Madurensins A, B, and C, Tetra-Aryl Cyclobutanes, from Crotalaria madurensis

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J. Nat. Prod., **1991**, 54 (1), 104-109• DOI: 10.1021/np50073a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 104-109, Jan-Feb 1991

MADURENSINS A, B, AND C, TETRA-ARYL CYCLOBUTANES, FROM *CROTALARIA MADURENSIS*

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ABSTRACT.—Three isomeric tetra-aryl cyclobutanes, madurensins A [**1**], B [**2**], and C [**3**], have been isolated from *Crotalaria madurensis*. Their structures were determined by spectral analyses and X-ray studies.

Crotalaria madurensis R. Wight (Leguminosae) is an ornamental shrub that grows in the Nilgiris and Madura Hills in India (1). During a program of screening Indian plants for broad biological activity at the Central Drug Research Institute, EtOH extracts of the leaves and stems exhibited antifungal activity. Activity-directed fractionation led to localization of biological activity in the $CHCl_3$-soluble and EtOAc-soluble fractions, which were then selected for detailed chemical studies. The structures of several constituents from the EtOAc-soluble fraction were reported earlier (2), and recently we described the structure of two isoflavones, crotarin and crotalarin, isolated from the $CHCl_3$-soluble fraction of this plant material (3). We have now isolated three more compounds, which have been named madurensins A, B, and C. We report here our studies on the structures of these novel compounds.

The most abundant of the three compounds, madurensin B [**2**], showed ir spectral bands at 1600, 1520, and 1030 cm^{-1}, indicating the aromatic nature of the compound and the absence of hydroxy and carbonyl groups. The aromatic nature was also evident from the uv spectrum (λ max 280 nm). The molecular formula was deduced to be $C_{36}H_{40}O_8$ on the basis of cims data (*m/z* 601 $[M+H]^+$) and nmr spectra. The eims displayed the base peak and the heaviest ion at *m/z* 300. This abundant fragment spectrum (Table 1) was in agreement because it indicated a symmetric character by corresponding to a proton count of 20 instead of 40.

TABLE 1. ^{1}H-nmr Spectra of Madurensins A [**1**], B [**2**], and C [**3**].

Compound		
1	**2**	**3**
3.52 (4H, brs, w½ = 11 Hz)	4.29 (4H, brs, w½ = 5.5 Hz)	4.29 (brs, 4H, w½ = 5.5 Hz
3.74 (12H, s, OMe × 4)	3.60 (12H, s, OMe × 4)	3.64 (12H, s, OMe × 4)
3.81 (6H, s, OMe × 2)	3.65 (6H, s, OMe × 2)	3.66 (6H, s, OMe × 2)
3.85 (6H, s, OMe × 2)	3.82 (6H, s, OMe × 2)	3.79 (6H, s, OMe × 2)
6.33 (2H, d, *J* = 2.1 Hz)	6.21 (2H, d, *J* = 1.8 Hz)	6.19 (2H, d, *J* = 1.3 Hz)
6.45 (4H, d, *J* = 1.8 Hz)	6.31 (4H, d, *J* = 1.8 Hz)	6.27 (4H, d, *J* = 1.3 Hz)
6.77 (2H, d, *J* = 1.9 Hz)	6.55 (2H, d, *J* = 1.8 Hz)	6.54 to 6.73 (6H, m)
6.84 (2H, d, *J* = 9 Hz)	6.65 to 6.8 (4H, m)	
6.86 (2H, d, d, *J* = 1.9; 9 Hz)		

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A key feature of the ^{1}H-nmr spectrum was the presence of methoxy methyl signals at 3.60, 3.65, and 3.82 ppm in the proportion of 2:1:1. A coupling constant of 1.8 Hz, which is characteristic of meta coupling, was associated with the aromatic proton signals.

