Secondary Mould Metabolites. Part 16.[1] Stemphyltoxins, New Reduced Perylenequinone Metabolites from *Stemphylium botryosum* var. Lactucum

Alberto Arnone and Gianluca Nasini *
Dipartimento di Chimica del Politecnico, Centro del C.N.R. per le Sostanze Organiche Naturali, Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy
Lucio Merlini
Istituto di Biochimica Generale, Università di Milano, Via Celoria 2, I 20133 Milano
Gemma Assante
Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, I 20133 Milano

The structure and stereochemistry of stemphyltoxins I—IV (**1**)—(**4**), four epoxy derivatives of reduced perylenequinones isolated from the fungus *Stemphylium botryosum* var. Lactucum, have been elucidated, mainly on the basis of n.m.r. evidence. A further metabolite, stemphyperylenol (**5**), is a hexahydro-1,4,7,10-tetrahydroxyperylene-3,9-quinone, a structure which suggests an unusual head-to-tail biosynthetic coupling of two pentaketide units.

Stemphylium botryosum Wallr. is a mould which causes leaf spot of lettuce, a disease of economic importance in many countries. The toxicity of moulds is usually related to the production of one or more phytotoxins, and this is the case of *Stemphylium botryosum* var. Lactucum, which has been reported to produce toxins when cultivated on a potato–dextrose–agar medium.[2]

One of them, named stemphylin,[2] was recently found to be identical[3] with altersolanol A, a well-known metabolite of *Alternaria* and *Dactylaria*,[4] many species of which produce toxins. The structure of another toxin, stemphyloxin I, from *S. botryosum* var. Licopersicon, has also been elucidated.[5]

This paper reports the isolation and structural elucidation of five new metabolites of *S. botryosum* var. Lactucum. Cultures of the fungus were grown on a Sabouraud–maltose–agar medium and extracted with ethyl acetate. Flash chromatography of the extracts gave mixtures, which were purified by repeated preparative thin-layer chromatography, to give the pure compounds (**1**)—(**5**). Substances (**1**)—(**4**), which we propose to call stemphyltoxins I—IV, are rather unstable, being quickly transformed, even in the solid state, into black insoluble products; owing to the nature of these compounds, this material resembles the natural black pigment aspergillin.[6]

The structural elucidation of compounds (**1**)—(**5**) is based on ^{1}H and ^{13}C n.m.r. studies as well as chemical evidence. ^{1}H and ^{13}C Spectral parameters are summarized in Tables 1—3. The ^{13}C n.m.r. spectrum of (**1**) showed 20 carbon atom resonances. Fourteen signals were indicative of the presence of two tetrasubstituted aromatic rings and two carbonyl carbon atoms, while six resonances were attributed to one methylene, one oxygen-bearing quaternary and four methine sp^3-hybridized carbon atoms (three of them oxygen-bearing). ^{1}H N.m.r. analysis, as corroborated by ^{1}H-{^{1}H} nuclear Overhauser effects (n.O.e.s) in [$^2H_6$]acetone + D_2O, and ^{13}C spectral data, permitted the fragments A—C to be constituted.

Fragment A. The ^{1}H n.m.r. spectrum revealed two pairs of *ortho* coupled aromatic protons. Irradiation of the proton at δ_H 7.90 (7-H) gave 10% n.O.e. to 8-H and 12.5% n.O.e. to 6-H (Figure 1); this suggested the presence of a biphenyl moiety, a deduction in agreement with the u.v. spectral characteristics of the compound. The spectrum also exhibited two chelated phenolic hydroxy protons which were assigned to C-4 and C-9 since these carbon atoms show two- and three-bond coupling constants to aromatic protons [^2J(CH) 2.5 and 2.5, ^3J(CH) 10 and 10 Hz] in the fully ^{1}H coupled ^{13}C n.m.r. spectrum. As a consequence, it was necessary for the two carbonyl groups to be located at C-3a and C-9a respectively. Similar arguments allowed the other aromatic carbons to be assigned.

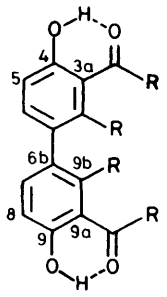
Fragment B. The chemical shift values and the magnitude[7] of

Table 1. ^{1}H N.m.r. data for compounds (**1**)—(**4**)

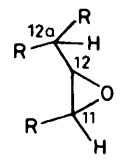
Proton	(1)[a]	(2)[a]	(3)[a]	(4)[b]
	δ_H			
1	4.72	2.95 (α) 2.59 (β)	7.85	4.62
2	3.22 (α) 2.89 (β)	3.25 (α) 2.77 (β)	6.54	3.91
5	7.02	7.06	7.08	7.08
6	8.04	8.13	8.13	8.15
7	7.90	8.04	8.13	8.15
8	6.93	6.99	7.02	7.00
11	3.66	3.72	3.77	3.82
12	5.28	4.44	4.60	4.69
12a	3.75	3.62	3.89	3.97
1-OH	4.89			
4-OH	12.60	12.79	12.40	12.02[c]
9-OH	11.94	12.09	12.17	12.04[c]
12b-OH	4.76	4.80	5.14	5.79

	(1)	(2)	(3)	(4)
J/Hz				
1α,1β		13.3		
1α,2α		5.0		
1α,2β		2.5		
1β,2α	11.9	14.1	10.3	3.8
1β,2β	4.5	4.1		
1,1-OH	8.2			
2α,2β	16.4	17.6		
5,6	8.8	8.8	8.6	8.8
7,8	8.8	8.8	8.6	8.8
8,12a	0.9	1.0	0.9	0.9
11,12	3.8	3.7	3.6	3.8
11,12a	0.9	0.9	0.8	0.7
12,12a	0.8	0.4	*ca.* 0	*ca.* 0

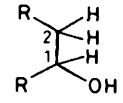
[a] In [$^2H_6$]acetone. [b] In [$^2H_6$]DMSO. [c] Assignments may be interchanged.



