

HANDBOOK OF BIOLOGICAL DYES AND STAINS

SYNTHESIS AND INDUSTRIAL APPLICATIONS

R. W. SABNIS

Pfizer Inc.
Madison, NJ



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Dedicated to
My Father
Late Mr. Wasudeo S. Sabnis
&
My Mother
Late Mrs. Suhasini W. Sabnis

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Preface

Color has been a fascination for individuals for a long time. The book is intended as a reference guide for dyes used in biology, chemistry, histology, cytology, medicine, microscopy, and all color- and medical-related allied fields.

Even though the use of biological dyes is widespread, it is growing rapidly, and has exploded in the last decade, there is no book available in the market directly on these dyes that provides information, such as CAS registry numbers, safety/toxicity data, and various applications, in one source. Hence, there was a need to publish a book that provided an immediate incentive for compiling the notes to update the scientific community with the wealth of information on biological dyes and stains. The dyestuff literature, particularly on biological dyes and stains, is largely in patents. This book, as a reference handbook, provides systematic and up-to-date library of information on 200 + biological dyes and stains. The book is compiled as a resource guide for biologists, chemists, histologists, cytologists, medical professionals, and nonchemists in industry as well as in university.

Biological dyes and stains are arranged alphabetically by the most commonly used name. Again, the choice of primary name is somewhat arbitrary, but an effort has been made to strike a balance between names that are easily recognizable and names that are chemically informative. The detailed information on each biological dye or stain is covered in the following order: CAS registry number, chemical structure, CA index name, other names, Merck index number (Merck Index 14th Edition, 2006), chemical/dye class, molecular formula, molecular weight, physical form, solubility, melting point, boiling point, pH range, color change at pH, pK_a , absorption (λ_{max}), emission (λ_{max}), synthesis, staining applications, biological applications, industrial applications, safety/toxicity, certification/approval, and references. Where there are discrepancies between different values, the author has used his judgment on selecting the most likely value.

Numerous recent references have been provided on various synthetic methods, staining applications, biological applications, industrial applications, and safety/toxicity

data. Space and format limitations prevent giving all the references for each dye. This is the first ever book that provides safety/toxicity data with reference to acute toxicity, aquatic toxicity, carcinogenicity, cytotoxicity, chronic toxicity, ecotoxicity, genotoxicity, hematotoxicity, hepatotoxicity, immunotoxicity, microbial toxicity, mutagenicity, nephrotoxicity, neurotoxicity, nucleic acid damage, oral toxicity, phototoxicity, phytotoxicity, skin toxicity, reproductive toxicity, and so on. The book also provides Biological Stain Commission (BSC)-certified dyes and Food & Drug Administration (FDA)-approved dyes.

Several appendixes have been provided at the end of the book for scientists to conveniently and easily find a dye as per their need. These appendixes include CAS registry numbers, BSC-certified dyes, FDA-approved dyes, metal indicators, nucleic acid stains, organelle probes, and pH indicators.

Omissions as well as errors of fact and interpretation are inevitable in dealing with so vast a subject as biological dyes. I shall be glad to have my attention drawn to errors and to incorporate suggestions for improvement when a revision becomes possible.

I express my profound respect and appreciation to my guru/mentor/advisor, Prof. D. W. Rangnekar, who brought me to this wonderful world of color science in the Dyestuff Technology Department of Mumbai University Institute of Chemical Technology (MUICT), where I laid the foundation stone for my research career in dye chemistry.

It is my pleasure to make grateful acknowledgement to Dr. Alan Fanta, Dr. Ganapati Shankarling, and Dr. Jeffrey Talkington for their extremely useful discussions, encouragement, and inspiration.

Words are inadequate to express my sincere appreciation for my wife Madhuri and daughter Anika. It would not have been possible to write this book without their encouragement and patience. It is a great pleasure to express my gratitude to John Wiley & Sons, Inc. for giving me an opportunity to write this book.

