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Siderophores of highly phytopathogenic *Alternaria longipes*

Structures of hydroxycoprogens

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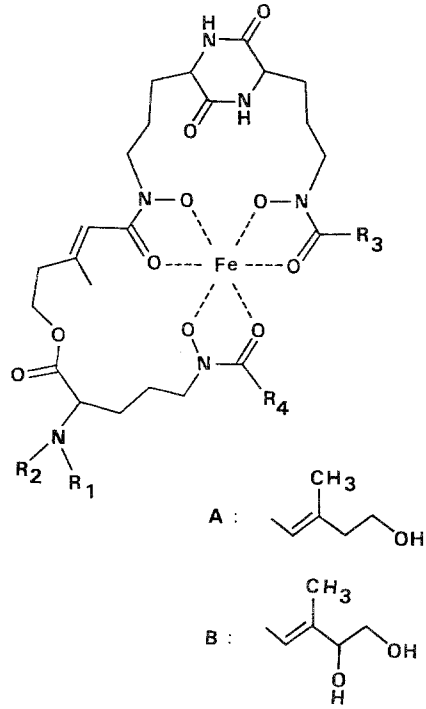
Summary. *Alternaria longipes* ATCC 26293, a highly phytopathogenic fungus, has been found to produce a large number of siderophores under iron-deficient conditions. Most of the compounds are members of the coprogen family. Structures of three novel siderophores, termed hydroxycoprogens, have been determined by ^{1}H and ^{13}C NMR, FAB mass spectrometry and partial hydrolysis. The compounds are analogs of coprogen, neocoprogen I and isoneocoprogen I, in which one of the terminal *trans*-anhydromevalonic acid residues is replaced by a *trans*-4,5 dihydroxy-3-methyl-2-pentenoic acid residue.

Key words: Siderophores — Hydroxycoprogens — Coprogens — Phytopathogenic fungi — *Alternaria longipes*

Introduction

Siderophores are specific iron(III)-chelating compounds, naturally produced by aerobic microorganisms to acquire iron under iron-deficient conditions (Neilands 1981). Fungi, in general, produce ornithine-based hydroxamate-type siderophores belonging to various structural families (van der Helm et al. 1987). Pathogenic microorganisms represent a special group which have to cope with iron starvation after infecting a host body, where iron is usually bound tightly in various proteins and compounds in a form not easily available to the pathogen. *Alternaria longipes* ATCC 26293 (previously *A. alternata* ATCC 26293) is a phytopathogenic fungus which is known for its high virulence (Stavely and Slana 1973; Simmons 1981); it readily produces leaf brown spot disease in *Nicotiana tabaccum*. The purpose of this study is to investigate the structural variety of siderophores produced by this phytopathogenic fungus under iron-deficient conditions. Most of the siderophores produced by this fungus belong to the coprogen family **(1–10).** We have reported the structures of three novel coprogens, N^{α}-dimethylcoprogens **(5–7),** isolated from this fungus, in an earlier paper. This report describes the structures of the other siderophores isolated and identified so far from its culture



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broth. Among these compounds are three new siderophores, the hydroxycoprogens.

1. $R_1=H$ $R_2=COCH_3$ $R_3=R_4=A$
2. $R_1=H$ $R_2=COCH_3$ $R_3=CH_3$ $R_4=A$
3. $R_1=H$ $R_2=COCH_3$ $R_3=A$ $R_4=CH_3$
4. $R_1=H$ $R_2=COCH_3$ $R_3=R_4=CH_3$
5. $R_1=R_2=CH_3$ $R_3=R_4=A$
6. $R_1=R_2=R_3=CH_3$ $R_4=A$
7. $R_1=R_2=R_4=CH_3$ $R_3=A$
8. $R_1=H$ $R_2=COCH_3$ $R_3=A$ $R_4=B$
9. $R_1=H$ $R_2=COCH_3$ $R_3=CH_3$ $R_4=B$
10. $R_1=H$ $R_2=COCH_3$ $R_3=B$ $R_4=CH_3$

Formulae 1–10.

