Spectroscopy 17 (2003) 725–734
IOS Press

New chaetoglobosins from maize infested by *Phomopsis leptostromiformis* fungi. Production, identification, and semi-synthesis

Ludovic Burlot [a], Jean-Claude Cherton [a,*], Odile Convert [b], Isabelle Correia [b] and Bruno Dennetiere [c]

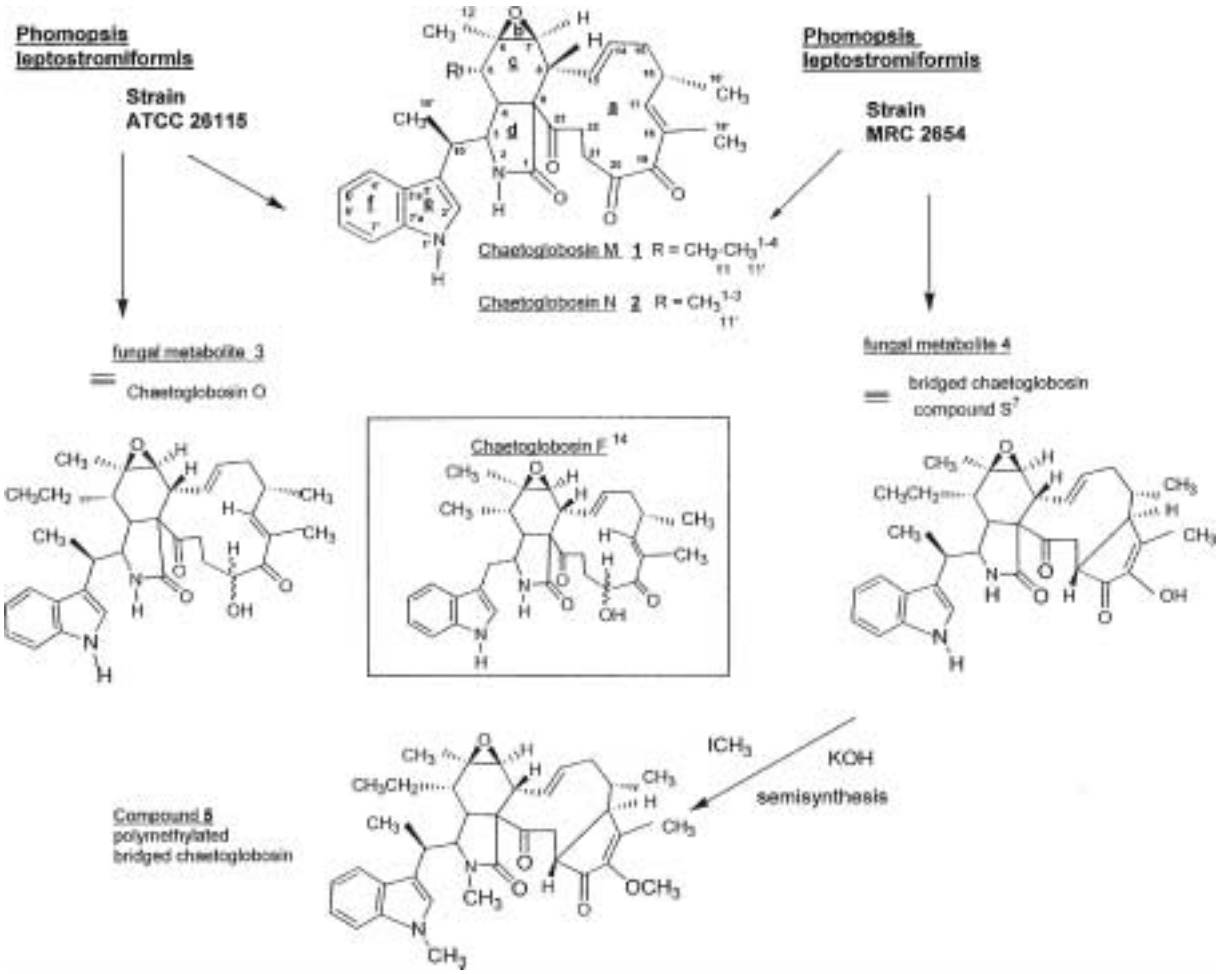
[a] *Laboratoire SIRCOB, Département de Chimie, Université de Versailles Saint-Quentin en Yvelines, 45 avenue des Etats-Unis, 78035 Versailles, France*
[b] *Université Pierre et Marie Curie, 4 place Jussieu, boite 45, 75252 Paris, Cedex 05, France*
[c] *Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, 12 rue Buffon, 75005, Paris, France*

Abstract. This paper deals with the cytotoxic chaetoglobosins obtained from culture on maize of the strains ATCC 26115 and MRC 2654 of the fungus *Phomopsis leptostromiformis*. Both strains produced, in more or less important amounts, the known chaetoglobosins M (**1**) and N (**2**). In addition, the new chaetoglobosin O (**3**), was isolated from the ATCC 26115 strain and characterized by HRMS and 2D NMR experiments. With the MRC 2654 strain, a fungal metabolite **4**, more polar than the chaetoglobosins M and N, was extracted and purified. Spectroscopic and chromatographic data of metabolite **4** led to its identification to the bridged chaetoglobosin named compound **S**, which previously has been obtained by intramolecular Michael reaction on the chaetoglobosin M. Moreover, a methylation under Williamson conditions of the chaetoglobosin **4** resulted in the formation of the trimethylated chaetoglobosin **5**.

