Rubrolides A–H, Metabolites of the Colonial Tunicate *Ritterella rubra*

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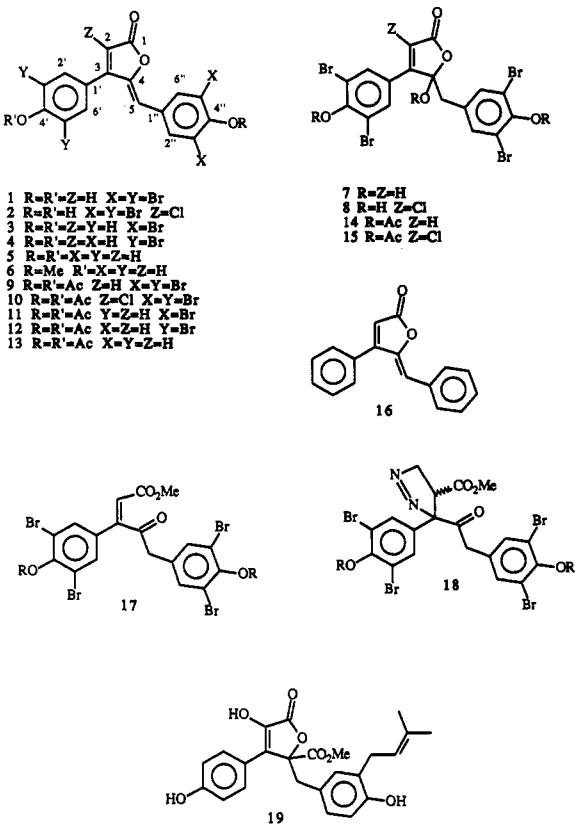
Rubrolides A (1) to H (8), a new family of biologically active tunicate metabolites, have been isolated from *Ritterella rubra*. The structures of the rubrolides were solved by a combination of spectroscopic analysis and chemical interconversions. Rubrolides B (2) and H (8) represent some of the first chlorinated metabolites known from tunicates. The rubrolides are potent in vitro antibiotics and they show moderate but selective inhibition of protein phosphatases 1 and 2A.

Marine tunicates (phylum Chordata) have proven to be a rich source of biologically active nitrogenous secondary metabolites.[1] One major group of tunicate metabolites, which includes the didemnins,[2] the tunichromes,[3] the patellamides,[4] and the diazonamides,[5] are peptides that frequently contain phenylalanine- or tyrosine-derived residues. A second major group of tunicate metabolites, which includes the eudistomins,[6] shermilamine A,[7] 2-bromoleptoclinidinone,[8] the lamellarins,[9] and the ecteinascidins,[10] can be classified as alkaloids. Several of the tunicate alkaloids such as shermilamine A,[7] 2-bromoleptoclinidinone,[8] and eudistomin K sulfoxide[11] are brominated and there has been one report of an iodinated metabolite from a *Didemnum* sp.[12] Diazonamides A and B from *Diazonia chinensis* represent the first examples of chlorinated tunicate metabolites.[5] A screening program designed to detect compounds with potential pharmaceutical activity in Northeastern Pacific marine invertebrates[13] showed that extracts of the colonial tunicate *Ritterella rubra* (Abbott and Trason 1968) had potent antibacterial activity and that they were capable of moderate but differential inhibition of protein phosphatases 1 and 2A.[14] Bioassay-guided fractionation demonstrated that the compounds responsible for the antibacterial[15] and phosphatase inhibition activities of *R. rubra* extracts were the rubrolides A (1) to H (8). The rubrolides represent a new family of biologically active tunicate metabolites that do not contain nitrogen and two members of the family, rubrolides B (2) and H (8), join the diazonamides[5] as examples of chlorinated metabolites from the phylum Chordata (Chart I).

Chart I



R. rubra is a scarlet to crimson colored colonial tunicate that is only rarely encountered in Northeastern Pacific subtidal habitats. Specimens of *R. rubra* (400 g) were collected by hand using SCUBA (−3 m) near Anthony Island in the Queen Charlotte Island chain, British Columbia, and kept frozen (−5 °C) until workup. Thawed samples of *R. rubra* were homogenized and extracted with methanol to give a crude extract. The dichloromethane-soluble material in the crude methanol extract was repeatedly fractionated by means of Sephadex LH20 chromatography to give pure samples of rubrolides A (1) (132 mg), B (2) (68 mg), and C (3) (48 mg). The remaining rubrolides could be obtained only as mixtures from Sephadex LH20 chromatography. Rubrolide F (6) (3 mg) was taken to final purity by silica gel preparative TLC.

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 551; **1986**, *3*, 1; **1987**, *4*, 539; **1988**, *5*, 513.
(2) Rinehart, K. L.; Gloer, J.; Cook, J. C.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
(3) Bruening, R. C.; Olitz, E. M.; Furukawa, K.; Nakanishi, K.; Kustin, K. *J. Am. Chem. Soc.* **1985**, *107*, 5298.
(4) Ireland, C. M.; Durso, A. R.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.
(5) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303.
(6) Lake, R. J.; Blunt, J. W.; Munro, H. G. *Aust. J. Chem.* **1989**, *42*, 1201.
(7) Cooray, N. M.; Scheuer, P. J.; Parkanyi, L.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4619.
(8) de Guzman, F. S.; Schmitz, F. J. *Tetrahedron Lett.* **1989**, *30*, 1069.
(9) Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492.
(10) (a) Rinehart, K. L.; Holt, T. G.; Fregzau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512. (b) Wright, A. E.; Foreleo, D. A.; Gunawardana, G. P.; Koehn, F. E.; McConnell, O. L. *J. Org. Chem.* **1990**, *55*, 4508.
(11) Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, H. G.; Panell, L. K. *Tetrahedron Lett.* **1988**, *29*, 2255.
(12) Sesin, D. F.; Ireland, C. M. *Tetrahedron Lett.* **1984**, *25*, 403.
(13) See, for example: Burgoyne, D. L.; Miao, S.; Pathirana, C.; Andersen, R. J.; Ayer, W. A.; Singer, P. P.; Kokke, W. C. M. C.; Ross, D. M. *Can. J. Chem.* **1991**, *69*, 20.
(14) Enzyme inhibition assays were conducted by Dr. Charles Holmes, Department of Biochemistry, University of Alberta. The protein phosphatase inhibition activity of the rubrolides will be described elsewhere.
(15) The major rubrolides show the following antibacterial activities in a standard disc assay (MICs in μg/disc): *Staphylococcus aureus*, rubrolide A (9 μg), rubrolide B (2 μg), rubrolide C (11 μg); *Bacillus subtilis*, rubrolide A (9 μg), rubrolide B (2 μg), rubrolide C (11 μg).