Materials and methods

Production and isolation. A. longipes was maintained on agar slants made of Grimm-Allen's iron-deficient medium and cultured in the liquid medium of the same composition as described before (Jalal et al. 1984a). Siderophores were extracted as their ferric complexes with an XAD-2 column according to the procedure described earlier (Jalal et al. 1988). The extract was subjected to chromatography on silica gel columns (solvent, chloroform/methanol/water, 35:12:2, CMW) and on a C_{18} silica gel reversed-phase column (solvent, 0–40% methanol in water gradient) until the compounds were obtained in pure form (Jalal et al. 1984b). Alkaline hydrolysis was carried out in methanolic solutions with ammonia according to the published method (Frederick et al. 1981). Hydrolysis products were purified by chromatographic procedures using LH 20/ethanol and silica gel/CMW systems.

Spectroscopic methods. ^1H and ^{13}C-NMR spectra were determined at 300 MHz and 75.4 MHz respectively with a Varian XL300 instrument. Assignment was done on the basis of decoupling experiments, deuterium exchange studies and chemical shift correlation with known siderophores of analogous structures (Hossain et al. 1987; Jalal et al. 1986). Additionally $-CH_3$, $-CH_2-$, $-CH-$, $=C-$ and $-C=O$ carbon atoms were distinguished by an attached proton best (APT) pulse sequence program (Patt and Shoolery 1982). Fast-atom-bombardment (FAB) mass spectra were determined with a VG ZAB-E mass spectrometer. To determine accurate mass by peak matching, coprogen (MH^+ *m/z* 822.30982) and neocoprogen I (MH^+ *m/z* 752.2680) were used as internal standards. Spectra were calibrated externally with CsI.

Thin-layer chromatography and paper electrophoresis. Purification steps were followed by thin-layer chromatography on silica gel 60H with solvent systems (a) chloroform/methanol/water (35:12:2) (CMW), (b) butanol/acetic acid/water (4:1:5, upper phase) and (c) chloroform/methanol/hexane (1:1:1). Paper electrophoresis was carried out along with authentic coprogen and coprogen B on Whatman 3MM paper at a field strength of 1 kV for 1 h.

Deferriation of ferric siderophores. Deferriation was carried out by the 8-hydroxyquinoline method described previously (Jalal et al. 1985).

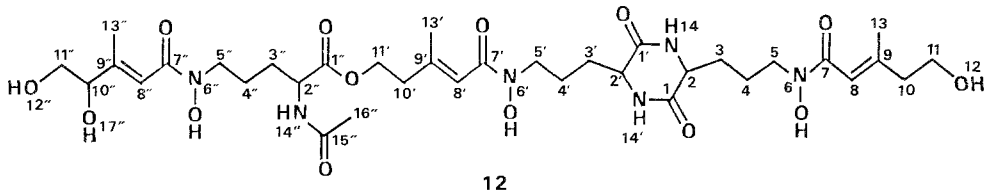
Results and discussion

Identification of siderophores

A large number of siderophores have been purified and identified from the culture filtrate of iron-starved *A. longipes*. Most of the compounds belong to the coprogen family of siderophores. In addition to the known members of the family [coprogen **(1),** neocoprogen I **(2)** and neocoprogen II **(4)**], six novel compounds are also produced by the fungus. Three of these compounds, the N^{α}-dimethylcoprogens (N^{α}-dimethylcoprogen **(5),** N^{α}-dimethylneocoprogen I **(6)** and N^{α}-dimethylisoneocoprogen I **(7)**] have been described in a previous paper (Jalal et al. 1988). The other three compounds **(8–10),** which are produced as major siderophores by the fungus, are termed hydroxycoprogens. Evidence for their structures will be presented in the following sections.