1. Introduction

Recent investigations on maize incubated with MRC 2654 strain of *Phomopsis leptostromiformis* allowed the characterization in the methanolic extract of some chaetoglobosins which are fungal metabolites rather unexpected from such a fungus [2–4]. Thus, beside the chaetoglobosin M (**1**) (mp 185°C, amorphous powder [2–4]) previously described as an uncrystallized compound [5], the novel chaetoglobosin N (**2**) (mp 205°C, amorphous powder [2–4]) was formed (see Scheme 1). Both the chaetoglobosins M and N displayed acute toxicity to rats during *in vivo* assays and cytotoxicity towards rat liver cells [4]. This latter property is not surprising since chaetoglobosins represent an important sub-group of the cytochalasins, a family of toxins known for their inhibition of the cytoplasmic cleavage of animal cells [6]. In view of these properties, it was important to improve production of the chaetoglobosins by optimizing the culture conditions, either in liquid medium or on maize and lupin seeds [1,2,4,6] and to realize semi-synthesis [1,7] on the most abundant chaetoglobosin M. As a result of a progressive degeneracy of the MRC 2654 strain, the chaetoglobosin production was dramatically decreasing. Consequently, after a screening based on FABMS and HPLC monitorings, the ATCC 26115 strain of the same fungus *P. leptostromiformis* was selected as a second source for this production [1]. This report presents the scheme

*Corresponding author. Tel.: +331 39 25 44 54; Fax: +331 39 25 44 52; E-mail: cherton@chimie.uvsq.fr.



Scheme 1. Studied chaetoglobosins. Chaetoglobosins **1** to **4** are resulting from incubation of maize *by Phomopsis leptostromiformis* strains and chaetoglobosin **5** is obtained by semi-synthesis from **4**. The numbering for chaetoglobosins **1**–**5** and chaetoglobosin F [14] is that proposed by Binder and Tamm [12], and the relative configurations are assumed the same as that established by X-ray diffraction experiments [15,16] for chaetoglobosins K and L.

isolation–purification and the structure elucidation of the compounds obtained from both *P. leptostromiformis* strains or by semi-synthesis.

2. Experimental

2.1. General experimental procedures

Melting points were measured on a Reichert microscope. IR spectra were recorded in KBr disks on a IFS 25 Bruker infrared FT spectrophotometer in the transmittance mode, and UV spectra in methanol solution on a Hewlett-Packard HP 84-50 instrument. Positive ion (<Xe>, 200 μA, 8 keV, copper target) FABMS and HR-FABMS were obtained with a JEOL AX500 (EB geometry) mass spectrometer, at the "Laboratoire de spectrométrie de masse", Centre Régional Universitaire de Spectroscopie, Université

de Rouen or at the "Centre de Micro-analyses de Lyon". ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM500 spectrometer equipped with an Aspect 3000 computer and operating at 500 MHz for the proton and at 125 MHz for ^{13}C, or on a Bruker Avance DMX 500 spectrometer for the HMBC spectra. The solutions were obtained by dissolving 3–10 mg of the samples in 0.5 ml of CD_3OD or DMSO-d_6. ^{1}H and ^{13}C chemical shifts δ were reported in ppm by using the residual solvent signals as internal references (3.31 and 49 ppm in CD_3OD, 2.5 and 39.5 ppm in DMSO). Two-dimensional experiments were acquired using the standard Bruker software for 2D H–H COSY [8], TOCSY [9] and C–H COSY [10]. Successive TOCSY spectra were realized with different mixing times, in order to determine direct and long range correlations. HMBC [11] spectra using gradient pulses for selection were optimized for $^{n}J_{CH} = 7$–8 Hz. NOE measurements were performed by 1-D NOE difference spectra (one spectrum with irradiation and another one with irradiation off-resonance) by using an irradiation time of 800 ms and a relaxation delay of 3 s.

Analytical and semi-preparative HPLC were performed on a KONTRON equipment consisting of pump systems 422 and 420, a diode array detector 440 and a data system DS450-MT2/DAD. Injections were performed with a Rheodyne model 7725i valve with 20 or 100 μl loops. A C_{18} 300 Å Nucleosil column (5 μm, 150 mm $\times$ 4.6 mm i.d.) and a C_{18} 300 Å Nucleosil cartridge (5 μm, 10 mm $\times$ 3 mm i.d.), purchased from Interchim (Montluçon, France) were used for the RP analytical separations. For the semi-preparative experiments, C_4 300 Å columns (5 μm, 30 cm $\times$ 2 cm i.d.) and precolumns (5 μm, 5 cm $\times$ 2 cm i.d.) were used and purchased from Life Sciences International (Cergy-Pontoise, France). Preparative HPLC was performed as previously described [2] and Preparative TLC on precoated silica plates with concentration zone (Kieselgel 60 F 254 S, Merck Nogent sur Marne, France) by using petroleum ether : ethyl acetate (1 : 1, v/v) for the prepurification of the chaetoglobosins issued from both strains. Chromatographic solvents used for all the separations were HPLC grade and purchased from SDS (Solvent, Documentation, Synthèse, Vitry, France)

2.2. Organisms

Two strains of the fungus *Phomopsis leptostromiformis* were used. The strain MRC (Medical Research Council) 2654 was originally isolated in 1969 from a field outbreak of lupinosis in sheep from South Africa and was kindly provided by W.F.O. Marasas (Tygerbourg, South Africa). The strain ATCC 26115 was purchased from the American Type Culture Collection, Manasas, USA.