R. W. SABNIS

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About the Author



Ram W. Sabnis is currently a patent agent at Pfizer Inc. in Madison, NJ. His interests include dyes, pigments, organic chemistry, heterocycles, polymers, synthesis, formulations, coatings, biotechnology, medicinal chemistry, medical devices, and patents. Presently, he focuses on drafting and prosecuting

U.S. and international patents. He is a registered patent agent with U.S. Patent & Trademark Office (USPTO) and is also the inventor of more than 50 U.S. and international patents (issued/published). Before entering the legal (patents) field, he was a research chemist for Ascadia, General Electric, Brewer Science, U.S. Textiles, and Invitrogen in the United States. He had also worked as a patent agent at Squire, Sanders & Dempsey L.L.P. in San Francisco, CA.

Dr. Sabnis was born and raised in Mumbai, India. He received his M.Sc. in Organic Chemistry from University of Mumbai and Ph.D. in Organic Chemistry (Dyes) from University Institute of Chemical Technology (UICT),

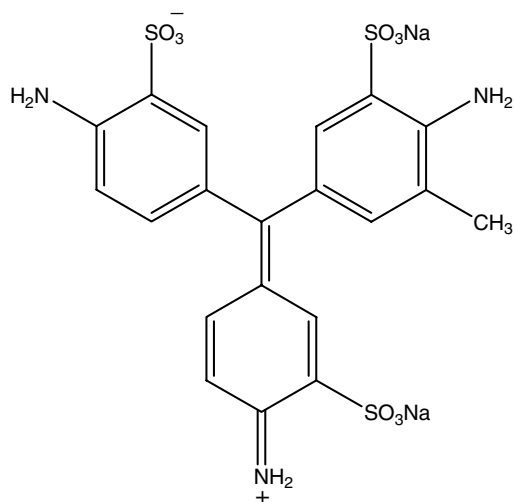
University of Mumbai, India. He received FAIC from American Institute of Chemists, USA. He was awarded CCol FSDC (Chartered Colourists, Fellow of Society of Dyers & Colourists), Society of Dyers & Colourists, UK.

Dr. Sabnis is one of the world's foremost experts in dyes, inventing world's first colored bubbles (nonstaining) and color changing dye system with many applications. He has more than 25 years of industrial and academic research experience in dye chemistry, particularly, dyes for biomedical (fluorescent probes), personal care products, health/beauty products, displays, inks, paints, plastics, textiles, and toys. He has over 150 publications that include books, book chapters, encyclopedia chapters, patents, reviews, papers, and symposium presentations. Dr. Sabnis is the recipient of Perkin Innovation Award by Society of Dyers & Colourists (SDC), UK; Grand Innovation Award by Popular Science, USA; Six Sigma Green Belt & Competitive Spirit Award by GE, USA; and Best Doctoral Thesis Award by University of Mumbai, India. He will continue to focus his activities on fascinating dye chemistry as well as demanding intellectual property in the years to come.

ACID FUCHSIN

CAS Registry Number 3244-88-0

Chemical Structure



CA Index Name Benzenesulfonic acid, 2-amino-5-[(4-amino-3-sulphophenyl)(4-imino-3-sulfo-2,5-cyclohexadien-1-ylidene)methyl]-3-methyl-, sodium salt

Other Names Benzenesulfonic acid, 2-amino-5-[(4-amino-3-sulphophenyl)(4-imino-3-sulfo-2,5-cyclohexadien-1-ylidene)methyl]-3-methyl-, disodium salt; C.I. Acid Violet 19; C.I. Acid Violet 19, disodium salt; Rubine S; Acid Fuchsine; Acid Fuchsine FB; Acid Fuchsine N; Acid Fuchsine O; Acid Fuchsine S; Acid Leather Magenta A; Acid Magenta; Acid Magenta O; Acid Violet 19; Acid fuchsin sodium salt; Acid rosein; Acid rubin; Acidal Fuchsine; Acidal Magenta; Albion Acid Magenta; Andra-

deindicator; C.I. 42685; Fuchine Acid Photo Grade; Fuchsin S; Fuchsin acid; Fuchsine acid; Kiton Magenta A; Triacid Magenta; *p*-Fuchsine acid