In light of our previous isolation of *trans*-3,4,3′,5′-tetramethoxystilbene [**4**] from an EtOAc-soluble fraction from this plant material, the monomeric unit of madurensin B appeared to be **4**. The details of the ^{1}H-nmr spectra of madurensin B supported this point. The absence of olefinic proton signals and the presence of a broad singlet at 4.29 ppm corresponding to 4 protons (in the dimer) suggested a cyclobutane ring with an aryl substituent and a hydrogen at each ring carbon. Such a structure would easily arise by the dimerization of **4** (we are making the reasonable assumption that the trans stilbene monomer would not change to the cis configuration during dimerization), which could lead to four isomeric structures of types S_1, S_2, S_3, and S_4 (see Figure 1). It would be expected that S_1 and S_2 would have very similar ^{1}H-nmr spectra and so would S_3 and S_4. Definitive structural information was obtained by single crystal X-ray diffraction studies, and madurensin B was shown to be **2** which corresponds to S_2.

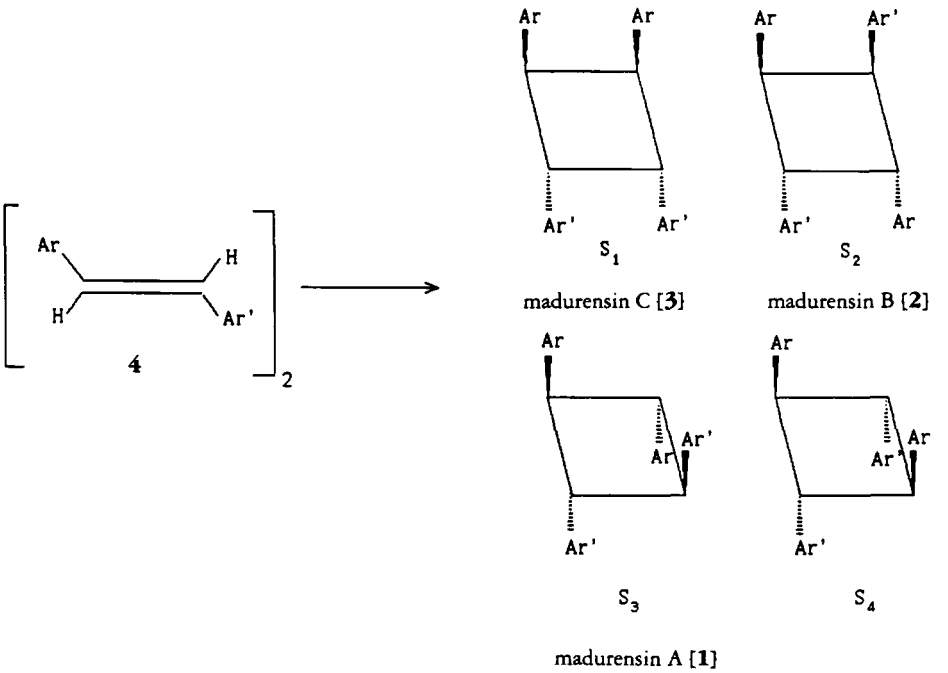

FIGURE 1. Dimerization of **4**.

The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) of madurensin C [**3**] were nearly identical with those of madurensin B [**2**], which corresponds to S_2. Therefore, madurensin C [**3**] should be of type S_1, and the corresponding structure **3** could now be assigned to it. Only a very small crystal of madurensin C [**3**] was available for X-ray diffraction studies. It was possible to gather very limited data (only 6 reflections were found after 6 h of a search procedure) that were fully compatible with this structure for madurensin C.

Madurensin A [**1**] failed to yield crystals suitable for X-ray studies. The structure of this compound, which is isomeric with madurensin B, was deduced from nmr spectral studies. The nmr spectra of madurensin A [**1**] were fairly similar to those of madurensin B [**2**], but there were a few distinct differences.

