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Antiprotozoal and Antimicrobial Compounds from the Plant Pathogen *Septoria pistaciarum*

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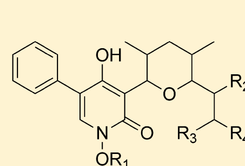
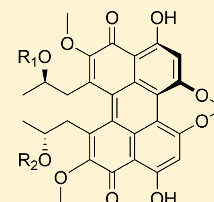
Supporting Information

ABSTRACT: Four new 1,4-dihydroxy-5-phenyl-2-pyridinone alkaloids, 17-hydroxy-*N*-(*O*-methyl)septoriamycin A (**1**), 17-acetoxy-*N*-(*O*-methyl)septoriamycin A (**2**), 13-(*S*)-hydroxy-*N*-(*O*-methyl)septoriamycin A (**3**), and 13-(*R*)-hydroxy-*N*-(*O*-methyl)septoriamycin A (**4**), together with the known compounds (+)-cercosporin (**5**), (+)-14-*O*-acetylcercosporin (**6**), (+)-di-*O*-acetylcercosporin (**7**), lumichrome, and brassicasterol, were isolated from an ethyl acetate extract of a culture medium of *Septoria pistaciarum*. Methylation of septoriamycin A (**8**) with diazomethane yielded three di-*O*-methyl analogues, two of which existed as mixtures of rotamers. We previously reported antimalarial activity of septoriamycin A. This compound also exhibited significant activity against *Leishmania donovani* promastigotes. Compounds **5**−**7** showed moderate in vitro activity against *L. donovani* promastigotes and chloroquine-sensitive (D6) and -resistant (W2) strains of *Plasmodium falciparum*, whereas compound **5** was fairly active against methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. Compounds **5**−**7** also displayed moderate phytotoxic activity against both a dicot (lettuce, *Lactuca sativa*) and a monocot (bentgrass, *Agrostis stolonifera*) and cytotoxicity against a panel of cell lines.



	R_1	R_2	R_3	R_4
1	CH_3	CH_2OH	H	CH_3
2	CH_3	CH_2OAc	H	CH_3
3	CH_3	CH_3	OH (S)	CH_3
4	CH_3	CH_3	OH (R)	CH_3
8	H	CH_3	H	CH_3
9	CH_3	CH_3	H	CH_3



	R_1	R_2
5	H	H
6	OAc	H
7	OAc	OAc

Phytotoxins from plant pathogenic fungi may act as inhibitors of plant-like metabolic pathways in the apicoplast, a chloroplast-like organelle, essential for the survival of plasmodium species.[1] Previously we reported[2] the isolation and identification of the 2-pyridinone alkaloid septoriamycin A[3] (**8**) and three of its derivatives from the causative agent of septoria leaf spot disease in pistachio, *Septoria pistaciarum* (Ascomycetes), as part of our program to identify antimalarial compounds from plant pathogenic fungi. Although we detected several minor alkaloids of the same class in the original extract, their structural elucidation was not possible due to insufficient quantities. In order to isolate the minor compounds, we recultured the same fungus under the identical conditions at a larger scale. In the ethyl acetate extract of this fermentation culture broth, a series of pigments were detected with 2-pyridinone alkaloidal architecture. The extract showed herbicidal, antimicrobial, antiplasmodial, and antileishmanial activities albeit with low selectivity indices. From this extract, four more new minor septoriamycin A analogues, 17-hydroxy-*N*-(*O*-methyl)septoriamycin A (**1**), 17-acetoxy-*N*-(*O*-methyl)-septoriamycin A (**2**), 13-(*S*)-hydroxy-*N*-(*O*-methyl)-septoriamycin A (**3**), and 13-(*R*)-hydroxy-*N*-(*O*-methyl)-septoriamycin A (**4**), in addition to the parent and its previously reported analogues were identified. Septoriamycin A (**8**), the major 2-pyridinone alkaloid in this extract, showed significant antileishmanial activity in addition to its reported antiplasmodial activity. The pigments were identified as three known perylenequinones, (+)-cercosporin (**5**),[4] (+)-14-*O*-acetylcercosporin (**6**),[4] and (+)-di-*O*-acetylcercosporin (**7**).[5] These pigments have previously been identified as phytotoxins produced by a number of phytopathogenic *Cercospora* species and have been linked to their pathogenicity.[6] Their biosynthesis appeared to be controlled by numerous environmental and physiological factors, and the presence of even small amounts of certain compounds in the medium was found to have a strong stimulatory or inhibitory effect on their production.[6,7] Their ability to generate reactive oxygen species in the presence of light has been attributed to their phytotoxic activity.[6] Cercosporin and its esters have also been reported to have antibacterial and antifungal[8] activities as well as growth-inhibitory effects on lettuce[4] and tomato seeds.[8] In this study, the perylenequinones showed antileishmanial, antiplasmodial, and cytotoxic activities in addition to antibacterial and antifungal activities. Two more known compounds, lumichrome[9] and brassicasterol,[10] were also isolated and identified.