Identification of coprogen **(1),** neocoprogen I **(2)** and neocoprogen II **(4),** which have also been reported from other fungi (Diekmann 1970; Frederick et al. 1981, 1982; Hossain et al. 1987), have been carried out by co-chromatography with authentic samples and confirmed by FAB mass spectroscopy (relative intensity in parentheses): [coprogen, *m/z* 822 (100) MH^+, *m/z* 844 (16) MNa^+; neocoprogen I, *m/z* 752 (100) MH^+, *m/z* 774 (19) MNa^+; neocoprogen II, *m/z* 682 (100) MH^+, *m/z* 704 (17) MNa^+]. Identification of neocoprogen I **(2)** was further based on the production of the dihydroxamate **18** after hydrolysis of the ester bond [^1H NMR of **18** identical to that of the same compound reported by Jalal et al. (1988)].

The only siderophore isolated from *A. longipes* which does not belong to the coprogen family is identified as ferricrocin (Barnes et al. 1984) on the basis of cochromatography and FAB mass spectroscopy [*m/z* 771 (64) MH^+; *m/z* 793 (60) MNa^+]. Another Fe(III) chelator produced in large quantity by the fungus has been identified as the toxin tenuazonic acid **(11).** This compound is also produced by other *Alternaria* species (Stickings and Townsend 1961). It possesses the ability to chelate most biologically available metal ions and shows a range of biological activities (Holzapfel 1980). Identification of the compound was based on (a) its NMR spectra [^1H-NMR, CD_3OD, δ/ppm = 0.87 (t, 3 H, CH_3, 1), 1.0 (d, 3 H, CH_3, 10), 1.16, 1.37 (m, 2 H, CH_2, 2), 1.89 (m, 1 H, CH, 3), 2.34 (s, 3 H, CH_3, 9), 3.61 (m, 1 H, CH, 4); ^{13}C NMR, CD_3OD, δ/ppm = 12.38 (CH_3, 1), 16.60 (CH_3, 10), 24.16 (CH_2, 2), 27.03 (CH_3, 9), 38.15



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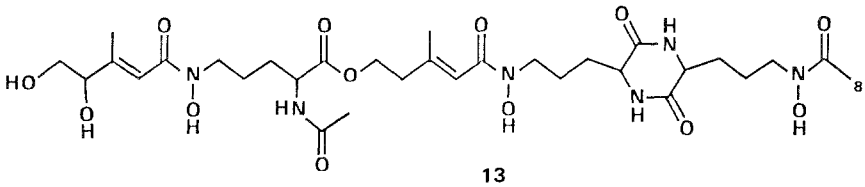

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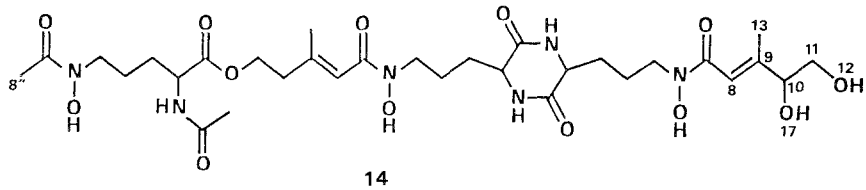

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Table 1. ^1H (300-MHz) chemical shifts of deferrihydroxycoprogen **(12)**, deferrihydroxyneocoprogen I **(13)** and deferrihydroxyisoneocoprogen I **(14)** in CD_3SO