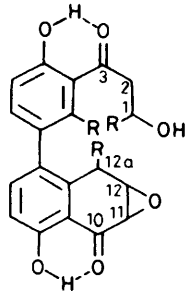
A



B



C



D

the one-bond (C,H) couplings exhibited by C-11 and C-12 indicated that they are part of an epoxide ring [δ_C 54.94, ^1J(CH) 185.5; δ_C 58.03, ^1J(CH) 187 Hz]. Specific ^{13}C-{^{1}H} low-power decoupling experiments permitted the correlation of these two resonances with signals at δ_H 3.66 and 5.28. They were both coupled to the C-12a methine proton [^4J(HH) 0.9 and ^3J(HH) 0.8 Hz] which, in turn, was long-range coupled to all the carbon atoms of ring D, with coupling constants ranging from 1 to 7 Hz; this indicated its benzylic nature (Table 3).

Fragment C. The ^{1}H n.m.r. spectrum contained an ABXY spin system which was ascribed to the 1-H, 2-H_2, and 1-OH protons on the basis of ^{1}H-{^{1}H} homonuclear decoupling experiments. The presence of the 1-OH function was inferred from disappearance of the signals at δ_H 4.89 and removal of an 8.2 Hz coupling from the 1-H resonance on addition of deuterium oxide. Irradiation of 2-H and 11-H resulted in decoupling of C-3 and C-3a, and of C-10 and C-9a respectively,

Table 2. ^{1}H N.m.r. data for compounds (**5**), (**5a**), and (**5b**)

	(5)[a]	(5a)[b]	(5b)[a]		(5a)[c]	(5b)
		δ_H				
Proton				J/Hz		
1	4.76	6.03	4.72	1,2α	4.2	4.1
2α	3.07	3.16	2.90	1,2β	10.6	12.5
2β	3.17	2.99	3.07	1,12b	7.5	9.0
5	6.81	7.05	7.01	1,1-OH		5.1
6	8.14	7.45	8.13	2α,2β	14.1	13.0
6b	3.75	4.07	3.79	5,6	8.3	8.9
7	4.76	6.03	4.81	5,12b	*d*	0.8
8α	3.07	3.16	3.06	5,4-OMe		<0.2
8β	3.17	2.99	3.19	6,6b	*d*	1.1
11	6.81	7.05	6.81	6b,7	7.5	9.1
12	8.14	7.45	8.02	6b,11	*d*	0.9
12b	3.75	4.07	3.75	6b,12b	3.3	3.3
1-OR	4.97	2.15	4.81	7,8α	4.2	4.7
4-OR	12.09	2.38	3.82	7,8β	10.6	11.9
7-OR	4.97	2.15	4.94	7,OH-7		5.6
10-OR	12.09	2.38	12.00	8α,8β	14.1	15.5
				11,12	8.3	8.8
				12,12b	*d*	1.0

[a] In [$^2H_6$]acetone. [b] In $CDCl_3$. [c] Coupling constants determined by computer simulation. [d] Not assigned.

Table 3. ^{13}C N.m.r. data for compounds (**1**) and (**5**)

	(1)[a]			(5)[b]		
Carbon atom	δ_C[c]/p.p.m.	^1J(CH)/Hz	$^{>1}J$(CH)/Hz[d]	δ_C[c]/p.p.m.	^1J(CH)/Hz	$^{>1}J$(CH)/Hz[d]
1	71.02 (Ddd)	144	8 and 4 (2-H_2)	68.32 (Dm)	141.5	*e*
2	43.56 (DDd)	135.5 and 126	1.5 (1-H)	47.84 (DD)	133 and 125	
3	203.96 (St)		6.5 (2-H_2)	204.01 (St)		6.5 (2-H_2)
3a	114.46 (Sm)		3.5 (2-H_2), 5 (5-H), and 1 (6-H)	115.92 (Sm)		*ca.* 3 (2-H_2), *ca.* 5 (5-H), *ca.* 3 (12b-H), and *ca.* 7 (4-OH)
4	161.93 (Sdd)		2.5 (5-H) and 10 (6-H)	161.03 (Sddd)		2.5 (5-H), 10 (6-H), and 5 (4-OH)
5	119.09 (D)	163.5		115.55 (Dd)	160	7 (4-OH)
6	133.87 (D)	159		135.58 (D)	159	
6a	125.67 (Sdd)		8 (5-H) and 3.5 (7-H)	130.90 (Sm)		8 (5-H), 3 (7-H), and 5 and 4 (6b- and 12b-H)
6b	125.99 (Sddd)		3 (6-H), 8 (8-H), and 3 (12a-H)	46.05 (Dm)	127	*e*
7	133.43 (Dd)	158	1 (12a-H)	68.32 (Dm)	141.5	*e*
8	117.02 (Dd)	163	1 (12a-H)	47.84 (DD)	133 and 125	
9	162.63 (Sddd)		10 (7-H), 2.5 (8-H), and 1 (12a-H)	204.01 (St)		6.5 (8-H_2)
9a	114.75 (Sm)		1 (7-H), 5 (8-H) 3 and 2.5 (11- and 12a-H)	115.92 (Sm)		*ca.* 3 (6b-H), *ca.* 3 (8-H_2), *ca.* 5 (11-H), and *ca.* 7 (10-OH)
9b	136.85 (Sddd)		7.5 (7-H), 7 (12-H), and 7 (12a-H)	143.73 (Sddd)		8 and 4 (6b- and 12b-H) and 7.5 (12-H)
10	199.24 (Sdd)		4 (11-H) and 1 (12-H)	161.03 (Sddd)		2.5 (11-H), 10 (12-H), and 5 (10-OH)
11	54.94 (Ddd)	185.5	2 (12-H) and 4.5 (12a-H)	115.55 (Dd)	160	7 (10-OH)
12	58.03 (Dd)	187	9[e]	135.58 (D)	159	
12a	47.04 (Ddd)	124	2 (1-H) and 6 (12-H)	130.90 (Sm) 46.05 (Dm)		3 (1-H), 8 (11-H), and 5 and 4 (6b- and 12b-H)
12b	71.75 (Sm)		*e*		127	*e*
12c	141.04 (Sdd)		7.5 (6-H) and 2.5 (12a-H)	143.73 (Sddd)		7.5 (6-H) and 8 and 4 (6b- and 12b-H)