2.3. Production of the chaetoglobosins from maize inoculated by P. leptostomiformis

The maize seeds were distributed in 3 Erlenmeyers of 1 l [3 $\times$ (100 g in 80 ml of water)], sterilized at 120°C for 20 minutes, then incubated at 25°C for 4 weeks with the strains of *P. leptostomiformis*. The inoculum of fungus consisted in samples of mycelium (of 10 mm diameter) obtained from 10 days old PDA (potato-dextrose-agar) culture.

2.4. Isolation and purification of the chaetoglobosins

For both strains ATCC 26115 and MRC 2654 the isolation procedure started after 28 days of incubation at 25°C by a MeOH extraction of the ground seeds followed by precipitation of polar compounds with isopropylic ether and filtering, resulting in crude residues. From the ATCC 26115 strain this procedure led after TLC separation (silica, petroleum ether/ethyl acetate 1 : 1 v/v) to a crude mixture of chaetoglobosins M (**1**) and N (**2**) (24 mg, Rf = 0.55), and crude fungal metabolite **3** (12 mg, Rf = 0.35). After TLC (*vide*

supra) and semi-preparative HPLC (C_4, 300 Å packing, MeOH/H_2O 70 : 30 v/v) pure chaetoglobosins M (6 mg), N (3.6 mg) and O (**3**), (3.6 mg) were obtained. From the MRC 2654 strain, the same procedure led after preparative HPLC [2] (silica, petroleum ether/ethyl acetate 1 : 1 v/v) to a crude mixture (380 mg) of chaetoglobosins M (**1**) and N (**2**), and crude A_3 fraction (1 g). This fraction was purified by TLC (*vide supra*), yielding pure fungal metabolite **4** (15 mg, HPLC retention factor $k' = 1.2$, analytical column C_{18} 5 μm, 300 Å, H_2O/CH_3CN 30 : 70 v/v.

*Chaetoglobosin O (**3**)* $mp_{decomp} \sim 200°C$, UV(MeOH) λ_{max} (logε) 225 (2.83) 282 (2.27) nm; IR (KBr) ν_{max} 3335, 1693 (strong), 1633, 979 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-FABMS m/z $[M + H]^+$ 559.3166 (calculated for $C_{37}H_{46}N_2O_5$, 559.3171).

*Bridged chaetoglobosin (**4**) ≡ compound S [7]* $mp_{decomp} = 254°C$, UV (MeOH) λ_{max} (logε) 224 (2.81), 272 (2.37) nm; IR (KBr) ν_{max}: 3335, 3016, 2930, 1693, 1633, 1450, 979, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-FABMS, m/z $[M + H]^+$ 557.3016 (calculated for $C_{34}H_{40}N_2O_5$, 557.3015).

*2.5. Methylation of the bridged chaetoglobosin (**4**)*

100 mg of powdered KOH was added to an acetonitrile solution (10 ml) of compound 4 (10 mg) and ICH_3 (100 mg). The mixture was stirred for 3 hours at room temperature. Preparative TLC on silica allowed the isolation of 7 mg of pure compound **5**. Trimethylated chaetoglobosin (**5**): $m.p._{decomp} = 235°C$, UV (MeOH) λ_{max} (logε) 224 (2.78), 272 (2.28) nm; IR (KBr) ν_{max}: 2963, 2935, 1707, 1680, 1437, 1389, 1341, 1100, 978 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-FABMS, m/z $[M + H]^+$ 599.3463 (calculated for $C_{37}H_{46}N_2O_5$, 599.3484).

3. Results and discussion

3.1. Chaetoglobosins obtained from ATCC 26115 strain of Phomopsis leptostromiformis

The isolation–purification of the toxins produced in maize incubated with ATCC 26115 strain, involved the extraction of the ground seeds by MeOH, then a precipitation of a polar fraction by isopropylic ether and preparative TLC (on silica) followed by reversed-phase HPLC (C_4 300 Å packing [1]) for the less polar residue. The conditions for production of these fungal metabolites were optimized with monitoring by FAB-MS and HPLC. An incubation period of 28 days appeared to be a good compromise for a reasonable yield [1]. Three main products were isolated from the MeOH extract: chaetoglobosin M (**1**) (MW = 556; mp 185°C [2–4]), chaetoglobosin N (**2**) (MW = 542; mp 205°C [2–4]) and a new fungal metabolite **3** (MW = 558; mp $\sim$ 200° C), more polar than chaetoglobosins M and N. The low amount of **3** prevented a determination of elemental composition by combustion analysis, and positive-ion HRFABMS was used to establish the empirical formula of $C_{37}H_{46}N_2O_5$ (MW = 558). Given the size of the monocrystals it was not possible to record X-ray diffraction spectrum of this fungal metabolite. As for chaetoglobosin M, the IR spectrum exhibited absorption bands at 3333 and 1693 cm^{-1} for the NH and CO groups and at 1633 and 979 cm^{-1} for the trans ethylenic bonds present in the macrocycle **a** of the chaetoglobosin skeleton (cf. the numbering proposed by Binder and Tamm [12], Scheme 1). By using CD_3OD as NMR solvent a large dispersion in the ^1H signals of **3** was observed for the 39 non-exchangeable protons and a spectrum recorded in DMSO-d_6 revealed the presence of three additional labile protons. A combination of 2D H–H COSY [8] and TOCSY [9] spectra (see respectively Figs 1 and 2) allowed the determination of a series of correlations and to characterize typical fragments of a chaetoglobosin structure: indole ring

