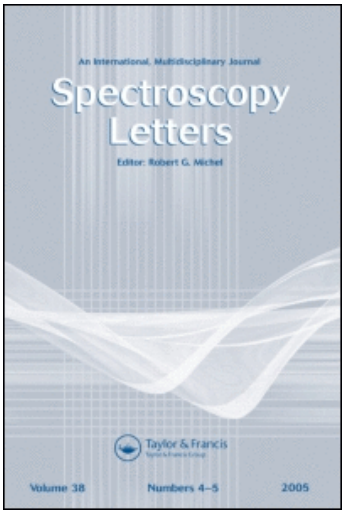


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Acrodontiolamide, a Chlorinated Fungal Metabolite from *Acrodontium salmoneum*

N. Buarque de Gusmáo[a]; M. Kaouadji[b]; F. Seigle-Murandi[a]; R. Steiman[a]; F. Thomasson[c]
[a] GEDEXE, Laboratoire de Botanique, Cryptogamie, Biologie Cellulaire et Génétique, UFR de Pharmacie, Université Joseph Fourier-Grenoble l, Meylan Cédex, France [b] Laboratoire de Pharmacognosie, UFR de Pharmacie, Université Joseph Fourier-Grenoble l, Domaine de La Merci, La Tronche Cédex, France [c] Service Commun de RMN, UFR de Pharmacie, Université Joseph Fourier-Grenoble l, Domaine de La Merci, La Tronche Cédex, France

To cite this Article de Gusmáo, N. Buarque , Kaouadji, M. , Seigle-Murandi, F. , Steiman, R. and Thomasson, F.(1993) 'Acrodontiolamide, a Chlorinated Fungal Metabolite from *Acrodontium salmoneum*', Spectroscopy Letters, 26: 8, 1373 — 1380
To link to this Article: DOI: 10.1080/00387019308011616
URL: http://dx.doi.org/10.1080/00387019308011616

SPECTROSCOPY LETTERS, 26(8), 1373-1380 (1993)

ACRODONTIOLAMIDE, A CHLORINATED FUNGAL METABOLITE FROM *ACRODONTIUM SALMONEUM*

Key Words : *Acrodontium salmoneum*, Mucedinaceae, Hyphomycetes, Moniliales, acrodontiolamide, 3-(*p*-nitrophenyl)-3-hydroxy-4-dichloromethoxy-isobutanamide, NMR, MS.

N. Buarque de Gusmão,[a] M. Kaouadji,*[b] F. Seigle-Murandi,*[a] R. Steiman,[a] and F. Thomasson[c]

[a] GEDEXE, Laboratoire de Botanique, Cryptogamie, Biologie Cellulaire et Génétique, UFR de Pharmacie, Université Joseph Fourier-Grenoble I, BP 138, 38243 Meylan Cédex, France

[b] Laboratoire de Pharmacognosie, UFR de Pharmacie, Université Joseph Fourier-Grenoble I, Domaine de La Merci, 38706 La Tronche Cédex, France

[c] Service Commun de RMN, UFR de Pharmacie, Université Joseph Fourier-Grenoble I, Domaine de La Merci, 38706 La Tronche Cédex, France

ABSTRACT

Acrodontiolamide, a new fungal metabolite has been isolated from the cell-free culture medium of *Acrodontium salmoneum*. Its purification was achieved by centrifugal TLC on silica gel and the structural analysis was based on spectroscopic data relative to both the natural product and its diacetyl derivative. This secondary metabolite, identified with 3-(*p*-nitrophenyl)-3-hydroxy-4-dichloromethoxy-isobutanamide exhibited antifungal properties.

INTRODUCTION

As a part of a program directed toward the isolation of biologically active metabolites from fungi, we previously selected fungal strains producing antifungal compounds without production of patulin and devoid of cytotoxicity[1, 2]. Gliotoxin was isolated from *Dichotomomyces cejpii* (Mil'ko) Scott and reported with corrections to previously ^{1}H- and ^{13}C NMR assignments[3, 4]. More recently, an unusual polyketide named Coniothyriol was isolated from the broth of *Coniothyriol sporulosum* (W. Gams & Domsch) van der Aa[5]. We have here investigated upon *Acrodontium salmoneum* de Hoog (class Hyphomycetes, order Moniliales, family Mucedinaceæ). This species was not previously reported to be a producer of known secondary metabolites. It was selected according to the strong antifungal activity exhibited by crude culture medium extracts. Fractionation of the ethyl acetate soluble portion of *A. salmoneum* broth by centrifugal TLC on silica gel led to the isolation of the major compound responsible of the biological activity. Named acrodontiolamide, this natural product was a nitro compound characterized by a C_6-C_4 structure as well as by chlorination. This paper deals with the isolation and the structure elucidation of the investigated isolate not previously described in the literature.

