Miotoxin-A: A Novel Macrocyclic Trichothecene from the Brazilian Plant *Baccharis coridifolia*

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Miotoxin-A, Trichothecene, Compositae, Poisonous Plants, NMR- and X-Ray Structure Analysis

The isolation of Miotoxin-A*, a novel macrocyclic trichothecene from *Baccharis coridifolia* DC. (Compositae) is described. The structure has been determined to be 4-hydroxy-roridin E by means of NMR-spectroscopy and X-ray structure analysis.

Introduction

Macrocyclic trichothecenes are well-known mycotoxins, which have been isolated from cultures of various species of Fungi Imperfecti [1]. In the course of isolating tumor inhibitors of plant origin, Kupchan *et al.* [2] and later Jarvis and Mazzola [3] described new macrocyclic trichothecenes, which were separated from extracts of the shrub *Baccharis megapotamica*. These particular compounds, called baccharinoids, show significant activity *in vivo* against P-388 leukemia in mice. In contrast to the mycotoxins from fungi cultures, the baccharinoids show additional oxygen functions in the macrocyclic and trichothecane ring systems.

In our search for the active constituents of hazardous plants of South America, we recently found the two macrocyclic trichothecenes Roridin E (**1**) und Roridin A (**2**) in extracts of the Brazilian shrub *Baccharis coridifolia* [4], a plant which has been proven to be highly toxic to livestock in Brazil and Argentina [5]. This is the first known case of the appearance of genuine macrocyclic trichothecene mycotoxins in a higher plant. The relatively high concentrations of these compounds suggest that *Baccharis coridifolia* is able to absorb trichothecenes from soil fungi and store them structurally unchanged to large extent. This is especially interesting in consideration of the highly phytotoxic character of these trichothecenes; only a few plants are able to survive a contamination with a few ppm of such substances [6].

We now wish to report the structural features of one new minor trichothecene component, designated by us as Miotoxin-A (**3**).

Results and Discussion

Miotoxin-A (**3**) was isolated from the chloroform extract of dried plant material from *Baccharis coridifolia* by silica gel column chromatography, preparative thin layer chromatography, preparative high performance liquid chromatography, and finally thin layer chromatography. Mass spectral data of **3** agree with $C_{29}H_{38}O_9$. The ^{1}H-NMR and ^{13}C-NMR spectra, including a DEPT-experiment for the determination of the ^{13}C-multiplicities, suggest a structure derived from **1**, and distinguished from this in containing one more OH-group at C(4′) (NMR-data shown in Table I). Significant differences between the two trichothecene spectra are: 1) an upfield deviation of the C(4′) and C(5′) signals in the ^{13}C-NMR spectrum, 2) the presence of a broad singulet at 4.3 ppm in the proton NMR spectrum, and variations in the unresolved parts at 2.1 and 3.7 ppm.

The assumption of a 4′-hydroxy-derivative of **1** was established by application of two-dimensional Fourier transform NMR-techniques [7]. The 2D-^{1}H-^{13}C shift correlation spectrum (Fig. 1) connects proton and carbon shifts for every CH_n-group.

Dedicated to Prof. Dr. Phil. Alfons Schöberl on the occasion of his 80th birthday.

* With reference to the Brazilian colloquial name of *Baccharis coridifolia*: "mio-mio".

Reprint requests to Prof. Dr. G. Habermehl.

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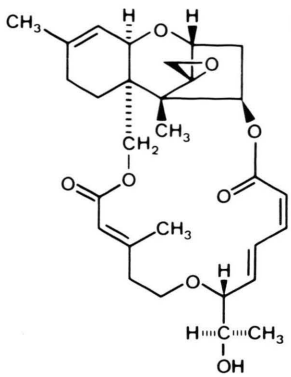
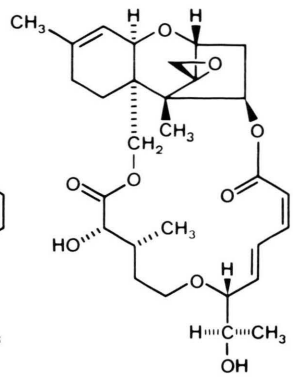
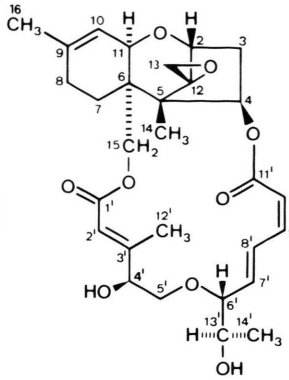