Table 1
^{1}H and ^{13}C[^{1}H] NMR data[a] for chaetoglobosins **1**, **3**–**5**

Ring[b]	Site[b]	^{1}H NMR				^{13}C NMR			
		1	**3**	**4**	**5**	**1**	**3**	**4**	**5**[c]
d	1					176.7	177.4	176.5	173.8
d	3	3.72 (d)	3.62 (d)	3.72 (d)	3.72 (bs)	60.1	60.1	59.6	66.5
d	4	2.60 (dd)	2.61 (dd)	2.91 (dd)	2.91 (dd)	48.1	48.3	47.0	44.4
d	9					64.7	65.6	69.0	69.5
	Me2				3.16				30.2
	10	3.08 (m)	2.87 (m)	2.98 (m)	3.53 (m)	37.8	38.9	38.0	32.4
	10′	1.35 (d)	1.35 (d)	1.34 (d)	1.36 (d)	18.9	19.2	18.3	19.6
c	5	1.41 (m)	1.29 (m)	1.40 (m)	1.53 (m)	45.9	46.1	46.1	46.1
c	6					58.3	58.9	58.5	58.3
c	7	2.63 (d)	2.68 (d)	2.83 (d)	2.70 (d)	62.1	63.1	61.8	61.5
c	8	2.19 (dd)	2.20 (dd)	2.19 (dd)	2.13 (dd)	50.6	49.6	51.9	52.6
	11	1.58 (m)	1.47 (m)	1.51 (m)	1.80 (m)	22.5	22.6	22.3	22.9
		1.09 (m)		1.12 (m)	1.60 (m)				
	11′	0.85 (t)	0.67 (t)	0.84 (t)	1.14 (t)	12.9	13.2	12.9	13.3
	12	1.27	1.24	1.25	1.28	19.4	19.8	19.4	18.9
a	13	6.15 (dd)	6.33 (dd)	6.12 (dd)	6.04 (dd)	128.2	130.0	129.9	130.0
a	14	5.07 (m)	5.25 (m)	5.16 (dd)	5.09 (ddd)	135.6	135.6	135.1	134.6
a	15	2.35 (m)	2.42 (m)	2.27 (m)	2.21 (m)	41.2	42.4	45.2	45.1
		1.87 (m)	2.06 (m)	1.88 (m)	1.80 (m)				
a	16	2.74 (m)	2.79 (m)	1.48 (m)	1.38 (m)	34.4	35.0	43.9	43.7
a	17	5.95 (d)	6.19 (d)	2.03	1.87 (d)	157.7	150.6	55.3	52.2
a	18					133.1	138.3	149.6	153.5
a	19					198.4	206.2	150.9	160.2
a	20		4.69 (m)			206.8	62.6	205.5	205.8
a	21	1.85 (m)	1.75 (m)	2.11 (m)	1.78 (m)	33.4	31.9	51.5	52.5
		3.03 (m)							
a	22	1.65 (m)	2.60 (m)	2.83 (m)	2.20 (m)	39.2	38.4	42.9	41.4
		2.47 (m)	2.03 (m)	0.84 (dd)	−0.40 (dd)				
a	23					209.7	211.2	212.8	212.1
	16′	1.00 (d)	1.04 (d)	1.00 (d)	0.93 (d)	19.6	20.6	21.9	21.8
	18′	1.75	1.78	1.97	1.92	10.3	12.8	16.9	17.3
	Me19′				3.85				59.0
	Me1′				3.91				33.1
e	2′	6.97	6.96	6.94	6.86	124.6	124.6	124.7	130.7
e	3′					115.8	117.2	115.8	112.9
e	3′a					128.7	128.9	128.5	129.5
e	7′a					137.6	137.3	137.9	137.7
f	4′	7.53 (d)	7.44 (d)	7.50 (d)	7.28 (d)	119.8	120.1	119.9	118.9
f	5′	7.06 (t)	7.01 (t)	6.98 (t)	6.93 (t)	120.4	120.5	120.1	120.0
f	6′	7.12 (t)	7.08 (t)	7.00 (t)	6.95 (t)	122.7	123.0	122.3	121.9
f	7′	7.32 (d)	7.33 (d)	7.24 (d)	7.16 (d)	112.4	113.2	112.7	111.1

[a] Values in (δ) ppm. Spectra were taken in MeOD-d_4. All signals corresponding to proton were observed as a singlet, unless otherwise stated (d), (bs), (m) and (t) correspond to doublet, broad singlet, multiplet and triplet, respectively.
[b] See the numbering adopted in Scheme 1.
[a] Only the methyl signals resulting from the methylation are labelled as Me in compound **5**, the other methyl being designed by the corresponding carbon number.

