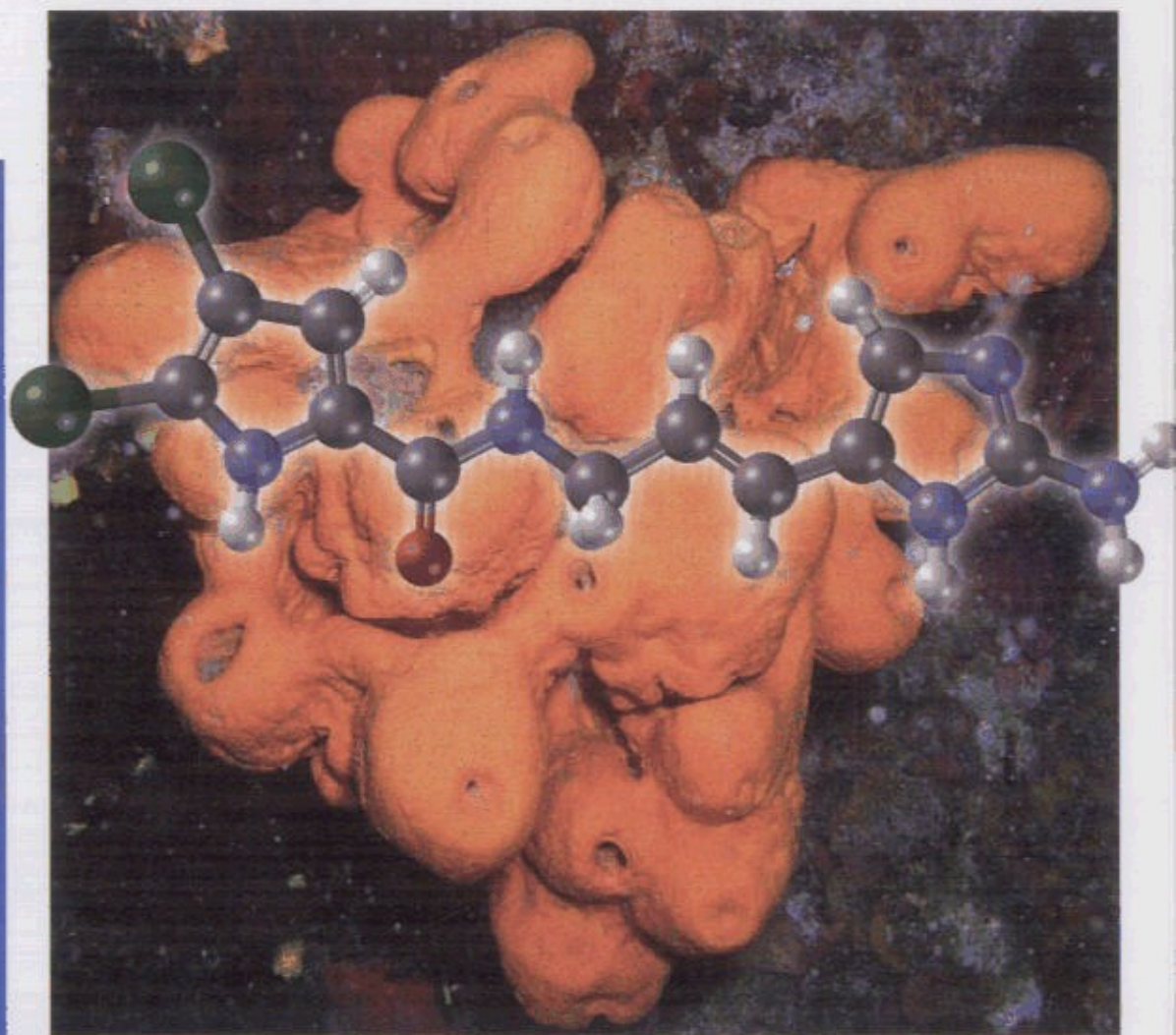


Edited by Ernesto Fattorusso and
Orazio Tagliatela-Scafati

 WILEY-VCH

Modern Alkaloids

Structure, Isolation, Synthesis and Biology



Contents

Preface XVII

List of Contributors XIX

I	Bioactive Alkaloids: Structure and Biology	1
1	Ecological Roles of Alkaloids	3
	<i>Michael Wink</i>	
1.1	Introduction: Defense Strategies in Plants	3
1.2	Ecological Roles of Alkaloids	4
1.3	Modes of Action	9
1.3.1	Unspecific Interactions	11
1.3.2	Specific Interactions	12
1.3.3	Cytotoxicity of Alkaloids	16
1.4	Evolution of Alkaloidal Defense Systems	19
1.5	Conclusions	23
2	Antitumor Alkaloids in Clinical Use or in Clinical Trials	25
	<i>Muriel Cuendet, John M. Pezzuto</i>	
2.1	Introduction	25
2.2	Antitumor Alkaloids in Clinical Use	25
2.2.1	Vinca Alkaloids	25
2.2.1.1	Vinblastine (VLB, 1)	28
2.2.1.2	Vincristine (VCR, 2)	28
2.2.1.3	Vindesine (VDS, 3)	28
2.2.1.4	Vinorelbine (VRLB, 4)	29
2.2.1.5	Vinflunine (VFL, 5)	29
2.2.2	Camptothecin and Analogs	29
2.2.2.1	Camptothecin (CPT, 6)	31
2.2.2.2	Irinotecan (CPT-11)	31
2.2.2.3	Topotecan	32
2.2.2.4	Exatecan	32
2.2.2.5	Gimatecan	32

2.2.2.6	Karenitecin	32
2.2.2.7	Lurtotecan	32
2.2.2.8	Rubitecan (9-nitrocamptothecin)	33
2.2.3	Taxanes	33
2.2.3.1	Paclitaxel	33
2.2.3.2	Docetaxel	35
2.3	Antitumor Alkaloids in Clinical Trials	36
2.3.1	Ecteinasidin-743 (Yondelis, Trabectedin)	36