[a] In [$^2H_6$]acetone. [b] In [$^2H_6$]acetone–[$^2H_6$]DMSO. [c] Capital letters refer to one-bond couplings observed in single frequency off-resonance decoupled ^{13}C n.m.r. spectra: S, singlet; D, doublet. Lower case letters refer to long-range couplings observed in fully ^{1}H coupled ^{13}C n.m.r. spectra: d, doublet; t, triplet; m, multiplet. [d] Coupling constants <1 Hz were not available. [e] Not assigned.

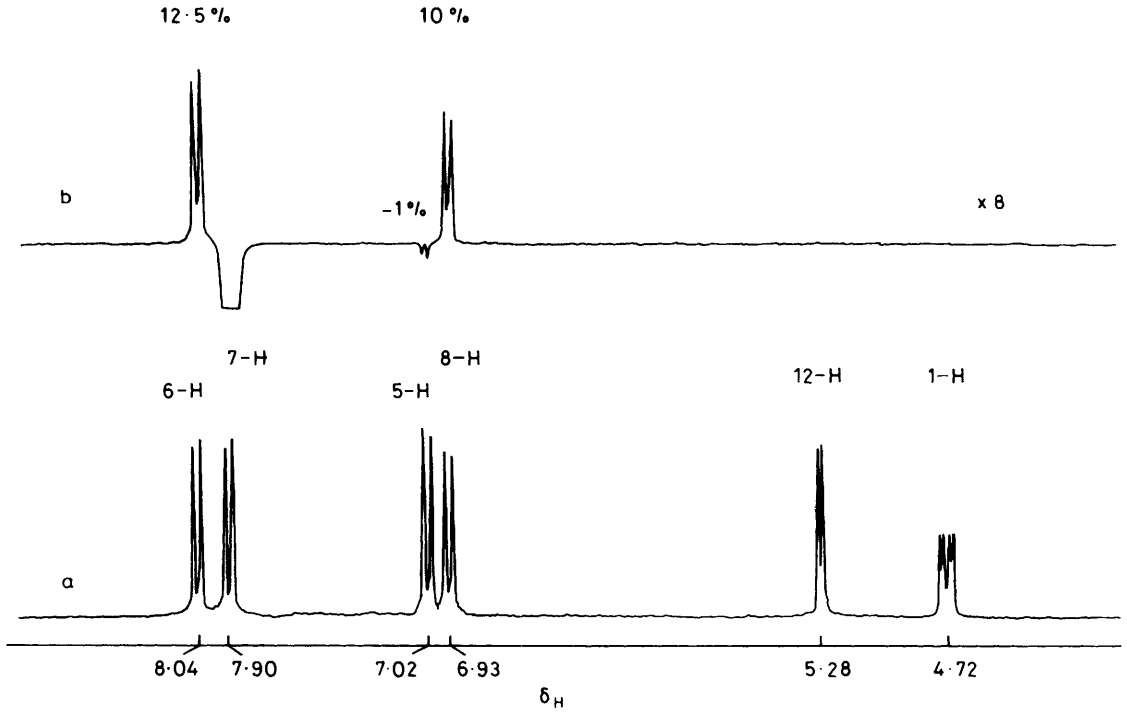
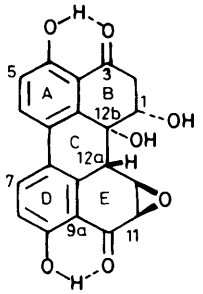