MATERIAL AND METHODS

Fungal source and culture conditions : Metabolites were extracted from the culture broth of a strain of Micromycetes obtained from our laboratory collection (CMPG : Collection Mycologie Pharmacie Grenoble). The strain was isolated from a grotto soil[6] and identified as *Acrodontium salmoneum*. It was recorded as CMPG 280 and maintained on solid malt extract medium (1.5 %) at 4°C. Subcultures were grown on solid malt extract medium for a week at 24°C to provide sufficient inoculum. Culture plates were scrapped and inoculated in 3 L of liquid yeast extract (2%) saccharose (4%) medium, pH 6.5 in a 8 L fermentor. The strain was grown for 5 days at 24°C with aeration (1 L/L/min).

Isolation of acrodontiolamide : The mycelium of the fungal strain was removed by filtration at the end of the growth period and the metabolites in the filtrate were extracted with ethyl acetate (3 x 3.5 L) at room temperature. The crude extracts of two fermentor broths were pooled, dried over anhydrous sodium sulfate and evaporated to dryness at 40°C under reduced pressure. This procedure afforded a brown viscous mass (2 g). A portion (0.75 g) of this residue was dissolved in $CHCl_3$-MeOH = 1:1 (0.5 mL) and then subjected to fractionation by centrifugal TLC on silica gel (layer thickness 2 mm) developed successively with $CHCl_3$ (20 mL) and the mixture $CHCl_3$-acetone-MeOH = 85:10:15 (100 mL). This procedure yielded 340 mg of pure acrodontiolamide in the middle fraction.

Analysis : Acrodontiolamide (**1**) : Colourless plates from MeOH, mp 145-147°C ; Rf 0.34 on silica gel TLC in $CHCl_3$-acetone-MeOH = 85:10:15 ; UV (MeOH) λ_{max} (ε) : 273 (386 000) nm ; IR (KBr) ν_{max} : 3500 (s), 3350 (s), 3250 (s), 3080 (w), 1695 (s), 1620 (w), 1570 (m), 1530 (s), *1420 (m), 1360 (s), 1260 (m), 1080 (s), 835 (s), 660 (s)* cm^{-1} ; EIMS [*m/z* (% rel. int.)] : 174 (4), 172 (24), 170 $[M\text{-}O_2NArCHOH]^+$ (34), 157 (7), 155 (40), 153 $[M\text{-}H_2NCOCHCH_2OCHCl_2+H]^+$ (100), 136 (27), 118 (22), 106 (24), 93 (23), 83 $[CHCl_2]^+$ (29), 85 (19), 87 (3), 77 (41), 70 (43), 65 (12), 60 (44) ; CIMS [*m/z* (% rel. int.)] : 344 (5), 342 (36), 340 $[M+NH_4]^+$ (52), 327 (15), 325 (73), 323 $[M+H]^+$ (100), 309 (5), 307 (24), 305 $[M+H\text{-}H_2O]^+$ (36), 289 $[M+H\text{-}Cl+H]^+$ (21), 275 (15), 200 (33), 174 (4), 172 (14), 170 (23), 157 (5), 155 (24), 153 (35), 135 (47), 122 (58), 117 (22), 107 (21), 93 (34), 77 (13), 65 (9), 60 (71) ; ^1H NMR (Table 1) ; ^{13}C NMR (Table 2). Acrodontiolamide diacetate (**2**) : Rf 0.24 on silica gel in toluene-MeOH = 97:3 ; EIMS [*m/z* (% rel. int.)] : 216 (4), 214 (19), 212 $[M\text{-}O_2NArCHOAc]^+$ (26), 195 $[O_2NArCHOAc+H]^+$ (19), 174 (4), 172 (17), 170 $[M\text{-}O_2NArCHOAc\text{-}Ac]^+$ (23), 153 $[O_2NArCHOH+H]^+$ (44), 135 (11), 118 (15), 106 (10), 89 (9), 87 (2), 85 (11), 83 $[CHCl_2]^+$ (16), 77 (19), 65 (13), 60 (21), 43 (100) ; CIMS [*m/z* (% rel. int.)] : 428 (18), 426 (76), 424 $[M+NH_4]^+$ (100), 411 (3), 409 (11), 407 $[M+H]^+$ (17), 351 (10), 349 (40), 347 $[M+H\text{-}AcOH]^+$ (57), 321 (4), 319 (16), 317 (20), 261 (5), 259 (18),