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The crude mixtures of rubrolides D (4), E (5), G (7), and H (8) were acetylated and the resulting acetates (12 (4 mg), 13 (3 mg), 14 (8 mg), and 15 (11 mg)) were purified on silica gel preparative TLC and normal-phase HPLC.

Rubrolide A (1) was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at m/z 595.7118, appropriate for a molecular formula of $C_{17}H_8O_4{}^{79}Br_2{}^{81}Br_2$ (ΔM –0.1 mmu). The ^{13}C NMR spectrum of 1 (Table III) contained only 13 resonances, indicating that there was some element(s) of symmetry in the molecule. Only five resonances, all singlets with chemical shifts greater than 6 ppm, were observed in the ^1H NMR spectrum of 1 (Table I) and integration showed that three of these singlets accounted for two protons apiece (δ 7.78, 8.05, and 10.52) and that the remaining two (δ 6.35 and 6.63) accounted for one proton apiece.

The broad two-proton resonance at δ 10.52 in the ^1H NMR spectrum was assigned to a pair of exchangable phenolic protons. Acetylation of rubrolide A (1) gave the diacetate 9 in agreement with this assignment. A HETCOR experiment showed that the protons resonating at δ 7.78 and 8.05 in the ^1H NMR spectrum of 1 were attached to carbons resonating at δ 132.5 and 134.2, respectively, and a gated decoupled ^{13}C NMR experiment showed that the carbons were both methines. FLOCK[16] correlations were observed between the proton resonance at δ 7.78 and carbon resonances at δ 152.8, 112.2, and 132.5 (Table I). The observation of both one-bond (HETCOR) and long-range correlations (FLOCK) between the proton resonance at δ 7.78 and the carbon resonance at δ 132.5 suggested the presence of two equivalent methine carbons situated meta to each other (i.e. 2′/6′) on a symmetrically substituted phenyl ring. The other ^{13}C NMR resonances that showed FLOCK correlations to the ^1H NMR resonance at δ 7.78 were assigned to a single phenolic carbon (δ 152.8: C4′) and to a pair of brominated carbons ortho to the phenol (δ 112.2: C 3′/5′) on the basis of a comparison of their chemical shifts to calculated values and the shifts of model compounds. A similar set of arguments led to the assignment of the proton resonance at δ 8.05 (H2″/H6″) and the carbon resonances at δ 134.2 (C 2″/6″), 111.9 (C 3″/5″), and 151.4 (C 4″) to a second 3,5-dibromo-4-hydroxyphenyl residue in 1. Two of the resonances in the ^{13}C NMR spectrum of 1 (δ 123.6, C1′, and 127.6, C1″) had chemical shifts appropriate for the C1 carbons of 3,5-dibromo-4-hydroxyphenyl groups attached to carbon atoms.

The remaining fragment of rubrolide A (1) had to account for an elemental composition of $C_5H_2O_2$ and four sites of unsaturation. A carbonyl stretching band at 1734 cm^{-1} in the IR spectrum and a resonance at δ 168.5 in the ^{13}C NMR spectrum indicated that the two oxygen atoms were present as an ester, and four olefinic carbon resonances (δ 110.2 (CH), 114.6 (CH), 146.6 (C), 155.0 (C)) and two deshielded proton resonances (δ 6.35 s and 6.63 s) could be assigned to two trisubstituted olefin functionalities. All of the the fragments of rubiolide A that could be identified from the spectral data were assembled in accordance with the FLOCK, HETCOR, and NOE data to give the final structure 1.

Important FLOCK data included the correlations between H2′/6′ (δ 7.78) and C3 (δ 155.0) that fixed the at-

(16) Reynolds, W. F.; McLean, S.; Perpick-Dumont, M.; Enriquez, R. *Magn. Reson. Chem.* **1989**, *27*, 162. The FLOCK sequence is an *n*-bond (n = 2 or 3) ^{13}C–^1H correlation experiment that totally suppresses correlations from one-bond connectivities. For a recent demonstration of the advantages of the experiment, see: Chan, W. R.; Tinto, W. F.; Laydoo, R. S.; Manchand, P. S.; Reynolds, W. F.; McLean, S. *J. Org. Chem.* **1991**, *56*, 1773.