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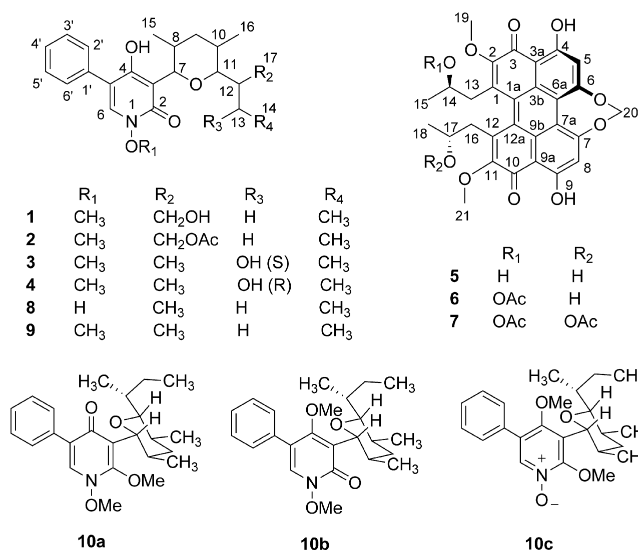
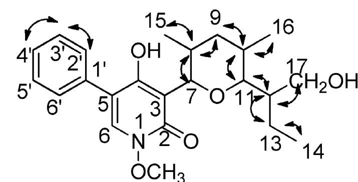


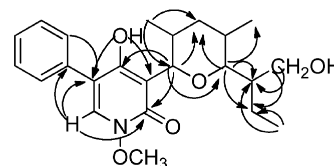
■ RESULTS AND DISCUSSION

Fractionation of an EtOAc extract of a culture medium of *S. pistaciarum* by Sephadex LH-20 gel column chromatography followed by purification using silica gel and RP C_{18} chromatography afforded four minor 2-pyridinone alkaloids (**1**−**4**) in addition to the known septoriamycin A (**8**) and its three derivatives,[2] three known perylenequinones, (+)-cercosporin (**5**), (+)-14-*O*-acetylcercosporin (**6**), and (+)-di-*O*-acetylcercosporin (**7**), lumichrome, and brassicasterol.

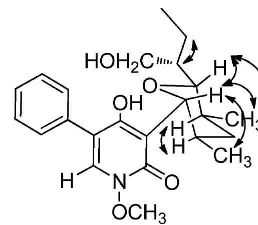
The molecular formula of compound **1** was determined as $C_{23}H_{31}NO_5$ by HRESIMS. UV maxima (205.0, 240.9, and 297.0 nm) and IR absorptions (3400, 3207, 1647, and 1555 cm^{-1}) of **1** were consistent with those of a 2-pyridinone moiety.[2] The aromatic regions of the ^1H NMR and COSY spectra were similar to those observed for 5-phenyl-2-pyridinones previously isolated from this species and consisted of a one-proton singlet and an A_2B_2C spin system due to a monosubstituted phenyl group.[2] The aliphatic region exhibited two oxymethine and two methyl doublets, indicating the presence of a 2,4-dimethyltetrahydropyran moiety and an *N*-methoxy group. HMBC correlations of H-2′ and H-6′ (δ_H 7.43) with C-3′ and C-5′ (δ_C 128.6), C-4′ (δ_C 127.8), C-1′ (δ_C 133.3), and C-5 (δ_C 114.5) and those of H-6 (δ_H 7.44) with C-2 (δ_C 158.0), C-4 (δ_C 161.9), C-5 (δ_C 114.5), and C-1′ (δ_C 133.3) supported the partial structure of the substituted pyridinone ring moiety. HMBC correlations of the H-7 oxymethine doublet (δ_H 4.73) with C-4 (δ_C 161.9), C-2 (δ_C 158.0), C-3 (δ_C 111.6), C-11 (δ_C 86.3), C-9 (δ_C 36.4), C-8 (δ_C 39.8), and C-15 (δ_C 17.8) supported the partial structure of the tetrahydropyran moiety. The major difference in the rest of the ^1H NMR resonances of compound **1** and the reported[2] *N*-(*O*-methyl)-septoriamycin A (**9**) is the replacement of the methyl doublet in the chain attached to C-11 of the tetrahydropyran ring by two diastereotopic oxymethylene hydrogens. This indicated that compound **1** was the C-17-oxygenated analogue of **9**. The COSY spectrum of compound **1** displayed cross-peaks between the C-17 oxymethylene (δ_H 3.60, 3.49) and C-12 methine proton (δ_H1.87). In the HMBC spectrum the oxymethylene protons showed cross-peaks with C-11 (δ_C 86.3), C-12 (δ_C 36.5), and C-13 (δ_C 22.3), and the H-11 oxymethine doublet at δ_H 3.36 with C-7 (δ_C 81.2), C-9 (δ_C 36.4), C-12 (δ_C 36.5), C-13 (δ_C 22.3), C-16 (δ_C 12.6), and C-17 (δ_C 63.6), further confirming that compound **1** was 17-hydroxy-*N*-(*O*-methyl)-septoriamycin A (Figure 1).



Key COSY ( ) correlations



Key HMBC ( ) correlations



Key ROESY ( ) correlations

Figure 1. COSY, HMBC, and ROESY correlations of compound **1**.

The HRESIMS data established the molecular formula of compound **2** as $C_{25}H_{33}NO_6$. The ^1H and ^{13}C NMR spectra were similar to those of **1** except for the presence of additional resonances [(δ_H 2.06) δ_C 21.1 (CH_3), δ_C 171.2 (CO)] originating from an *O*-acetyl group. HMBC correlations between the C-12 oxymethylene group and the acetyl carbonyl carbon indicated that compound **2** was 17-*O*-acetyl-*N*-(*O*-methyl)septoriamycin A. The remaining HMBC and COSY correlations were identical to those of compound **1**. Both $^3J_{7,8}$ and $^3J_{10,11}$ values were 10.2 Hz, indicating that these protons are *trans*-diaxially oriented. ROESY correlations of compound **1** (Figure 1) and **2** were identical to those observed for septoriamycin A (**8**), suggesting that these two compounds had the same relative configurations. Since we have previously assigned the absolute configuration of septoriamycin A on the basis of X-ray diffraction data,[2] and all these compounds presumably share a common biosynthetic origin, compounds **1** and **2** also have a 7*R*, 8*R*, 10*S*, 11*R*, and 12*R* absolute configuration. It is further supported by their dextrorotatory specific rotations.