Residue	^1H chemical shift, δ, in			Inventory of protons	Structural group	Position
	12	**13**	**14**			
Diketopiperazine ring	3.82 (m)	3.82 (m)	3.82 (m)	2	>CH—	2,2′
	8.14 (s)	8.14 (s)	8.14 (s)	2	—NH—	14,14′
Ornithyl residues	4.18 (m)	4.18 (m)	4.18 (m)	1	>CH—	2″
	8.27 (d, J=7.3 Hz)	8.27 (d, J=7.3 Hz)	8.27 (d, J=7.3 Hz)	1	—NH	14″
	1.60 (m)	1.60 (m)	1.60 (m)	12	—CH_2—CH_2—	3,3′,3″ 4,4′,4″
	3.50 (m)	3.50 (m)	3.50 (m)	6	—CH_2—	5,5′,5″
	9.68 (s)	9.70 (s)	9.75 (s)	3	—NOH—	6,6′,6″
N^{α}-Acetyl residue	1.84 (s)	1.84 (s)	1.84 (s)	3	—CH_3	16
Middle N^{δ}-anhydromevalonoyl residue	6.22 (s)	6.22 (s)	6.22 (s)	1	—CH=	8′
	2.39 (t, J=6.6 Hz)	2.39 (t, J=6.6 Hz)	2.39 (t, J=6.6 Hz)	2	—CH_2—	10′
	4.18 (m)	4.18 (m)	4.18 (m)	2	—CH_2O—	11′
	2.02 (s)	2.03 (s)	2.03 (s)	3	—CH_3	13′
Terminal N^{δ}-acyl residues	6.22 (s)		6.48 (s)	1	—CH=	8
	6.48 (s)	6.48 (s)		1	—CH=	8″
			3.92 (t)	1	—CH—	10
	3.92 (t)	3.92 (t)		1	—CH—	10″
	2.24 (t, J=6.7 Hz)			2	—CH_2—	10
	3.50 (m)		3.50 (m)	2	—CH_2—	11
	3.50 (m)	3.50 (m)		2	—CH_2—	11″
	4.56 (s)		4.64 (s)	1	—OH	12
	4.64 (s)	4.64 (s)		1	—OH	12″
			5.08 (s)	1	—OH	17
	5.08 (s)	5.08 (d)		1	—OH	17″
	2.02 (s)		1.95 (s)	3	—CH_3	13
	1.95 (s)	1.95 (s)		3	—CH_3	13″
		1.97 (s)		3	—CH_3	8
			1.97 (s)	3	—CH_3	8″

Table 2. ^{13}C (75.4-MHz) chemical shifts of deferrihydroxycoprogen **(12)**, deferrihydroxyneocoprogen I **(13)** and deferrihydroxyisoneocoprogen I **(14)** in CD_3SO

Residue	^{13}C chemical shift, δ, in			Inventory of carbon	Structural group	Position
	12	**13**	**14**			
Diketopiperazine	167.72	167.80	167.80	2	>C=O	1,1′
ring	53.84	53.74	53.74	2	>CH—	2,2′
Ornithyl	169.45	169.58	169.57	1	>C=O	1″
residues	52.01	51.94	51.94	1	>CH—	2″
	30.46	30.27	30.26	2	$-CH_2-$	3,3′
	28.10	27.97	27.98	1	$-CH_2-$	3″
	22.30	22.16	22.16	2	$-CH_2-$	4,4′
	23.19	23.05	23.07	1	$-CH_2-$	4″
	46.85	46.74	46.74	2	$-CH_2-$	5,5′
	46.51	46.42	46.40	1	$-CH_2-$	5″
N^{α}-Acetyl	171.92	172.05	172.04	1	>C=O	15
residue	22.22	22.04	22.05	1	$-CH_3$	16
Middle N^{δ}-anhydro-	166.15	166.20	166.20	1	>C=O	7′
mevalonoyl residue	117.10	117.10	117.19	1	—CH=	8′
	148.60	148.65	148.68	1	=C<	9′
	40.00[a]	40.00[a]	40.00[a]	1	$-CH_2-$	10′
	62.36	62.28	62.29	1	$-CH_2-$	11′
	18.13	17.98	17.99	1	$-CH_3$	13
Terminal N^{δ}-acyl	166.45	170.21	166.82	1	>C=O	7
residues	166.78	166.82	170.22	1	>C=O	7″
	116.21		114.89	1	—CH=	8
	114.89	114.89		1	—CH=	8″
	150.95		153.46	1	=C<	9
	153.45	153.22		1	=C<	9″
			76.57	1	—CH—	10
	76.61	76.59		1	—CH—	10″
	43.83			1	$-CH_2-$	10
	59.21		64.54	1	$-CH_2-$	11
	64.60	64.53		1	$-CH_2-$	11″
	18.35		14.81	1	$-CH_3$	13
	14.95	14.81		1	$-CH_3$	13″
		20.26		1	$-CH_3$	8
			20.25	1	$-CH_3$	8″