2.3.2	7-Hydroxystaurosporine (UCN-01)	37
2.3.3	Ellipticine and Analogs	37
2.3.4	Acronycine and Analogs	38
2.3.5	Colchicine and Analogs	39
2.3.6	Ukrain	40
2.4	Alkaloids Used for MDR Reversal	40
2.4.1	<i>Cinchona</i> Alkaloids	40
2.4.2	Dofequidar Fumarate (MS-209)	41
2.5	Alkaloids Used for Cancer Prevention	42
2.6	Conclusions	43
2.7	Acknowledgments	44
3	Alkaloids and the Bitter Taste	53
	<i>Angela Bassoli, Gigliola Borgonovo, Gilberto Busnelli</i>	
3.1	Introduction	53
3.2	The Bitter Taste Chemoreception Mechanism	54
3.3	Bitter Alkaloids in Food	58
3.4	The Bitter Taste of Alkaloids in Other Drugs and Poisons	63
3.5	Alkaloids and Taste in Insects	66
3.6	The Bitter Taste of Alkaloids: Should We Avoid, Mask, or Understand?	69
3.7	Acknowledgments	70
4	Capsaicin and Capsaicinoids	73
	<i>Giovanni Appendino</i>	
4.1	Introduction	73
4.2	What Is an Alkaloid? Is Capsaicin an Alkaloid?	73
4.3	Diversity, Biosynthesis, and Metabolism of Capsaicinoids	77
4.4	Quantization of Capsaicinoids and Their Distribution in Chili Pepper	83
4.5	Isolation and Synthesis of Capsaicin	86
4.6	TRV1 as the Biological Target of Capsaicin and the Ecological Raison d'être of Capsaicinoids: A Molecular View	90
4.7	Naturally Occurring Analogs and Antagonists of Capsaicin and Endogenous Vanilloids	93
4.8	Structure–Activity Relationships of Capsaicinoids	94