The HRESIMS data of **3** established its molecular formula as $C_{23}H_{31}NO_5$. Comparison of the NMR spectra of **3** with those of **8** showed that the major difference was the replacement of a methyl triplet (δ_H 0.89) of the latter by a methyl doublet (δ_H 1.16) and an oxymethine doublet (δ_H 3.78) in the former. These changes could be attributed to the substitution of one of the C-13 diastereotopic methylene hydrogens in **8** with a hydroxy group, indicating that compound **3** is the 13-hydroxy analogue of *N*-(*O*-methyl)septoriamycin A (**9**). The methyl doublet at δ_H 0.93 (17-CH_3) showed HMBC cross-peaks with

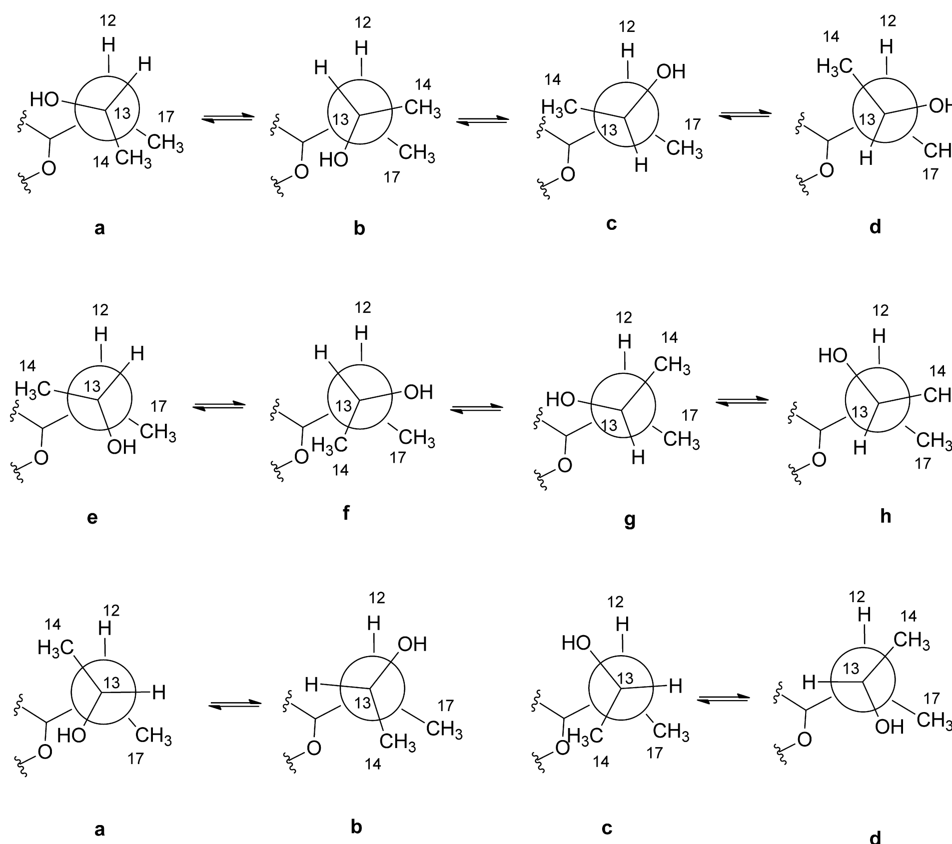


Figure 2. Rotamer representation of compounds **3** and **4**.

C-11 (δ_C 88.9), C-12 (δ_C 41.4), and C-13 (δ_C 68.9), and the oxymethine multiplet at δ_H 3.78 with C-11 (δ_C 88.9), C-12 (δ_C 41.4), and C-17 (δ_C 15.9), confirming the C-13 location of the secondary hydroxy group, and thus, compound **3** was 13-hydroxy-*N*-(*O*-methyl)septoriamycin A.

ROESY data and ^{1}H NMR coupling constants of compound **3** showed close correlations to those reported[2] for septoriamycin A (**8**). Our attempts to determine the absolute configuration at C-13 by Mosher analysis were unsuccessful. Treatment of compound **3** with the *R*- and *S*-Mosher's acid chlorides afforded a mixture not worth resolving. Methylation prior to acylation also yielded a mixture of products. The absolute configuration of compound **3** was proposed by a $^3J_{12,13}$-based comparison of compounds **3** and **4** (vide infra).