Table I. ^1H NMR Data (400 MHz)

C	rubrolide A (1), DMSO-d_6			rubrolide B (2), DMSO-d_6			rubrolide C (3), DMSO-d_6			rubrolide F (6) MeOH-d_4
	^1H δ	COSY	NOE[a]	^1H δ	COSY	NOE[a]	^1H δ	COSY	NOE[a]	^1H δ
2	6.63, s	H5[b]	H2′/H6′				6.42, s	H5[b]	H2′/H6′	6.04, s
5	6.35, s	H2,[b] H2″/H6″[b]	H2′/H6′, H2″/H6″	6.32, s	H2″/H6″[b]	H2′/H6′, H2″/H6″	6.37, s	H2,[b] H2″/H6″[b]	H2′/H6′, H2″/H6″	6.37, s
2′/6′	7.78, s		H2, H5	7.73, s		H5	7.50, d, J = 8.5 Hz	H3′/H5′	H2,H5, H3′/H5′	7.36, d, J = 8.6 Hz
3′/5′							6.93, d, J = 8.5 Hz	H2′/H6′	H2′/H6′	6.77, d, J = 8.6 Hz
2″/6″	8.05, s	H5[b]	H5	8.05, s	H5[b]	H5	8.05, s	H5[b]	H5	7.78, d, J = 8.8 Hz
3″/5″										6.97, d, J = 8.8 Hz
ArOH 4′,4″	10.52, bs			10.6, bs						
OMe										3.83, s, 3 H

[a] Resonance in C column irradiated. [b] Correlations observed in long-range COSY only.

tachment of one 3,5-dibromo-4-hydroxyphenyl residue to C3 and between H2″/6″ (δ 8.05) and C5 (δ 110.2) and between H5 (δ 6.35) and C2″/6″ (δ 134.2) that fixed the attachment of the second 3,5-dibromo-4-hydroxyphenyl residue to C5. Additional FLOCK correlations between each of the H2 (δ 6.63) and H5 (δ 6.35) resonances and both of the carbon resonances at δ 155.0 and 146.6, in conjunction with the lack of appreciable scalar coupling between the two protons, supported the placement of H2 and H5 on the terminal carbons of a conjugated diene, and a FLOCK correlation between H2 (δ 6.63) and C1′ (δ 123.6) located a 3,5-dibromo-4-hydroxyphenyl residue vicinal to H2 (i.e. at C3). A FLOCK correlation between H2 (δ 6.63) and the ester carbonyl carbon (δ 167.8) established the connection between C1 and C2 and the ring formed by attachment of the ester alkoxy oxygen to C4 supplied the final site of unsaturation required in this fragment. Irradiation of H5 induced NOEs in the resonances assigned to H2′/H6′ (δ 7.78) and to H2″/H6″ (δ 8.05), demonstrating that the $\Delta^{4,5}$ olefin had the *Z* configuration, while irradiation of H2 induced a NOE only in the resonance assigned to H2′/H6′ (δ 7.78). A correlation between H2 and H5 was observed in a long-range COSY experiment even though the H2 and H5 resonances appeared as sharp singlets in the ^{1}H NMR spectrum. Further support for the structure of rubrolide A (1) came from the similarity of its ^{13}C NMR chemical shifts (Table III) to those published for the model compound **16** (δ 168.5 (C1), 114.3 (C2 or C5), 158.6 (C3), 147.6 (C4), 113.6 (C2 or C5)).[17]

Rubrolide B (2) was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at *m/z* 629.6726, corresponding to a molecular formula of $C_{17}H_7O_4{}^{79}Br_2{}^{81}Br_2{}^{35}Cl$ (Δ*M* −0.3 mmu). The elemental composition of 2 differed from that of 1 simply by the replacement of one hydrogen atom by a chlorine atom, suggesting that rubrolide B was a monochloro derivative of rubrolide A. The ^{1}H NMR spectrum of 2 (Table I) was nearly identical with the ^{1}H NMR spectrum of 1 except for the absence of any resonance that could be assigned to H2. Therefore, it was concluded that rubrolide B (2) was the 2-chloro derivative of rubrolide A (1). The FLOCK, NOE, and ^{13}C NMR data (Tables I and III) collected on 2 were in complete agreement with this assignment.

Rubrolide C (3) was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at *m/z* 437.8928, corresponding to a molecular formula of $C_{17}H_{10}O_4{}^{79}Br{}^{81}Br$ (Δ*M* 0.1 mmu). Examination of the ^{1}H NMR spectrum of 3 showed a close correspondence to the ^{1}H NMR spectrum of rubrolide A (1), except that the two-proton singlet resonance assigned to H2′/H6′ in 1 was replaced by a pair of mutually coupled two-proton doublets (δ 6.93, d, *J* = 8.5 Hz and 7.50, d, *J* = 8.5 Hz). This ^{1}H NMR evidence indicated that the 3,5-dibromo-4-hydroxyphenyl residue attached to C3 in 1 was replaced by a 4-hydroxyphenyl residue at C3 in rubrolide C (3) (Table I). The MS, COSY, FLOCK, NOE, and ^{13}C NMR data (Tables I and III) collected on 3 were in complete agreement with this assignment.

Rubrolide D (4) was isolated as its diacetate 12. A parent ion was observed at *m/z* 521.9132 ($C_{21}H_{14}O_6{}^{79}Br{}^{81}Br$: Δ*M* 0.6 mmu) in the EIHRMS of 12. The ^{1}H NMR data (Table II) for 12 revealed that rubrolide D (4) differed from rubrolide A (1) only in the replacement of the 3,5-dibromo-4-hydroxyphenyl residue attached to C5 in 1 with a 4-hydroxyphenyl residue in 4. COSY, HMBC,[18] NOE, and ^{13}C NMR experiments (Tables II and

(17) Saalfrank, R. W.; Hafner, W.; Markmann, J.; Bestmann, H.-J. *Tetrahedron* **1988**, *44*, 5095.

