Secondary Mould Metabolites. Part 15.[1] Structure Elucidation of Rubellins A and B, two Novel Anthraquinone Metabolites from *Mycosphaerella rubella*

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The structure of rubellins A (**1**) and B (**2**), two novel anthraquinone metabolites isolated from *Mycosphaerella rubella*, have been assigned by detailed analysis of their ^1H and ^{13}C n.m.r. spectra and chemical evidence. Their relative configuration and conformation were deduced from the observed $^1H-\{^1H\}$ nuclear Overhauser effects (n.O.e.s) and the value of $^1H-^1H$ coupling constants.

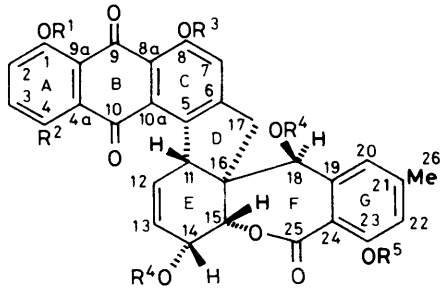
During the last few years we have screened, for secondary metabolites, a number of strains belonging to the genus *Mycosphaerella*[2-5] as it includes many phytopathogenic species and it is a source of interesting metabolites (*viz.*, epoxydon, rosigenin, mycochromone, mycoxanthone, dothistromin, *etc.*), some of them presenting phytotoxic and pharmacological activity.[6-8]

The present paper concerns the isolation and the structural elucidation of rubellins A and B, two novel anthraquinonoid metabolites produced by *M. rubella* which is the causal agent of a necrotic spot disease in *Angelica silvestris*, a medicinal plant of the Umbelliferae family. The fungus was grown on MPG-agar medium and the metabolites were extracted with ethyl acetate. Purification of the crude extract gave an orange mixture of the metabolites (**1**) and (**2**), which were separated by reverse-phase chromatography.

The extract of the fungus was tested on living plants of *Nicotiana tabacum*, *Beta vulgaris* v. *esculenta*, *Solanum lycopersicum* cv. *S. Marzano*, *Petroselinum hortense*, and *Daucus carota* cultivated in our greenhouse. After 24 h, leaves showed necrotic areas enlarging proportionally with the toxin concentration. Rubellins A (**1**) and B (**2**) showed photodynamic activity, inducing a lipoperoxidative degradation of pea stem and rat liver mitochondria and microsomes.[9]

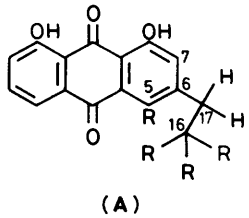
Rubellin A (**1**) is a yellow solid, m.p. 208—210 °C, $[\alpha]_D^{20}$ +285°. Elemental analysis and high-resolution mass spectrometry (M^+, 526.1257) showed that it had a molecular formula $C_{30}H_{22}O_9$. U.v. and i.r. spectra indicated the presence of an anthraquinone system in the molecule, which was also confirmed by decolorization of a methanolic solution of rubellin A by reductive treatment with aqueous sodium hydrosolphite (disodium dithionite) and successive oxidation in air. The structure (**1**) was assigned on the basis of the following data. The 300.13 MHz ^1H n.m.r. spectrum of rubellin A (**1**) possessed the following features: an ABX spin system due to the three vicinal aromatic protons of ring A, an aromatic (7-H), and two *meta*-coupled aromatic protons of ring G both benzylically coupled to the C-26 methyl group. Moreover it showed a sequence such as C(11)H–C(12)H=C(13)H–C(14)H–C(15)H–O, two geminally coupled protons (17-H_2), a methine proton (18-H) coupled to 18-OH (^3J 5.1 Hz), an aliphatic 14-OH coupled to 14-H (^3J 4.9 Hz), and three chelated phenolic OH signals at δ_H 12.53, 12.02, and 9.83 respectively (see Table 1). The presence of five OH groups in rubellin A (**1**) was confirmed by the formation of the penta-acetate (**3**) upon acetylation.

Methylation of rubellin A (**1**) with Ag_2O–MeI gave the partial methylated derivatives (**5**)—(**7**), while methylation of the mixture of the two metabolites (**1**) and (**2**) with K_2CO_3–MeI yielded the trimethyl ether (**8**) and the tetramethyl ether (**9**). ^1H N.m.r. chemical shifts and coupling constants of the aforementioned products are summarized in Tables 1 and 2.

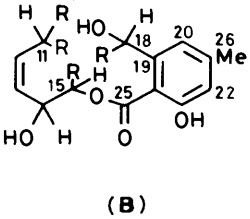


(**1**) $R^1 = R^2 = R^3 = R^4 = R^5 = H$
(**2**) $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = OH$
(**3**) $R^1 = R^3 = R^4 = R^5 = COMe$, $R^2 = H$
(**4**) $R^1 = R^3 = R^4 = R^5 = COMe$, $R^2 = OCOMe$
(**5**) $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Me$
(**6**) $R^1 = R^5 = Me$, $R^2 = R^3 = R^4 = H$
(**7**) $R^1 = R^2 = R^4 = H$, $R^3 = R^5 = Me$
(**8**) $R^1 = R^3 = R^5 = Me$, $R^2 = R^4 = H$
(**9**) $R^1 = R^3 = R^5 = Me$, $R^2 = OMe$, $R^4 = H$

The 75.47 MHz ^{13}C n.m.r. spectrum of compound (**1**) in $CDCl_3$–[$^2H_6$]DMSO exhibited signals due to 23 sp^2 and 7 sp^3-hybridized carbon atoms. The sp^2 resonances indicated the presence of 8 methine and 15 quaternary carbon atoms while the sp^3 resonances were assigned to one methyl, one methylene, four methine (three of them oxygen-bearing), and one quaternary carbon atom. Chemical-shift criteria and the analysis of $^1H-^{13}C$ coupling constants in the fully ^1H-coupled ^{13}C n.m.r. spectrum of compounds (**1**) and (**3**), in conjunction with low-power specific ^1H decouplings which enabled us to correlate ^1H and ^{13}C resonances, permitted their rigorous assignments (see Table 3). From the above spectral data, fragments (**A**) and (**B**) could be constituted.