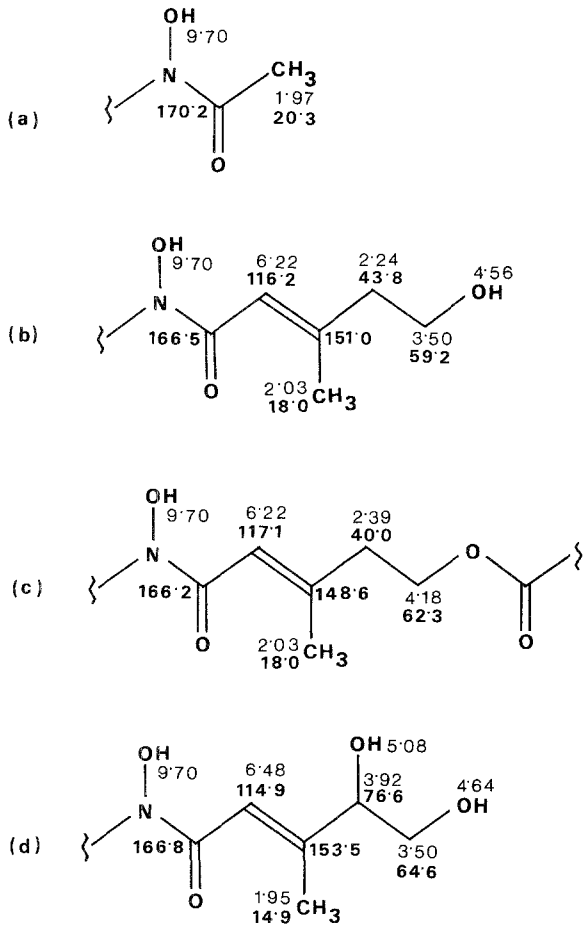
[a] Underneath solvent peak

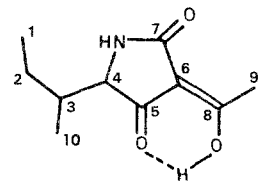
(CH, 3), 66.79 (CH, 4), 103.74 (=C—, 6), 178.59 (CO, 5), 194.60 (=CO, 7), 198.38 (=COH, 8). (b) FAB mass spectra of its deferri form [*m/z* 636.2885(55), calculated for $3M-H+2Na^+$, $C_{30}H_{44}N_3O_9Na_2$ 636.2873; 417 (100), $2M+Na^+$; 220.0945(82) MNa^+ (calculated for MNa^+, $C_{10}H_{15}NO_3Na$ 220.0950)] and its Fe(III) chelate [*m/z* 448 (100), $2M-2H+Fe^+$, $C_{20}H_{28}N_2O_6Fe$].

Structure of hydroxycoprogen **(8)**

Hydroxycoprogen **(8)** shows two distinct peaks in the FAB mass spectrum, at *m/z* 838 (100) and 860 (17), which correspond to the MH^+ and MNa^+ ions respectively. The molecular formula of **8** has been found to be $C_{35}H_{53}N_6O_{14}Fe$ on the basis of the accurate mass of the MH^+ ion (observed, 838.3062; calculated for MH^+, 838.3047). This molecular formula contains one oxygen atom more than that of coprogen **(1)**, which indicates that the compound is an hydroxylated form of coprogen. Similar to coprogen **(1)**, it is neutral in paper electrophoresis at pH 5.0 and 2.0 and its visible spectra (absorption maximum at 434 nm) is stable in the pH range 7.0–2.0. In thin-layer chromatography with silica gel/CMW, it moves slower ($R_F=0.36$) than coprogen ($R_F=0.52$), which is a further indication of its higher polarity.