Table II. ^{1}H NMR Data (400 MHz, $CDCl_3$)

	rubrolide D diacetate (12)			rubrolide E diacetate (13)			rubrolide G triacetate (14)			rubrolide H triacetate (15)
C	^{1}H δ	COSY	NOE[a]	^{1}H δ	COSY	NOE[a]	^{1}H δ	COSY	NOE[a]	^{1}H δ
2	6.25, s	H5[b]	H2′/H6′	6.21, s	H5[b]	H2′/H6′	6.33, s	H5[b]	H2′/H6′	
5	6.11, s	H2,[b] H2″[b]/H6″[b]	H2′/H6′, H2″/H6″	6.15, s	H2,[b] H2″/H6″[b]	H2′/H6′, H2″/H6″	3.55, d, *J* = 14.2 Hz	H2,[b] H2″/H6″[b]	H2′/H6′, H2″/H6″	3.48, d, *J* = 14.3 Hz
							3.08, d, *J* = 14.2 Hz			3.05, d, *J* = 14.3 Hz
2′/6′	7.69, s	H3′/H5′	H2, H5 H3′/H5′	7.52, d, *J* = 8.6 Hz	H3′/H5′	H2, H5, H3′/H5′	7.71, s		H2, H5	7.87, s
3′/5′		H2′/H6′	H2′/H6′	7.27, d, *J* = 8.6 Hz	H2′/H6′	H2′/H6′				
2″/6″	7.85, d, *J* = 8.7 Hz	H5,[b] H3″/H5″	H5, H3″/H5″	7.83, d, *J* = 8.7 Hz	H5,[b] H3″/H5″	H5, H3″/H5″	7.22, bs	H5[b]	H5	7.22, bs
3″/5″	7.16, d, *J* = 8.7 Hz	H2″/H6″	H2″/H6″	7.14, d, *J* = 8.7 Hz	H2″/H6″	H2″/H6″				
4′-OAc	2.45, s, 3 H			2.36, s, 3 H			2.43, s, 3 H			2.45, s, 3 H
4″-OAc	2.32, s, 3 H			2.31, s, 3 H			2.38, s, 3 H			2.40, s, 3 H
4-OAc							2.18, s, 3 H			2.20, s, 3 H

[a] Resonance in C column irradiated. [b] Correlations observed in long-range COSY only.

III) carried out on the diacetate **12** confirmed the assignment of structures **12** to the diacetate and **4** to rubrolide D.

Rubrolide E (**5**) was isolated as its diacetate **13**, a colorless solid that gave a parent ion at m/z 364.0945 ($C_{21}H_{16}O_6$: ΔM −0.2 mmu) in the EIHRMS. COSY, NOE, HMBC, ^{1}H NMR, and ^{13}C NMR experiments (Tables II and III) carried out on rubrolide E diacetate (**13**) showed that rubrolide E (**5**) was simply the tetradebromo derivative of rubrolide A (**1**).

Rubrolide F (**6**) was obtained as a colorless solid that gave a parent ion in the EIHRMS at m/z 294.0885, appropriate for a molecular formula of $C_{18}H_{14}O_4$ (ΔM −0.7 mmu). The ^{1}H NMR data (Table I) for rubrolide F showed that it was a monomethyl ether of rubrolide E (**5**). NOE difference experiments revealed that the methoxy residue in **6** was located at C4″. Thus, irradiation of the doublet at δ 6.97 (H3″/H5″) that was scalar coupled to a doublet at δ 7.78 (H2″/H6″) induced NOEs in the methoxy resonance at δ 3.83 (OMe 4″) and in the H2″/H6″ resonance at δ 7.78, while irradiation of the H2″/H6″ (δ 7.78) resonance induced NOEs in an olefinic resonance at δ 6.37 (H5) and in the H3″/H5″ resonance at δ 6.97. Irradiation of the H2′/H6′ resonance at δ 7.36 induced NOEs in the H3′/H5′ resonance at δ 6.77, in the H2 resonance at δ 6.04, and in the H5 resonance at δ 6.37.

Rubrolides G (**7**) and H (**8**) proved to be inseparable in their natural forms. Therefore, a crude mixture of the two compounds was acetylated and the pure triacetates **14** and **15** were isolated from the reaction mixture. Rubrolide G triacetate (**14**) was obtained as an optically inactive colorless solid that gave a parent ion in the EIHRMS at m/z 739.7532, corresponding to a molecular formula of $C_{23}H_{16}{}^{79}Br_2{}^{81}Br_2O_8$ (ΔM −0.9 mmu). Analysis of the ^{1}H NMR, ^{13}C NMR, NOE, and HMBC data (Tables II and III) collected on **14** revealed that the molecule was closely related to rubrolide A diacetate (**9**). In particular, ^{1}H and ^{13}C NMR resonances could be routinely assigned to two 3,5-dibromo-4-acetoxyphenyl residues (C1′ to C6′ and C1″ to C6″, Tables II and III) and to an α,β unsaturated ester (C1 to C3: Tables II and III). HMBC correlations (H2 to C1, C3 and C1′, H2′/H6′ to C3: Table III) confirmed that one of the 3,5-dibromo-4-acetoxyphenyl residues (C1′ to C6′) was attached to the β carbon (C3) of the unsaturated ester. The remaining resonances in the ^{1}H NMR spectrum of **14** could be assigned to a pair of geminal methylene protons (δ 3.08, d, J = 14.2 Hz and 3.55, d, J = 14.2 Hz) and to a set of acetate methyl protons (δ 2.18, s). The remaining carbon resonances were assigned to an aliphatic methylene carbon (δ 42.0, t), to an acetate residue (δ 21.6, q and 166.6, s), and to an acetal carbon (δ 105.5). HMBC ($H5_a$, $H5_b$ to C1″ and C2″/C6″), LRCOSY ($H5_a$, $H5_b$ to H2″/H6″), and NOE (H2″/H6″ to $H5_a$, $H5_b$) data showed that the aliphatic methylene carbon was attached to the second 3,5-dibromo-4-acetoxyphenyl residue and the HMBC data also showed that the acetal carbon (C4) was situated between the β carbon of the α,β unsaturated ester (H2 correlated to C4: Table II) and the methylene carbon ($H5_a$, $H5_b$ correlated to C4: Table II). Attachment of the alkoxy oxygen of the unsaturated ester and the alkoxy oxygen of the acetate residue to the acetal carbon completed the structure of rubrolide G triacetate (**14**).