Merck Index Number 107

Chemical/Dye Class Triphenylmethane

Molecular Formula C₂₀H₁₇N₃Na₂O₉S₃

Molecular Weight 585.54

Physical Form Olive to dark olive-green crystals or powder

Solubility Very soluble in water; slightly soluble to insoluble in ethanol; insoluble in xylene

Melting Point >250 °C

pH Range 12.0–14.0

Color Change at pH Red (12.0) to colorless (14.0)

Absorption ($\lambda_{\max}$) 546 nm

Emission ($\lambda_{\max}$) 630 nm

Synthesis Synthetic methods^{1–3}

Staining Applications Antigen;⁴ bacteria;⁵ collagen;⁶ fungi;⁷ fats;⁸ neurons;^{9,10} paraffin sections;¹¹ proteins;⁸ starch;⁸ processed food;¹² tumor cells;^{1,13} decayed teeth;¹⁴ lips;^{1,15,16} hairs^{1,17}

Biological Applications Detecting enzyme activity,¹⁸ proteins,¹⁹ tumor cells^{1,13}

Industrial Applications Color filter;^{1,20} recording material;^{1,21} photographic film;²² inks;^{1,23} highlighters;^{1,24} paints;^{1,23} explosives;^{1,25} corrosion inhibitors;^{1,26} leathers;²⁷ textiles^{1,28}

Safety/Toxicity Acute oral toxicity;^{1,29} genotoxicity;^{1,30} neurotoxicity^{1,31,32}

Certification/Approval Certified by Biological Stain Commission (BSC)

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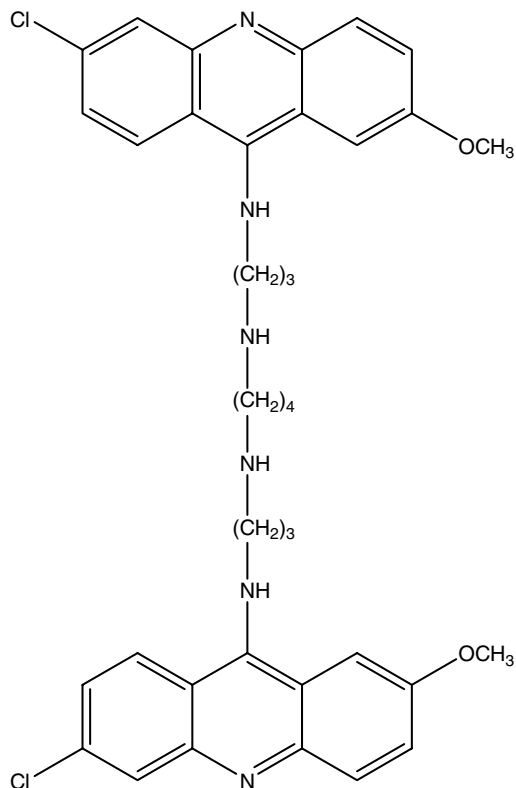
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ACRIDINE HOMODIMER

CAS Registry Number 57576-49-5

Chemical Structure



CA Index Name 1,4-Butanediamine, *N,N'*-bis[3-[(6-chloro-2-methoxy-9-acridinyl)amino]propyl]-

Other Names Acridine homodimer; NSC 219743

Merck Index Number Not listed

Chemical/Dye Class Acridine

Molecular Formula C₃₈H₄₂Cl₂N₆O₂

Molecular Weight 685.69

Physical Form Orange-brown powder

Solubility Soluble in *N,N*-dimethylformamide, dimethyl sulfoxide

Melting Point 169–170 °C

Boiling Point (Calcd.) 885.4 ± 65.0 °C, pressure: 760 Torr

pK_a (Calcd.) 10.63 ± 0.19, most basic, temperature: 25 °C

Absorption (λ_{max}) 431 nm

Emission (λ_{max}) 498 nm

Synthesis Synthetic methods^{1–4}

Staining Applications Nucleic acids;^{3–8} chromosomes⁹

Biological Applications Diagnosis of tissue necrosis;¹⁰ treating malformed proteins causing neurodegenerative disease¹¹