The ^1H- and ^{13}C-NMR spectroscopy of its deferri derivative **(12)** shows that it is a close analog of coprogen, in which one of the N^{δ}-*trans*-anhydromevalonic acid residues is hydroxylated. The





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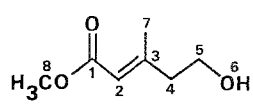

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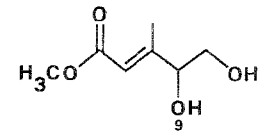

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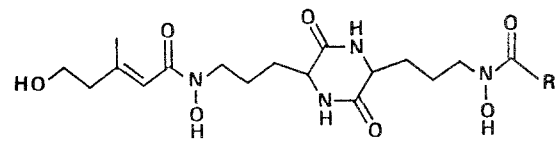
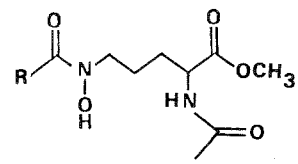


17 R = A
18 R = CH_3
19 R = B



20 R = B

^{1}H- and the ^{13}C-NMR spectra of deferri hydroxycoprogen and deferri coprogen are virtually identical except in the resonances of one of the terminal N^{δ}-acyl groups (Tables 1 and 2). In deferrihydroxycoprogen **(12)** the intensities of NMR signals arising from the terminal *trans*-5-hydroxy-3-methyl-2-pentenoyl (*trans*-anhydromevalonoyl) group are reduced to half compared to those of deferricoprogen **(1)** (Hossain et al. 1987), which indicates that the compound **(12)** contains this acyl group in only one end of the molecule. The new signals present in **12** show that its other terminal N^{δ}-acyl group is derived from *trans*-4,5-dihydroxy-3-methyl-2-pentenoid acid. The differences in the chemical shifts of ^{1}H and ^{13}C signals of this group and various other N^{δ}-acyl groups found in the coprogen-type siderophores are presented in Scheme 1.

Treatment of **12** with ammonia/methanol cleaves the ester bond and produces three compounds, one of which is chromatographically and spectroscopically identical to dimerum acid **(17)** (Jalal et al. 1986) [**17**, FAB *m/z* 507 (32) MNa^+, 529 (16) $M-H+2Na^+$; ^{1}H-NMR CD_3OD, δ/ppm = 1.8 (m, 8H, β and γ CH_2), 2.07 (s, 6H, CH_3), 2.37 (t, 4H, —CH_2—), 3.66 (m, 4H, δCH_2), 3.70 (t, 4H, —CH_2—), 4.01 (t, 2H, αCH), 6.32 (s, 2H, —CH=)]. The other two compounds are identified as the methyl ester and the free carboxylic acid forms of N^{α}-acetyl-*trans*-hydroxyfusarinine. The methyl ester form **(20)** produces an MH^+ ion peak at *m/z* 333 (99) and an MNa^+ peak at *m/z* 355 (65). Its ^{1}H-NMR spectrum is compatible with the structure shown [^{1}H NMR, CD_3OD, δ/ppm = 1.75 (m, 4H, β and γ CH_2), 1.99 (s, 6H, =CCH_3 and N^{α}-acetyl CH_3), 3.50, 3.63 (m, 2H, —CH_2O—) 3.65 (m, 2H, δ-CH_2), 3.70 (s, 3H, OCH_3), 4.09 (t, 1H, —CHO), 4.38 (t, 1H, αCH), 6.57 (s, 1H, =CH—)]. The free carboxylic acid form of **20** which runs slower on silica gel/CMW thin layers, shows peaks at *m/z* 340 (70) ($M-H+Na^+$), 341 (11) (MNa^+) and 362 (20) ($M-H+2Na^+$). Its ^{1}H-NMR spectrum is identical with that of **20,** except that the OCH_3 singlet at δ = 3.70 ppm is absent and the αCH triplet is slightly upfield (δ = 4.32 ppm). Based on these ob-