Support for the proposed structure of **14** came from the reaction of the inseparable mixture of rubrolides G and H with diazomethane to give as one of the major products

Table III. ^{13}C NMR Data

C	rubrolide A (1)[a]		rubrolide B (2)[a]		rubrolide C (3)[a]		rubrolide D diacetate (12)[b]		rubrolide E diacetate (13)[b]		rubrolide G triacetate (14)[b]		rubrolide H triacetate (15)[b]
	^{13}C δ	FLOCK	^{13}C δ	FLOCK	^{13}C δ	FLOCK	^{13}C δ	HMBC	^{13}C δ	HMBC	^{13}C δ	HMBC	^{13}C δ
1	167.76	H2	163.40		168.27	H2	167.74	H2	168.51	H2	167.60	H2	163.33
2	114.57		117.96		111.30		116.00		114.72		119.81		124.66
3	154.96	H2, H5, H2′/H6′	147.25	H5, H2′/H6′	157.71	H5, H2′/H6′	154.91	H5, H2′/H6′	157.75	H2, H5, H2′/H6′	158.55	H2, H5, H2′/H6′	147.52
4	146.58	H2, H5	145.19	H5	146.31	H2	147.12	H2, H5	147.78	H2, H5	105.46	H2, H5	104.86
5	110.17	H2″/H6″	111.41	H2″/H6″	110.56	H2″/H6″	113.18		112.85	H2″/H6″	41.97		41.82
1′	123.59	H2	121.05		120.28	H3′/H5′	130.61		127.90	H3′/H5′	129.33	H2	128.18
2′/6′	132.45	H6′/H2′	132.86	H6′/H2′	130.48	H6′/H2′	132.18[c]	H6′/H2′	129.73	H6′/H2′	131.06	H6′/H2′	131.72
3′/5′	112.16	H2′/H6′	112.08	H2′/H6′	115.89	H5′/H3′	118.72	H2′/H6′	122.48		119.30	H2′/H6′	119.14
4′	152.77	H2′/H6′	152.78	H2′/H6′	159.90	H2′/H6′	147.98	H2′/H6′	152.34	H2′/H6′	148.85	H2′/H6′	148.62
1″	127.57		126.98		126.13		130.23	H3″/H5″	130.60	H3″/H5″	132.26	H5	131.68
2″/6″	134.22	H5, H6″/H2″	134.33	H5, H6″/H2″	134.16	H5, H6″/H2″	132.11[c]	H5, H6″/H2″	132.00	H5, H6″/H2″	134.18	H5, H6″/H2″	134.19
3″/5″	111.87	H2″/H6″	111.97	H2″/H6″	112.38	H2″/H6″	122.17		122.05		117.58		117.67
4″	151.44	H2″/H6″	151.88	H2″/H6″	152.82	H2″/H6″	151.56	H2″/H6″	51.27	H2″/H6″	146.02		146.15
4-OAc											166.64[c]		166.65[c]
											21.63[d]		21.69[d]
4′-OAc							167.00[d]				167.00[c]		166.99[c]
							20.51[e]		21.12		20.47[d]		20.54[d]
4″-OAc							169.05[d]		169.10[c]		166.87[c]		167.66[c]
							21.15[e]		21.12		20.47[d]		20.46[d]

[a] ^{13}C NMR recorded in DMSO-d_6 at 50 MHz, FLOCK in DMSO-d_6 at 75 MHz. [b] Recorded in $CDCl_3$ at 125 MHz. [c-e] Assignments may be interchanged within the same column.

(18) (a) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285. (b) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.

the ring-opened methyl ester 17. A second product obtained in this reaction was compound 18, a diazomethane addition product. The spectral data obtained for compounds 17 and 18 were in complete agreement with the structures shown. Formation of the methyl ester 17 provided evidence that rubrolide G (7) must exist as an equilibrium mixture of cyclic and open-chain forms, in agreement with the lack of optical activity observed for the triacetate 14. It is interesting to note that the proton resonance assigned to H2″/H6″ in the room-temperature ^{1}H NMR spectrum of 14 was very broad and that it sharpened up at 50 °C. The broadening was attributed to hindered rotation about the C5/C1″ bond.

Rubrolide H triacetate (15) was isolated as an optically inactive colorless solid that gave a parent ion in the EIHRMS at m/z 773.7158, appropriate for a molecular formula of $C_{23}H_{15}O_8{}^{79}Br_2{}^{81}Br_2{}^{35}Cl$ (ΔM 0.6 mmu). The elemental composition of 15 differed from that of 14 simply by the replacement of one hydrogen atom by a chlorine atom, suggesting that rubrolide H triacetate was a monochloro derivative of rubrolide G triacetate. Examination of the ^{1}H NMR spectrum (Table II) of 15 showed that it was nearly identical with the ^{1}H NMR spectrum of 14 except for the absence of any resonance that could be assigned to H2, indicating that rubrolide H triacetate (15) was the 2-chloro derivative of rubrolide G triacetate (14). The ^{13}C NMR data (Table III) collected on 15 was in complete agreement with this assignment.

The rubrolides 1 to 8 are a structurally novel family of biologically active tunicate metabolites. They are related to a series of butenolides (i.e. 19) that have been isolated from cultures of the fungus *Aspergillus terreus*.[19] Isotope incorporation studies have demonstrated that the *A. terreus* metabolite 19 is biosynthesized from two molecules of phenylalanine.[20] A related biogenesis from either phenylalanine or tyrosine appears most likely for the rubrolides. Therefore, even though the rubrolides are part of the very small minority of tunicate metabolites that do not contain nitrogen,[21] they probably share an amino acid biogenetic origin with the majority of other tunicate metabolites. A series of brominated β-carbolines (i.e. the eudistomins) have been reported from *Ritterella sigillinoides*,[11] the only other tunicate in the genus *Ritterella* that has been subjected to chemical investigation. Even though brominated and iodinated[12] metabolites have been known from tunicates for some time, chlorinated metabolites have only been encountered very recently. Rubrolides B (2) and H (8), along with the diazonamides,[5] represent some of the first examples of chlorinated tunicate metabolites.