Compound **4** had the same molecular formula, $C_{23}H_{31}NO_5$, as that of **3** based on HRESIMS data. The ^{1}H NMR spectra of these compounds were similar except for the downfield shift of the oxymethine proton (δ_H 4.20) and the upfield shift of a methyl doublet (δ_H 1.00). The COSY and HMBC spectra of compound **4** showed the same correlations as those observed for compound **3**, suggesting that both compounds have the same gross structure. Identical coupling constants and ROESY correlations for the protons in the tetrahydrofuran moiety indicated that they had the same relative configuration except at C-13. Since compounds **3**, **4**, and **8** presumably share a common biosynthetic origin, compounds **3** and **4** also have a 7*R*, 8*R*, 10*S*, 11*R*, 12*R* absolute configuration. As described earlier for compound **3**, our attempts to determine the absolute configuration of the C-13 stereogenic center by Mosher analysis were unsuccessful. Thus, we used a *J*-based approach relying on ^{1}H NMR coupling constants combined with ROESY correlations, to assign the C-13 absolute configuration of compounds **3** and **4**. An observed $^3J_{12,13}$ value of 7.2 Hz for **3** suggested a dihedral angle of ca. 30° or 150° between H-12 and H-13. Eight rotamers are possible for **3** with these dihedral angles for the *S* (**3a**–**3d**) and *R* (**3e**–**3h**) epimers (Figure 2). Observed ROESY correlations between H-12 and H-13, H-13 and CH_3-17, and CH_3-14–CH_3-17 ruled out all conformers except **3a** as the probable most abundant rotamer for compound **3** in solution, indicating a 13*S* absolute configuration. Similarly, an observed $^3J_{12,13}$ of 1.0 Hz for **4** supported the fact that the dihedral angle between H-12 and H-13 was 90°, as shown in Figure 2 for the 13*S* (**4a**, **4b**) and *R* (**4c**, **4d**) epimers. In the ROESY spectrum, H-12 showed correlation with H-13 and CH_3-14 and the absence of interaction between CH_3-14 and CH_3-17, indicating **4a** as the dominant rotamer and, hence, a 13*R* absolute configuration.

Methylation of compounds **3** and **4** with diazomethane afforded several products. Treatment of septoriamycin A (**8**) with diazomethane as a model gave three products, whereas methylation with MeI and Cs_2CO_3 afforded a single compound, which was identified as analogue **9**. The products of diazomethane methylation of septoriamycin A were separated by chromatography. All these products had the same molecular formula, $C_{24}H_{33}NO_4$, by HRESIMS, suggesting that they were di-*O*-methyl derivatives. The ^{1}H NMR spectrum of the least polar compound (**10a**) was similar to that of **9** except for the presence of an additional *O*-methyl resonance in the former. The ^{13}C NMR spectrum of this compound showed changes in resonances in the pyridone ring. A downfield shift of C-4 (δ_C 177) indicated that the carbonyl group now resided at this carbon, suggesting **10a** was *N*,2-di-*O*-methylseptoriamycin A. A hypsochromic shift of the UV absorption (λ_{max} 278.9 nm) when

Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−4 in $CDCl_3$−Methanol-d_4

	1		2		3		4	
position	δ_C [b]	δ_H [a] (J in Hz)	δ_C [b]	δ_H [a] (J in Hz)	δ_C [b]	δ_H [a] (J in Hz)	δ_C [b]	δ_H [a] (J in Hz)
2	158.0		158.0		158.0		158.2	
3	111.6		111.4		112.0		112.0	
4	161.9		161.8		161.6		161.7	
5	114.5		114.3		114.5		114.8	
6	133.0	7.43, s	133.1	7.44, s	132.9	7.42, s	132.8	7.43, s
7	81.2	4.73, d (10.2)	81.3	4.74, d (10.2)	80.8	4.70, d (10.2)	80.9	4.66, d (10.2)
8	39.8	1.87, m	36.4	1.91, m	36.2	1.91, m	36.7	1.91, m
9	36.4	1.34, q (12.6)	36.5	1.30, q (12.6)	42.6	1.06, q (12.6)	42.2	1.16, q (12.0)
		1.98, brd (13.2)		2.00, (overlap)		1.80, dt (13.8, 3.6)		1.84, dt (13.2, 3.6)
10	35.8	1.69, m	36.0	1.64, m	34.1	2.03, m	32.9	1.89, m
11	86.3	3.36, d (10.2)	86.2	3.33, d (10.2)	88.9	3.14, d (10.2)	88.5	3.12, dd (10.2, 2.8)
12	36.5	1.87, m	37.0	2.00, m	41.4	2.05, m	38.7	1.75, m
13	22.3	1.02, m, 1.58, m	22.3	1.02, m, 1.55, m	68.9	3.78, m (7.2, 7.2)	66.0	4.20, m (6.6, 1.0)
14	12.6	0.88, t (7.2)	12.6	0.89, t (7.2)	23.1	1.16, d (6.6)	22.8	1.16, d (6.3)
15	17.8	0.91, d (6.6)	17.7	0.91, d (7.2)	17.9	0.85, d (6.6)	17.9	0.87, d (6.6)
16	17.1	0.95, d (6.6)	17.0	0.97, d (7.2)	17.7	0.83, d (6.6)	17.7	0.84, d (6.6)
17	63.6	3.49, dd (10.6, 3.6)	65.3	3.91, dd (11.2, 5.4)	15.9	0.93, d (7.2)	10.5	1.01, d (7.2)
		3.60, dd (10.6, 3.6)		3.98, dd (11.6, 4.5)				
1′	133.3		133.4		133.4		133.7	
2′, 6′	129.2	7.44, d (7.3)	129.3	7.44, d (7.3)	129.2	7.43, d (7.3)	129.4	7.43, d (7.3)
3′, 5′	128.6	7.39, t (7.2)	128.6	7.40, t (7.3)	128.5	7.38, t (7.8)	128.6	7.36, d (7.2)
4′	127.8	7.32, t (7.2)	127.9	7.34, t (7.3)	127.7	7.32, t (7.2)	127.8	7.30, t (7.2)
OH		9.63, s		9.47, s		9.63, s		9.65, s
OCH_3	65.0	4.04, s	65.0	4.06, s	65.0	4.04, s	65.0	4.05, s
$COCH_3$			21.1	2.06, s				
$COCH_3$			171.2					

[a] ^{1}H NMR spectra recorded at 600 MHz. [b] ^{13}C NMR spectra recorded at 100 MHz.