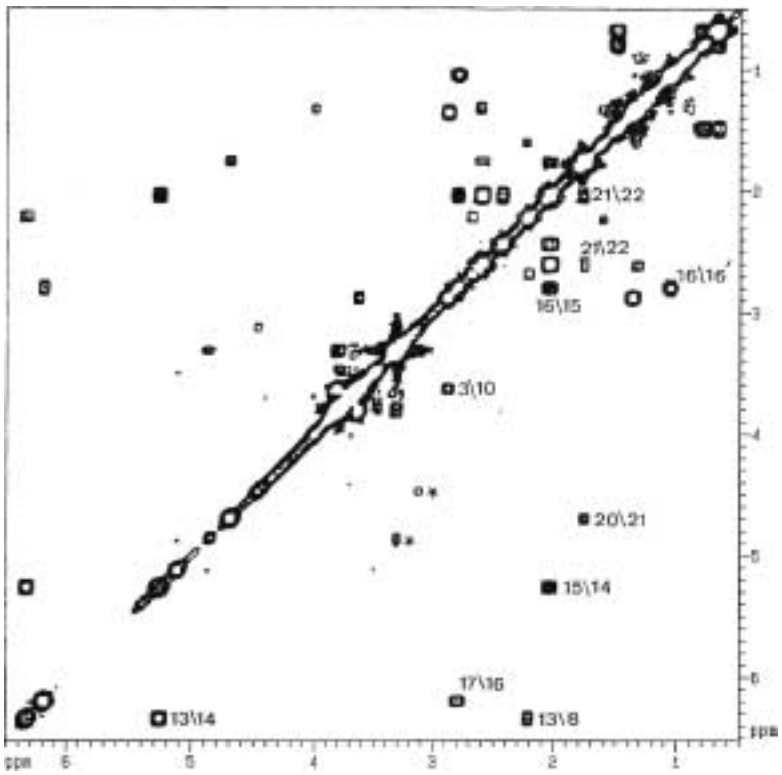


Figure 1. 2D NMR ^{1}H:^{1}H COSY 45 of fungal metabolite **3** = chaetoglobosin O (0.035 M CD_3OD solution, 500.13 MHz).

(rings **e** and **f**), rings **b**, **c** and **d**, north region of the macrocycle **a** (containing three ethylenic protons, cf. Scheme 1). Among the 34 ^{13}C signals of fungal metabolite **3**, assigned by the correlations in the C–H COSY [10] spectrum, the greatest part displayed chemical shifts similar to those observed for chaetoglobosin M. However, a comparison of the two spectra made evident the presence of one additional methine group at 62.6 ppm correlated to a proton at 4.69 ppm, at the same time as the disappearance of one carbonyl group. The chemical shift value of this methine group strongly suggested the presence of an alcohol group in the fungal metabolite **3** resulting from the reduction of one carbonyl group [C(19), C(20) or C(23)]. This hypothesis is consistent with the two units difference observed between HR-FABMS spectra of the two compounds. Three possible structures (**a** to **c** in Scheme 2) for the south region of the macrocycle may account for such a difference. The possibilities **a** and **b** were rejected on the basis of the ^{13}C carbonyl chemical shifts and of the multiplicity for the additional methine proton at 4.69 ppm: in hypothesis **b**, the proton H(19) should have shown only small allylic coupling constants as in chaetoglobosin A [13]. In fact, the multiplicity (doublet of doublets, 7.0 and 4.5 Hz) may be explained by the hypothesis **c** compatible with the large changes in C(17) and C(18) chemical shifts observed between compounds **1** and **3**.

So, a chaetoglobosin skeleton with an alcohol function in position C(20) of the macrocycle **a**, i.e. a structure very similar to the known chaetoglobosin F [14], has been proposed for the fungal metabolite **3** named chaetoglobosin O (cf. Scheme 1). The complete assignment of the ^{1}H and ^{13}C signals (cf. Table 1) is made comparatively with the chaetoglobosin M. The structure proposed for the chaetoglobosin O accounts for all the ^{13}C signals: particularly the signal at 62.6 ppm corresponds to the methine alcoholic