Experimental Section

Isolation and Purification. Frozen specimens of *R. rubra* (400 g) were immersed in methanol (1 L), allowed to thaw, homogenized in a Warring-brand blender, and then extracted at rt for 2 days. The methanol extract was filtered and concentrated in vacuo to give a red aqueous suspension, which was diluted with water to 300 mL and then extracted sequentially with hexanes (400 mL × 3), dichloromethane (400 mL × 3), and ethyl acetate (400 mL × 3). The organic layers were dried over anhydrous sodium sulfate and evaporated in vacuo. ^{1}H NMR experiments indicated that the dichloromethane and ethyl acetate soluble fractions contained similar aromatic compounds. These fractions were combined and chromatographed on Sephadex LH20 (eluent MeOH) to give seven major fractions containing rubrolides G/H, F, E, D, B, C, and A in sequence. Rubrolides A, B, and C were independently rechromatographed on Sephadex LH20 (eluent 40:10:4 EtOAc/MeOH/H_2O) to afford pure rubrolides A (1) (132 mg), B (2) (68 mg), and C (3) (48 mg). Fractionation on silica gel TLC (1:1 EtOAc/hexane) yielded 3 mg of pure rubrolide F (6). Samples of the impure rubrolides D, E, and G/H were acetylated with Ac_2O/pyridine (5 h at rt), and the resulting acetates were purified on silica gel TLC (MeOH/CH_2Cl_2 1:99) followed by HPLC (EtOAc/hexane 3:1) to give the pure acetates of rubrolides D (12, 4 mg), E (13, 3 mg), G (14, 8 mg), and H (15, 11 mg).

Rubrolide A (1): obtained as a red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; UV (MeOH) 439 (ε 14 000), 355 (19 000), 324 (17 000, sh), 257 (23 000) nm; IR (film) ν_{max} 1734, 1717, 1654, 1559, 1508, 1474, 1458, 1219, 732 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz), see Table I; ^{13}C NMR (DMSO-d_6, 50 MHz), see Table III; EIMS, m/z (rel intensity) 600 (16.9), 598 (65.2), 596 (100.0), 594 (67.1), 592 (17.4), 540 (2.3), 538 (1.6), 520 (6.1), 518 (19.1), 516 (19.5), 514 (6.8), 463 (7.0), 461 (19.8), 459 (19.5), 457 (6.5), 440 (2.0), 438 (5.0), 436 (4.7), 434 (1.5), 410 (6.4), 408 (13.6), 406 (6.5), 381 (6.9), 294 (16.8), 292 (28.6), 290 (14.5), 279 (14.5), 204 (16.2), 185 (27.9); EIHRMS 597.7104 (M$^+$, $C_{17}H_8O_4{}^{79}Br^{81}Br_3$, ΔM 0.4 mmu).

Rubrolide B (2): obtained as a red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; ^{1}H NMR (DMSO-d_6, 400 MHz), see Table I; ^{13}C NMR (DMSO-d_6, 50 MHz), see Table III; EIMS, m/z (rel intensity) 634 (30.0), 632 (81.5), 630 (100.0), 628 (60.7), 626 (14.8), 600 (0.3), 598 (1.8), 596 (3.0), 594 (2.0), 592 (0.3), 552 (8.5), 550 (6.3), 541 (12.6), 539 (18.6), 537 (12.6), 535 (3.7), 518 (3.2), 516 (4.7), 497 (3.9), 495 (7.7), 493 (6.0), 444 (6.1), 442 (7.9), 440 (10.8), 438 (15.4), 436 (8.6), 310 (7.3), 294 (19.4), 292 (31.1), 279 (21.5), 262 (6.1), 185 (38.1), 183 (38.5), 134 (18.0), 121 (26.0), 82 (71.0), 80 (70.3), 43 (32.9), 32 (56.7); EIHRMS 629.6726 (M$^+$, $C_{17}H_7O_4{}^{79}Br_2{}^{81}Br_2{}^{35}Cl$, ΔM –0.3 mmu).

Rubrolide C (3): obtained as a red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; ^{1}H NMR (DMSO-d_6, 400 MHz), see Table I; ^{13}C NMR (DMSO-d_6, 50 MHz), see Table III; EIMS, m/z (rel intensity) 440 (21.8), 438 (46.0), 436 (22.1), 384 (2.8), 382 (5.7), 380 (2.8), 303 (23.9), 301 (24.6), 294 (12.6), 292 (25.2), 290 (13.1), 278 (5.8), 264 (6.4), 262 (5.6), 250 (10.3), 222 (7.1), 185 (23.2), 183 (22.6), 165 (16.1), 118 (35.0), 32 (100); EIHRMS 437.8928 (M$^+$, $C_{17}H_{10}O_4{}^{79}Br^{81}Br$, ΔM 0.1 mmu);

Rubrolide F (6): obtained as a yellow solid; IR (CH_2Cl_2) ν_{max} 2927, 2856, 1750, 1603, 1510, 1420, 1172, 1086, 1031, 896, 832 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table I; EIMS, m/z (rel intensity) 295 (25.4), 294 (100.0), 279 (5.1), 266 (4.4), 223 (13.2), 165 (4.9), 148 (42.2), 133 (21.8), 120 (23.9), 77 (19.6); EIHRMS 294.0885 (M$^+$, $C_{18}H_{14}O_4$, ΔM –0.7 mmu), 279.0667 (M$^+$ – CH_3, $C_{17}H_{11}O_4$, ΔM 0.9 mmu).