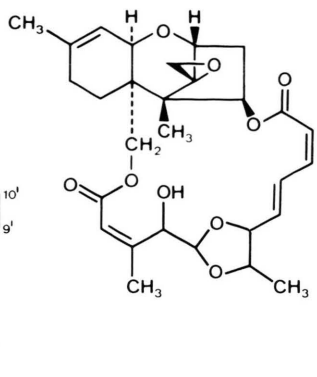
Roridin E (**1**)



Roridin A (**2**)



Miotoxin A (**3**)



Roridin J (**4**)

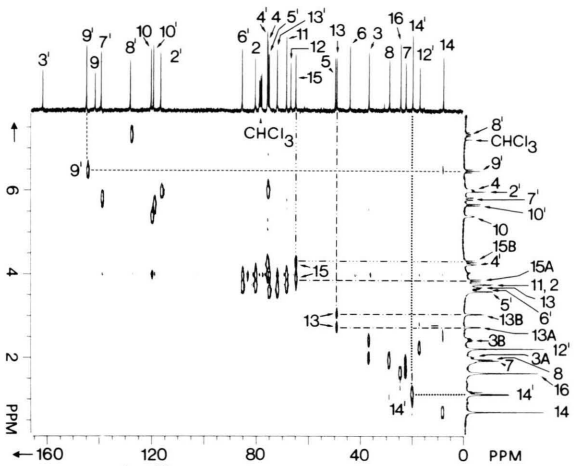


Fig. 1. 2D-^{1}H-^{13}C shift correlation contour plot of Miotoxin-A (**3**) with selected assignments. The 1D-^{1}H-(300 MHz) and the 1D-^{13}C-(75 MHz; signals for C(1′) and C(11′) at $\delta = 165.9$ and 166.5 ppm respectively, are not delinated) spectra are given along the respective frequency axes.

Profiles of cross-sections through the spectra permitted an assignment of the complete spectrum of **3**. Thus it also was possible to correlate tightly neighbouring ^{13}C-resonances, for example at $\delta = 74.2$ and 74.4 ppm to C(4) and C(4′) respectively.

A 2D-proton-proton shift correlation spectrum (Fig. 2) was then used to identify the pairs of resonances that are coupled together.

In order to determine the remaining configurations at C(4′), C(6′), C(13′) and at the C(2′)–C(3′) double bond, an X-ray single crystal diffraction analysis was accomplished [12]. The crystal structure of **3** is orthorhombic, space group $P22_12_1$, with

Table I. ^{1}H and ^{13}C chemical shifts for Roridin E (**1**) and Miotoxin-A (**3**).

Position	Roridin E (**1**)[a]			Miotoxin-A (**3**)[b]		
	^{1}H	J [Hz]	^{13}C	^{1}H	J [Hz]	^{13}C
2	3.86 d	5	79.2	3.84 d		79.1
3A	2.09 m		35.8	2.10 ddd		35.5
3B	2.54 m		–	2.51 dd	8/15.5	–
4	6.23 dd	4/8	74.2	6.11 dd	4/8	74.2
5	–		48.4	–		48.5
6	–		42.8	–		42.8
7	1.9–2.1 m		21.6	2.01 m		21.2
8	1.9–2.1 m		27.7	2.03 m		27.6
9	–		140.0	–		140.1
10	5.50 d	5	117.8	5.47 d	5	118.8
11	3.82 d	5	67.3	3.84 m		67.2
12	–		65.6	–		65.5
13A	2.83 d	4	48.1	2.81 d	4	48.0
13B	3.15 d	4	–	3.13 d	4	–
14	0.80 s		6.7	0.78 s		6.7
15A	3.95 d	12.5	63.7	3.93 d	12	63.6
15B	4.33 d	12.5	–	4.38 d	12	–
16	1.72 s		23.2	1.71 s		23.2
1′	–		165.8[c]	–		165.9[c]
2′	5.97 m		119.0	6.07 m		115.1
3′	–		159.0	–		159.5
4′	2.4–2.71 m		41.3	4.32 bs		74.4
5′	3.5–3.76 m		69.8	3.72 d	6.7	73.7
6′	3.5–3.76 m		83.9	3.74 t	7	83.9
7′	5.92 dd	3/16	138.0	5.88 dd	3/16	137.7
8′	7.54 dd	11.5/16	126.6	7.43 dd	11/16	126.6
9′	6.59 t	11.5	143.6	6.55 t	11.4	143.4
10′	5.76 d	11	117.2	5.75 d	11	117.9
11′	–		166.4[c]	–		166.4[c]
12′	2.27 d	1.5	20.2	2.30 d	1.5	15.8
13′	3.5–3.76 m		70.6	3.79 m		70.7
14′	1.20 d	6	18.3	1.20 d	6	18.6