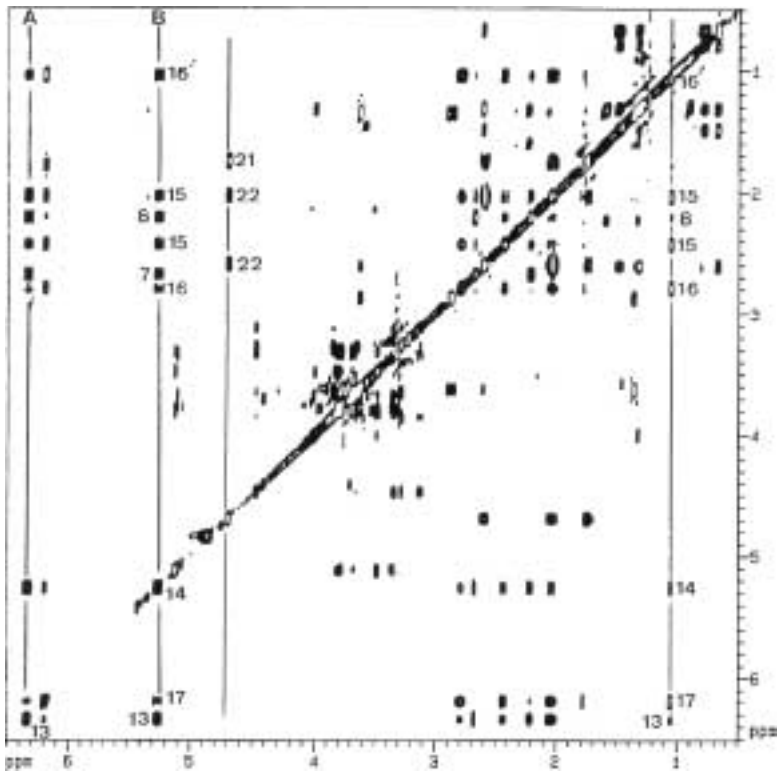
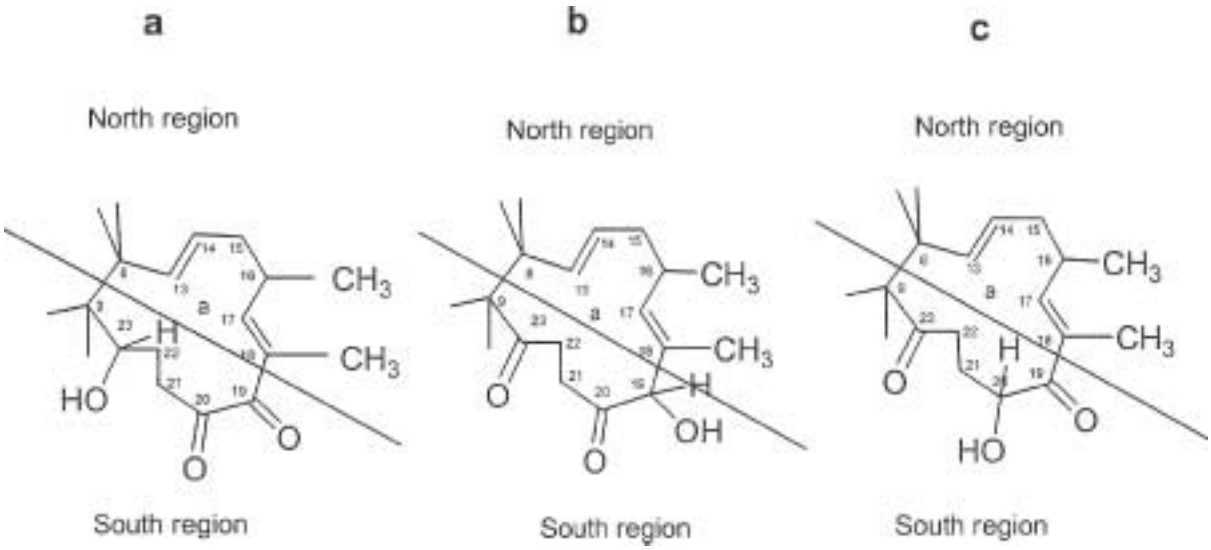


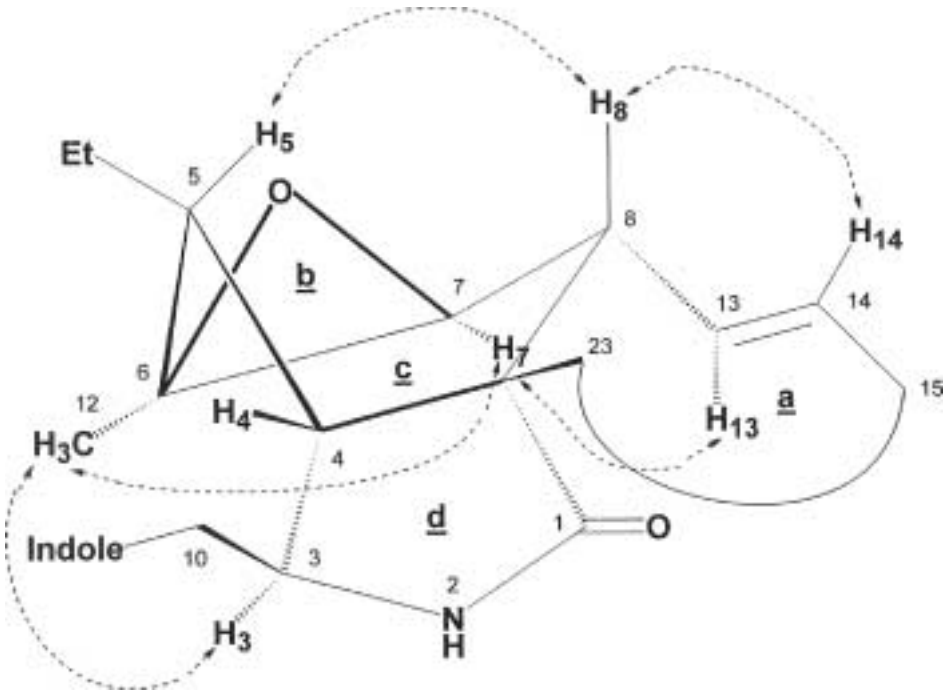
Figure 2. 2D NMR ^{1}H:^{1}H TOCSY of fungal metabolite **3** = chaetoglobosin O (0.035 M CD_3OD solution, 500.13 MHz).

group and those at 177.4, 206.2 and 211.2 ppm to the C(1) amide group, the conjugated C(19) and the non-conjugated C(23) carbonyl groups, respectively.