Acetylation of Rubrolides. Individual rubrolides or mixtures of rubrolides were dissolved in 1 mL of dry pyridine. To this red solution was added 1 mL of dry acetic anhydride (The color of the reaction mixture immediately changed to a greenish hue.). The reaction mixture was stirred at rt for 5 h and then evaporated to dryness in vacuo. Chromatography on TLC (silica gel, MeOH/CH_2Cl_2 1:99) followed by HPLC (silica gel, EtOAc/hexane 1:3) afforded pure rubrolide acetates.

Rubrolide A diacetate (9): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 1784, 1772, 1544, 1455, 1371, 1181, 1010, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (s, H2″/H6″), 7.68 (s, H2′/H6′), 6.30 (s, H2), 5.96 (s, H5), 2.45 (s, 4′-OAc), 2.40 (s, 4″-OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.0 (2 × C), 166.9 (C), 154.7 (C), 148.5 (C), 148.2 (C), 146.7 (C), 134.0 (2 × CH), 133.0 (C), 132.0 (2 × CH), 130.1 (C), 118.9 (2 × C), 118.2 (2 × C), 117.1 (C), 110.0 (C), 20.5 (2 × CH_3); EIHRMS 637.7226 (M$^+$ – C_2H_2O, $C_{19}H_{10}O_5{}^{79}Br_2{}^{81}Br_2$, ΔM 0.2 mmu), 595.7125 (M$^+$ – $2C_2H_2O$, $C_{17}H_8O_4{}^{79}Br_2{}^{81}Br_2$, ΔM 0.6 mmu).

Rubrolide B diacetate (10): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 1790, 1544, 1455, 1371, 1182, 1008, 898 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (s, H2″/H6″), 7.68 (s, H2′/H6′), 5.98 (s, H5), 2.47 (s, 4′-OAc), 2.40 (s, 4″-OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.9 (C), 166.8 (C), 162.8 (C), 148.3 (C), 147.0 (C), 146.8 (C), 146.0 (C), 134.1 (2 × CH), 132.6 (2 × CH), 132.5 (C), 127.7 (C), 122.0 (C), 118.9 (2 × C), 118.3 (2 × C), 110.7 (C), 20.5

(19) Golding, B. T.; Rickards, R. W.; Vanek, Z. *J. Chem. Soc., Perkin Trans. I* **1975**, 1961.
(20) Kiriyama, N.; Nitta, K.; Sakaguchi, Y.; Taguchi, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1977**, *25*, 2593.
(21) For other examples of non-nitrogenous tunicate metabolites, see: Lindquist, N.; Fenical, W.; Sesin, D. F.; Ireland, C. M.; van Duyne, G. D.; Forsyth, C. J.; Clardy, J. *J. Am. Chem. Soc.* **1988**, *110*, 1308.

(2 × CH_3); EIHRMS 713.6925 (M^+, $C_{21}H_{11}O_6{}^{79}Br_3{}^{81}Br^{37}Cl$, ΔM −0.6 mmu).

Rubrolide C diacetate (11): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 2925, 2854, 1775, 1503, 1458, 1371, 1213, 1200, 1180, 1168, 1065, 912 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.00 (s, H2″/H6″), 7.50 (d, J = 8.5 Hz, H2′/H6′), 7.28 (d, J = 8.5 Hz, H3′/H5′), 6.26 (s, H2), 6.01 (s, H5), 2.36 (s, 4′-OAc), 2.40 (s, 4″-OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1 (C), 167.8 (C), 167.1 (C), 157.5 (C), 152.5 (C), 149.2 (C), 146.4 (C), 133.9 (2 × CH), 133.5 (C), 129.7 (2 × CH), 127.4 (C), 122.6 (2 × CH), 118.1 (2 × C), 115.7 (C), 109.7 (C), 21.1 (CH_3), 20.5 (CH_3); EIHRMS 479.9024 (M^+ − C_2H_2O, $C_{19}H_{12}O_5{}^{79}Br^{81}Br$, ΔM −0.9 mmu).

Rubrolide D diacetate (12): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 1766, 1506, 1455, 1370, 1217, 1198, 1183, 1169, 946 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EIMS, m/z (rel intensity) 524 (3.6), 522 (7.1), 520 (3.7), 482 (37.6), 480 (74.6), 478 (37.2), 440 (51.0), 438 (100.0), 436 (51.7), 382 (2.4), 358 (3.1), 330 (2.2), 301 (5.3), 274 (5.0), 250 (17.6), 221 (7.7), 134 (36.7), 43 (91.0), 32 (81.4); EIHRMS 521.9132 (M^+, $C_{21}H_{14}O_6{}^{79}Br^{81}Br$, ΔM −0.7 mmu).

Rubrolide E diacetate (13): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 2927, 2854, 1764, 1609, 1501, 1370, 1201, 1166, 1085, 1016, 960, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 364 (15.0), 322 (72.3), 280 (100.0), 252 (5.2), 224 (7.4), 134 (38.1), 133 (35.2), 77 (18.3), 43 (87.8); EIHRMS 364.0945 (M^+, $C_{21}H_{16}O_6$, ΔM −0.2 mmu).

Rubrolide G triacetate (14): obtained as a colorless solid; $[\alpha]_D$ 0° ($CHCl_3$, c 0.3); IR (film) ν_{max} 1778, 1459, 1370, 1183, 1034, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 702 (3.8), 700 (13.4), 698 (19.7), 696 (13.2), 694 (3.3), 660 (1.2), 658 (4.1), 656 (6.2), 654 (4.3), 652 (1.3), 642 (19.5), 640 (58.7), 638 (78.9), 636 (55.2), 634 (13.0), 600 (21.1), 598 (58.0), 596 (75.4), 594 (47.7), 592 (11.6), 482 (16.9), 480 (34.7), 478 (17.5), 440 (20.2), 438 (40.3), 436 (21.2), 349 (11.4), 334 (10.0), 332 (15.6), 330 (7.9), 310 938.7), 308 (76.9), 306 (39.8), 278 (12.4), 276 (24.2), 274 (13.0), 267 (53.3), 265 (100.0), 263 (53.0), 185 (12.4), 43 (29.9); EIHRMS 739.7532 (M^+, $C_{23}H_{16}O_8{}^{79}Br_2{}^{81}Br_2$, ΔM −0.9 mmu).

