

Contents

Preface	v
Abbreviations, Symbols and Definitions	xv
Introduction	xvii
The Beginning of the Chemical Study of Bioluminescence	xix
Luciferin	xx
Photoprotein	xxi
Chemical Studies on Bioluminescence in the Last One Hundred Years	xxii
Chemical Study of Bioluminescence in the Future	xxiv
The Contents of this Book	xxvi
1 The Fireflies and Luminous Insects	1
1.1 The Fireflies	3
1.1.1 Essential Factors in the Firefly Luminescence Reaction	3
1.1.2 Firefly Luciferin and Oxyluciferin	5
1.1.3 Firefly Luciferase	8
1.1.4 Assays of Luciferase Activity, ATP and Luciferin	11
1.1.5 General Characteristics of the Bioluminescence of Fireflies	12
1.1.6 Mechanisms of the Bioluminescence	15

1.1.7	Light Emitters in the Firefly Luminescence System	17
1.1.8	A Note on the Dioxetanone Pathway and the ^{18}O -incorporation Experiment	19
1.2	Phengodidae and Elateroidae	23
1.2.1	Phengodidae	24
1.2.2	Elateridae	24
1.3	Diptera	25
1.3.1	The Glow-worm <i>Arachnocampa</i>	25
1.3.2	The American Glow-worm <i>Orfelia</i>	27
2	Luminous Bacteria	30
2.1	Factors Required for Bioluminescence	31
2.2	Bacterial Luciferase	33
2.3	Long-chain Aldehyde	35
2.4	Mechanism of Luminescence Reaction	37
2.5	Assay of Luciferase Activity	39
2.6	Quantum Yield of Long-chain Aldehydes	41
2.7	<i>In vivo</i> Luminescence of Luminous Bacteria	41
3	The Ostracod <i>Cypridina</i> (<i>Vargula</i>) and Other Luminous Crustaceans	47
3.1	The Ostracod <i>Cypridina</i>	49
3.1.1	Overview of Ostracoda	49
3.1.2	<i>Cypridina hilgendorffii</i> Müller	51
3.1.3	Research on <i>Cypridina</i> Luminescence before 1955	53
3.1.4	Purification and Crystallization of <i>Cypridina</i> Luciferin	55
3.1.5	Properties of <i>Cypridina</i> Luciferin	58
3.1.6	Oxyluciferin and Etioluciferin	62
3.1.7	Purification and Molecular Properties of <i>Cypridina</i> Luciferase	62
3.1.8	Luciferin-luciferase Luminescence Reaction	64
3.1.9	Quantum Yield	69

3.2	Euphausiids <i>Euphausia pacifica</i> and <i>Meganctiphanes norvegica</i>	71
3.2.1	Involvement of the Fluorescent Compound F and Protein P	71
3.2.2	Fluorescent Compound F	74
3.2.3	Protein P	79
3.2.4	Luminescence Reaction	80
3.3	The Decapod Shrimp <i>Oplophorus gracilirostris</i> . .	82
3.3.1	<i>Oplophorus</i> Luciferase	82
3.3.2	Coelenterazine-luciferase Reaction	83
3.4	Copepoda	88
4	The Jellyfish <i>Aequorea</i> and Other Luminous Coelenterates	90
4.1	The Hydrozoan Medusa <i>Aequorea aequorea</i>	92
4.1.1	History of the Biochemical Study of <i>Aequorea</i> Bioluminescence	94
4.1.2	Extraction and Purification of Aequorin . .	95
4.1.3	Properties of Aequorin	100
4.1.4	Discovery of the Coelenterazine Moiety in Aequorin	111
4.1.5	Regeneration of Aequorin from Apoaequorin	113
4.1.6	Recombinant Aequorin	116
4.1.7	Semisynthetic Aequorins	118
4.1.8	The <i>In Vivo</i> Luminescence of <i>Aequorea</i> . .	129
4.2	The Hydroid <i>Obelia</i> (Hydrozoan)	133
4.2.1	Natural Obelins	133
4.2.2	Recombinant Obelin	134
4.3	The Hydrozoan Medusa <i>Phialidium gregarium</i> . .	137
4.4	Other Bioluminescent Hydrozoans	138
4.5	The Scyphozoans <i>Pelagia</i> and <i>Periphylla</i>	140
4.5.1	<i>Pelagia noctiluca</i>	140
4.5.2	<i>Periphylla periphylla</i>	140
4.6	The Anthozoan <i>Renilla</i> (Sea Pansy)	147
4.7	Green Fluorescent Protein (GFP)	151
4.8	The Ctenophores	154