Taking into account the great analogy observed between most ^{1}H and ^{13}C chemical shifts of chaetoglobosin O and chaetoglobosin M (cf. Table 1) and also chaetoglobosin F [14] we assumed that the different rings of this new chaetoglobosin present the same relative configurations as depicted in the formulas in Scheme 1, i.e., those previously established by X-ray diffraction for the chaetoglobosins K and L [15,16]. In order to check this hypothesis nOe experiments were carried out on chaetoglobosin O. The strong nOe observed between H(5) and H(8) ascertains their 1–4 relation in a twisted boat conformation of the six-membered ring **c**. The expected cis junction of the epoxide ring **b** and ring **c** is evidenced by the nOe observed between H(7) and CH_3(12). The existence of a nOe between the same methyl and H(3) establishes a "trans" disposition of the ring **b** and the five-membered ring **d** relatively to the plane defined by the carbons (4), (6), (7) and (9) of the ring **c**. Consequently the rings **c** and **a** are trans fused with pseudo-equatorial junctions, while rings **c** and **d** are *cis* fused, as previously noticed with chaetoglobosin M [3] (see Scheme 3). Although some other nOes were detected between protons of macrocycle **a**, especially between H(20) and H(17), the C(20) stereochemistry was not made clear, that is also the case for chaetoglobosin F [14] (cf. Scheme 1). Concerning the stereochemistry of the C(10) and C(16) carbons of chaetoglobosin O, we adopt the relative configuration deduced from X-ray analysis on other members of the series [13a] as depicted in Scheme 1.



Scheme 2. Propositions of structures for the south region of the macrocycle **a** for fungal metabolite **3** (chaetoglobosin O). Hypothesis **a**: Alcohol function at C_{23} and $CHOH–CH_2–CH_2–CO–CO$ sequence. Hypothesis **b**: Alcohol function at C_{19}, $CO–CH_2–CH_2–CO–CHOH$ sequence, cf. chaetoglobosin A [13]. Hypothesis **c**: Alcohol function at C_{20} and $CO–CH_2–CH_2–CHOH–CO$ sequence, cf. chaetoglobosin F [14].



Scheme 3. nOe results on the chaetoglobosin O, ←- - -→ significant nOe.

3.2. Chaetoglobosins obtained from ATCC 26115 strain of Phomopsis leptostromiformis

A similar extraction-precipitation was used (*vide supra*) for the fungal metabolites produced by the MRC 2654 strain. The resulting residue contained, besides the crude fractions of chaetoglobosins M and N previously named A_4 and A_4' fractions, a more polar fraction [2] named fraction A_3, the toxicity of which to rats was approximatively half that of A_4 [2]. In the present work, the fraction A_3 purification was carried out by successive TLC on silica and resulted in the isolation of pure fungal metabolite **4**. FABMS spectra indicated for this compound a molecular weight of 556 Da, suggesting an isomeric relationship with the chaetoglobosin M (**1**). ^{1}H and ^{13}C NMR spectra as well as melting points and RP-HPLC retention times demonstrated that fungal metabolite **4** corresponds to a bridged chaetoglobosin named compound S [7] and previously described as a semi-synthetic product obtained from chaetoglobosin M. Metabolite **4** is characterized by the existence of a bridge between the carbons C(17) and C(21) in the macrocycle **a** (cf. Scheme 1). It would be interesting to determine the production mode of this bridged chaetoglobosin **4**, either directly by the fungus or *via* chaetoglobosin M (**1**) as an intermediate.

In the perspective of a structure/activity relationship study on chaetoglobosins, a modification of the chaetoglobosin **4** by methylation was realized. This reaction was carried out under Williamson methylation conditions. Analytical RP-HPLC made evident the rapid conversion of chaetoglobosin **4** to a more lipophilic compound **5**, purified by preparative TLC on silica. FABMS and HR-FABMS spectra indicated for **5** a molecular weight of 598 Da and an empirical formula of $C_{37}H_{46}N_2O_5$. The molecular weight difference observed between compounds **4** and **5** implicates the simultaneous methylation of the three possible sites: enol, indole and cyclic amido group. This is consistent with the absence of hydroxyl absorption bands around 3400 cm^{-1} in IR spectrum and the observation of new methyl signals at 3.85, 3.91 and 3.16 ppm in ^{1}H, 59.0, 33.1 and 30.2 ppm in ^{13}C spectra. The complete assignment of the ^{1}H and ^{13}C signals (cf. Table 1) for compound **5** was established by comparison with the bridged chaetoglobosin **4** and based for the new ^{13}C signals [Me(1′), Me(**2**) and Me(19′)], on the known chemical shift difference between NCH_3 and OCH_3 groups [17], and on the long range correlations observed in the HMBC spectra [11]. To notice is the spectacular non-equivalence existing between the chemical shift of the geminal protons (22) of compound **5**, one of which being dramatically shielded. With compound **4**, such a non-equivalence (see Table 1) has been previously explained on the basis of molecular modeling, by the hypothesis of double bond anisotropy effects exercised by C(13)=C(14) and/or the C(23)=O [7].

