer Defendants’ isosulfan blue in the United States, including in Texas and in this district. Further, on information and belief, Defendants have approached and

contracted with group purchasing organizations ("GPOs") to provide Defendants' isosulfan blue to their members throughout the United States, including in Texas and in this district

37. On March 2, 2016, Plaintiffs sent Aurobindo India and Aurobindo USA each a letter identifying the '992 patent and the '616 patent and advising that Plaintiffs believe that Aurobindo India and/or Aurobindo USA infringe one or more claims of the '992 patent and the '616 patent.

38. On March 3, 2016, Plaintiffs sent a similar letter to AuroMedics identifying the '992 patent and the '616 patent and advising that Plaintiffs believe that Aurobindo India and/or Aurobindo USA infringe one or more claims of the '992 patent and the '616 patent. This letter provided constructive notice to AuroMedics that to sell or offer for sale Defendants' isosulfan blue product would also constitute an act of infringement.

39. On March 7, 2016, Defendants replied that they believed they did not infringe the '992 patent and the '616 patent.

40. On March 8, 2016, Plaintiffs sent Defendants a letter seeking more information regarding Defendants' process steps and specifically requesting, among other things, "whether any silver-containing ingredients are utilized [and] what oxidizing agents are employed . . . in the oxidation step to yield Aurobindo's isosulfan blue product." Also, the letter requested a sample of Defendants' isosulfan blue product. Further, a copy of the '007 Pub'n was enclosed with the letter in which Plaintiffs informed Defendants of their belief that the claims of the '019 App'n in their current form would be allowed by the USPTO without additional amendments and that the current claims of the '019 App'n are substantially identical to the claims in the '007 Pub'n. The '019 App'n was issued as the '050 patent on May 31, 2016 with the same claims as those presented to Defendants on March 8, 2016.

41. On March 14, 2016, Plaintiffs and Defendants entered into a non-disclosure agreement concerning the exchange of additional information from Defendants for outside counsel and expert eyes only, and for the sole purpose of Plaintiffs' infringement analyses. Following execution of the agreement, Defendants replied on March 14, 2016, disclosing an oxidizing agent it allegedly used, but absent any documentation to support the claim.

42. On March 17, 2016, Plaintiffs sent another letter to Defendants requesting additional information and supporting documentation for the purpose of Plaintiffs' infringement analyses.

43. On March 18, 2016, Defendants responded with a letter and a single-paged document that did not disclose the additional information requested by Plaintiffs.

44. On March 23, 2016, Plaintiffs sent a letter to Defendants detailing the specific information needed for analyses under both the theories of literal infringement and the doctrine of equivalents.

45. On March 28, 2016, Defendants replied, asserting their position that they had disclosed sufficient information, and refusing to disclose further information without explanation from Plaintiffs concerning their theories of infringement under the doctrine of equivalents.

46. On March 29, 2016, Plaintiffs sent a final letter to Defendants reiterating their request for additional information and describing why the requested information was needed.

47. On April 1, 2016, Defendants sent a few additional documents that did not address the scope of Plaintiffs' prior requests for information or product samples.

ACTS GIVING RISE TO THIS ACTION

48. On information and belief, Defendants have directly, or through affiliates and subsidiaries, made and/or imported Defendants' isosulfan blue product into the United States.

49. On information and belief, Defendants have directly, or through affiliates and subsidiaries, provided notice to potential customers in the United States, that Defendants' isosulfan blue product is commercially available throughout the United States, including in Texas and in this district.

50. On information and belief, Defendants have directly, or through affiliates and subsidiaries, offered for sale and/or sold Defendants' isosulfan blue product in the United States, including in Texas and in this district.

51. On information and belief, Defendants have directly, or through affiliates and subsidiaries, offered to sell and/or sold Defendants' isosulfan blue product to Mylan's customers at a price significantly below Mylan's contract price with those customers.

52. On information and belief, Defendants knew that wholesalers would sell Defendants' isosulfan blue product to their customers, who would then use Defendants' isosulfan blue product during sentinel lymph node mapping; thus these wholesalers and customers directly infringe the '050 patent. Because Defendants had knowledge of the '050 patent, and specifically intended that the wholesalers would sell Defendants' isosulfan blue product and thus infringe the '050 patent, Defendants induced infringement.

53. On information and belief, Defendants' isosulfan blue product has been used in lymphatic mapping procedures in the United States, including in Texas and in this district.

COUNT 1
INFRINGEMENT OF THE '050 PATENT

54. Plaintiffs repeat and reallege each of the foregoing paragraphs as if fully set forth herein.

55. Defendants infringe (literally and/or under the doctrine of equivalents) the '050 patent, including at least Claims 1, 11, and 15, by making, using, offering to sell and/or selling

within the United States and/or importing into the United States isosulfan blue products without authorization, and/or by contributing to or inducing infringement.