Table 2. ^{1}H and ^{13}C NMR Data Methylated Compounds 10a, 10b, and 10c in $CDCl_3$−Methanol-d_4

	10a		10b				10c			
position	δ_C [b]	δ_H [a] (J in Hz)	δ_C [b]		δ_H [a] (J in Hz)		δ_C [b]		δ_H [a] (J in Hz)	
2	155.0		159.2	157.2			158.0	156.0		
3	119.5		124.3	122.8			125.6	125.3		
4	177.0		164.9	164.8			155.7	157.0		
5	134.6		117.2	115.9			128.5	128.5		
6	132.7	7.51, s	133.4	134.0	7.44, s	7.49, s	139.6	139.7	8.15, s	8.14, s
7	78.4	4.63, d (10.2)	79.4	79.5	4.56, d (10.2)	4.21, d (10.2)	79.4	80.7	4.33, d (10.4)	4.33, d (10.4)
8	36.3	2.0, m	33.8	33.8	2.3, m	2.3, m	32.6	32.9	2.44, m	2.52, m
9	43.4	1.78, m	43.2	43.1	1.79, m	1.84, m	43.1	43.1	1.86, m	1.86, m
		1.02, m			1.04, m	1.06, m			0.96, m	0.96, m
10	33.7	1.69, m	32.3	31.9	1.72, m	1.72, m	32.4	32.5	1.88, m	1.88, m
11	89.2	2.92, d (12.0)	89.0	88.9	2.97, d (12.0)	2.87, d (12.0)	89.8	90.0	2.92, d (10.0)	2.94, d (10.0)
12	34.2	1.54, m	36.1	36.7	1.57, m	1.57, m	36.0	36.0	1.60, m	1.60, m
13	22.8	1.54, m	22.6	21.9	1.53, m	1.53, m	22.5	22.5	1.5, m	1.5, m
		0.82, m			0.85, m	0.85, m			1.1, m	1.1, m
14	13.0	0.84, t (7.2)	12.7	12.4	0.84, t (6.6)	0.84, t (6.6)	12.9	12.8	0.87, t (6.6)	0.87, t (6.6)
15	17.6	0.68, d (6.6)	17.5	17.8	0.80, d (6.6)	0.80, d (6.6)	17.6	17.6	0.81, d (6.6)	0.81, d (6.6)
16	17.7	0.77, d (6.6)	17.3	17.6	0.75, d (6.6)	0.68, d (6.6)	17.7	17.8	0.64, d (6.4)	0.67, d (6.6)
17	17.0	0.89, d (6.6)	16.8	17.0	0.91, d (6.6)	0.91, d (6.6)	17.2	17.2	0.90, d (6.6)	0.90, d (6.6)
1′	126.6		133.8	133.8			133.0	133.0		
2′, 6′	128.9	7.56, d (7.2)	128.6	128.5	7.43, d (7.2)	7.43, d (7.2)	129.0	128.9	7.48, d (6.8)	7.48, d (7.5)
3′, 5′	128.3	7.33, t (7.2)	128.4	128.5	7.39, t (7.2)	7.39, t (7.2)	128.9	128.7	7.42, t (6.8)	7.42, d (7.5)
4′	127.8	7.25, t (7.2)	127.6	127.5	7.34, t (7.2)	7.34, t (7.2)	129.8	129.8	7.39, t (7.3)	7.39, t (7.3)
OCH_3	66.8	4.01, s	64.5	64.5	4.08, s	4.08, s	61.7	62.1	3.36, s	3.37, s
OCH_3	64.4	4.0, s	61.5	61.2	4.01, s	3.34, s	60.6	60.4	4.16, s	4.14, s

[a] ^{1}H NMR spectra recorded at 600 MHz. [b] ^{13}C NMR spectra recorded at 100 MHz.

compared to **9** (λ_{max} 295 nm)[11] and COSY and HMBC correlations further supported this structure. The ^{1}H and ^{13}C NMR spectra of compound **10b** indicated that it existed as a 3:1 mixture of two rotamers about the C-3−C-7 bond. Their ^{1}H and ^{13}C NMR spectra were similar to those of compound **9** but had an additional methoxy resonance. HMBC correlations showed that the additional methoxy group correlated to C-4, indicating that **10b** was *N*,4-di-(*O*-methyl)septoriamycin A. At 100 °C in pyridine, duplicated ^{1}H NMR resonances coalesced but were restored to the original ratio when the temperature returned to ambient conditions. Atropisomers of 3-cyclohexyl- or 3-cycloheptyl-*N*,4-dihydroxy-2-pyridone alkaloids have previously been isolated from several fungal species.[11−15] Even though a number of 3-pyrano-*N*,4-dihydroxy-2-pyridone alkaloids similar to septoriamycin A analogues have been identified[16] from fungal sources, none have been reported to exist as rotamers. The introduction of a 4-methoxy group appears to cause restricted rotation about the C-3−C-7 bond, giving rise to rotamers. The most polar compound (**10c**) showed ionic characteristics. ^{1}H and ^{13}C NMR data indicated that the compound also existed as a 3:1 mixture of two diastereomeric rotamers. HMBC correlations showed that the methoxy resonance correlated to C-2 and C-4, suggesting that **10c** is the 2,4-di-*O*-methylpyridinone-*N*-oxide analogue of septoriamycin A. The presence of the 4-methoxy group appears to cause restricted rotation about the C-3−C-7 bond. At 100 °C in pyridine duplicated ^{1}H NMR resonances coalesced. Even though the original ratio of rotamers reappeared at ambient temperature, some decomposition of **10c** was also observed.