Table 1. ^{1}H NMR spectra (δ ppm, 200 MHz) of acrodontiolamide (**1**) and its diacetate **2**.

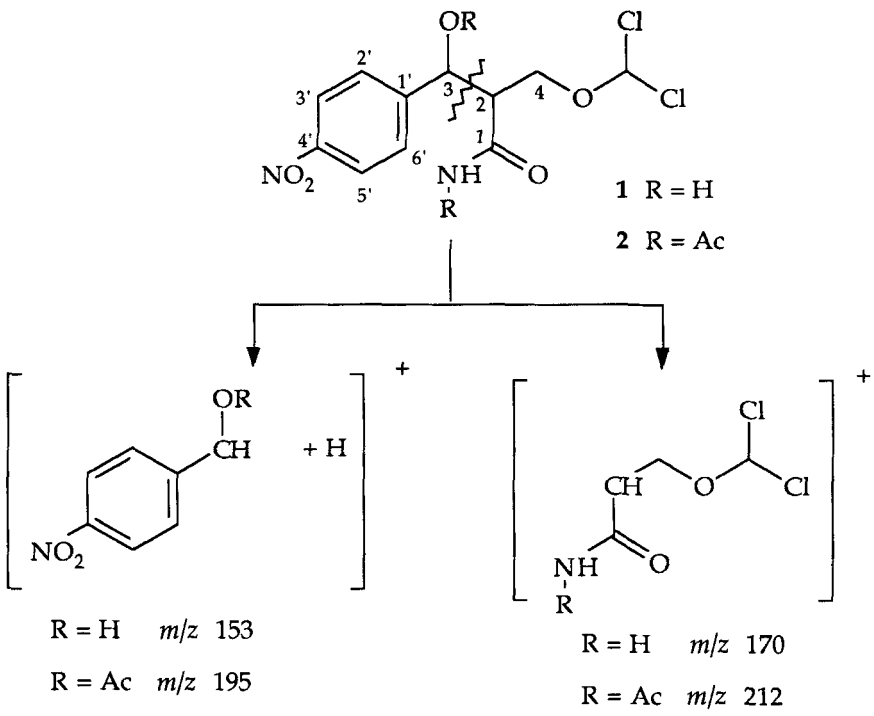
H	1[a]	2[b]
2	4.20 br m	4.61 m
3	5.31 br d J = 2.4 Hz	6.06 br d J = 5.6 Hz
4_A	3.83 dd J = 10.8 and 6.8 Hz	4.04 dd J = 11.6 and 6.3 Hz
4_B	3.71 dd J = 10.8 and 5.7 Hz	4.17 dd J = 11.6 and 5.2 Hz
2',6'	7.68 br d J = 8.8 Hz	7.52 d J = 8.8 Hz
3',5'	8.15 d J = 8.8 Hz	8.20 d J = 8.8 Hz
Cl_2CHO-4	6.35 s	5.86 s
H_2N-1	5.10 br s	
HO-3	7.62 s	
HNAc		6.87 s
		2.06[c] s
OAc		2.15[c] s

[a]In acetone-d_6.
[b]In $CDCl_3$.
[c]Assignments may be reversed.

Table 2. ^{13}C NMR data (δ ppm, 50 MHz) of acrodontiolamide (**1**) and its diacetate **2**.

C	1[a]	2[b]	C	1[a]	2[b]
1	164.7	164.1	3',5'	123.7	123.9
2	57.7	52.6	4'	147.8	148.0
3	71.7	72.6	Cl_2CHO-4	67.3	65.9
4	62.1	62.1	HNAc		170.4[c]
1'	151.0	143.0			20.5[d]
2',6'	127.9	127.5	OAc		169.5[c]
					20.7[d]

[a]In acetone-d_6.
[b]In $CDCl_3$.
[c, d]Assignments may need interchanging.