56. Defendants infringe at least claim 1 of the ’050 patent because Defendants’ isosulfan blue, which it sells without authority in the U.S., has a purity of at least 99.0% by HPLC. Moreover, Defendants infringe at least claims 11 and 15 of the ’050 patent because Defendants package their isosulfan blue having a purity of at least 99.0% by HPLC in a solution (i.e., isosulfan blue injection 1%), which it sells without authority in the U.S.

57. Defendant Aurobindo India had knowledge of the ’050 patent at least as early as May 31, 2016 and no later than the service of this First Amended Complaint (“FAC”).

58. Defendant Aurobindo USA had knowledge of the ’050 patent at least as early as May 31, 2016, and no later than the service of this FAC.

59. Defendant AuroMedics had knowledge of the ’050 patent at least as early as May 31, 2016, and no later than the service of this FAC.

60. Defendants have had constructive notice of the ’050 patent as of its date of issuance on May 31, 2016. Furthermore, Defendants were aware of the ’007 Pub’n, which issued as the ’050 patent with substantially identical claims, at least as early as March 8, 2016, when Plaintiffs sent to Defendants the a copy of the ’007 Pub’n.

61. Upon information and belief, Defendants were and are aware of the existence of the ’050 patent and acted without a reasonable basis for believing that it would not be liable for infringement of the ’050 patent, thus rendering this case “exceptional” under 35 U.S.C. § 285.

62. Plaintiffs have and will continue to be substantially and irreparably damaged and harmed if Defendants’ continued infringement of the ’050 patent is not enjoined by this Court.

63. Defendants' infringement of the '050 patent has caused Plaintiffs substantial harm.

COUNT 2
RECOVERY UNDER 35 U.S.C. § 154(d)

64. Plaintiffs repeat and reallege each of the foregoing paragraphs as if fully set forth herein.

65. The invention as claimed in the '050 patent is substantially identical to the invention as claimed in the '007 Pub'n.

66. Prior to the issuance of the '050 patent, Defendants had actual notice of the published '007 Pub'n.

67. With actual notice of the published '007 Pub'n, and before the issuance of the '050 patent on May 31, 2016, Defendants violated Plaintiffs' rights under 35 U.S.C. §154(d), by making, using, selling, offering for sale and/or importing the invention as claimed in the '007 Pub'n and/or by contributing to or inducing violations.

68. Defendants' violation of Plaintiffs' rights under 35 U.S.C. §154(d) was willful and caused Plaintiffs to suffer substantial damages.

COUNT 3
INFRINGEMENT OF THE '992 PATENT

69. Plaintiffs repeat and reallege each of the foregoing paragraphs as if fully set forth herein.

70. Defendants infringe (literally and/or under the doctrine of equivalents) the '992 patent, including at least Claim 1, by making, using, offering to sell and/or selling within the United States and/or importing into the United States isosulfan blue products without authorization, and/or by inducing infringement.

71. Defendants infringe the ’992 patent because Defendants’ isosulfan blue product is prepared by the same process, or its equivalent, as claimed in the ’992 patent. Defendants’ isosulfan blue product is not materially changed by subsequent processes, nor is it a trivial and nonessential component of another product.

72. Defendant Aurobindo India cited the ’992 patent in its Indian Patent application published as Publication Number IN3509/CHE/2012, which was filed on August 27, 2012.

73. Defendant Aurobindo India had knowledge of the ’992 patent at least as early as August 23, 2013 and no later than the service of the Complaint (ECF No. 1).

74. Defendant Aurobindo USA had knowledge of the ’992 patent at least as early as March 3, 2016, and no later than the service of the Complaint (ECF No. 1).

75. Defendant AuroMedics had knowledge of the ’992 patent at least as early as March 4, 2016, and no later than the service of the Complaint (ECF No. 1).

76. Defendants have had constructive notice of the ’992 patent as of its date of issuance on February 16, 2010.

77. Upon information and belief, Defendants were and are aware of the existence of the ’992 patent and acted without a reasonable basis for believing that it would not be liable for infringement of the ’992 patent, thus rendering this case “exceptional” under 35 U.S.C. § 285.

78. Plaintiffs have and will continue to be substantially and irreparably damaged and harmed if Defendants’ continued infringement of the ’992 patent is not enjoined by this Court.