4.9	Molecular Gastronomy of Hot Food	98
4.9.1	Biomedical Relevance of Capsaicin-Induced Trigeminal Responses	98
4.9.2	Effect of Capsaicin on Taste	98
4.9.3	Gustatory Sweating	99
4.9.4	Gustatory Rhinitis	99
4.9.5	Hot Food Mitridatism	99
4.9.6	Effect of Capsaicin on Digestion	100
4.9.7	Capsaicin and Stomach Cancer	100
4.9.8	The Effect of Age and Sex on the Sensitivity to Capsaicin	100
4.9.9	Capsaicin as a Slimming Agent	101
4.9.10	Quenching Capsaicin	101
4.9.11	Chilies and Olive Oil	102
4.9.12	Who Should Avoid Chilies?	102
4.9.13	How can the Pungency of Chilies be Moderated?	102
4.9.14	Psychology of Pepper Consumption	102
4.10	Conclusions	103
4.11	Acknowledgments	103
5	Glycosidase-Inhibiting Alkaloids: Isolation, Structure, and Application	111
	<i>Naoki Asano</i>	
5.1	Introduction	111
5.2	Isolation and Structural Characterization	111
5.2.1	Deoxynojirimycin and Related Compounds	112
5.2.1.1	Isolation from <i>Morus</i> spp. (Moraceae)	112
5.2.1.2	Isolation from Thai Medicinal Plants “Thopthaep” and “Cha Em Thai”	113
5.2.2	α -Homonojirimycin and Related Compounds	115
5.2.2.1	Isolation from Garden Plants	115
5.2.2.2	Isolation from the Thai Medicinal Plant “Non Tai Yak”	117
5.2.2.3	Isolation from <i>Adenophora</i> spp. (Campanulaceae)	117
5.2.3	Indolizidine and Pyrrolizidine Alkaloids	117
5.2.3.1	Isolation from the Leguminosae Family	118
5.2.3.2	Isolation from the Hyacinthaceae Family	120
5.2.4	Nortropane Alkaloids	122
5.2.4.1	Isolation from the Solanaceae Family	123
5.2.4.2	Isolation from the Convolvulaceae Family	124
5.3	Biological Activities and Therapeutic Application	125
5.3.1	Antidiabetic Agents	125
5.3.1.1	α -Glucosidase Inhibitors	125
5.3.1.2	Glycogen Phosphorylase Inhibitors	128
5.3.1.3	Herbal Medicines	128
5.3.2	Molecular Therapy for Lysosomal Storage Disorders	129

5.3.2.1	Substrate Reduction Therapy	130
5.3.2.2	Pharmacological Chaperone Therapy	130
5.4	Concluding Remarks and Future Outlook	133
6	Neurotoxic Alkaloids from Cyanobacteria	139
	<i>Rashel V. Grindberg, Cynthia F. Shuman, Carla M. Sorrels, Josh Wingerd, William H. Gerwick</i>	
6.1	Introduction	139
6.2	Neurotoxic Alkaloids of Principally Freshwater and Terrestrial Cyanobacteria	141
6.2.1	Anatoxin-a, Homoanatoxin-a, Anatoxin-a(s), and Analogs	141
6.2.1.1	Anatoxin-a	142
6.2.1.2	Homoanatoxin-a	145
6.2.1.3	Anatoxin-a(s)	145
6.2.2	β -Methylaminoalanine	146
6.2.3	Saxitoxin	151
6.3	Neurotoxic Alkaloids of Marine Cyanobacteria	156
6.3.1	Antillatoxin A and B	156
6.3.2	Jamaicamide A, B, and C	158
6.3.3	Kalkitoxin	161
6.4	Conclusion	162
7	Lamellarin Alkaloids: Structure and Pharmacological Properties	171
	<i>Jérôme Kluza, Philippe Marchetti, Christian Bailly</i>	
7.1	Introduction	171
7.2	The Discovery of Lamellarins	172
7.3	Modulation of Multidrug Resistance	174
7.4	Antioxidant Properties	176
7.5	Inhibition of HIV-1 Integrase	176
7.6	Cytotoxicity	177
7.7	Topoisomerase I Inhibition	178
7.8	Targeting of Mitochondria and Proapoptotic Activities	180
7.9	Conclusion	184
8	Manzamine Alkaloids	189
	<i>Jiangnan Peng, Karumanchi V. Rao, Yeun-Mun Choo, Mark T. Hamann</i>	
8.1	Introduction	189
8.2	Manzamine Alkaloids from Marine Sponges	191
8.2.1	β -Carboline-containing Manzamine Alkaloids	191
8.2.1.1	Manzamine A Type	191
8.2.1.2	Manzamine B Type	195
8.2.1.3	Manzamine C Type	196
8.2.1.4	Other β -Carboline-containing Manzamines	196
8.2.2	Ircinal-related Alkaloids	198
8.3	Source and Large-scale Preparation of Manzamine Alkaloids	202