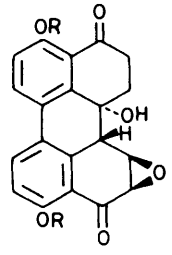
Figure 1. The 300 MHz spectra of compound (**1**) in the region δ 4.7—8.1 in [$^2H_6$]acetone + D_2O; (a) normal; (b) n.O.e. difference spectrum obtained by irradiation of 7-H

thus indicating that they are on carbon atoms linked to C-3 and C-10 carbonyl groups. All these findings are consistent with the partial structure D. The remaining quaternary carbon atom (C-12b, δ_C 71.75), which bears the tertiary hydroxy group at δ_H 4.76, must then be connected to C-1, C-12a, and C-12c, leading to structure (**1**). The magnitude of the coupling constants between 1-H, assumed as β, and 2-H_2 protons ($^3J_{1\beta,2\alpha}$ 11.9 and $^3J_{1\beta,2\beta}$ 4.5 Hz) well accounts for a preferred half-chair conformation of the cyclohexenone ring with 1-OH in an equatorial position. The large n.O.e. observed for 12a-H upon irradiation of 1β-H (10.5%) is indicative of a 1,3-*cis* diaxial relationship between these two protons, all these data being consistent with a *trans* orientation of 12a-H and 12b-OH. The oxirane ring must be β placed, the small coupling constant between 12-H and 12a-H [^3J(HH) 0.8 Hz] suggesting a dihedral angle near 90° which is in agreement with the planarity of the C(9)–C(9a)–C(10) fragment required by the hydrogen bond. On this evidence, the most probable relative configuration of (**1**) appears to be 1*S**,11*R**,12*R**,12a*S**,12b*R**.

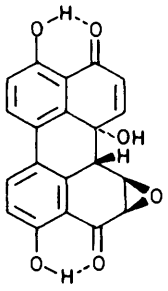
Comparison of the mass spectra of (**1**) and (**2**) indicates that (**2**) lacks an oxygen atom with respect to (**1**). The ^1H n.m.r. spectrum showed an ABCM spin system due to the C(1)H_2–C(2)H_2 fragment, the other resonances being very similar to those of (**1**), except for 12-H which exhibited an upfield shift ($\Delta\delta_H$ −0.84 p.p.m.) because of the absence of 1-OH. Irradiation of the axial 1-methylene proton at δ_H 2.59, assumed as β, in [$^2H_6$]acetone + D_2O, resulted in a 6.5% enhancement of 12a-H, whilst irradiation of 1α-H gave an 11% enhancement of 12-H. Moreover irradiation of 12b-OH in [$^2H_6$]DMSO led to a 2 and 1% enhancement of 2α-H and 1α-H respectively. These results, which indicate a 1,3-*cis* diaxial relationship between 1β-H and 12a-H, and 2α-H and 12b-OH, establish a *trans* orientation between 12b-OH and 12a-H. Therefore formula (**2**) must represent the structure of the second metabolite, whose relative configuration is 11*R**,12*R**,12a*S**,12b*R**. Comparison of the circular dichroism (c.d.) spectra of (**1**) and (**2**) shows a



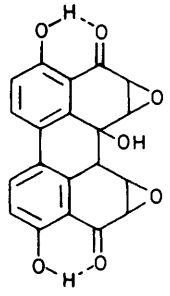
(**1**)



(**2**) R = H
(**2a**) R = Ac



(**3**)



(**4**)

strict similarity between the curves, which is consistent with (**1**) and (**2**) having also the same absolute configuration. Acetylation of (**2**) with pyridine–Ac_2O afforded the diacetate (**2a**).

Compound (**3**) showed a molecular formula with two protons less than in compound (**2**). ^1H and ^{13}C N.m.r. spectra revealed the presence of a CO-conjugated double bond [δ_H 7.85 (1-H) and 6.54 (2-H); δ_C 148.01 (C-1) and 127.64 (C-2)].

Hydrogenation of (**3**) with Pd 10% on $BaSO_4$ gave (**2**), clearly showing the relationship between these two compounds.

The mass spectrum of (**4**) presented a molecular peak at *m/z* 364, corresponding to a formula $C_{20}H_{12}O_7$, with one oxygen atom more than in (**3**). Comparison of the ^1H n.m.r. spectrum of (**4**) with that of (**3**) revealed the presence of two vicinally coupled [^3J(HH) 3.8 Hz] C-1 and C-2 protons of an epoxide ring in place of C-1 and C-2 vinylic protons of (**3**). No stereochemical evidence for this compound resulted from spectroscopic experiments.

High-resolution mass spectrometry established the molecular formula $C_{20}H_{16}O_6$ for (**5**), which we propose to call stemphyperylenol. The ^1H and ^{13}C n.m.r. spectra, which contained signals due to only eight protons and ten carbon atoms, indicated that the molecule was a C_{10} dimer. The ^1H resonances were assigned to an AX spin system of *ortho* coupled aromatic protons, and to a chelated phenolic hydroxy group plus a C(2)H_2–C(1)HOH–C(12b)H fragment. The ^{13}C resonances were attributed to a tetra-substituted aromatic ring and a carbonyl carbon atom, the remaining signals being assigned to one methylene and two methine (one of which is oxygen-bearing) sp^3-hybridized carbons. The above spectral data in conjunction with the analysis of (C,H) long-range coupling constants (Table 3) led to the partial structure E.