[a] ^{1}H-NMR: 300 MHz; ^{13}C-NMR: 75 MHz.
[b] ^{1}H-NMR: 400 MHz; ^{13}C-NMR: 75 MHz.
[c] Assignments may be interchanged.
Chemical shifts are in ppm downfield from tetramethylsilane (TMS; $\delta = 0$ ppm). All spectra were taken in $CDCl_3$.

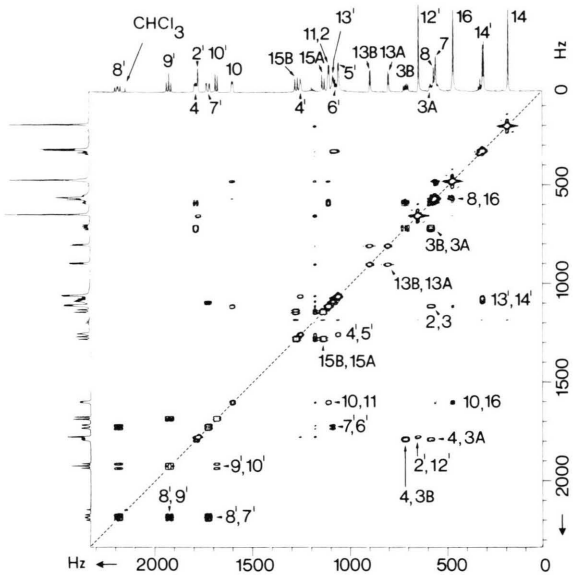


Fig. 2. Homonuclear scalar correlated 2D-^{1}H-NMR spectrum and 1D-^{1}H-NMR spectrum (300 MHz) of Miotoxin-A (**3**).

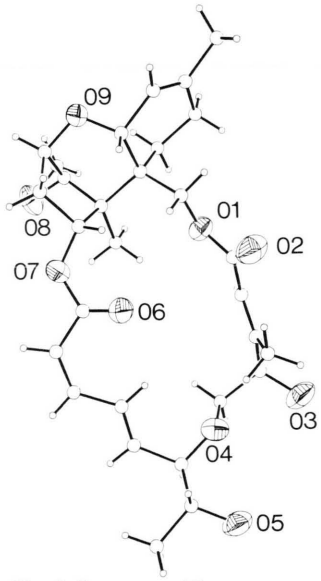


Fig. 3. Structure of **3**.

$a = 10.130(2)$, $b = 10.184(2)$, $c = 30.456(5)$ Å, $V = 3142.0$ Å^3, $Z = 4$, $D_c = 1.12$ Mg/m^3, $\mu = 0.77$ mm^{-1}. The structure (Fig. 3) was solved by direct methods with SHELXTL [8].

Blocked-matrix least-squares refinement of the atomic coordinates and anisotropic thermal parameters reduced R to 0.14; H-atoms were then positioned. With isotropic H-atom thermal parameters, the final R was 0.079 for 2903 reflections. None of the atom parameters shifted more than 0.04 σ in the last cycle. Positional parameters are listed in Table II. The determined bond lengths and angles of **3** are given in Tables III and IV.