5	The Coelenterazines	159
5.1	Discovery of Coelenterazine	159
5.2	Occurrence of Coelenterazine	160
5.3	Properties of Coelenterazine and its Derivatives . .	165
5.4	Chemi- and Bioluminescence Reactions of Coelenterazine	168
5.5	Chemical Reactions of Coelenterazine	173
5.6	Synthesis of Coelenterazines	176
5.7	Coelenterazine Luciferases	176
6	Luminous Mollusca	180
6.1	The Limpet <i>Latia</i>	182
6.2	The Clam <i>Pholas dactylus</i>	192
6.3	Luminous Squids (Cephalopoda)	199
6.3.1	The Firefly Squid <i>Watasenia scintillans</i> . . .	200
6.3.2	The Purpleback Flying Squid <i>Symplectoteuthis oualaniensis</i> (Tobi-ika) . .	204
6.3.3	The Luminous Flying Squid <i>Symplectoteuthis luminosa</i> (Suji-ika)	210
7	Annelida	216
7.1	The Tubeworm <i>Chaetopterus variopedatus</i>	216
7.1.1	Biochemistry of the Luminescence of <i>Chaetopterus variopedatus</i>	218
7.1.2	Properties of the <i>Chaetopterus</i> Photoprotein and its Luminescence Reaction	221
7.2	The Bermuda Fireworm <i>Odontosyllis</i>	225
7.3	Luminous Earthworms (Oligochaeta)	234
7.4	Polynoid Scaleworm <i>Harmothoë lunulata</i>	242
7.5	The Polychaete <i>Tomopteris</i>	246
8	Dinoflagellates and Other Protozoa	248
8.1	Radiolarians	248

8.2	Dinoflagellates	249
8.2.1	Cultivation and Harvesting of Dinoflagellates	251
8.2.2	Scintillons	251
8.2.3	The Luciferase of <i>Gonyaulax polyedra</i>	252
8.2.4	Extraction and Purification of Dinoflagellate Luciferin	256
8.2.5	Properties of Dinoflagellate Luciferin	258
8.2.6	Chemical Structures of Dinoflagellate Luciferin and its Oxidation Products	260
8.2.7	Chemical Mechanism of Dinoflagellate Bioluminescence	263
8.2.8	Luciferin Binding Protein of Dinoflagellates	264
9	Luminous Fungi	266
9.1	An Overview on Fungal Bioluminescence	266
9.2	Early Studies on the Biochemistry of Luminous Fungi	268
9.3	Role of Superoxide in Fungal Luminescence	271
9.4	Studies on <i>Panellus stipticus</i>	275
9.4.1	Panal	277
9.4.2	Activation Products of Panal	279
9.4.3	PS-A and PS-B	282
9.4.4	Activation of PS-A and PS-B	283
9.4.5	Mechanism of the <i>in vivo</i> Bioluminescence of <i>P. stipticus</i>	289
9.4.6	Synthetic Studies of Panellus Luciferin	291
9.5	Studies on <i>Mycena citricolor</i>	294
9.5.1	Luciferin Obtained by Kuwabara and Wassink	294
9.5.2	Studies on the <i>Mycena citricolor</i> Luminescence by the Author	294
9.6	Summary on the Chemistry of Fungal Luminescence	298

10	Other Luminous Organisms	301
10.1	Ophiuroidea: Brittle Stars	301
10.1.1	The Brittle Star <i>Ophiopsila californica</i> . . .	302
10.1.2	The Brittle Star <i>Amphiura filiformis</i>	307
10.2	Millipede <i>Luminodesmus sequoiae</i> (Diplopoda) . .	307
10.3	Centipede <i>Orphaneus brevilabiatus</i> (Chilopoda) . .	314
10.4	Hemicordata	315
10.4.1	The Acorn Worm <i>Balanoglossus</i> <i>biminiensis</i>	315
10.4.2	The Luciferin of <i>Ptychodera flava</i>	318
10.5	Tunicates (Phylum Chordata)	319
10.6	The Luminous Fishes	322
10.6.1	Coastal and Shallow-water Fishes that Utilize <i>Cypridina</i> Luciferin	323
10.6.2	Oceanic Deep-sea Luminous Fishes	327
10.6.3	Future Research on Fish Bioluminescence	330
	Appendix	333
A	Taxonomic Classification of Selected Luminous Organisms	333
B	Lists of Luciferins, Luciferases and Photoproteins Isolated	340
C	Miscellaneous Technical Information	349
C1	Basic Principle of the Isolation of Bioluminescent Substances	349
C1.1	Reversible Inhibition of Bioluminescence	350
C1.2	Extraction of Luciferin-luciferase Systems	353
C1.3	Solubilization of Proteins	353
C1.4	Purification	355
C2	Storage of Samples	356
C3	Measurement of Luminescence	360

C4	Calibration of Luminometer and the Measurement of Quantum Yield	361
C5	Measurements of Coelenterazine, its Derivatives, and other Important Substances in Bioluminescence	363
C5.1	Assay of Coelenterazine	363
C5.2	Assay of the Coelenterazine Luciferase Activity	364
C5.3	Assay of the Stabilized Forms of Coelenterazine	365
C5.4	Assay of Dehydrocoelenterazine	366
C5.5	Assay of <i>Cypridina</i> Luciferin	366
C5.6	Assay of <i>Cypridina</i> Luciferase	367
C5.7	Assay of Ca^{2+} -sensitive Photoproteins	368
C5.8	Measuring Bioluminescence in the Field	369
C6	^{18}O -Labeling of the Reaction Product CO_2	370
C7	Glass Blowing	374
D	Advice to Students Who are Interested in Studying the Chemistry of Bioluminescence	375
References		379
Index		455