(**A**)



(**B**)

Table 1. ^{1}H N.m.r. chemical shifts (δ) of rubellin A (**1**), rubellin B (**2**), and some derivatives

Proton	(1)[a]	(2)[a]	(3)[b]	(4)[b]	(5)[c]	(6)[c]	(7)[c]	(8)[c]	(9)[c]	(13)[c]	(16)[c]
2	7.36 (7.27)[d]	7.40[f]	7.58 (7.42)[d]	7.58	7.25	7.34	7.29	7.29	7.27	7.30	7.30
3	7.79 (7.66)	7.41[f]	7.93 (7.78)	7.58	7.62	7.70	7.63	7.63	7.27	7.65	7.62
4	7.71 (7.77)		8.18 (8.18)		7.76	7.92	7.75	7.80		7.75	7.66
7	7.26 (7.12)	7.29	7.45 (7.27)	7.39	7.06	7.04	7.27	7.19	7.11	7.25	7.32
11	4.37 (4.69)	4.45	4.84 (4.75)	4.49	4.55	4.53	4.62	4.57	4.60	4.83	5.01
12	5.54 (5.80)	5.54	5.98 (5.86)	5.97	5.78	5.81	5.83	5.82	5.83	5.98	6.98
13	5.62 (5.73)	5.59	5.79 (5.80)	5.77	5.72	5.69	5.74	5.69	5.65	5.86	6.31
14	4.31 (4.50)	4.31	5.63 (5.67)	5.59	4.48	4.49	4.50	4.51	4.49	4.67	
15	4.19 (4.46)	4.19	4.82 (4.75)	4.75	4.29	4.30	4.29	4.35	4.31	5.24	4.85[h]
17α	4.73 (4.78)	4.73	4.50 (4.40)	4.49	4.74	4.71	4.81	4.81	4.80	4.46	3.84
17β	2.95 (3.03)	2.95	3.18 (3.18)	3.15	2.97	2.95	3.02	3.02	3.01	3.27	2.75
18	4.69 (5.03)	4.70	6.18 (6.30)	6.10	4.88	4.92	4.91	5.02	5.01		
20	6.75 (6.92)	6.78[g]	7.18 (6.99)	7.18	6.97	7.02	6.97	6.98	7.09	7.07[f]	7.36
22	6.75 (6.79)	6.75[g]	7.12 (6.99)	7.10	6.73	6.76	6.76	6.80	6.79	6.87[f]	7.14
26	2.29 (2.34)	2.29	2.41 (2.40)	2.43	2.35	2.36	2.39	2.43	2.42	2.36	2.39
1-OR	12.02 (12.13)	12.17	2.41 (2.40)	2.37[f]	12.14	4.02	12.08	3.98[f]	3.95[f]	12.13	12.01
4-OR		12.97		2.36[f]					3.95[f]		
8-OR	12.53 (12.58)	12.66	2.41 (2.40)	2.35[f]	12.58	12.58	4.03	4.01[f]	3.96[f]	12.63	12.54
14-OR	5.66 *e*	5.64	2.14 (2.14)	2.12	*e*	*e*	*e*	*e*	*e*	*e*	
18-OR	5.38 *e*	5.41	1.49 (1.46)	1.43	*e*	*e*	*e*	*e*	*e*		
23-OR	9.83 (9.35)	9.83	2.25 (2.29)	2.23	3.84	3.84	3.86	3.91	3.91	10.05	11.40

[a] In [$^2H_6$]DMSO. [b] In [$^2H_6$]acetone. [c] In $CDCl_3$. [d] Values in parentheses are chemical shifts in $CDCl_3$. [e] Not assigned. [f,g] Assignments within each column may be interchanged. [h] OH resonance.

Table 2. ^{1}H–^{1}H Coupling constants (*J*/Hz) for compounds (**1**), (**3**), (**4**), (**8**), and (**9**)

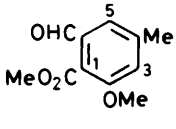
J	(1)	(3)	(4)	(8)	(9)
2,3	8.1	8.0	*a*	8.4	*a*
2,4	1.3	1.3		1.1	
3,4	7.5	7.9		7.7	
$7,17_\alpha$	1.5	1.4	1.4	1.4	1.4
$7,17_\beta$	0.6	0.6	0.6	0.6	0.6
11,12	2.5	2.6	2.6	2.7	2.7
11,13	2.0	2.1	2.1	1.9	1.9
11,14	1.5	1.6	1.6	1.6	1.6
12,13	10.0	10.1	10.1	10.2	10.2
12,14	*ca.* 0.5	0.8	0.8	0.8	0.8
12,18	*ca.* 0.5	0.9	0.9	0.9	0.9
13,14	5.2	5.4	5.4	5.5	5.5
14,15	4.3	4.8	4.8	4.3	4.3
$17_\alpha,17_\beta$	18.4	18.2	18.2	17.8	17.8
18,20	0.9	0.8	0.8	0.8	0.8
18,22	0.4	0.4	0.4	0.4	0.4
20,22	1.6	1.7	1.7	1.7	1.7
20.26	0.7	0.8	0.8	0.8	0.8
22.26	0.9	0.9	0.9	0.9	0.9

[a] A value of 9.3 Hz was obtained from the spectrum of compound (**2**).

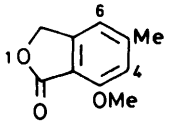
Fragment A.—The presence of a substituted 1,8-dihydroxyanthraquinone system was indicated by the ^{1}H and ^{13}C spectra which were interpreted both by inspection and by reference to published data for related anthraquinone compounds.[10] Moreover the coupling constant between C-7 and the chelated 8-OH group [^3J(CH) 7.0 Hz] and the lack of ^{1}H–^{1}H *ortho* and *meta* coupling constants of 7-H revealed that the anthraquinone ring is disubstituted at C-5 and C-6. The 17-H_2 group was placed at C-6 because the 17-methylene protons were found to be benzylically coupled to 7-H as well as to all carbon atoms of ring C with coupling constants ranging between 1.5—7.0 Hz in the ^{13}C spectrum of compound (**3**). In addition, irradiation of the 17-H_2 protons in compound (**3**) resulted in Overhauser (n.O.e.) enhancement of 7-H, thus confirming their spatial proximity, while the lack of vicinal ^{1}H–^{1}H and other ^{1}H–^{13}C sp^2 coupling constants indicated that 17-H_2 had to be connected to the quaternary sp^3-hybridized C-16 carbon atom (δ_C 53.89 p.p.m.).