Evidently, the C(2′)–C(3′) double bond is of the same *trans* configuration between the H(2′) and the methyl group at C(3′) as found in **1** [9]. In Roridin J (**4**) [10], until now the only known macrocyclic trichothecene where an allylic hydroxy group occurs at C(4′), the C(2′)–C(3′) bond shows the opposite configuration. The stereochemistry at C(6′), C(13′), and at C(4′) is determined as (R) in all three cases.

Table II. Atom coordinates ($\times 10^4$) and temperature factors (Å $\times 10^3$) of **3**.

Atom	*x*	*y*	*z*	U^a
C(7)	847(7)	1652(7)	1822(3)	46(2)
C(8)	1089(8)	164(8)	1798(3)	52(3)
C(9)	2199(9)	−186(7)	1501(3)	52(3)
C(16)	2292(10)	−1618(8)	1379(3)	70(3)
C(10)	3095(8)	671(7)	1380(3)	48(2)
C(11)	3072(7)	2097(7)	1498(2)	42(2)
O(9)	2722(5)	2780(5)	1098(2)	50(2)
C(2)	2526(8)	4166(7)	1163(3)	49(3)
C(12)	1354(8)	4342(7)	1458(3)	48(3)
C(13)	3(9)	4435(8)	1312(3)	67(3)
O(8)	718(6)	5611(5)	1420(2)	68(2)
C(5)	1875(7)	3943(7)	1905(2)	44(2)
C(14)	951(8)	4395(8)	2284(3)	55(3)
C(4)	3268(8)	4649(7)	1905(3)	47(2)
C(3)	3655(8)	4840(8)	1430(2)	50(3)
O(7)	3171(5)	5935(5)	2107(2)	51(2)
C(11′)	3549(8)	6034(8)	2537(3)	51(3)
O(6)	3926(7)	5124(6)	2750(2)	66(2)
C(10′)	3395(8)	7401(7)	2680(3)	54(3)
C(9′)	3268(8)	7789(8)	3091(3)	57(3)
C(8′)	3239(8)	6978(8)	3491(3)	54(3)
C(7′)	3030(9)	7463(9)	3874(3)	61(3)
C(6′)	3039(9)	6737(9)	4308(3)	61(3)
C(13′)	4300(10)	7025(9)	4576(3)	64(3)
C(14′)	4484(12)	8485(10)	4663(4)	90(5)
O(5)	4238(6)	6346(7)	4988(2)	74(2)
O(4)	2971(5)	5345(5)	4247(2)	55(2)
C(5′)	1662(8)	4880(10)	4160(3)	67(3)
C(4′)	1658(8)	3378(9)	4166(3)	57(3)
O(3)	1931(7)	2881(7)	4584(2)	79(3)
C(3′)	2534(8)	2776(8)	3812(3)	53(3)
C(12′)	3973(9)	2705(12)	3914(4)	97(5)
C(2′)	1977(8)	2374(7)	3437(2)	47(2)
C(1′)	2722(8)	1849(8)	3068(3)	54(3)
O(2)	3760(7)	1266(7)	3075(2)	83(3)
O(1)	2087(5)	2088(5)	2681(2)	51(2)
C(15)	2860(7)	1901(8)	2299(2)	46(2)
C(6)	2120(7)	2410(7)	1886(2)	39(2)

[a] Equivalent isotropic *U* defined as one third of the trace of the orthogonalised *U* tensor.

Table III. Bond lengths (Å) of **3**.