8.3.1	Source of Manzamine Alkaloids	202
8.3.2	Large-scale Preparation of Manzamines	204
8.3.3	Supercritical Fluid Chromatography Separation of Manzamine Alkaloids	205
8.4	Synthesis of Manzamine Alkaloids	206
8.4.1	Total Synthesis of Manzamine A and Related Alkaloids	206
8.4.2	Total Synthesis of Manzamine C	208
8.4.3	Total Synthesis of Nakadomarin A	214
8.4.4	Synthetic Studies of Manzamine Alkaloids	216
8.4.5	Studies on Biomimetic Synthesis	217
8.4.6	Synthesis of Manzamine Analogs	219
8.5	Biological Activities of Manzamines	220
8.5.1	Anticancer Activity	220
8.5.2	Antimalarial Activity	222
8.5.3	Antimicrobial and Antituberculosis Activity	224
8.5.4	Miscellaneous Biological Activities	225
8.6	Concluding Remarks	226
9	Antiangiogenic Alkaloids from Marine Organisms	233
	<i>Ana R. Diaz-Marrero, Christopher A. Gray, Lianne McHardy, Kaoru Warabi, Michel Roberge, Raymond J. Andersen</i>	
9.1	Introduction	233
9.2	Purine Alkaloids	235
9.3	Terpenoid Derivatives	236
9.3.1	Avinosol	236
9.3.2	Cortistatins A–D	237
9.3.3	Squalamine	238
9.4	Motuporamines	240
9.5	Pyrrole-Imidazole Alkaloids: “Oroidin”-Related Alkaloids	244
9.5.1	Agelastatin A	245
9.5.2	Ageladine A	247
9.6	Tyrosine-derived Alkaloids	250
9.6.1	Aeropylsinin-1	250
9.6.2	Psammaphin A	254
9.6.3	Bastadins	256
9.7	Tryptophan-derived Alkaloids	259
9.8	Ancorinosides	262
9.9	Concluding Remarks	263
10	A Typical Class of Marine Alkaloids: Bromopyrroles	271
	<i>Anna Aiello, Ernesto Fattorusso, Marialuisa Menna, Orazio Tagliatela-Scafati</i>	
10.1	Introduction	271
10.2	Oroidin-like Linear Monomers	273
10.3	Polycyclic Oroidin Derivatives	278

10.3.1	C-4/C-10 Derivatives	278
10.3.2	N-1/C-9 Derivatives	281
10.3.3	N-7/C-11 + N-1/C-12 Derivatives	281
10.3.4	N-7/C-11 + C-4/C-12 Derivatives	284
10.3.5	N-1/C-12 + N-7/C-12 Derivatives	285
10.3.6	N-1/C-9 + C-8/C-12 Derivatives	285
10.4	Simple or Cyclized Oroidin-like Dimers	286
10.5	Other Bromopyrrole Alkaloids	291
10.6	Conclusions	296

11 Guanidine Alkaloids from Marine Invertebrates 305

Roberto G.S. Berlinck, Miriam H. Kossuga

11.1	Introduction	305
11.2	Modified Creatinine Guanidine Derivatives	305
11.3	Aromatic Guanidine Alkaloids	307
11.4	Bromotyrosine Derivatives	309
11.5	Amino Acid and Peptide Guanidines	310
11.6	Terpenic Guanidines	320
11.7	Polyketide-derived Guanidines	321

II New Trends in Alkaloid Isolation and Structure Elucidation 339

12 Analysis of Tropane Alkaloids in Biological Matrices 341

Philippe Christen, Stefan Bieri, Jean-Luc Veuthey

12.1	Introduction	341
12.2	Extraction	343
12.2.1	Plant Material	343
12.2.2	Supercritical Fluid Extraction	343
12.2.3	Microwave-assisted Extraction	344
12.2.4	Pressurized Solvent Extraction	345
12.2.5	Solid-phase Microextraction	345
12.2.6	Biological Matrices	346
12.3	Analysis of Plant Material and Biological Matrices	348
12.3.1	Gas Chromatography	348
12.3.2	High-performance Liquid Chromatography	355
12.3.3	Capillary Electrophoresis	359
12.3.4	Desorption Electrospray Ionization Mass Spectrometry	361
12.4	Conclusions	362