Industrial Applications Not reported

Safety/Toxicity Neurotoxicity¹¹

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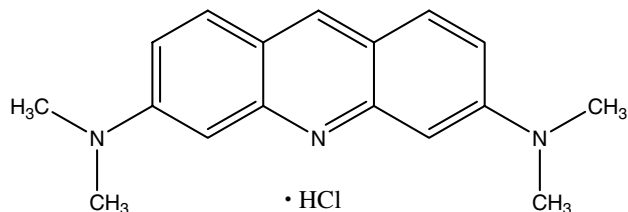
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ACRIDINE ORANGE

CAS Registry Number 65-61-2

Chemical Structure



CA Index Name 3,6-Acridinediamine, *N*3,*N*3,*N*6,*N*6-tetramethyl-, hydrochloride (1 : 1)

Other Names 3,6-Acridinediamine, *N,N,N',N'*-tetramethyl-, monohydrochloride; Acridine Orange R; Acridine, 3,6-bis(dimethylamino)-, hydrochloride; Acridine, 3,6-bis(dimethylamino)-, monohydrochloride; 3,6-Bis(dimethylamino)acridine hydrochloride; Acridine Orange; Acridine Orange N; Acridine Orange NO; Acridine Orange NS; Basic Orange 14; Basic Orange 3RN; C.I. 46005; C.I. Basic Orange 14; Euchrysine 3R; Rhoduline Orange NO; Sumitomo Acridine Orange NO; Sumitomo Acridine Orange RK conc

Merck Index Number Not listed

Chemical/Dye Class Acridine

Molecular Formula C₁₇H₂₀ClN₃

Molecular Weight 301.81

Physical Form Burnt orange to brick-red powder

Solubility Soluble in water, ethanol, dimethyl sulfoxide

Melting Point 284–287 °C

pK_a −3.2, 10.5

Absorption (λ_{max}) 500 nm

Emission (λ_{max}) 526 nm

Synthesis Synthetic methods^{1–8}

Staining Applications Lysosomes;⁹ Golgi apparatus;⁹ acidic compartments;⁹ secretory granules (SGs);¹⁰ synaptic-like microvesicles (SLMVs);¹⁰ liposomes;¹⁰ acidic organelle membranes;¹⁰ bacteria;¹¹ bacterial endospores;¹² apoptotic cells;¹³ blood smears;¹⁴ nucleic acids;^{15–17} cells;¹⁸ malignant musculoskeletal tumors;¹⁹ micronucleus;²⁰ microorganisms;^{21,22} nuclei;²³ peptides;²⁴ proteins;²⁴ antibodies;²⁴ parasites;²⁵ sperms²⁶

Biological Applications Detecting cancer cells;^{27,28} spores;²⁸ human papilloma virus (HPV);²⁹ stress biomarkers;³⁰ treating amyloid associated diseases;³¹ lupus;³² pathogen infections;³³ apoptosis assays;³⁴ cytotoxicity assays³⁵

Industrial Applications Thin films;³⁶ wiring boards³⁷

Safety/Toxicity Carcinogenicity;^{38,39} cytotoxicity;⁴⁰ DNA damage;⁴¹ embryotoxicity;⁴² genotoxicity;^{43,44} mutagenicity;^{45,46} phototoxicity⁴⁷