The chelated phenolic hydroxy group was located at C-4, this carbon atom presenting two- and three-bond coupling constants to 4-OH, 5-H (δ_H 6.81), and 6-H (δ_H 8.14) [^2J(COH) 5, ^2J(CH) 2.5, and ^3J(CH) 10 Hz]. Consequently the carbonyl group was established as being at C-3a. Finally, irradiation of the 2-methylene protons caused decoupling of the C-3 and C-3a resonances [^2J(CH) 6.5 and ^3J(CH) *ca.* 3 Hz], thereby establishing the C(2)–C(3) bond. Thus, the two moieties (E) could be joined together to give structure (**5**), the latter being consistent with the following n.O.e. and spin decoupling experiments carried out on the tetra-acetate (**5a**) and monomethyl ether (**5b**), respectively. The ^1H-{^1H} homonuclear decouplings on (**5b**) gave insight into the type of long-range coupling constants present between the aromatic and benzylic protons. In fact 6b-H and 12b-H exhibited, respectively, *ortho* benzylic coupling constants to the lower field C-6 and C-12 aromatic protons [^3J(HH) 1.1 and ^3J(HH) 1.0 Hz] and *para* benzylic coupling constants to the higher field C-11 and C-5 aromatic protons [^6J(HH) 0.9 and ^6J(HH) 0.8 Hz]. Irradiation of 6-H (and 12-H) at δ_H 7.45 in (**5a**) resulted in a 16.5% enhancement of the signal of the *ortho* coupled 5-H (and 11-H) at δ_H 7.05 and a 17% enhancement of 7-H (and 1-H), thus indicating that the two aromatic rings are part of a dihydroanthracene moiety (Figure 2). The hypsochromic shift of the u.v. and c.d. spectra with respect to (**1**) is also consistent with a less conjugated structure, the two phenyl rings here being separated.

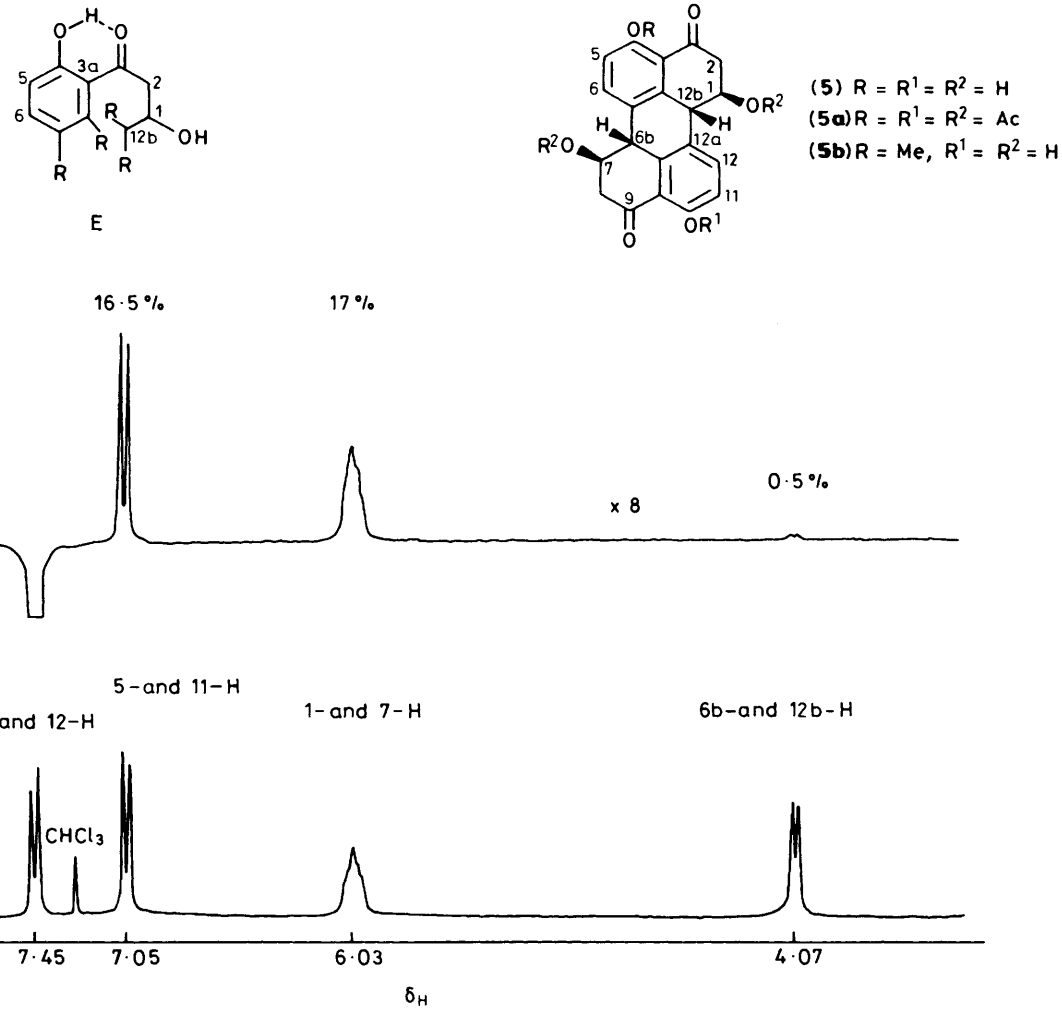
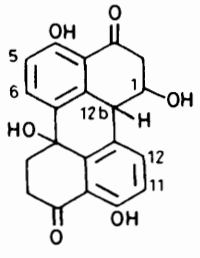


Figure 2. The 300 MHz spectra of compound (**5a**) in the region δ 4.0—7.5 in $CDCl_3$: (a) normal; (b) n.O.e. difference spectrum obtained by irradiation of 6-H (and 12-H)

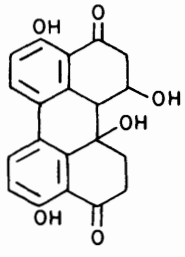
The relative configuration and the preferred conformation of (**5**) were derived from the following evidence. Compound (**5**) showed $[\alpha]_D^{23}$ +411.5° (*c* 0.2 in MeOH). This fact, combined with the above mentioned ^1H and ^{13}C n.m.r. observations, can be accounted for only by the existence of a C_2 axis perpendicular to the plane of the molecule, requiring a *cis* relationship between 6b-H and 12b-H, notwithstanding the planar or helix-shaped nature of (**5**).