C(7)–C(8)	1.536(11)	C(7)–C(6)	1.516(10)
C(8)–C(9)	1.486(12)	C(9)–C(16)	1.509(11)
C(9)–C(10)	1.312(11)	C(10)–C(11)	1.496(10)
C(11)–O(9)	1.446(9)	C(11)–C(6)	1.558(10)
O(9)–C(2)	1.439(9)	C(2)–C(12)	1.499(11)
C(2)–C(3)	1.564(11)	C(12)–C(13)	1.443(12)
C(12)–O(8)	1.449(9)	C(12)–C(5)	1.516(11)
C(13)–O(8)	1.437(10)	C(5)–C(14)	1.555(11)
C(5)–C(4)	1.584(11)	C(5)–C(6)	1.581(10)
C(4)–C(3)	1.511(11)	C(4)–O(7)	1.450(9)
O(7)–C(11′)	1.370(10)	C(11′)–O(6)	1.193(10)
C(11′)–C(10′)	1.467(11)	C(10′)–C(9′)	1.318(12)
C(9′)–C(8′)	1.472(12)	C(8′)–C(7′)	1.284(12)
C(7′)–C(6′)	1.516(12)	C(6′)–C(13′)	1.544(13)
C(6′)–O(4)	1.432(10)	C(13′)–C(14′)	1.523(14)
C(13′)–O(5)	1.433(11)	Q(4)–C(5′)	1.433(10)
C(5′)–C(4′)	1.530(13)	C(4′)–O(3)	1.399(10)
C(4′)–C(3′)	1.525(12)	C(3′)–C(12′)	1.493(12)
C(3′)–C(2′)	1.338(11)	C(2′)–C(1′)	1.455(11)
C(1′)–O(2)	1.207(11)	C(1′)–O(1)	1.366(9)
O(1)–C(15)	1.414(9)	C(15)–C(6)	1.553(10)

Table IV. Bond angles (deg.) of **3**.

C(8)–C(7)–C(6)	111.9(6)
C(8)–C(9)–C(16)	115.4(7)
C(16)–C(9)–C(10)	122.1(8)
C(10)–C(11)–O(9)	105.5(6)
O(9)–C(11)–C(6)	112.8(6)
O(9)–C(2)–C(12)	108.0(6)
C(12)–C(2)–C(3)	102.4(6)
C(2)–C(12)–O(8)	114.2(6)
C(2)–C(12)–C(5)	103.3(6)
O(8)–C(12)–C(5)	117.9(7)
C(12)–O(8)–C(13)	60.0(5)
C(12)–C(5)–C(4)	100.9(6)
C(12)–C(5)–C(6)	106.6(6)
C(4)–C(5)–C(6)	108.0(6)
C(5)–C(4)–O(7)	110.5(6)
C(2)–C(3)–C(4)	104.6(6)
O(7)–C(11′)–O(6)	123.6(7)
O(6)–C(11′)–C(10′)	127.6(8)
C(10′)–C(9′)–C(8′)	128.3(8)
C(8′)–C(7′)–C(6′)	127.2(8)
C(7′)–C(6′)–O(4)	111.6(7)
C(6′)–C(13′)–C(14′)	112.3(8)
C(14′)–C(13′)–O(5)	108.9(8)
O(4)–C(5′)–C(4′)	109.3(7)
C(5′)–C(4′)–C(3′)	113.1(7)
C(4′)–C(3′)–C(12′)	116.1(8)
C(12′)–C(3′)–C(2′)	125.2(8)
C(2′)–C(1′)–O(2)	128.1(8)
O(2)–C(1′)–O(1)	120.9(7)
O(1)–C(15)–C(6)	110.7(6)
C(7)–C(6)–C(5)	112.0(6)
C(7)–C(6)–C(15)	110.2(6)
C(5)–C(6)–C(15)	112.0(6)
C(7)–C(8)–C(9)	112.7(6)
C(8)–C(9)–C(10)	122.3(7)
C(9)–C(10)–C(11)	124.5(7)
C(10)–C(11)–C(6)	113.0(6)
C(11)–O(9)–C(2)	112.9(5)
O(9)–C(2)–C(3)	113.7(6)
C(2)–C(12)–C(13)	125.0(8)
C(13)–C(12)–O(8)	59.6(5)
C(13)–C(12)–C(5)	128.7(7)
C(12)–C(13)–O(8)	60.4(5)
C(12)–C(5)–C(14)	112.1(6)
C(14)–C(5)–C(4)	113.7(6)
C(14)–C(5)–C(6)	114.5(6)
C(5)–C(4)–C(3)	106.8(6)
C(3)–C(4)–O(7)	107.9(6)
C(4)–O(7)–C(11′)	116.9(6)
O(7)–C(11′)–C(10′)	108.8(7)
C(11′)–C(10′)–C(9′)	125.1(7)
C(9′)–C(8′)–C(7′)	122.6(8)
C(7′)–C(6′)–C(13′)	112.0(7)
C(13′)–C(6′)–O(4)	107.3(7)
C(6′)–C(13′)–O(5)	109.5(7)
C(6′)–O(4)–C(5′)	113.3(6)
C(5′)–C(4′)–O(3)	111.8(7)
O(3)–C(4′)–C(3′)	112.5(7)
C(4′)–C(3′)–C(2′)	118.7(7)
C(3′)–C(2′)–C(1′)	123.5(8)
C(2′)–C(1′)–O(1)	110.9(7)
C(1′)–O(1)–C(15)	115.2(6)
C(7)–C(6)–C(11)	108.9(6)
C(11)–C(6)–C(5)	109.1(6)
C(11)–C(6)–C(15)	104.2(5)