Fragment B.—The sequence C(11)—C(15) in compounds (**1**) and (**3**) was firmly established by extensive ^{1}H–{^{1}H} and ^{13}C–{^{1}H} decoupling experiments (see later). The lactone carbonyl atom C-25, which showed a three-bond coupling constant to 15-H [^3J(CH) 5.2 Hz] and a sharpening by irradiation of C-20 and C-22 aromatic protons, was assigned as bonded to C-24 of ring G and must be responsible for the hydrogen bond with 23-OH. Irradiation of the C-26 methyl protons caused enhancement of both *meta*-coupled aromatic protons which must therefore be *ortho* to the methyl group. Their location at C-20 and C-22 respectively is also confirmed because they both exhibited long-range coupling constants through three bonds to C-24; moreover 22-H showed a two-bond coupling to C-23 [^2J(CH) 3.5 Hz]. Finally the C(18)HOH group must be bonded to C-19 because 18-H presented *ortho* and *para* benzylic coupling constants to 20- and 22-H [^4J(HH) 0.8 and ^6J(HH) 0.4 Hz] and to C-19, C-20, and C-24 carbon atoms [^2J(CH) 4.5, ^3J(CH) 3.0, and ^3J(CH) 3.0 Hz].

The substitution pattern of ring G was also supported by the reaction of the mixture of the aromatic methyl ethers (**8**) and (**9**) with methanolic KOH which yielded the compounds (**10**)



(**10**)



(**11**)

and (**11**), whose structure was elucidated by ^{1}H–{^{1}H} decoupling and n.O.e. experiments (see Experimental section). The formation of the aldehyde (**10**) may proceed from hydrolysis of the lactone ring F and a retro-aldol mechanism on C-18, while the formation of lactone (**11**) could be explained by a Cannizzaro reaction which reduced the aldehyde group of

Table 3. ^{13}C N.m.r. data for compounds (**1**), (**2**), and (**3**)

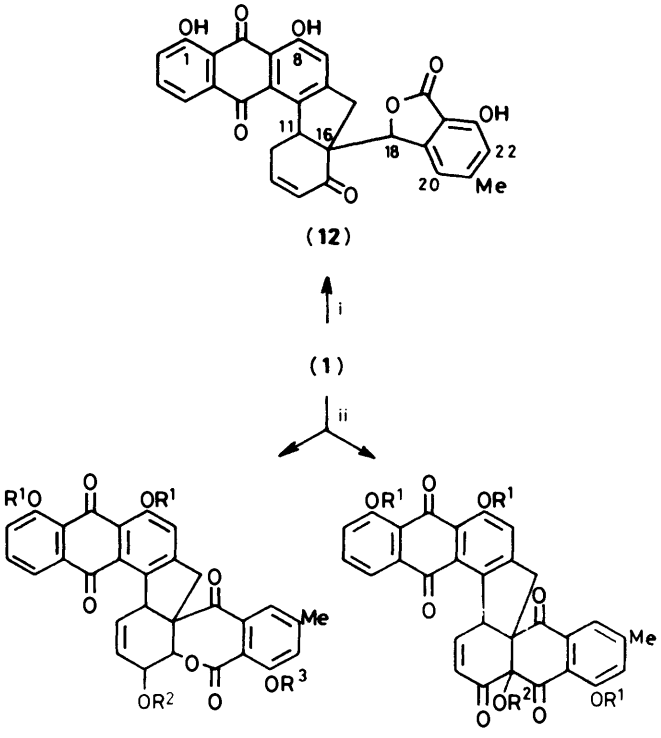
	(1)[a]	(2)[a]	(3)[b,e,f]				
Carbon	δ_C/p.p.m.	δ_C/p.p.m.	δ_C/p.p.m.	J(CH)/Hz[g]			
1	161.78 S	156.71 S	149.98 S	C-1,2-H	3.5	C-14,14-H	154.0
2	123.76 D	128.77 D	130.28 D	C-1,3-H	10.0	C-15,15-H	140.0
3	136.60 D	129.10 D	134.54 D	C-1,4-H	1.3	C-17,17-H	134.5
4	119.44 D	157.49 S	125.35 D	C-2,2-H	164.0	C-18,18-H	148.0
4a	133.87 S	113.20 S	134.75 S	C-2,3-H	1.5	C-19,18-H	4.5
5	141.65 S	141.68 S	143.62 S	C-2,4-H	8.0	C-19,20-H	≤1
6	157.69 S	157.65 S	151.44 S	C-3,3-H	163.5	C-20,18-H	3.0
7	119.37 D	119.68 D	126.18 D	C-4,2-H	7.5	C-20,20-H	161.5
8	163.18 S	163.27 S	150.33 S	C-4,3-H	1.5	C-20,22-H	6.5
8a	113.48 S	113.58 S	124.74 S	C-4,4-H	166.0	C-20,26-H	5.0
9	191.94 S	190.08 S	180.66 S	C-4a,3-H	8.0	C-21,26-H	6.0
9a	115.71 S	112.51 S	125.70 S	C-5,7-H	7.5	C-22,18-H	1.5
10	182.84 S	187.99 S	183.44 S	C-5,11-H	6.0	C-22,20-H	6.7
10a	125.38 S	125.48 S	127.71 S	C-5,12-H	2.0	C-22,22-H	163.0
11	48.05 D	48.27 D	48.29 D	C-6,17-H_α	2.0	C-22,26-H	6.0
12	128.96[c] D	128.87[c] D	130.54 D	C-5,17-H_β	5.0	C-23,20-H	≤1
13	125.48[c] D	125.62[c] D	122.29 D	C-6,11-H	5.5	C-23,22-H	3.5
14	64.94 D	64.94 D	66.17 D	C-6,17-H_α	7.0	C-24,18-H	3.0
15	81.36 D	81.31 D	76.68 D	C-6,17-H_β	4.5	C-24,20-H	8.0
16	53.41 S	53.45 S	53.89 S	C-7,7-H	164.5	C-24,22-H	5.5
17	39.59 T	39.59 T	38.04 T	C-7,17-H_α	2.0	C-24,26-H	≤1
18	78.26 D	78.26 D	77.40 D	C-7,17-H_β	2.0	C-25,15-H	5.2
19	142.70 S	142.68 S	135.57 S	C-8,7-H	3.5	C-25,20-H	1.5
20	118.03 D	118.03 D	121.88 D	C-8,17-H_α	1.5	C-25,22-H	1.5
21	145.62 S	145.66 S	144.62 S	C-8a,7-H	5.0	C-26,20-H	4.5
22	116.74 D	116.74 D	123.70 D	C-8a,17-H_α	≤2	C-26,22-H	4.5
23	159.37 S	159.37 S	149.50 S	C-9a,2-H	5.5	C-26,26-H	127.5
24	109.19 S	109.19 S	119.32 S	C-9a,3-H	1.2	C-27,28-H	7.0
25	172.28 S	172.26 S	165.64 S	C-9a,4-H	7.0	C-28,28-H	130.5
26	21.93 Q	21.93 Q	21.80 Q	C-10,4-H	4.0	C-29,30-H	7.0
27			169.60[c] S	C-10a,11-H	≤2	C-30,30-H	130.5
28			20.94[d] Q	C-10a,17-H_α	≤2	C-31,14-H	3.5
29			169.32[c] S	C-11,11-H	142.0	C-13,32-H	7.0
30			20.94[d] Q	C-12,11-H	8.0	C-32,32-H	130.5
31			169.72 S	C-12,12-H	165.0	C-33,18-H	4.2
32			21.10[d] Q	C-12,14-H	5.0	C-33,34-H	7.0
33			168.86 S	C-13,11-H	6.0	C-34,34-H	130.5
34			19.69 Q	C-13,13-H	168.0	C-35,36-H	7.0
35			169.12[c] S	C-13,14-H	6.0	C-36,36-H	130.5
36			20.94[d] Q				