79. Defendants’ infringement of the ’992 patent has caused Plaintiffs substantial harm.

COUNT 4
INFRINGEMENT OF THE '616 PATENT

80. Plaintiffs repeat and reallege each of the foregoing paragraphs as if fully set forth herein.

81. Defendants infringe (literally and/or under the doctrine of equivalents) the '616 patent, including at least Claim 1, by making, using, offering to sell and/or selling within the United States and/or importing into the United States isosulfan blue products without authorization, and/or by inducing infringement.

82. Defendants infringe the '616 patent because Defendants' isosulfan blue product is prepared by the same process, or its equivalent, as claimed in the '616 patent. Defendants' isosulfan blue product is not materially changed by subsequent processes, nor is it a trivial and nonessential component of another product.

83. Defendant Aurobindo India had knowledge of the '616 patent at least as early as March 7, 2016, and no later than the service of the Complaint (ECF No. 1).

84. Defendant Aurobindo USA had knowledge of the '616 patent at least as early as March 3, 2016, and no later than the service of the Complaint (ECF No. 1).

85. Defendant AuroMedics had knowledge of the '616 patent at least as early as March 4, 2016, and no later than the service of the Complaint (ECF No. 1).

86. Defendants have had constructive notice of the '616 patent as of its date of issuance on March 3, 2015.

87. Upon information and belief, Defendants were and are aware of the existence of the '616 patent and acted without a reasonable basis for believing that it would not be liable for infringement of the '616 patent, thus rendering this case "exceptional" under 35 U.S.C. § 285.

88. Plaintiffs have and will continue to be substantially and irreparably damaged and harmed if Defendants' continued infringement of the '616 patent is not enjoined by this Court.

89. Defendants' infringement of the '616 patent has caused Plaintiffs substantial harm.

JURY DEMAND

90. Under Rule 38(b) of the Federal Rules of Civil Procedure, Plaintiffs respectfully request a trial by jury on all issues.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs request the following relief:

A. A judgment that Defendants have infringed the '050 patent under 35 U.S.C. §§ 271(a), 271(b), 271(c), and/or 271(g) by making, using, selling, offering to sell within the United States and/or importing into the United States Defendants' isosulfan blue products and/or by contributing to the infringement of or inducing others to infringe the Patents-in-Suit;

B. A judgment that Defendants have infringed the '992 patent under 35 U.S.C. §§ 271(a), 271(b), and/or 271(g) by making, using, selling, offering to sell within the United States and/or importing into the United States Defendants' isosulfan blue products and/or by inducing others to infringe the Patents-in-Suit;

C. A judgment that Defendants have infringed the '616 patent under 35 U.S.C. §§ 271(a), 271(b), and/or 271(g) by making, using, selling, offering to sell within the United States and/or importing into the United States Defendants' isosulfan blue products and/or by inducing others to infringe the Patents-in-Suit;

D. An order preliminarily and/or permanently enjoining Defendants, their officers, agents, servants, employees, parents, subsidiaries, affiliate corporations, other business entities and all other persons acting or attempting to act in concert or privity with them, their successors, and assigns, or acting on their behalf, from infringing, contributorily infringing, or inducing others to infringe the Patents-in-Suit, including engaging in the offer to sell and selling in the United States, and/or importation into the United States, of Defendants' isosulfan blue products that are the subject of ANDA No. 206831 until the expiration of the Patents-in-Suit, inclusive of any extension(s) and additional period(s) of exclusivity to which Plaintiffs are or may become entitled;

E. A judgment awarding Plaintiffs damages or other monetary relief under 35 U.S.C. § 281 as appropriate;

F. A judgment ordering Defendants to pay damages to Plaintiffs to compensate for their infringement of each of the Patents-in-Suit, including supplemental damages for any post-verdict infringement up until entry of the final judgment with an accounting as needed, together with pre-judgment and post-judgment interest on the damages awarded, with all of these damages to be enhanced in an amount up to treble the amount of the calculated compensatory damages as justified under 35 U.S.C. § 284;

G. A judgment ordering Defendants to pay damages to Plaintiffs based on violations of Plaintiffs' rights under 35 U.S.C. § 154(d);

H. A judgment declaring that Defendants' infringement of the Patents-in-Suit was willful, and awarding treble damages under 35 U.S.C. § 284;

I. That this is an exceptional case under 35 U.S.C. § 285, and that Plaintiffs be awarded reasonable attorneys' fees and costs; and

J. Such further and other relief as this Court may deem just and proper.

Dated: May 31, 2016

Respectfully submitted,

/s/ Melissa R. Smith

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CERTIFICATE OF SERVICE

The undersigned hereby certifies that the foregoing document was filed electronically in compliance with Local Rule CV-5(a). As such, this document was served on all counsel who have consented to electronic service, on May 31, 2016.

/s/ Melissa Smith

Melissa Smith