4. Conclusion

The ATCC 26115 strain of *Phomopsis leptostromiformis* produces in moderate yields the known chaetoglobosins M and N [2–4] as well as a new one, the chaetoglobosin O, related to chaetoglobosin F [14]. The production of chaetoglobosins M and N is approximately 15-fold more important from the MRC 2654 strain. This last strain also produces a small amount of the bridged chaetoglobosin **4**, previously described as the compound S [7] obtained synthetically by intramolecular reaction from chaetoglobosin M.

Acknowledgement

We thank Pr. W.F.O. Marasas (Tygerbourg, South Africa) for the gift of a sample of the strain MRC 2654 *Phomopsis leptostromiformis*.

References

[1] L. Burlot, Nouvelles chaetoglobosines produites naturellement par culture sur maïs de différentes souches du champignon *Phomopsis leptostromiformis* ou par hémisynthèse. Isolement et étude structurale, Ph.D. Thesis, Versailles University, 1998.

[2] J.-C. Cherton, A. Jellal, G. Lhommet, C. Loutelier, F. Dardoize, L. Lacoste and C. Subilleau, *Analusis* **22** (1994), 210–216.

[3] O. Convert, A. Jellal, I. Correia, F. Dardoize, L. Menguy and J.-C. Cherton, *Analusis* **22** (1994), 217–221.

[4] A. Jellal, Production, isolement et purification de deux chaetoglobosines produites par le champignon *Phomopsis leptostromiformis*. Etudes structurale et toxicologique, Ph.D. Thesis, Paris VII University, 1993.

[5] C. Spondolin and C. Tamm, *Helv. Chim. Acta* **71** (1998), 1881–1884.

[6] (a) M. Umeda, K. Ohtsubo, M. Saito, S. Sekita, Y. Yoshihira, S. Natori, S. Udagawa, F. Sakabe and H. Kurata, *Experientia* **31** (1975), 435–438; (b) T. Hirose, Y. Izawa, K. Koyama, S. Natori, K. Iida, I. Yahara, S. Shimaoka and K. Maruyama, *Chem. Pharm. Bull*. **38** (1990), 971–974; (c) J.P. Springer, J. Clardy, J.M. Wells, R.J. Cole, J.W. Kirksey, R.D. Macfarlane and D.F. Torgerson, *Tetrahedron Lett.* **17** (1976), 1355–1358.

[7] L. Burlot, J.-C. Cherton, O. Convert, C. Larpent, B. Dennetiere and F. Dardoize, *Magn. Resonance in Chemistry* **34** (1996), 538–544.

[8] A. Bax and R. Freeman, *J. Magn. Reson.* **44** (1981), 542–561.

[9] A. Bax and D.G. Davis, *J. Magn. Reson.* **65** (1985), 355–360.

[10] H. Friebolin, *Basic One and Two-Dimensional NMR Spectroscopy*, VCH New York, 1991, Chapter 9, pp. 218–250.

[11] A. Bax and D. Marion, *J. Magn. Reson.* **78** (1988), 186–191.

[12] M. Binder and C. Tamm, *Angew Chem. Internat. Ed*. **12** (1973), 370–380.

[13] (a) J.V. Silverton, T. Akiyama, C. Kabuto, S. Sekita, K. Yoshihira and S. Natori, *Tetrahedron Lett.* **17** (1976), 1349–1350; (b) H. Oikawa, Y. Murakami and A. Ichiara, *Tetrahedron Lett.* **32** (1991), 4533–4536; (c) H. Oikawa, Y. Murakami and A. Ichiara, *J. Chem. Soc. Perkin Trans. I.* (1992), 2949–2953.

[14] (a) S. Sekita, K. Yoshihira, S. Natori and H. Kuwano, *Tetrahedron Lett.* (1973), 2109–2112. (b) S. Sekita, K. Yoshihira and S. Natori, *Tetrahedron Lett.* (1976), 1351–1354.

[15] (a) H.G. Cutler, F.G. Crumley, R.H. Cox, R.J. Cole, J.W. Dorner, J.P. Springer, F.M. Latterell, J.E. Tehan and A.E. Rossi, *Agric. Food Chem.* **28** (1980), 139–142; (b) J.P. Springer, R.H. Cox, G.H. Cutler and F.G. Crumley, *Tetrahedron Lett.* **21** (1980), 1905–1908.

[16] C. Spondolin and C. Tamm, *Heterocycles* **28** (1989), 453–465.

[17] E. Breitmaier and W. Voelter, *Carbon-13 NMR Spectroscopy. High-Resolution Methods and Applications in Organic Chemistry and in Biochemistry*, 3rd edn, VCH New York, 1987, Chapter 4, pp. 282–284.