13 LC-MS of Alkaloids: Qualitative Profiling, Quantitative Analysis, and Structural Identification 369

Steven M. Colegate, Dale R. Gardner

13.1	Introduction	369
13.2	LC-MS Overview	369

13.2.1	Optimization	370
13.2.1.1	Modification of Mobile Phases and Ionization Parameters	370
13.2.1.2	HPLC Versus UPLC	372
13.2.1.3	Fluorinated HPLC Solid Phases	372
13.2.1.4	Reduction of Ion Suppression	373
13.3	Clinical Chemistry and Forensic Applications	374
13.3.1	Extraction and Analytical Considerations	375
13.3.2	Forensic Detection of Plant-derived Alkaloids	375
13.3.2.1	Plant-associated Intoxications	375
13.3.2.2	Illicit Drug Use: Multiple Reaction Monitoring	376
13.3.2.3	Quality Control of Herbal Preparations: APCI-MS	376
13.4	Metabolite Profiling and Structure Determination	376
13.4.1	LC-MS/MS Approaches to the Identification/Structural Elucidation of Alkaloid Drug Metabolites	377
13.4.1.1	Tandem MS	377
13.4.1.2	Accurate Mass Measurement	378
13.4.1.3	Chemical Modification	378
13.4.2	Minimization of Sample Treatment	378
13.4.3	Structure Determination	380
13.4.3.1	Nudicaulins from <i>Papaver nudicaule</i> : High-resolution MS	380
13.4.3.2	Endophyte Alkaloids: An MS Fragment Marker	380
13.5	Pyrrolizidine Alkaloids and Their N-Oxides	382
13.5.1	Solid Phase Extraction	383
13.5.2	Qualitative Profiling	383
13.5.2.1	<i>Echium plantagineum</i> and <i>Echium vulgare</i>	385
13.5.2.2	<i>Senecio ovatus</i> and <i>Senecio jacobaea</i>	387
13.5.3	Quantitative Analysis	392
13.5.3.1	Calibration Standards	393
13.5.3.2	Honey	394
13.6	Alkaloids from <i>Delphinium</i> spp. (Larkspurs)	395
13.6.1	Flow Injection (FI) Mass Spectrometry	396
13.6.1.1	Qualitative FI Analysis	397
13.6.1.2	Quantitative FI Analyses	398
13.6.1.3	Chemotaxonomy of <i>Delphinium</i> Species	399
13.6.2	LC-MS Analysis of Diterpene Alkaloids	400
13.6.2.1	Toxicokinetics and Clearance Times	400
13.6.2.2	Diagnosis of Poisoning	401
13.6.3	Structural Elucidation of Norditerpenoid Alkaloids	402
13.6.3.1	Stereochemical Indications	402
13.6.3.2	Isomeric Differentiation Using Tandem Mass Spectrometry	403
13.6.3.3	Novel Diterpene Alkaloid Identification: Application of Tandem Mass Spectrometry	405
13.7	Conclusions	405