The magnitude of the vicinal coupling constants between C-1 (and C-7), assumed as α, and C-2 (and C-8) protons ($^3J_{1\alpha,2\beta}$ 10.6 and $^3J_{1\alpha,2\alpha}$ 4.2 Hz) in (**5a**) suggests that the cyclohexenone rings are preferentially in the half-chair conformation with the hydroxy groups in an equatorial position. This is confirmed by the n.O.e. observed between C-12b (and C-6b) and the axial C-2β (and C-8β) protons (3%), but not with C-2α (and C-8α). These facts place the C-12b (and C-6b) proton in a β axial position as well. The relative configuration at C-1 (and C-7) and at C-12b (and C-6b) is thus established as *R**. The absolute configuration at C-1 (and C-7) was then established as *R* by the application of the Horeau method of kinetic resolution[8] on the monomethyl ether (**5b**). As a consequence, the absolute configuration at C-6b and C-12b is also *R*.

From the n.m.r. spectral parameters of the biphenyl (**1**) and dihydroanthracene (**5**) structures we conclude that the aromatic protons *meta* to the chelated phenolic hydroxy groups and *para* to the carbonyl groups resonate at lower field than the protons *ortho* to the chelated hydroxy groups and *meta* to the carbonyl groups. Moreover, in all biphenyl-type compounds (**1**)—(**4**), a *para* benzylic coupling constant between 8-H and 12a-H [^6J(HH) 0.9—1.0 Hz] has been found (Table 1). Our results are therefore different from those obtained by Stinston *et al.*,[9] who reversed the assignment of the above-mentioned aromatic protons in the structure of altertoxin I (**6**). The alleged *ortho* benzylic coupling constants between 12b-H and the higher field aromatic proton (δ_H 7.03) signal, wrongly attributed[9] to 12-H [^3J(HH) 1.0 Hz] is really a *para* benzylic coupling constant between 12b-H and 5-H. Furthermore, the lack of a sizeable *ortho* benzylic coupling for 12b-H, such has been observed in compound (**5b**), suggests that the structure of altertoxin I should be revised to (**7**). It follows that the structure of the dehydration product of altertoxin I, proposed by Stinson,[9] must also be revised.



(**6**)



(**7**)

The structure (**7**), apart from its stereochemistry, is identical with that given by Okuno[10] for dihydroalterperylenol. It is also to be noted that the structures of alterperylenol[10] and alteichin[11] appear to be identical from *X*-ray studies, although different physical constants such as $[\alpha]_D$ have been reported.[10,11] Additionally, it is possible that each of these compounds is the same as altertoxin II, already isolated by Pero[12] and Chu.[13] There is no doubt that such a confused situation needs clarification, and that accurate perusal of the literature cannot be too strongly recommended.

Compounds (**1**)—(**5**) constitute a conspicuous addition to the group of reduced perylenequinones recently found in Nature.[9–11] They have been so far identified in fungi of the genus *Alternaria*, to which *Stemphylium* is closely related morphologically.[14] Their biosynthesis occurs most probably *via* a phenol coupling of two pentaketide-derived moieties[10] (as happens for similar perylenequinone metabolites, *e.g.* cercosporin[15] and elsinochromes[16]), followed by reduction and hydroxylation or epoxidation in different positions. It is remarkable that, whereas all the compounds (including perylenequinones[17]) so far found appear to derive from a so-called head-to-head coupling (*e.g.* stemphyltoxins I—IV), stemphyperylenol (**5**) seems to be the first example of a head-to-tail coupling of these moieties.

Compounds (**1**)—(**5**) showed antibacterial activity *in vitro* against *Bacillus subtilis*, *B. cereus*, and *E. coli*. The presence of epoxy groups in (**1**)—(**4**) may well support the hypothesis that they are phytotoxic compounds. Experiments along these lines are in progress.

Experimental

M.p.s are uncorrected. U.v. spectra were measured in 95% EtOH on a Beckman DK-2 spectrophotometer, i.r. spectra with a Perkin-Elmer 137 instrument. Flash chromatography was performed with Merck silica gel (0.040—0.063 mm), and t.l.c. with Merck HF_{254} silica gel. C.d. spectra were recorded on a JASCO 500A spectropolarimeter. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^1H (300.13 MHz) and ^{13}C (75.47 MHz) N.m.r. spectra were recorded on a Bruker CXP-300 spectrometer. Chemical shifts are in p.p.m. (δ) from $SiMe_4$ as internal standard. N.O.e. difference spectra were obtained by substracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS. N.O.e. values reported in the test have only qualitative significance.

Isolation and Purification of Metabolites (**1**)—(**5**).—The mycelium of a strain of *Stemphylium botryosum* var. Lactucum, CBS 273.55, obtained from Centraal Bureau voor Schimmelcultures, Baarn, grown on Sabouraud–maltose–agar (2:20:15 g l^{-1}) in 50 Roux flasks, was extracted twice with EtOAc containing 3% of MeOH after 2 weeks of growth at room temperature. The extracts (1.5 g) were dried and evaporated under reduced pressure to give a brown mixture of crude metabolites. This mixture was adsorbed on a chromatographic column and eluted with CH_2Cl_2–MeOH (30:1) to give the metabolites (**2**)—(**4**) as a mixture; this was subjected to preparative t.l.c. (p.l.c.) with CH_2Cl_2–MeOH (15:1) to give the pure compounds. Compound (**4**) was obtained in very poor yield and is very unstable. Finally by using a 15:1 ratio the two metabolites (**1**) and (**5**) were eluted, but a further p.l.c. purification with the same solvent was necessary.