servations, hydroxycoprogen **(8)** has been assigned a structure in which *trans*-4,5-dihydroxy-3-methyl-2-pentenoyl residue is present in the ester-linked ornithine residue and the *trans*-anhydro-mevalonoyl moiety is present in the diketopiperazine end. Acid hydrolysis of a methanolic solution of hydroxycoprogen **(8)** releases the N^{δ}-acylating acids in their methyl ester forms **(15, 16).** The two compounds are separated by ether and ethyl acetate extraction, respectively, and purified by preparative thin-layer chromatography with silica gel/CMW system. Their identification is based on the mass and the ^{1}H-NMR spectra [compound **15,** *m/z* 145.0858 (52) $C_7H_{13}O_3$, MH^+; *m/z* 126 (74), $M^+ - H_2O$; ^{1}H NMR, $CDCl_3$, δ/ppm = 2.20 (s, 3 H, CH_3, 7), 2.30 (broad, 1 H, OH, 6), 2.41 (t, J = 6.3 Hz, 2 H, CH_2, 4), 3.69 (s, 3 H, OCH_3, 8), 3.79 (t, J = 6.3 Hz, 2 H, CH_2O, 5), 5.75 (s, 1 H, CH, 2); compound **16,** *m/z* 161.0731(19), $C_7H_{13}O_4$, MH^+; *m/z* 142 (38), $M^+ - H_2O$. ^{1}H-NMR $CDCl_3$, δ/ppm = 2.13 (s, 3 H, CH_3, 7), 2.60 (broad, OH, 6, 9), 3.55 (q, J = 7.1 and 11.3 Hz, 1 H) and 3.78 (q, J = 3.3 and 11.3 Hz, 1 H) (CH_2O, 5), 3.71 (s, 3 H, OCH_3, 8), 4.24 (q, J = 3.3 and 7.1 Hz, 1 H, CHO, 4), 6.06 (s, 1 H, =CH—, 2)]. *Trans*-4,5-dihydroxy-3-methyl pentenoic acid (free acid form of **16**) is a new acylating acid not reported from any other siderophore. The *trans*-4,5-dihydroxy-3-methyl-2-pentenoyl group contains a chiral center at position 4, but the absolute configuration of this position has not been determined.

Structure of hydroxyneocoprogen I **(9)** *and hydroxyisoneocoprogen I* **(10)**

These two compounds are found to be structural isomers of each other as they produce similar FAB mass spectra. Hydroxyneocoprogen I **(9)** produces two prominent peaks at 768 (100) and 790 (17) corresponding to the MH^+ and MNa^+ ions. The accurate mass of the MH^+ ion (observed 768.2506, calculated 768.2629) suggests that **9** has a molecular formula of $C_{31}H_{47}N_6O_{13}Fe$. The isomeric compound **10** also produces these MH^+ (100) and the MNa^+ (18) ions and have an identical accurate mass of the MH^+ ion (observed, 768.2565). Both of these compounds (**9** and **10**) contain one oxygen atom more than neocoprogen I **(2),** which indicates that they are hydroxylated forms of neocoprogen I. Similar to neocoprogen I and other trihydroxamate siderophores, these compounds have a visible absorption bands (maximum at 428 nm) stable in the pH range 2.0–7.0. In silica gel thin-layer chromatography with CMW as the solvent system, both compounds (**9** and **10**) run slower (R_F = 0.36) than neocoprogen I (R_F = 0.52). Although all three hydroxycoprogens **(8–10)** move together as a single band in silica gel column eluted with CMW, they separate from each other in the reserved-phase column and elute in the order hydroxyisoneocoprogen I **(10),** hydroxyneocoprogen I **(9)** and hydroxycoprogen **(8).**