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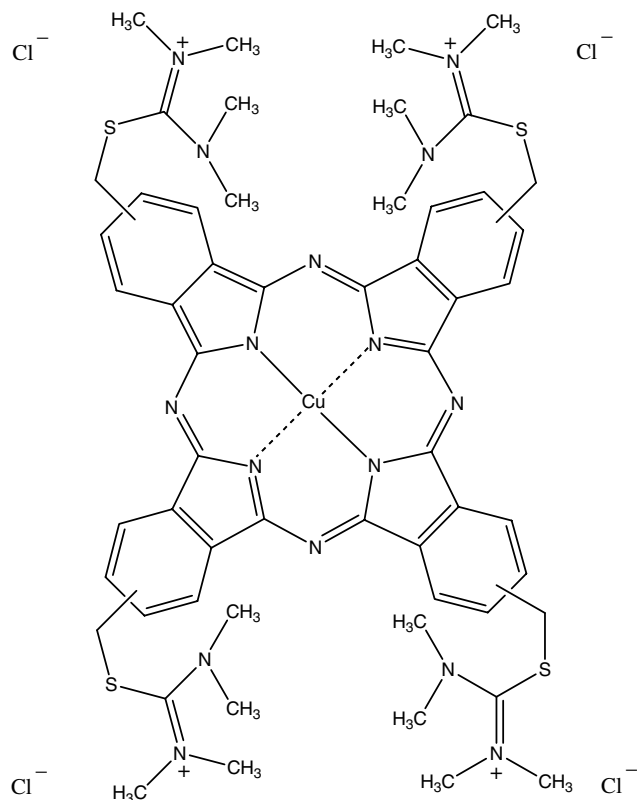
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ALCIAN BLUE 8GX

CAS Registry Number 33864-99-2

Chemical Structure



CA Index Name Copper(4+), [[N,N',N'',N''']-(29H,31H-phthalocyanine-C,C,C,C-tetrayl-κN29,κN30,κN31,κN32)tetrakis[methylenethio[(dimethylamino)methylidene]]]tetrakis[N-methylmethanaminiumato]](2-)-, chloride (1:4)

Other Names Alcian Blue; Alcian Blue 8G; Alcian Blue 8GN; Alcian Blue 8GS; Alcian Blue 8GX; C.I.

74240; C.I. Ingrain Blue 1; Chloromethylated copper phthalocyanine-thiourea reaction products; Copper (4+), [[N,N',N'',N''']-(29H,31H-phthalocyaninetetrayl-κN29,κN30,κN31,κN32)tetrakis[methylenethio[(dimethylamino)methylidene]]]tetrakis[N-methylmethanaminiumato]](2-)-, tetrachloride; Copper(4+), [[N,N',N'',N''']-(29H,31H-phthalocyaninetetrayltetrakis[methylenethio[(dimethylamino)methylidene]]]tetrakis[N-methylmethanaminiumato]](2-)-N29,N30,N31,N32]-, tetrachloride; Methanaminium, N,N',N'',N'''-[29H,31H-phthalocyaninetetrayltetrakis[methylenethio[(dimethylamino)methylidene]]]tetrakis[N-methyl-, copper complex

Merck Index Number 218

Chemical/Dye Class Copper phthalocyanine

Molecular Formula C₅₆H₆₈Cl₄CuN₁₆S₄

Molecular Weight 1298.93

Physical Form Dark blue-violet powder

Solubility Soluble in water, ethanol

Melting Point 148 °C

Absorption (λ_{max}) 615 nm

Synthesis Synthetic methods¹⁻⁶

Staining Applications Bonghan threads inside lymphatic vessels;⁷ chromatin;^{8,9} glycoproteins;¹⁰ glycosaminoglycans;¹¹ lung goblet cells;¹² neurons;¹³ nuclear basic proteins;¹⁴ surface coat of cells;¹⁵ urine sediments;¹⁶

Biological Applications Glycoconjugates assay;² hyaluronic acid assay;¹⁷ hybridization assay;¹⁸ cell agglutination;^{19,20} detecting humic acid,^{21,22} detecting pancreatic β-granules;²³ locomotion of amoeba,²⁴ quantitative determination of glycosaminoglycans,^{25,26}

Industrial Applications Inks;⁴ leather products;²⁷ textiles²⁸

Safety/Toxicity No data available

Certification/Approval Certified by Biological Stain Commission (BSC)

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