[a] In $CDCl_3$–[$^2H_6$]DMSO. Capital letters refer to the pattern resulting from one-bond (C,H) coupling constants; S = singlet, D = doublet, T = triplet, and Q = quartet. [b] In $CDCl_3$. [c,d] Assignments within each column may be interchanged. [e] C(27), C(29), C(31), C(33), C(35) and C(28), C(30), C(32), C(34), C(36) are respectively carbonyl and methyl carbon atoms of the 1-, 8-, 14-, 18-, and 23-acetate groups. [f] C-11, C-14, C-15, C-16, and C-17 carbon atoms appeared as complex multiplets precluding long-range coupling assignments. [g] Values are not intended to correlate with the carbon number in column 1.

compound (**10**) to the benzylic alcohol, thus yielding the stable γ-lactone (**11**).

To confirm the complete structure of rubellin A (**1**) we have only to link the quaternary atom C-16 to the C-11, C-15, and C-18 carbon atoms, and C-11 to C-5. Further evidence for rubellin A having structure (**1**) came from the coupling constants found between 11-H and C-5, C-6, and C-10a [^2J(CH) 6.0, ^3J(CH) 5.5, and ^3J(CH) 2 Hz] and between 12-H and C-5 [^3J(CH) 2.0 Hz]. Owing to the proximity of the resonances due to 11- and 15-H in [$^2H_6$]acetone, the above experiments were performed in [$^2H_6$]benzene (11-H, δ_H 4.93; 15-H, δ_H 5.01), giving unambiguous results.

Some reactions were performed in order to obtain additional information on the reactivity of rubellin A. Treatment of compound (**1**) with trifluoroacetic acid (TFA) or dry HCl afforded the α,β-unsaturated ketone (**12**), which arose from transesterification of the ε-lactone ring F to the more stable γ-lactone and loss of water from the resulting diol followed by keto–enol rearrangement of the enolic intermediate. Oxidation of compound (**1**) with $K_2Cr_2O_7$ in conc. H_2SO_4 yielded compounds (**13**) and (**16**), as shown in the Scheme. The ^1H n.m.r. spectrum of product (**13**) revealed no 18-H resonance, indicating the oxidation of the secondary 18-OH to a carbonyl group, and its ^{13}C n.m.r. spectrum showed a signal at δ_C 206.49 p.p.m. assigned to the C-18 carbonyl carbon atom. The formation of compound (**16**) may be attributed to successive oxidation of the secondary 14-OH and enolization of the resulting α,β-carbonyl group followed by nucleophilic intramolecular rearrangement of C-15 onto the lactone carbonyl carbon atom. Its ^1H n.m.r. spectrum showed signals at δ_H 6.98 and 6.31 due to two vinylic protons which are part of an α,β-unsaturated ketone, and a tertiary OH group at δ_H 4.85, as well



(13) $R^1 = R^2 = R^3 = H$
(14) $R^1 = R^3 = Me$, $R^2 = H$
(15) $R^1 = R^2 = R^3 = COMe$
(16) $R^1 = R^2 = H$
(17) $R^1 = Me$, $R^2 = H$
(18) $R^1 = R^2 = Me$

Scheme. *Reagents*: i, dry HCl or TFA; ii, $K_2Cr_2O_7$–H^+ or PCC

as a lack of resonances due to 14-, 15-, and 18-H protons with respect to the spectrum of rubellin A (**1**). The ^{13}C n.m.r. spectrum of compound (**16**) revealed three new carbonyl carbon atoms at δ_C 196.57, 193.90, and 193.68 p.p.m. and a quaternary sp^3-hybridized oxygen-bearing carbon atom at δ_C 82.16 p.p.m. (C-15).

Methylation of compounds (**13**) and (**16**) gave the trimethyl ether (**14**) and the tri- and tetra-methyl ethers (**17**) and (**18**). Acetylation of compound (**13**) afforded the expected tetra-acetate (**15**).