The known perylenequinones, (+)-cercosporin (**5**),[4] (+)-14-*O*-acetylcercosporin (**6**),[4,5] and (+)-di-*O*-acetylcercosporin (**7**),[5] lumichrome,[9] and brassicasterol[10] were identified by comparing their spectroscopic data with literature data. The absolute configurations of **5**−**7** were determined by comparing experimental and reported electronic circular dichroism data.[17,18] The helicity of compounds **5**−**7** was confirmed as *M* (*aS*), and the absolute configuration of both C-14 and C-17 as *R*.[19]

Compounds **1**−**4** showed no antimicrobial, antifungal, antiprotozoal, phytotoxic, or cytotoxic activity in vitro. Compounds **5**−**7** showed moderate in vitro antiplasmodial activity (Table 3) but were cytotoxic to Vero cells. Their low selectivity indices (ratio of cytotoxicity vs antiplasmodium activity) preclude them as antimalarial drug leads. Even though the plant pathogen *S. pistaciarum* is host-specific to pistachio, compounds **5**−**7** showed nonspecific moderate phytotoxic activity toward both bentgrass (*A. stolonifera*) and lettuce (*L. sativa* cv. L., iceberg) in the presence of light (Table 4). General phytotoxcity of phytotoxins from host-specific pathogens is very common. Biosynthesis of cercosporin (**5**) appeared to be controlled by numerous environmental and physiological factors, and its production has been linked to the pathogenicity of fungi.[6,7] The possible mechanism of phytotoxic activity of this type of compound has previously been attributed to their ability to generate reactive oxygen species in the presence of light.[6] This suggested that the selective inhibition of the plant-like metabolic pathways in the apicoplast of malaria parasite[1] is not responsible for the observed antimalarial activity of compounds **5**−**7**. Septoriamycin A (**8**), with demonstrated antiplasmodial and antifungal activities,[2] exhibited significant antileishmanial activity, with an IC_{50} of 0.11 μM and an IC_{90} of 0.29 μM (Table 5), and was more potent than the positive controls pentamidine and amphotericin B. Compounds **5**−**7** also showed significant antileishmanial activity, with IC_{50} values of 1.14, 1.7, and 3.1 μM, respectively (Table 5).

Compound **5** was moderately active against both methicillin-sensitive and methicillin-resistant *Staph. aureus*, with MIC values 2.5 and 5.0 μg/mL (4.7 and 9.4 μM), respectively. In the same assay, the positive control, ciprofloxacin, had a MIC value of 0.37 μg/mL (1.11 μM) against both methicillin-sensitive and methicillin-resistant *Staph. aureus*.

The cytotoxic potential of compounds **5**−**7** was further evaluated against a panel of human solid tumor cell lines (SK-MEL, KB, BT-549, SK-OV-3) and pig kidney epithelial cells (LLC-PK_{11}) (Table 6). Moderate cytotoxicity was observed against all the cell lines. The di-*O*-methyl derivatives of septoriamycin A, **10a**, **10b**, and **10c**, were also evaluated for the above activities, and compound **10c** exhibited weak antiplasmodial activity (Table 3).

Table 3. Antiplasmodial Activity of Compounds 5−7 and 10c

	chloroquine-sensitive (D6) clone		chloroquine-resistant (W2) clone		cytotoxicity to Vero cells
compound	IC_{50},[b] μM	S.I.[c]	IC_{50},[b] μM	S.I.[c]	IC_{50},[b] μM
5	1.08	4.8	1.62	3.2	5.24
6	2.78	1.9	3.12	1.7	5.21
7	2.75	1.6	1.94	2.3	4.53
10c	6.76	>1.1	6.51	>1.2	NC[d]
chloroquine[a]	0.03		0.31		NC
artemisinin[a]	0.02		0.01		NC

[a]Positive controls. [b]IC_{50}: concentration causing 50% growth inhibition. [c]S.I. (selectivity index) = IC_{50} for cytotoxicity/IC_{50} for antiplasmodial activity. [d]NC: not cytotoxic.

Table 4. Phytotoxic Activity of Compounds 5−7[a]

compound	concentration (mM)	lettuce	bentgrass
5	1.87	3	2
6	1.73	2	4
7	1.62	3	4

[a]Concentration (mM) = 1 mg/mL. Ranking based on scale of 0 to 5: 0 = no effect; 5 = no growth.

Table 5. Antileishmanial Activity of Compounds 5−8

compound	IC_{50},[b] μM	IC_{90},[c] μM
5	1.14	2.81
6	1.7	8.5
7	3.1	9.7
8	0.11	0.29
pentamidine[a]	2.9	5.58
amphotericin B[a]	0.18	0.38

[a]Positive controls. [b]IC_{50}: concentration causing 50% growth inhibition. [c]IC_{90}: concentration causing 90% growth inhibition.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a Rudolph Research Analytical Autopol IV automatic polarimeter model 589-546. Melting points were measured with a Unimelt, Thomas-Hoover capillary melting point apparatus. UV and

Table 6. Cytotoxic Activity [IC_{50} (μM)] of Compounds 5–7[a]

	SK-MEL	KB	BT-549	SK-OV-3	LLC-PK_{11}
5	3.6	7.1	10.8	3.7	3.9
6	3.8	8.5	8.6	4.0	10.1
7	4.9	8.7	8.7	4.8	9.4
doxorubicin[b]	1.6	2.6	2.6	1.5	1.6

[a]IC_{50} = concentration causing 50% growth inhibition. SK-MEL = human malignant melanoma. KB = human epidermal carcinoma. BT-549 = human breast carcinoma (ductal). SK-OV-3 = human ovary carcinoma. LLC-PK_{11} = pig kidney epithelial. [b]Positive control.