Scheme 1

257 (24), 216 (9), 214 (46), 212 [AcNHCOCHCH$_2$OCHCl$_2$]$^+$ (67), 195 [O$_2$NArCHOAc+H]$^+$ (24), 178 (10), 170 (7), 164 (13), 152 (39), 135 (47), 122 (27), 102 (17), 60 (18), 43 (42) ; ^{1}H NMR (Table 1) ; ^{13}C NMR (Table 2).

RESULTS AND DISCUSSION

The CI mass spectrum of acrodontiolamide (**1**) showed peaks at *m/z* 340 [M+NH$_4$]$^+$ and *m/z* 323 [M+H]$^+$ consistent with a molecular formula $C_{11}H_{12}N_2O_5Cl_2$. The presence of two chlorine atoms was confirmed by the isotope pattern of the above mentioned ions and those at *m/z* 305 [M+H-H$_2$O]$^+$ and *m/z* 170 (Scheme 1) as well as by the strong IR band at 660 cm^{-1} [7].

Treatment of the related compound with Ac_2O in pyridine gave a diacetate which CI mass spectrum also exhibited two peaks corresponding to quasi-molecular ions at *m/z* 424 $[M+NH_4]^+$ and *m/z* 407 $[M+H]^+$. The 200 MHz ^1H NMR spectrum of **1** in acetone-d_6 (Table 1) revealed a *p*-disubstituted aromatic ring (4H), an aliphatic C_3 chain (5H) and an isolated H as well as three exchangeable protons [δ 7.62 (1H) and δ 5.10 (2H)] in CD_3OD. The *p*-disubstituted aromatic nucleus was evidenced by two *ortho*-doublets (J = 8.8 Hz) integrating for two protons each at δ 8.15 (H-3',5') and δ 7.68 (H-2',6'). The two substituents were identified respectively with a nitro group and a lowfield methine (δ 5.31, H-3) belonging to the alkyl C_3 chain. The conjugated nitro function responsible of the strong IR absorptions at 1530 and 1360 cm^{-1} [7] caused both deshielding of the *ortho*-protons (δ 8.15) and shielding of the corresponding carbons (δ 123.7)[8, 9]. Parallely, the peak broadening affecting H-2',6' (δ 7.68) and H-3 (δ 5.31) was due to a four bond coupling[10, 11]. This result was confirmed by the ^1H-^1H shift correlation spectra of both the natural product and the acetylated derivative showing a cross peak between the above related protons. Acetylation induced the lowfield shift for H-3 (Δδ +0.75 ppm) pointing thus an hydroxy group at this position and, as expected, the upfield shift of the aromatic quaternary carbon C-1' at the α position (Δδ -8.0 ppm) (Table 2)[8, 9]. In this molecule, the remaining two C of the C_3 chain corresponded respectively to a methine group characterized by a complex multiplet at δ 4.20 (H-2) and to a methylene oxy bearing two non equivalent protons (J_{gem} = 10.8 Hz) appearing as double doublets at δ 3.83 ($H\text{-}4_A$) and δ 3.71 ($H\text{-}4_B$)[7]. A primary amide function was indicated by three IR bands at 3350 and 3250 cm^{-1} (N-H stretching) and 1695 cm^{-1} (CO stretching) as well as by the medium absorption at 1570 cm^{-1} (N-H bending)[7]. Consequent to the cross peak between HO-3 (δ 7.62) and the carbonyl (δ 164.7) in the long range ^1H-^{13}C shift correlation 2D NMR spectrum, the amide group must be linked to C-2 to give rise to a 3,3,4-trisubstituted isobutanamide. Finally, the 4-*O*-substituent of the C_4 chain was defined as a methine (δ 67.3) which lowfield isolated proton appearing as a sharp singlet (δ 6.35) agreed with dichlorination of the related C atom. This analysis was confirmed in the mass spectra of both

the natural product and the acetyl derivative by the isotope pattern of the $HCCl_2^+$ ion at *m/z* 83, 85, and 87 in the approximate ratio 9:6:1[7]. Thus acrodontiolamide was identified with 3-(*p*-nitrophenyl)-3-hydroxy-4-dichloromethoxy-isobutanamide, a new fungal metabolite.