Rubellin B (**2**), as orange needles, m.p. 214—215 °C, $[\alpha]_D^{20}$ +280°, was identified as 4-hydroxyrubellin A on the basis of spectral and chemical evidence. Its mass spectrum (M^+, 542) required one oxygen atom more than compound (**1**). The ^1H n.m.r. spectrum of rubellin B (**2**) contained an AB spin system due to two *ortho* aromatic protons of ring A instead of the ABX spin system observed in rubellin A (**1**), and a new chelated 4-OH group at δ_H 12.97, whose presence was confirmed by the formation of the hexa-acetate (**4**). Comparison of the ^{13}C n.m.r. spectra of rubellins (**1**) and (**2**) indicated a close similarity between the two compounds, the only difference being in the resonances of ring A and B which was accounted for by the introduction of a chelated OH group at C-4. Confirmation of the structure again came from the identical values of the ^1H–^1H coupling constants of the alicyclic rings in compounds (**3**),(**4**) and (**8**),(**9**) (see Table 2), thus requiring that rubellin A and B possess the same relative configuration and preferred conformation.

In order to ascertain the relative configuration and the preferred conformation of rubellins A and B, significant data were derived from ^1H–^1H coupling-constant values as well as from ^1H–{^1H} n.O.e. experiments on compound (**3**) (see Tables 2 and 4). For the basis of the following discussion, it is assumed that C-11 has the (*R*) configuration, *i.e.* 11-H occupies the β position in the cyclohexane ring E.

Table 4. ^1H–{^1H} N.O.e. enhancements (%) for compound (**3**) in [$^2H_6$]acetone[a]

Proton irradiated	Proton observed
7	17α (0.6), 17β (2.0)
11[b]	12 (5.5), 15 (4.5), 20 (5.7)
12	11 (6.2), 13 (7.8)
15[b]	11 (6.4), 14 (12.0), 18 (1.8)
17α	7 (0.7), 17β (14.2), 18 (−2.9)
17β	7 (3.7), 17α (17.0), 18 (12.1)
18	15 (1.6), 17α (−2.7), 17β (9.4), 20 (1.6)
20	11 (3.0), 18 (1.2), 26 (2.5)
22[b]	26 (2.8)
26	20 (11.2), 22 (13.7)

[a] N.O.e. values have only qualitative significance. [b] In [$^2H_6$]benzene.

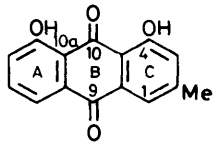
The magnitude of the vicinal and allylic coupling constants of 11- and 14-H with the vinylic protons 12- and 13-H [^3J(11,12) 2.6, ^4J(11,13) 2.1, ^3J(13,14) 5.4, and ^4J(12,14) 0.8 Hz] requires that 11- and 14-H occupy respectively pseudo-axial and pseudo-equatorial positions in ring E.[11] In addition the significant n.O.e. observed between 11- and 15-H (4.5%) establishes that 15-H is β. A wealth of stereochemical information was obtained by the n.O.e. observed between 11- and 20-H as follows. This result indicates that 20-H is spatially close to 11 β-H, thus requiring that the E,F junction be *trans*. As a consequence the junction between rings D and E must be *cis* and ring D must take up an envelope conformation with C-16 above the plane C(11)–C(5)–C(6)–C(17). Furthermore 15-H_β must be axially positioned, ring E assuming a half-chair conformation.

The (*S*) chirality to both C-15 and C-16 is also proved. Moreover the pseudo-equatorial 14-H must be β because of the coupling constant of 4.8 Hz arising through interaction with pseudo-axial 15-H_β which is indicative of a *gauche* arrangement of these two protons. As a consequence the 14-OCOMe group must be α-axially disposed, pointing toward 17-H_α, and is responsible for the large downfield shift exhibited by 17-H_α with respect to the geminal 17-H_β. Irradiation of 18-H resulted in sizeable n.O.e. (9.4%) of the signal for 17-H_β, this experiment suggesting that these two protons are in close spatial proximity, as expected if C-18 has (*S*) chirality. The high-field chemical shift (δ_H 1.49) exhibited by 18-OCOMe which may be shielded by ring C supported the C-18 chirality assignment.

Thus rubellins A and B possess the relative configuration 11*R*, 14*R*, 15*S*, 16*S*, 18*S*, the enantiomeric ring being equally probable if the 11*S* configuration is used in the discussion.

From a biogenetic point of view, it is interesting to observe that rubellin A may be considered as a dimer of two units of the anthraquinone chrysophanol (**19**); the lowest part being derived by oxidative cleavage of the C(10)–C(10a) bond.[12]

Work is in progress to obtain evidence for the biosynthesis of



(19)

rubellins either from chrysophanol or *via* a single polyketide chain.

The rubellins show no activity in antibacterial and antitumour tests.

Experimental

U.v. spectra were recorded for solutions in 95% EtOH ($\lambda_{max.}$ in nm) on a Beckmann DK-2 apparatus. I.r. spectra were measured on a Perkin-Elmer 177 spectrophotometer. Mass spectra were taken at 70 eV on a Hitachi RMU6D spectrometer. ^{1}H N.m.r. spectra were determined on Bruker CXP-300 (300.13 MHz) and Varian XL-100-15 (100 MHz) spectrometers with $SiMe_4$ as internal standard. ^{13}C N.m.r. spectra were obtained at 75.47 MHz (Bruker CXP-300) and at 25.2 MHz (Varian XL-100-15) with $SiMe_4$ as internal standard. N.O.e. difference spectra were obtained by subtracting alternatively right-off-resonance free-induction decays (FIDS) from right-on-resonance-induced FIDS.

Column chromatography was performed with Merck silica gel (0.04—0.063 mm) at medium pressure. Unless otherwise indicated, the purity of products was checked by t.l.c., n.m.r., and mass spectra, and was deemed sufficient for the purpose of structural elucidation. M.p.s were measured on a Kofler apparatus and are uncorrected.

Isolation and Purification of Rubellins A *and* B.—A strain of *Mycosphaerella rubella* obtained from Centraalbureau voor Schimmelcultures, Baarn, grown on malt–peptone–glucose–agar (20:2:20:15 g l^{-1}) in Roux flasks was extracted twice with EtOAc after three weeks growth at room temperature. The extract was dried over Na_2SO_4 and evaporated to give a red-brown mixture of crude pigments (*ca.* 250 mg from each 2-l flask). This mixture was adsorbed on the top of a chromatographic column and eluted with a mixture of hexane–EtOAc. By using a 1:1 eluant ratio two metabolites (**1**) and (**2**) were obtained as a mixture which was chromatographed [reverse-phase; silica gel RP-18 (Merck)] with a 5:1 mixture of MeOH–water as eluant, to give the pure metabolites (**1**) and (**2**).