IR spectra were determined on a Varian-50 Bio UV visible spectrophotometer and a Bruker-Tensor-27 infrared spectrophotometer, respectively. NMR spectra were recorded on a Varian-Mercury-Plus-400 or Varian Unity-Inova-600 spectrometer using $CDCl_3$ and methanol-d_4 unless otherwise stated. MS data were obtained from an Agilent Series 1100 SL equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA). Column chromatography and preparative TLC were carried out using Merck silica gel 60 (230–400 mesh) and silica gel GF plates (20 × 20 cm, thickness 0.25 mm), respectively. HPLC analysis was conducted on a Hewlett-Packard Agilent 1100 with diode array detector.

Fermentation, Extraction, and Isolation. The plant pathogenic fungus *Septoria pistaciarum* Caracc. (ATCC 22201) was obtained from the American Type Culture Collection (Manassas, VA, USA), and it was grown as previously reported.[2]

The oily extract (3.3 g) was chromatographed over Sephadex LH-20 and eluted with 80% MeOH in $CHCl_3$ to give 16 fractions. Fractions 2–11, which showed antiplasmodial activity, were combined and chromatographed over a reversed-phase C_{18} column eluting with a gradient of 10% to 100% MeOH–H_2O to yield eight fractions. Subfractions 2, 3, and 4 were combined and separated on Sephadex LH-20 (MeOH) to give compounds **1** (5.0 mg) and **8** (35 mg). Subfraction 5 was chromatographed over a silica gel column using CH_2Cl_2–hexanes as the eluent to give compound **2** (8.0 mg). Combined fractions 6, 7, and 8 were chromatographed over a silica gel column using CH_2Cl_2–hexanes as the eluent to give 10 subfractions. Subfractions 2, 3, and 5 were combined and further separated using a C_{18} reversed-phase HPLC column eluting with MeCN–H_2O (1:1), flow rate 3.5 mL/min, to give compounds **3** (3.5 mg) and **4** (8.0 mg). Subfractions 6, 7, and 8 were combined and purified on Sephadex LH-20 (MeOH) to give compounds **5** (14.0 mg), **6** (12.0 mg), and lumichrome (3.0 mg). Subfractions 9 and 10 were combined and chromatographed over a silica gel column using CH_2Cl_2–hexanes as the eluent to give compound **7** (10.0 mg) and brassicasterol (8.0 mg).

*Compound **1**:* amorphous powder; $[\alpha]^{26}_{D}$ +112 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 205.0 (3.56), 240.9 (3.51), 297.0 (2.79) nm; IR ($CHCl_3$) ν_{max} 3400, 3207, 2967, 2929, 1647, 1555, 1454, 1049 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS $[M - H]^+$ *m/z* 400.2118 (calcd for $[C_{23}H_{31}NO_5 - H]^+$, 400.2124).

*Compound **2**:* amorphous powder; $[\alpha]^{26}_{D}$ +101 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 204.0 (3.44), 240.0 (3.25), 295.9 (2.56) nm; IR ($CHCl_3$) ν_{max} 3331, 2967, 2925, 1740, 1651, 1230, 1045 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS $[M + H]^+$ *m/z* 444.2370 (calcd for $[C_{25}H_{33}NO_6 + H]^+$, 444.2386).

*Compound **3**:* amorphous powder; $[\alpha]^{26}_{D}$ −38 (*c* 0.43, CH_3OH); UV (MeOH) λ_{max} (log ε) 208.2 (3.94), 241.9 (3.92), 297.0 (3.19) nm; IR ($CHCl_3$) ν_{max} 3405, 3201, 2965, 2929, 1644, 1551, 1455, 1219, 755 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS $[M + H + Na]^+$ *m/z* 425.2172 (calcd for $[C_{23}H_{31}NO_5 + H + Na]^+$, 425.2178).

*Compound **4**:* amorphous powder; $[\alpha]^{26}_{D}$ +130 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 207.1 (3.01), 241.0 (2.95), 295.1 (2.10) nm; IR ($CHCl_3$) ν_{max} 3422, 3159, 2926, 1641, 1541, 1456 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS $[M + H + Na]^+$ *m/z* 425.2174 (calcd for $[C_{23}H_{31}NO_5 + H + Na]^+$, 425.2178).

*Methylation of Septoriamycin A (**8**) with MeI.* A mixture of MeI (4 mL), Cs_2CO_3 (10.0 mg), and compound **8** (10.0 mg) in acetone was stirred at room temperature for 5 h. The reaction mixture was filtered, and the solvent was evaporated. The product was dissolved in CH_2Cl_2 and passed through a plug of Florisil to give *N*-(*O*-methyl)-septoriamycin A (**9**).