Up to now it has not been established, whether **3** is a native, and not yet isolated secondary metabolit of fungi, or the result of a plant induced hydroxylation of Roridin E, absorbed in *Baccharis coridifolia*. Elucidation of this aspect by feeding plant seedlings with Roridin E, as well as toxicological viewpoints of this work are under investigation.

Acknowledgements

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Experimental

General

Melting point (uncorr.): Kofler hot stage apparatus. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz): Nicolet NT 300 WB. ^{1}H-NMR (400 MHz): Bruker WM 400. IR: Perkin-Elmer 197 Spectrometer. MS: Varian MAT 311 A. UV: Varian Cary 17.

CC: Macherey Nagel silica gel MN 60. PTLC: 20×40 cm plates, coated with 1 mm Macherey Nagel silica gel P/UV 254 + 366. TLC: Merck aluminium-backed 60 F 254 silica gel plates. Spots being visualized under UV light (254 nm) and by spraying with *p*-anisaldehyde reagent [11], giving characteristic dark blue spots after brief heating at 110 °C. HPLC: Dupont Preparative HPLC; Zorbax SIL 2.12×25 cm. Elution systems in all cases: $CHCl_3/CH_3OH = 100/4$, v/v, or ethyl acetate.

X-ray analysis: Intensities were collected on a Nicolet four circle diffractometer (Mo-Kα radiation) equipped with a graphite monochromator. Crystals of **3** were grown as small transparent blocks with no special orientation by slow cooling of a warm saturated solution in ethyl acetate. Systematic absences of 0k0 and 001 for odd indices determined the space group as $P22_12_1$. 4194 symmetry-independent reflections h10 to h140 with $\theta \leqq 55°$ were measured in the $\theta - 2\theta$ scan mode. The data were corrected for background, Lorentz and polarization factors. 2914 reflections had $|E| > 3\delta_E$.

Miotoxin-A (**3**): 22.4 kg of the air dried plant material (shoots and seeds of *Baccharis coridifolia* collected in 1981 and 1982 from the region of Santa Maria, Rio Grande do Sul, Brazil) were extracted in 5.6 kg portions at room temperature, by using 5×60 l chloroform for each charge. After concentration of the extract by rotatory evaporation under reduced pressure, 2024 g of a green-black gum were obtained. This residue was chromatographed on several silica gel columns. The fractions obtained were tested for acute toxicity by intraperitoneal injection into mice after evaporation of the organic solvent and dissolving in physiological saline. The toxic fractions containing **3**, were further purified by PTLC, HPLC, and finally TLC to give 208 mg (9.3×10^{-4}%) of **3**.

A methanolic extract of 2.2 kg of *Baccharis coridifolia* yielded 55 mg (2.5×10^{-3}%) of **3** after a similar chromatographic purification. M.p. (ethyl acetate): 159–161 °C (decomp.). IR (KBr): 3410, 2960, 1710, 1635, 1600, 1220, 1175, 1085 cm^{-1}. UV (EtOH): $\lambda_{max} = 220$, 260 nm. FD-MS: $M^+ +$ H531, $C_{29}H_{38}O_9$.

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[12] Further details of the X-ray structure analysis are available by specification with CSD-No. 50775, authors-, and journals name at: Fachinformationszentrum Energie Physik Mathematik, D-7514 Eggenstein-Leopoldshafen.