Rubellin A (**1**) crystallized from benzene as yellow *needles*, m.p. 208—210 °C (decomp.); $[\alpha]_D^{20}$ +285° (*c* 0.16 in MeOH); $\lambda_{max.}$ 247, 288, 417 (sh), 440, and 466 (sh) nm (ε 25 000, 11 000, 8 200, 11 500, and 9 700); $\nu_{max.}$(KBr) 3 430 (OH), 1 695 (lactone CO), and 1 670 cm^{-1} (conjugated CO); *m/z* 526 (M^+), 508, 490, 361, 345, 328, 317, 205, 196, and 163 (Found: C, 68.4; H, 4.2%; M^+, 526.1257. $C_{30}H_{22}O_9$ requires C, 68.15; H, 4.40%; *M*, 526.1258 ± 0.002); ^{1}H and ^{13}C n.m.r. data are reported in Tables 1 and 3, respectively.

Rubellin B (**2**) crystallized from benzene as orange *needles*, m.p. 214—215 °C (decomp.); $[\alpha]_D^{20}$ +280° (*c* 0.1 in MeOH); $\lambda_{max.}$ 252, 288, 465 (sh), 483, 495, 517, and 530 nm (17 800, 8 800, 8 600, 9 800, 8 200, and 6 600); $\nu_{max.}$(KBr) 3 430 and 3 320 (OH), 1 725 (lactone CO), and 1 670 cm^{-1} (conjugated CO); *m/z* 542 (M^+), 430, 388, 361, and 345 (Found: C, 66.4; H, 3.9. $C_{30}H_{22}O_{10}$ requires C, 66.42; H, 4.09%); ^{1}H and ^{13}C n.m.r. data are reported in Tables 1 and 3, respectively.

Acetylation of the Mixture of the Metabolites (**1**) *and* (**2**).—The mixture (200 mg), dissolved in dry pyridine (1 ml)–Ac_2O (1 ml) was left for 12 h at 4 °C. The reaction mixture was dissolved in chloroform, and the solution was successively stirred with saturated aqueous $NaHCO_3$, water, saturated aqueous $KHSO_4$, and water, and was finally dried (Na_2SO_4). Preparative t.l.c. (p.l.c.) [silica; hexane–EtOAc (80:20)] yielded the two peracetates (**3**) and (**4**). Compound (**3**) was obtained as yellow *needles*, m.p. 204—205 °C, $[\alpha]_D^{20}$ +135.5° (*c* 0.16 in MeOH); $\lambda_{max.}$ 253, 274 (sh), and 352 nm (37 000, 17 500, and 7 000); $\nu_{max.}$($CHCl_3$) 1 780 (acetate CO), 1 752 (lactone CO), and 1 675 cm^{-1} (conjugated CO); *m/z* 736 (M^+), 676 (M^+ − 60), 634 (M^+ − 102), 592 (M^+ − 144), 574, 550, 532, 490, 472, 461, 429, 412, 387, 370, 345, 328, 316, 205, and 163 (Found: C, 64.9; H, 4.4. $C_{40}H_{32}O_{14}$ requires C, 64.77; H, 4.18%); ^{1}H and ^{13}C n.m.r. data are reported in Tables 1 and 3, respectively.

Compound (**4**) was obtained as yellow needles, m.p. 165—168 °C, $[\alpha]_D^{20}$ +129.4° (*c* 0.1 in $CHCl_3$); $\lambda_{max.}$ 250 and 350 nm (31 500 and 6 600); $\nu_{max.}$(KBr) 1 765 (acetate CO), 1 750 (lactone CO), and 1 675 cm^{-1} (conjugated CO); *m/z* 734 (M^+ − 60), 692 (M^+ − 102), 650 (M^+ − 144), 608, 590, 566, 548, 506, 445, 403, 344, 328, 205, and 163 (Found: C, 63.7; H, 4.6. $C_{42}H_{34}O_{16}$ requires C, 63.47; H, 4.31%); ^{1}H N.m.r. data are reported in Table 1.

Methylation of Rubellin A (**1**) *with* Ag_2O–MeI.—Rubellin A (400 mg) was dissolved in dry acetone (30 ml) and the solution was heated with Ag_2O (300 mg) and MeI (1 ml). The mixture was stirred at room temperature for 2 days. Filtration, followed by evaporation of the solvent, gave a yellow-brown residue which was purified by chromatography with a 1:1 mixture of hexane–EtOAc to give the methyl ethers (**5**)—(**7**).

Compound (**5**) (130 mg) (from EtOAc–hexane) as a yellow solid, m.p. 279—280 °C; *m/z* 540 (M^+), 361, 345, 328, 300, 272, 178, and 151 (Found: C, 68.6; H, 4.7. $C_{31}H_{24}O_9$ requires C, 68.88; H, 4.48%); ^{1}H n.m.r. data are reported in Table 1.

Compound (**6**) (90 mg) (from EtOAc–hexane) was a yellow-red solid, m.p. 270—272 °C; *m/z* 554 (M^+), 393, 377, 342, 331, 177, and 149 (Found: C, 68.9; H, 4.65. $C_{32}H_{26}O_9$ requires C, 69.31; H, 4.73%); ^{1}H n.m.r. data are reported in Table 1.

Compound (**7**) (100 mg) (from EtOAc–hexane) was a yellow solid, m.p. 242—243 °C; *m/z* 554 (M^+), 377, 359, 342, and 177 (Found: C, 69.6; H, 4.85%); ^{1}H n.m.r. data are reported in Table 1.