Rubrolide H triacetate (15): obtained as a colorless solid; $[\alpha]_D$ 0° ($CHCl_3$, c 0.4); IR (film) ν_{max} 1798, 1776, 1543, 1459, 1371, 1182, 1066, 992, 908, cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 776 (0.4), 774 (0.4), 740 (0.6), 738 (6.4), 736 (25.2), 734 (52.2), 732 (100.0), 730 (37.6), 692 (14.4), 690 (15.5), 688 (8.2), 686 (1.9), 634 (27.3), 632 (61.6), 630 (72.1), 628 943.0), 626 (10.6), 384 (6.5), 382 (8.1), 312 (18.1), 310 (72.5), 308 (100.0), 306 (45.0), 267 (56.4), 265 (76.0), 263 (35.0), 185 (11.1), 80 (20.4), 43 (37.6), 32 (87.9); EIHRMS 773.7158 (M^+, $C_{23}H_{15}O_8{}^{79}Br_2{}^{81}Br_2{}^{35}Cl$, ΔM 0.6 mmu).

Methylation of Rubrolides G and H. An acetone solution containing a mixture of rubrolides G and H (≈1 mg in 0.3 mL) was mixed with an ethereal solution of CH_2N_2 (0.2 mmol in 5 mL) and stirred at rt for 15 h. After evaporation of the solvent, the mixture was chromatographed on TLC (silica gel, MeOH/CH_2Cl_2 1:99) followed by HPLC (silica gel, EtOAc/hexane 1:3) to give two pure compounds, trimethylrubrolide G 17 (≈0.5 mg) and 18 (≈0.5 mg). **Trimethylrubrolide G (17)**: colorless solid; IR (CH_2Cl_2) ν_{max} 2926, 2854, 1719, 1712, 1655, 1560, 993 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.44 (s, 2 H), 7.34 (s, 2 H), 6.13 (s, 1 H), 3.91 (s, 3 H), 3.88 (s, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H); EILRMS, m/z (rel intensity) 660 (1.4), 658 (4.0), 656 (5.8), 654 (3.8), 652 (1.5), 628 (5.6), 626 (16.3), 624 (22.1), 622 (14.1), 620 (3.6), 379 (43.4), 377 (86.0), 375 (43.0), 351 (21.1), 349 (34.0), 291 (19.3), 281 (15.2), 279 (30.0), 277 (18.8), 270 (11.8), 268 (12.3), 183 (5.3), 154 (8.2). 18: colorless solid; IR ($CHCl_2$) ν_{max} 2949, 2923, 1736, 1729, 1605, 1472, 1423, 1211, 1177, 991, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.60 (s, 2 H), 7.22 (s, 2 H), 5.05 (dd, J = 18.3, 4.2 Hz), 4.61 (s, J = 18.2, 8.6 Hz), 4.15 (d, J = 17.1 Hz), 3.97 (d, J = 17.1 Hz), 3.90 (s, 3 H), 3.85 (s, 3 H), 3.71 (s, 3 H), 3.14 (dd, J = 8.6, 4.2 Hz); EILRMS, m/z (rel intensity) 700 (0.3), 698 (0.5), 696 (0.4), 694 (0.1), 674 (4.3), 672 (16.3), 670 (24.9), 668 (17.3), 666 (5.0), 615 (2.2), 613 (8.1), 611 (12.6), 609 (8.6), 607 (2.6), 393 (25.7), 391 (51.1), 389 (25.5), 365 (16.6), 363 (33.0), 361 (19.4), 284 (30.1), 282 (32.7), 281 (19.9), 279 (40.2), 277 (21.3), 225 (9.5), 223 (8.2), 203 (15.1).

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Supplementary Material Available: NMR spectra for compounds 1, 2, 3, 6, 12, 13, 14, and 15 and HETCOR and FLOCK spectra for 1 (17 pages). Ordering information is given on any current masthead page.

Use of Dihydroxyacetone Phosphate Dependent Aldolases in the Synthesis of Deoxyazasugars[1]

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The use of fructose-1,6-diphosphate (FDP), fuculose-1-phosphate (Fuc-1-P) and rhamnulose-1-phosphate (Rham-1-P) aldolases in organic synthesis is described. Fuc-1-P, Rham-1-P, and their phosphate-free species have been prepared and characterized. Both Fuc-1-P and Rham-1-P aldolases accept 3-azido-2-hydroxypropanal as a substrate to form L-ω-azidoketose phosphates, which upon dephosphorylation and hydrogenolysis on Pd/C, gave 1-deoxyazasugars structurally related to D-galactose and L-mannose. Hydrogenolysis of the enzyme products azidoketose 1-phosphates, however, gave 1,6-dideoxyazasugars structurally related to 6-deoxygalactose and L-rhamnose. Explanations for the stereoselectivity in the hydrogenolysis reactions were provided. Similarly, FDP aldolase catalyzed the aldol condensation reaction with 2-azido-3-hydroxypropanal to afford a new synthesis of 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine, a potent inhibitor of a number of glycosidases. A new empirical formula is developed to relate the inhibition constants and inhibitor binding for α- and β-glucosidases.

Naturally occurring and synthetic azasugars[2,3] and their derivatives are useful inhibitors of enzymes associated with carbohydrate processing. Synthesis of azasugars based on fructose-1,6-diphosphate (FDP) aldolase (EC 4.1.2.3) has

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