Stemphyltoxin I (1S*,11R*,12R*,12aS*,12bR*)-1,4,9,12b-*Tetrahydroxy*-3,10-*dioxo*-1,2,3,10,11,12,12a,12b-*octahydro*-11,12 *epoxyperylene*, (**1**) (10 mg), a yellow amorphous powder, had m.p. 135—140 °C (Found: C, 65.5; H, 3.9. $C_{20}H_{14}O_7$ requires C, 65.57; H, 3.85%); $[\alpha]_D$ +355.9° (*c* 0.05 in MeOH); c.d. (*c* 3.08 × 10^{-4} g ml^{-1} in EtOH) 388, 320, and 265 nm (Δε +4.5, +4.9, and +9.2); $\lambda_{max.}$ 257, 283 (sh), 295 (sh), and 364 nm (ε 23 850, 15 000, 12 100, and 5 100); $\nu_{max.}$(KBr) 3 450 (OH) and 1 650 cm^{-1} (conj. CO); *m/z* 366 (92%, M^+), 348 (17, $M^+ - 18$), 330 (100, $M^+ - 36$), 314 (88), and 302 (60).

Stemphyltoxin II (11R*,12R*,12aS*,12bR*)-4,9,12b-*Trihydroxy*-3,10-*dioxo*-1,2,3,10,11,12,12a,12b-*octahydro*-11,12-*epoxyperylene*, (**2**) (10 mg), a yellow powder, had m.p. 300 °C (decomp.) (Found: C, 68.65; H, 4.1. $C_{20}H_{14}O_6$ requires C, 68.57; H, 4.03%); $[\alpha]_D$ +480.7° (*c* 0.11 in MeOH); c.d. (*c* 3.2 × 10^{-4} g ml^{-1} in EtOH) 390, 325, and 267 nm (Δε +4.1, +2.0, and

+11.8); $\lambda_{max.}$ 260, 285 (sh), and 364 nm (ε 29 300, 19 700, and 6 200); $\nu_{max.}$(KBr) 3 460 (OH) and 1 650 cm^{-1} (conj. CO); *m/z* 350 (M^+), 332 ($M^+ - 18$) (Found: M^+, 350.0794 ± 0.005. $C_{20}H_{14}O_6$ requires *M*, 350.0790).

Acetylation of (**2**). Compound (**2**) (20 mg) dissolved in dry pyridine (0.8 ml) and Ac_2O (1.5 ml) was left overnight at 4 °C. The solution was poured onto ice, and the precipitate collected and chromatographed by p.l.c. using CH_2Cl_2–MeOH (30:1) as eluant to give the diacetate (**2a**) as a yellow solid (10 mg), m.p. 145—150 °C (Found: C, 66.25; H, 4.1. $C_{24}H_{18}O_8$ requires C, 66.36; H, 4.18%); *m/z* 434 (M^+), 392, ($M^+ - 42$), 350 ($M^+ - 84$), 332, and 316; δ_H (300 MHz, $CDCl_3$) 2.40 and 2.42 (2 × 3 H, s, 4- and 9-OAc), 2.41 and 2.77 (2 × 1 H, m, 1-H_2), 2.71 and 3.11 (2 × 1 H, m, 2-H_2), 3.64 (1 H, d, *J* 4 Hz, 11-H), 3.67 (1 H, br s, 12a-H), 4.15 (1 H, d, *J* 4 Hz, 12-H), 7.19 and 7.26 (2 × 1 H, d, *J* 9 Hz, 5- and 8-H), and 7.92 and 8.05 (2 × 1 H, d, *J* 9 Hz, 6- and 7-H).

Stemphyltoxin III (11R*,12R*,12aS*,12bR*)-4,9,12b-*Trihydroxy*-3,10-*dioxo*-3,10,11,12,12a,12b-*hexahydro*-11,12-*epoxyperylene*, (**3**) (15 mg), a brown solid, had m.p. >300 °C (decomp.); $[\alpha]_D$ +688.5° (*c* 0.13 in MeOH); $\lambda_{max.}$ 268, 274, 287 (sh), and 374 nm (ε 28 900, 26 000, 20 300, and 4 750); $\nu_{max.}$ 3 200 (OH), 1 650 (conj. CO), and 1 610 cm^{-1}; *m/z* 348 (M^+) and 330 ($M^+ - 18$) (Found: M^+, 348.0596 ± 0.005. $C_{20}H_{12}O_6$ requires *M*, 348.0633).

Hydrogenation of (**3**). Compound (**3**) (20 mg) was dissolved in acetone (5 ml) and hydrogenated in the presence of 10% Pd–$BaSO_4$ to give compound (**2**), identical with the natural one (t.l.c., n.m.r., and $[\alpha]_D$ comparison).

Stemphyltoxin IV 4,9,12b-*Trihydroxy*-3,10-*dioxo*-1,2,3,10,-11,12,12a,12b-*octahydro*-1,2,11,12-*diepoxyperylene*, (**4**) (5 mg) had m.p. >300 °C (decomp.); $\lambda_{max.}$ 268, 300 (sh), and 372 nm (ε 30 600, 13 250, and 5 500); *m/z* 364 (10%, M^+), 346 (100, $M^+ - 18$), 330 (60), and 318 (50) (Found: M^+, 364.0597 ± 0.002. $C_{20}H_{12}O_7$ requires *M*, 364.0583).