Degradation of the Mixture of the Methyl Ethers (**8**) *and* (**9**) *with* KOH.—A mixture of the metabolites (**1**) and (**2**) (300 mg) was dissolved in dry acetone (25 ml) and the solution was refluxed with MeI (1 ml) and K_2CO_3 (400 mg) for 4 h. The residue obtained after filtration and evaporation of the solvent, consisting of a mixture of the methyl ethers (**8**) and (**9**), was refluxed for 1.5 h with 0.5M-KOH in MeOH (20 ml). Dilution, neutralization with dilute HCl, extraction with EtOAc, evaporation of the solvent, and p.l.c. [silica; benzene–diethyl ether–formic acid (50:50:1)] of the residue gave, in very poor yield, two compounds (**10**) and (**11**). *Compound* (**10**) (from diethyl ether) was a white solid, m.p. 92—95 °C; $\nu_{max.}$(neat) 1 740 (ester CO) and 1 700 cm^{-1} (CHO); *m/z* 208 (M^+), 194, 179, and 166 (Found: M^+, 208.077 ± 0.004. $C_{11}H_{12}O_4$ requires *M*, 208.073); δ_H (300 MHz; [$^2H_6$]acetone) 2.46 (3 H, br s, 4-Me), 3.85 (3 H, s, CO_2Me), 3.88 (3 H, br s, 2-OMe), 7.29 (1 H, ddd, $J_{3,5}$ 1.4, $J_{3,2\text{-OMe}}$ 0.2, and $J_{3,4\text{-Me}}$ 0.8 Hz, 3-H), 7.36 (1 H, dd, $J_{5,3}$ 1.4 and $J_{5,4\text{-Me}}$ 0.8 Hz, 5-H), and 9.94 (1 H, s, CHO); n.O.e.s {4-Me} 3-H (4%) and 5-H (4), {2-OMe} 3-H (9.8), {6-CHO} 5-H (4.9), {5-H} 6-CHO (5.7), and 4-Me (1).

Compound (**11**) (from hexane) was a white solid, m.p. 77—79 °C; $\nu_{max.}$(Nujol) 1 760 cm^{-1} (lactone CO); *m/z* 178 (M^+), 159, 148, 131, 118, and 105 (Found: M^+, 178.065 ± 0.003. $C_{10}H_{10}O_3$ requires *M*, 178.063); δ_H (100 MHz; $CDCl_3$) 2.46 (3 H, s, Me), 3.98 (3 H, s, OMe), 5.20 (2 H, s, CH_2), 6.70 (1 H, s, 3-H), and 6.80 (1 H, s, 5-H).

Reaction of Rubellin A (**1**) *with* HCl.—Rubellin A (100 mg) was dissolved in dry methanol (10 ml); the solution was saturated with HCl, refluxed for 10 min, and again saturated with the acid. Evaporation, dilution of the residue with water, extraction with EtOAc, and p.l.c. of the residue [silica; chloroform–methanol (30:1)] gave *compound* (**12**) (from benzene) was a red solid, m.p. 225—226 °C; $\lambda_{max.}$ 282 (sh), 291, 442, 457, 522, and 535 nm (11 800, 13 700, 11 000, 10 300, 2 100,

and 1 900); $\nu_{max.}$(KBr) 3 400 (OH), 1 760 (lactone CO), and 1 662 and 1 625 cm^{-1} (conjugated CO); *m/z* 508 (M^+), 492, 464, 360, 344, 326, 299, 287, 271, 202, 180, and 163 (Found: C, 70.5; H, 3.75. $C_{30}H_{20}O_8$ requires C, 70.86; H, 3.96%); δ_H (100 MHz; [$^2H_6$]acetone) 2.08 (1 H, dddd, *J* 19, 9.5, 2.5, and 2.5 Hz, 12-H_α), 2.38 (3 H, s, 26-H_3), 3.07 (1 H, dddd, *J* 19, 7.5, 5.5, and 0.5 Hz, 12-H_β), 3.59 (2 H, s, 17-H_2), 4.01 (1 H, dd, *J* 9.5 and 7.5 Hz, 11-H), 5.98 (1 H, br s, 18-H), 6.22 (1 H, ddd, *J* 10, 2.5, and 0.5 Hz, 14-H), 6.56 and 6.78 (2 × 1 H, br s, 20- and 22-H), 7.14 (1 H, ddd, *J* 10, 5.5, and 2.5 Hz, 13-H), 7.31 (1 H, dd, *J* 8 and 1.5 Hz, 2-H), 7.35 (1 H, br s, 7-H), 7.67 (1 H, dd, *J* 8 and 1.5 Hz, 4-H), 7.77 (1 H, dd, *J* 8 and 8 Hz, 3-H), 10.5 (1 H, br s, 23-OH), 12.10 (1 H, s, 1-OH), and 12.62 (1 H, s, 8-OH). The same compound was obtained by treatment of rubellin A (**1**) with TFA.

Oxidation of Rubellin A (**1**) *with* $K_2Cr_2O_7$–H_2SO_4.—To a stirred mixture of rubellin A (200 mg) in a 1:1 mixture of MeOH–EtOAc (20 ml) was added dropwise during 1.5 h a solution of $K_2Cr_2O_7$ (1.6 g) in water (10 ml) containing 95% H_2SO_4 (1.6 ml). The mixture was refluxed for 2 h. Extraction with EtOAc and evaporation gave a red-brown residue which was purified by p.l.c. [silica; chloroform–methanol (95:5)] to give the products (**13**) and (**16**) (40 mg and 10 mg, respectively).

Compound (**13**) was a red solid, m.p. 188—190 °C; $\lambda_{max.}$ 252, 291, 443, and 462 (sh) nm (36 000, 23 700, 23 500, and 22 700); $\nu_{max.}$(KBr) 3 430 (OH), 1 735 (lactone CO), and 1 672 and 1 620 cm^{-1} (conjugated CO); *m/z* 524 (M^+), 488, 477, 460, 438, 361, 344, 328, 193, and 163 (Found: C, 68.4; H, 3.9. $C_{30}H_{20}O_9$ requires C, 68.70; H, 3.84%); ^1H n.m.r. data are reported in Table 1.

Compound (**16**) (from benzene) was a red solid, m.p. 219—221 °C; $\lambda_{max.}$ 254, 283 (sh), 358, 438, and 460 (sh) nm (22 300, 11 000, 5 100, 8 300, and 7 300); $\nu_{max.}$(KBr) 3 420 (OH), 1 700, and 1 620 cm^{-1} (conjugated CO); *m/z* 522 (M^+), 504, 478, 462, 360, 344, 278, 189, and 163 (Found: C, 68.8; H, 3.4. $C_{30}H_{18}O_9$ requires C, 68.96; H, 3.47%); ^1H n.m.r. data are reported in Table 1.