14	Applications of ^{15}N NMR Spectroscopy in Alkaloid Chemistry	409
	<i>Gary E. Martin, Marina Solntseva, Antony J. Williams</i>	
14.1	Introduction	409
14.1.1	^{15}N Chemical Shift Referencing	409
14.1.2	^{15}N Chemical Shifts	411
14.1.3	^{15}N Reviews and Monographs	411
14.2	Indirect-Detection Methods Applicable to ^{15}N	412
14.2.1	Accordion-optimized Long-range ^1H - ^{15}N Heteronuclear Shift Correlation Experiments	413
14.2.2	Pulse Width and Gradient Optimization	414
14.2.3	Long-range Delay Optimization	414
14.2.4	Establishing F_1 Spectral Windows	416
14.3	^{15}N Chemical Shift Calculation and Prediction	418
14.3.1	Structure Verification Using a ^{15}N Content Database	418
14.3.2	^{15}N NMR Prediction	419
14.3.3	Enhancing NMR Prediction With User-“trained” Databases	420
14.3.4	Validating ^{15}N NMR Prediction	420
14.4	Computer-assisted Structure Elucidation (CASE) Applications Employing ^{15}N Chemical Shift Correlation Data	422
14.5	Applications of ^{15}N Spectroscopy in Alkaloid Chemistry	428
14.6	Applications of Long-range ^1H - ^{15}N 2D NMR	430
14.6.1	Five-membered Ring Alkaloids	430
14.6.2	Tropane Alkaloids	436
14.6.3	Indoles, Oxindoles, and Related Alkaloids	437
14.6.3.1	Strychnos Alkaloids	437
14.6.3.2	Azaindoles	439
14.6.3.3	Indoloquinoline Alkaloids	439
14.6.3.4	Vinca Alkaloids	441
14.6.3.5	Other Indole Alkaloids	442
14.6.4	Carboline-derived Alkaloids	448
14.6.5	Quinoline, Isoquinoline, and Related Alkaloids	450
14.6.6	Benzo[c]phenanthridine Alkaloids	453
14.6.7	Pyrazine Alkaloids	456
14.6.8	Diazepinopurine Alkaloids	459
14.7	Pyridoacridine, Quinoacridine, and Related Alkaloids	460
14.8	Conclusions	465
III	New Trends in Alkaloid Synthesis and Biosynthesis	473
15	Synthesis of Alkaloids by Transition Metal-mediated Oxidative Cyclization	475
	<i>Hans-Joachim Knölker</i>	
15.1	Silver(I)-mediated Oxidative Cyclization to Pyrroles	475
15.1.1	Synthesis of the Pyrrolo[2,1- <i>a</i>]isoquinoline Alkaloid Crispine A	477

15.1.2	Synthesis of the Indolizidino[8,7- <i>b</i>]indole Alkaloid Harmicine 478	
15.2	Iron(0)-mediated Oxidative Cyclization to Indoles 478	
15.3	Iron(0)-mediated Oxidative Cyclization to Carbazoles 481	
15.3.1	3-Oxygenated Carbazole Alkaloids 482	
15.3.2	Carbazole-1,4-Quinol Alkaloids 483	
15.3.3	Furo[3,2- <i>a</i>]carbazole Alkaloids 483	
15.3.4	2,7-Dioxygenated Carbazole Alkaloids 485	
15.3.5	3,4-Dioxygenated Carbazole Alkaloids 487	
15.4	Palladium(II)-catalyzed Oxidative Cyclization to Carbazoles 488	
15.4.1	Carbazolequinone Alkaloids 489	
15.4.2	Carbazomadurins and Epocarbazolins 492	
15.4.3	7-Oxygenated Carbazole Alkaloids 493	
15.4.4	6-Oxygenated Carbazole Alkaloids 495	
16	Camptothecin and Analogs: Structure and Synthetic Efforts 503	
	<i>Sabrina Dallavalle, Lucio Merlini</i>	
16.1	Introduction: Structure and Activity 503	
16.2	Synthetic Efforts 507	
17	Combinatorial Synthesis of Alkaloid-like Compounds In Search of Chemical Probes of Protein–Protein Interactions 521	
	<i>Michael Prakesch, Prabhat Arya, Marwen Naim, Traian Sulea, Enrico Purisima, Aleksey Yu. Denisov, Kalle Gehring, Trina L. Foster, Robert G. Korneluk</i>	
17.1	Introduction 521	
17.2	Protein–Protein Interactions 523	
17.3	Alkaloid Natural Products as Chemical Probes of Protein–Protein Interactions 524	
17.4	Indoline Alkaloid Natural Product-inspired Chemical Probes 525	
17.4.1	Indoline Alkaloid-inspired Chemical Probes 526	
17.4.2	Tetrahydroquinoline Alkaloid-inspired Chemical Probes 528	
17.5	Alkaloid Natural Product-inspired Small-molecule Binders to Bcl-2 and Bcl-XL and <i>In Silico</i> Studies 532	
17.5.1	Alkaloid Natural Product-inspired Small-molecule Binders to Bcl-XL and NMR Studies 533	
17.5.2	Alkaloid Natural Product-inspired Small-molecule Probes for XIAP 535	
17.5.2.1	Cell Death Assay 535	
17.5.2.2	Caspase-3 Activation Assay 536	
17.5.2.3	Caspase-9 Release Assay 536	
17.5.3	Summary and Future Outlook 536	
17.6	Acknowledgments 538	