It is interesting to note that, as previously reported for other fungal secondary metabolites, acrodontiolamide presented a nitro function attached to an aromatic ring[12,13,14]. In the opposite, chlorination which usually affected the aromatic part of the molecule[12,15,16] was situated here on the aliphatic moiety. Such halogenation seems to be rare, since only one report described fifteen years ago an isocoumarin monochlorinated on its aliphatic C_3 chain[17].

ACKNOWLEDGEMENT

The authors are indebted to Dr. J. Ulrich (IBS-SMP, Grenoble) for running the mass spectra.

REFERENCES

1 R. Steiman, M.H. Bartoli, F. Seigle-Murandi, M. Boitard, H. Bériel, and J.Villard (1989) Antibacterial, antifungal and antitumoral activities of Micromycetes. I. Preliminary studies. *Microbiologica*, **12**, 307-316.

2 R. Steiman, F. Seigle-Murandi, L. Sage, and S. Krivobok (1989) Production of patulin by Micromycetes. *Mycopathologia*, **105**, 129-133.

3 M. Kaouadji, R. Steiman, F. Seigle-Murandi, S. Krivobok, and L. Sage (1990) Gliotoxin : uncommon ^1H couplings and revised ^1H- and ^{13}C-NMR assignments. *Journal of Natural Products*, **53**, 717-719.

4 R.J. Cole and R.H. Cox (1981) *Handbook of Toxic Fungal Metabolites*, Academic Press, New York.

5 N. Buarque de Gusmão, M. Kaouadji, R. Steiman, F. Seigle-Murandi and J. Ulrich (1993) Coniothyriol, an uncommon polyketide from *Coniothyrium sporulosum*. *Natural Products Letters* (submitted).

6 F. Seigle-Murandi, J. Nicot, L. Sorin and L. C. Genest (1980) Association mycologique dans la Salle de la Verna et le tunnel de l'EDF du réseau de la Pierre Saint Martin. *Revue d'Ecologie et Biologie du Sol*, **17**, 149-157.

7 I. Williams and D. H. Fleming (1973) *Spectroscopic Methods in Organic Chemistry*, McGraw-Hill, New York.

8 J. B. Stothers (1972) *Carbon-13 NMR Spectroscopy, a Series of Monographs*, Academic Press, New York.

9 E. Breitmaier and W. Voelter (1990) *Carbon-13 NMR spectroscopy*, VCH, Weinheim.

10 L. N. Lundgren and O. Theander (1987) *Cis*- and *trans*-dihydroquercetin glucosides from needles of *Pinus sylvestris*. *Phytochemistry*, **27**, 829-832.

11 A. Simon, A. J. Chulia, M. Kaouadji, D. P. Allais and C. Delage (1993) Further flavonoid glycosides from *Calluna vulgaris*. *Phytochemistry*, **32**, 1045-1049.

12 W.B. Turner and D.C. Alridge (1983) *Fungal Metabolites*, Academic Press, New York.

13 D. Butruille and X.A. Dominguez (1972) Un nouveau produit naturel : dimethoxy-1,4 nitro-2 trichloro-3,5,6 benzène. *Tetrahedron Letters*, 211-212.

14 V. Thaller and J.L. Turner (1972) Natural acetylenes. Part XXXV. Polyacetylenic acid and benzenoid metabolites from cultures of the fungus *Lepista diemii* Singer. *Journal of Chemical Society Perkin Trans I*, 2032-2034.

15 M. Séquin-Frey and C. Tamm (1971) Gentisinacetal und Chlorgentisinalkohol, zwei neue Metabolite einer *Phoma* species. *Helvetica Chimica Acta*, **54**, 851-861.

16 D.W. Cameron and M.D. Sidell (1978) 1,3,6,8,11,13-hexachloro-4,10-dihydroxydinaphtho[2,1-*b*:1',2'-*d*]furan-5,9-dione. A polychloro quinone from green soils. *Australian Chemical Journal*, 31, 1323-1333.

17 G.A. Ellestad, F.M. Lovell, N.A. Perkinson, R.T. Hargreaves and W.J. McGahren (1978) New zearalenone related macrolides and isocoumarins from an unidentified fungus. *Journal of Organic Chemistry*, **43**, 2339-2343.

Date Received: April 7, 1993
Date Accepted: June 7, 1993