The NMR spectra of the deferri derivatives (**13** and **14**) of **9** and **10** (Tables 1 and 2) show that these compounds are analogs of neocoprogen I **(2)** and its isomer **(3),** in which the terminal N^{δ}-*trans*-anhydro-mevalonoyl residue is replaced by an N^{δ}-*trans*-4,5-dihydroxy-3-methyl-2-pentenoyl group. The ^{1}H- and ^{13}C-NMR signals originating from the ornithyl residues and the middle N^{δ}-*trans*-anhydromevalonoyl group are common to these two novel compounds (**13** and **14**) and deferri neocoprogen I **(2)** (Hossain et al. 1987). The signals arising from the diketopiperazine ring, the N^{α}-acetyl group and the terminal N^{δ}-acetyl group are also present in the spectra of **13** and **14.** However, the ^{1}H- and ^{13}C-NMR signals of the terminal N^{δ}-*trans*-anhydromevalonoyl group of deferri neocoprogen I **(2)** are absent in both compounds and a new set of signals corresponding to the *trans*-4,5-dihydroxy-3-methyl-2-pentenoyl group (Scheme 1) is present.

Hydrolysis of the ester bond of compound **13** with ammonia/methanol produced the dihydroxamate **18**. The identity of compound **18** is established by FAB mass and ^{1}H-NMR spectroscopy [*m/z* 415 (30), MH^+; *m/z* 437 (15), MNa^+; ^{1}H-NMR, CD_3OD, δ/ppm = 1.80 (m, 8 H, β and γ CH_2), 2.07 (s, 3 H, =C—CH_3), 2.10 (s, 3 H, —CO—CH_3), 2.37 (t, 2 H, —CH_2—), 3.68 (m, 6 H, δ CH_2 and —CH_2O—), 4.01 (t, 2 H, α CH), 6.32 (s, 1 H, =CH—)]. This same compound is also produced from the hydrolysis of neocoprogen I. A second compound, chromatographically and spectroscopically identical to compound **20** is isolated from the alkaline methanolic hydrolysis of the compound **13**. The result of the partial hydrolysis suggests that the *trans*-4,5-dihydroxy-3-methyl-2-pentenoyl residue in compound **9** is present in the ester-linked ornithyl end. Hydrolysis of compound **14,** on the other hand, produces another dihydroxymate, which is identified as hydroxydimerum acid **(19)** [^{1}H-NMR, CD_3OD δ/ppm = 1.80 (m, 8 H, β and γ CH_2), 1.99 (s, 3 H, =CCH_3), 2.07 (s, 3 H, =CCH_3), 2.37 (t, 2 H, —CH_2—), 3.60 (m, 8 H, δ CH_2 and —CH_2O—), 4.01 (t, 2 H, α CH), 4.09 (t, 1 H, —CHO), 6.32 (s, 1 H, =CH—), 6.57 (s, 1 H, =CH—)]. Production

of this compound **(19)** from **14** indicates that the *trans*-4,5-dihydroxy-3-methyl-2-pentenoyl group is present in the diketopiperazine end in **10.** In summary, the chemical degradation and the spectral data suggest that compounds **9** and **10** are the hydroxylated forms of neocoprogen I **(2)** and isoneocoprogen I **(3)** respectively.

A. longipes ATCC 26293, which is known for its highly phytopathogenic nature (Stavely and Slana 1973) produces a large number of siderophores. Six of these are new coprogens, which have been previously unknown in nature. The biological significance of these siderophores, especially their role in the pathogenicity of the fungus, is yet to be determined. However, a remarkable ability of this fungus is that it biosynthesizes a variety of coprogens, some of which are more lipophillic (N^{α}-dimethylcoprogens) **(5–7)** than coprogen and neocoprogens **(1–3),** while the others are more hydrophilic (hydroxycoprogens) **(8–10).** The N^{α}-dimethylcoprogens modify the surface of the coprogen skeleton, especially the ester loop and also contribute a positive charge to the molecule, while the hydroxycoprogens modify the region occupied by the acyl groups surrounding the iron-chelation center. Based on the X-ray diffraction structure of neocoprogen I (Hossain et al. 1987), these modifications may be visualized. Further investigations involving the Fe(III) transport process may reveal the biological role of these structural modifications represented by the novel coprogens.

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