*Methylation of Septoriamycin A (**8**) with Diazomethane.* A solution of **8** (60.0 mg) in MeOH was treated with excess diazomethane in Et_2O at 0 °C for 2 h. The solvent was evaporated, and the mixture was separated by PTLC (40% EtOAc in hexanes) to yield compounds **10a** (8 mg), **10b**, and **10c**. Compounds **10b** and **10c** were further purified by HPLC using a reversed-phase Luna C_{18} column (1 × 25 cm) with MeOH–H_2O (92:8) as the mobile phase at a flow rate of 4 mL/min, to give compounds **10b** (5 mg) and **10c** (6.0 mg).

*Compound **10a**:* amorphous powder; $[\alpha]^{26}_{D}$ +14 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 205.0 (3.75), 232.0 (3.68), 278.9 (3.41) nm; IR ($CHCl_3$) ν_{max} 2959, 2929, 2873, 1627, 1547, 1469, 1046 cm^{-1}; ^1H and ^{13}C NMR data (see Table 2); HRESIMS $[M + Na]^+$ *m/z* 422.2307 (calcd for $[C_{24}H_{33}NO_4 + Na]^+$, 422.2307).

*Compound **10b**:* amorphous powder; UV (MeOH) λ_{max} (log ε) 204.0 (3.39), 235.0 (3.18), 308.0 (2.65) nm; IR ($CHCl_3$) ν_{max} 2958, 2929, 1740, 1656, 1524, 1457 cm^{-1}; ^1H and ^{13}C NMR data (see Table 2); HRESIMS $[M + H]^+$ *m/z* 400.2472 (calcd for $[C_{24}H_{33}NO_4 + H]^+$, 400.2487).

*Compound **10c**:* amorphous powder; UV (MeOH) λ_{max} (log ε) 207.0 (2.58), 240.9 (2.47), 307.1 (1.70) nm; IR ($CHCl_3$) ν_{max} 2900, 2850, 1575, 1450 cm^{-1}; ^1H and ^{13}C NMR data (see Table 2); HRESIMS $[M + Na]^+$ *m/z* 422.2279 (calcd for $[C_{24}H_{33}NO_4 + Na]^+$, 422.2307).

*Compound **5**:* red crystals; mp 239 °C (lit.[17] 240–241.5 °C); UV (MeOH) λ_{max} (log ε) 210.0 (3.71), 221.0 (3.72), 267.0 (3.55), 470.0 (3.42), 563.0 (2.98) nm; IR ($CHCl_3$) ν_{max} 3396, 2924, 1616, 1267 cm^{-1}; ^1H and ^{13}C NMR and CD data were consistent with those reported.[4,17,19]

*Compound **6**:* red crystals; mp 135 °C (lit.[5] 134 °C); UV (MeOH) λ_{max} (log ε) 222.0 (3.9), 269.0 (3.74), 471.0 (3.62), 563.0 (3.2) nm; IR ($CHCl_3$) ν_{max} 3338, 1735, 1616, 1266 cm^{-1}; ^1H and ^{13}C NMR and CD data were consistent with those reported.[4,5]

*Compound **7**:* red, amorphous powder; UV (MeOH) λ_{max} (log ε) 222.0 (3.9), 270.0 (3.84), 470.1 (4.0), 563.3 (3.26) nm; IR ($CHCl_3$) ν_{max} 2921, 1736, 1617, 1211 cm^{-1}; ^1H and ^{13}C NMR and CD data were consistent with those reported.[5]

Antiplasmodial Assay. The antiplasmodial activity was determined against D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of *Plasmodium falciparum* in an in vitro assay as described earlier.[20] Artemisinin and chloroquine were included as the drug controls, and IC_{50} values were computed from the dose–response curves using Microsoft Excel software.

Phytotoxicity Assay. The bioassay for phytotoxicity was carried out according to the procedure described by Dayan et al.[21] using bentgrass (*Agrostis stolonifera*) and lettuce (*Lactuca sativa* cv. L., iceberg), in 24-well plates. Test compounds (1 mg each) were dissolved in 100 μL of acetone, and a 20 μL aliquot of each solution was pipetted onto the filter paper and dried for 30 min by airflow in a sterile biohazard hood. Water (200 μL) was added after placing the dried and sample-impregnated filter paper in the well. The solvent controls were treated identically, using the solvent described above. Phytotoxicity was ranked visually. The ranking of phytotoxic activity was based on a scale of 0 to 5, with 0 showing no effect and 5 no growth.

Antileishmanial Assay. The in vitro antileishmanial activity of the compounds was carried out on a culture of *Leishmania donovani* promastigotes as described earlier.[22] Pentamidine and amphotericin B were used as standard antileishmanial agents. The IC_{50} values for each compound were computed from the growth inhibition curve using Microsoft Excel software.

Antimicrobial Assay. All organisms were obtained from the American Type Culture Collection (Manassas, VA, USA) and included the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria

Staphylococcus aureus ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods[23,24] as described by Samoylenko et al.[25] The drug controls ciprofloxacin (ICN Biomedicals, Aurora, OH, USA) for bacteria and amphotericin B (ICN Biomedicals) for fungi were included in each assay.

Cytotoxicity Assay for Mammalian Cells. In vitro cytotoxicity was determined against a panel of mammalian cells that included kidney fibroblast (Vero), kidney epithelial (LLC-PK_{11}), malignant melanoma (SK-MEL), oral epidermal carcinoma (KB), breast ductal carcinoma (BT-549), and ovary carcinoma (SK-OV-3) cells as described earlier.[26] The number of viable cells was determined by using Neutral Red dye, and IC_{50} values were obtained from dose−response curves. Doxorubicin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of compounds **1**−**4**, **10a**, **10b**, and **10c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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