Stemphyperylenol (1R,6bR,7R,12bR)-1,4,7,10-*Tetrahydroxy*-3,9-*dioxo*-1,2,3,6b,7,8,9,12b-*octahydroperylene*) (**5**). This was obtained as buff crystals (100 mg) from CH_2Cl_2–ether which decomposed without melting at 250 °C (Found: C, 68.05; H, 4.6. $C_{20}H_{16}O_6$ requires C, 68.18; H, 4.58%); $\nu_{max.}$(Nujol) 3 400 (OH) and 1 640 cm^{-1} (conj. CO); $\lambda_{max.}$ 212, 255, and 335 nm (ε 28 700, 25 600, and 6 800); c.d. (*c* 5.4 × 10^{-4} g ml^{-1} in EtOH) 334 (sh), 319 (sh), 310, and 260 nm (Δε +3.0, +6.4, and +12.1); *m/z* 352 (M^+), 334 ($M^+ - 18$), 316 ($M^+ - 36$), 308, 280, 262, and 236 (Found: M^+, 352.0962 ± 0.003. $C_{20}H_{16}O_6$ requires *M*, 352.0946).

Acetylation of (**5**). Compound (**5**) (50 mg), Ac_2O (2 ml) and H_2SO_4 (0.2 ml) were left for 0.5 h at 4 °C. The solution was poured onto ice, and the precipitate was purified by flash chromatography on silica gel with added 2% KH_2PO_4 with hexane–EtOAc (1:1) as eluant, to give the tetra-acetate (**5a**) as a yellow solid, m.p. 145—150 °C (Found: C, 64.55; H, 4.6. $C_{28}H_{24}O_{10}$ requires C, 64.61; H, 4.65%); *m/z* 400 ($M^+ - 2$ AcOH), 358, and 316.

Methylation of (**5**). A mixture of (**5**) (100 mg), Ag_2O (120 mg), and MeI (0.5 ml) in dry acetone (10 ml) was stirred for 2 days at room temperature in the dark and the residue was chromatographed by p.l.c. using CH_2Cl_2–MeOH (15:1) to give the monomethyl ether (**5b**), m.p. 176—178 °C (Found: C, 68.7; H, 4.85. $C_{21}H_{18}O_6$ requires C, 68.84; H, 4.95%); *m/z* 330 ($M^+ - 2H_2O$).

Reaction of (**5b**) *with* (±)-2-*Phenylbutyric anhydride*. (+)-2-Phenylbutyric anhydride (100 mg) was added to a solution of (**5b**) (40 mg) in dry pyridine (0.5 ml). The solution was kept for 20 h at room temperature; (+)-2-phenylbutyric acid with $[\alpha]_D$ +2.2° (*c* 0.1 in pyridine) was obtained upon work-up of the reaction mixture according to the literature method.[8]

Acknowledgements

This work was supported by Progetto Finalizzato per la Chimica Fine e Secondaria del C.N.R.

References

1 Part 15, A. Arnone, L. Camarda, G. Nasini, and G. Assante, *J. Chem. Soc., Perkin Trans. 1*, 1986, 255.
2 I. Barash, A. L. Karr, and G. A. Strobel, *Plant Physiol.*, 1975, **55**, 646.
3 G. Assante and G. Nasini, unpublished work.
4 W. B. Turner and D. C. Aldridge, in 'Fungal Metabolites II,' Academic Press, London, 1983, p. 145.
5 I. Barash, S. Manulis, Y. Kashman, J. P. Springer, M. H. M. Chen, J. Clardy, and G. A. Strobel, *Science*, 1983, **220**, 1065.
6 M. Barbetta, G. Casnati, and A. Ricca, *Rend. Ist. Lombardo Sci. Lett.*, 1967, **101**, 75.
7 J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972.
8 A. Horeau, in 'Stereochemistry,' ed. H. B. Kagan, Thieme, Stuttgart, 1977, vol. 3, p. 51.
9 E. E. Stinson, S. F. Osman, and P. E. Pfeffer, *J. Org. Chem.*, 1982, **47**, 4110.
10 T. Okuno, I. Natsume, K. Sawai, K. Sawamura, A. Furusaki, and T. Matsumoto, *Tetrahedron Lett.*, 1983, **24**, 5653.
11 D. Robeson, G. A. Strobel, G. K. Matusumoto, E. L. Fisher, M. H. Chen, and J. Clardy, *Experientia*, 1984, **40**, 1248.
12 R. W. Pero, H. Posner, M. Blois, D. Harvan, and J. W. Spalding, *EHP, Environ. Health Perspect.*, 1973, 87.
13 F. S. Chu, *J. Amer. Oil Chem. Soc.*, 1981, **58**, 1006A.
14 E. G. Simmons, *Mycologia*, 1967, **59**, 67.
15 A. Okubo, S. Yamazaki, and K. Fuwa, *Agric. Biol. Chem.*, 1975, **39**, 1173.
16 C. T. Chen, K. Nakanishi, and S. Natori, *Chem. Pharm. Bull.*, 1966, **14**, 1434; L. C. Vining, A. C. McInnes, D. G. Smith, and J. A. Welter, *Can. J. Chem.*, 1981, **59**, 422.
17 L. Merlini and G. Nasini, in 'Chemistry and Biotechnology of Biologically Active Natural Products,' ed. Cs. Szantay, Akadémiai Kiadó, Budapest 1984, p. 121.

Received 29*th July* 1985; *Paper* 5/1288