18	Daphniphyllum alkaloids: Structures, Biogenesis, and Activities	541
	<i>Hiroshi Morita, Jun'ichi Kobayashi</i>	
18.1	Introduction	541
18.2	Structures of Daphniphyllum Alkaloids	542
18.2.1	Daphnane-type Alkaloids	542
18.2.2	Secodaphnane-type Alkaloids	543
18.2.3	Yuzurimine-type Alkaloids	543
18.2.4	Daphnilactone A-type Alkaloids	543
18.2.5	Daphnilactone B-type Alkaloids	544
18.2.6	Yuzurine-type Alkaloids	544
18.2.7	Daphnezomines	545
18.2.8	Daphnicyclidins	551
18.2.9	Daphmanidins	557
18.2.10	Daphniglaucins	559
18.2.11	Calyciphyllines	560
18.2.12	Daphtenidines	560
18.2.13	Other Related Alkaloids	561
18.3	Biosynthesis and Biogenesis	564
18.3.1	Biosynthesis of Daphniphyllum Alkaloids	564
18.3.2	Biogenesis of the Daphnane and Secodaphnane Skeletons	564
18.3.3	Biogenesis of the Daphnezomines	565
18.3.4	Biogenesis of the Daphnicyclidins	568
18.3.5	Biogenesis of the Daphmanidins	569
18.3.6	Biogenesis of the Daphniglaucins	570
18.3.7	Biogenesis of the Calyciphyllines	573
18.3.8	Biogenesis of the Daphtenidines	573
18.4	Synthesis	575
18.4.1	Biomimetic Chemical Transformations	575
18.4.1.1	Transformation of an Unsaturated Amine to the Daphnane Skeleton	575
18.4.1.2	Transformation of Daphnicyclidin D to Daphnicyclidins E and J	575
18.4.2	Biomimetic Total Synthesis	576
18.4.2.1	Methyl Homosecodaphniphyllate and Protodaphniphylline	576
18.4.2.2	Secodaphniphylline	579
18.4.2.3	Methyl Homodaphniphyllate and Daphnilactone A	580
18.4.2.4	Codaphniphylline	582
18.4.2.5	Bukittingine	583
18.4.2.6	Polycyclization Cascade	583
18.5	Activities	585
18.6	Conclusions	586
19	Structure and Biosynthesis of Halogenated Alkaloids	591
	<i>Gordon W. Gribble</i>	
19.1	Introduction	591
19.2	Structure of Halogenated Alkaloids	591

19.2.1	Indoles	591
19.2.2	Carbazoles	596
19.2.3	β -Carbolines	596
19.2.4	Tyrosines	598
19.2.5	Miscellaneous Halogenated Alkaloids	603
19.3	Biosynthesis of Halogenated Alkaloids	605
19.3.1	Halogenation Enzymes	605
19.3.2	Indoles	606
19.3.3	Biosynthesis of Halogenated Tyrosines	609
19.3.4	Biosynthesis of Miscellaneous Alkaloids	612

20 **Engineering Biosynthetic Pathways to Generate Indolocarbazole Alkaloids in Microorganisms** 619

César Sánchez, Carmen Méndez, José A. Salas

20.1	Introduction	619
20.2	Studies Made Before the Identification of Biosynthetic Genes	620
20.3	Identification of Genes Involved in Indolocarbazole Biosynthesis	621
20.3.1	Genes Involved in Rebeccamycin Biosynthesis	621
20.3.2	Genes Involved in Staurosporine Biosynthesis	625
20.3.3	Genes Involved in Biosynthesis of Other Indolocarbazoles	625
20.4	Indolocarbazole Biosynthetic Pathways and Their Engineering	626
20.4.1	Tryptophan Modification	626
20.4.2	Formation of Bisindole Pyrrole	627
20.4.3	Formation of Carbazole	630
20.4.4	Formation of the Sugar Moiety	632
20.4.4.1	Sugar Moieties in Rebeccamycin and AT2433	632
20.4.4.2	The Staurosporine Sugar Moiety	634
20.4.5	Regulation and Self-resistance	636
20.5	Perspectives and Concluding Remarks	637
20.6	Acknowledgments	638

Index 641