The same products were obtained by treatment of rubellin A (**1**) with pyridinium dichromate (PDC) or pyridinium chlorochromate (PCC) in dry CH_2Cl_2 for 3 days at room temperature.

Acetylation of Compound (**13**).—Compound (**13**) was acetylated and worked up as described above for the acetylation of rubellin A (**1**). P.l.c. [silica; chloroform–methanol (99:1)] yielded the *tetra-acetate* (**15**) (from MeOH) as a yellow solid, m.p. 182—184 °C; $\lambda_{max.}$ 254, 320, and 445 nm (31 000, 6 700, and 3 000); $\nu_{max.}$(KBr) 1 770 (acetate CO), 1 740 (lactone CO) and 1 668 cm^{-1} (conjugated CO); *m/z* 632 (M^+ − 60), 589, 560, 546, 504, 488, 478, 460, 434, 386, 370, 344, 328, 235, 205, and 193 (Found: C, 66.2; H, 4.2. $C_{38}H_{28}O_{13}$ requires C, 65.89; H, 4.07%); δ_H (100 MHz; $CDCl_3$) 2.13, 2.32, 2.46, and 2.46 (each 3 H, s, OAc), 2.43 (3 H, s, 26-H_3), 3.40 (1 H, d, *J* 17.5 Hz, 17-H_β), 4.23 (1 H, d, *J* 17.5 Hz, 17-H_α), 4.81 (1 H, br s, 11-H), 5.42 (1 H, d, *J* 5 Hz, 15-H), 5.76 (1 H, dd, *J* 5 and 5 Hz, 14-H), 5.87 and 6.08 (2 × 1 H, m, 12- and 13-H), 7.22 and 7.22 (2 × 1 H, br s, 20- and 22-H), 7.40 (1 H, br s, 7-H), 7.40 (1 H, dd, *J* 8 and 1.5 Hz, 2-H), 7.76 (1 H, dd, *J* 8 and 8 Hz, 3-H), and 8.15 (1 H, dd, *J* 8 and 1.5 Hz, 4-H).

Methylation of Compound (**13**).—Compound (**13**) (20 mg) was dissolved in dry acetone (20 ml) and the solution was refluxed for 4 h with MeI over anhydrous potassium carbonate. Filtration, evaporation, and p.l.c. [silica; chloroform–methanol (95:5)] yielded the *trimethyl ether* (**14**) as a yellow solid, m.p. 278—280 °C; $\lambda_{max.}$ 280 (sh), 316, and 408 nm (9 800, 4 000, and 5 900); $\nu_{max.}$(KBr) 1 735 (lactone CO), 1 660, and 1 595 cm^{-1} (conjugated CO); *m/z* 566 (M^+), 372, 356, 341, 313, 192, and 177 (Found: C, 69.7; H, 4.5. $C_{33}H_{26}O_9$ requires C, 69.96; H, 4.63%).

Methylation of Compound (**16**).—Compound (**16**) (20 mg) was methylated as described above for the preparation of compound (**14**), and p.l.c. [silica; chloroform–methanol (98:2)] yielded the *trimethyl ether* (**17**) and the *tetramethyl ether* (**18**).

Trimethyl ether (**17**) was a yellow solid, m.p. 158—160 °C; *m/z* 564 (M^+), 509, 372, 207, 192, 177, and 148 (Found: C, 69.9; H, 4.5. $C_{33}H_{24}O_9$ requires C, 70.21; H, 4.29%); δ_H (100 MHz; $CDCl_3$) 2.44 (3 H, s, 26-H_3), 2.77 (1 H, d, *J* 17 Hz, 17-H_β), 3.80 (1 H, d, *J* 17 Hz, 17-H_α), 4.03, 4.00, and 4.00 (each 3 H, s, OMe), 4.67 (1 H, br s, 15-OH), 4.96 (1 H, dd, *J* 2.5 and 3 Hz, 11-H), 6.24 (1 H, dd, *J* 10.5 and 2.5 Hz, 13-H), 6.93 (1 H, dd, *J* 10.5 and 3 Hz, 12-H), 7.10 and 7.41 (2 × 1 H, br s, 22- and 20-H), 7.30 (1 H, br s, 7-H), 7.27 (1 H, dd, *J* 8.5 and 1 Hz, 2-H), 7.55 (1 H, dd, *J* 7.5 and 8.5 Hz, 3-H), and 7.65 (1 H, dd, *J* 7.5 and 1 Hz, 4-H).

Tetramethyl ether (**18**) was a yellow solid, m.p. 172—174 °C; *m/z* 578 (M^+), 564, 547, 504, 402, 387, 360, 289, 207, 177, and 148 (Found: C, 70.8; H, 4.45. $C_{34}H_{26}O_9$ requires C, 70.58; H, 4.53%); δ_H (100 MHz; $CDCl_3$) 2.45 (3 H, s, 26-H_3), 2.89 (1 H, d, *J* 17 Hz, 17-H_β), 3.77 (1 H, d, *J* 17 Hz, 17-H_α), 3.56 (3 H, s, 15-OMe), 3.98, 4.00, and 4.00 (each 3 H, s, OMe), 4.90 (1 H, dd, *J* 2.5 and 3 Hz, 11-H), 6.06 (1 H, dd, *J* 10.5 and 2.5 Hz, 13-H), 6.70 (1 H, dd, *J* 10.5 and 3 Hz, 12-H), 7.11 and 7.40 (2 × 1 H, br s, 22- and 20-H), 7.26 (1 H, br s, 7-H), 7.26 (1 H, dd, *J* 8.5 and 1 Hz, 2-H), 7.55 (1 H, dd, *J* 7.5 and 8.5 Hz, 3-H), and 7.61 (1 H, dd, *J* 7.5 and 1 Hz, 4-H).

Biological Tests.—Treatment of test plants was performed by application of 9.5×10^{-5}M and 1.9×10^{-4}M solutions of the crude extract in water–EtOH (95:5) at neutral pH on puncture wounds in the upper faces of the leaves.

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