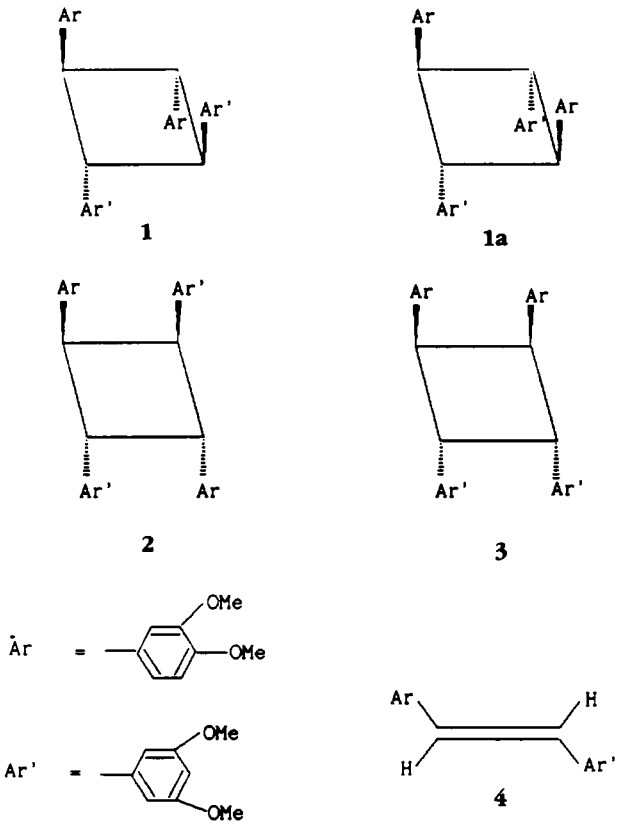

FIGURE 2. Configurational isomers of madurensins.

In case of madurensin A [**1**], therefore, the choice lay between the similar structures S_3 and S_4. These structures are characterized by the all trans nature of the aryl groups on the cyclobutane ring. The cis aryl groups in **2** and **3** would be subject to steric interaction with each other, unlike the trans aryl groups in S_3 and S_4. The shielding effect of the aryl groups on the cyclobutane ring protons would thus be expected to be different in **1** and **2** (3). This indeed is the case: the cyclobutane protons in madurensin A [**1**] resonate at 3.52 ppm as compared to 4.29 ppm in the case of madurensins B [**2**] and C [**3**]. The ^{13}C-nmr spectra also show marked differences: four distinct signals (at 52.21, 51.72, 51.62, and 51.11 ppm) are observed for the cyclobutane carbons in **1**. In case of S_4, only two separate cyclobutane carbon signals can be expected, whereas in case of S_3, different conformations of the aryl rings could make the cyclobutane carbons nonequivalent and give rise to four distinct signals in the ^{13}C-nmr spectrum. Therefore, the S_3 type structure **1** can be assigned to madurensin A.

Madurensins A–C are the first known examples of tetra-aryl substituted cyclobutanes (stilbene dimer) from plants. Earlier a dimethyl-diaryl cyclobutane (β-methylstyrene dimer) had been reported by Yamamura *et al.* (4) from a plant in the Aristolochiaceae family.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, uv spectra were obtained in MeOH, ir spectra were run as KBr discs, and nmr spectra were obtained in $CDCl_3$ with TMS as internal standard. Tlc was carried out on Si gel GF 254 and cc over Si gel (BDH).

TABLE 2. ^{13}C nmr of Madurensins A [**1**], B [**2**], and C [**3**].

Carbon	Compound		
	1	**2**	**3**
C-7, 7′	52.21; 51.72	48.18	48.30
C-8, 8′	51.62; 51.11	46.97	47.10
Ar(*ortho*-dimethoxyphenyl)			
1,1′	135.99; 135.44	133.40	133.61
2,2′	111.88	111.28	111.26
3,3′	148.21	148.44	148.17
4,4′	148.10	147.29	147.62
5,5′	110.97	110.73	112.35
6,6′	119.18	119.98	120.21
Ar′(*meta*-dimethoxyphenyl)			
9,9′	145.22; 145.19	143.31	143.37
10,10′	105.43	106.53	106.76
11,11′	161.06	160.45	160.63
12,12′	98.41	97.81	98.14
13,13′	161.06	160.45	160.76
14,14′	105.43	106.53	106.76
OMe	56.06	55.81	55.97
	56.04	55.71	55.89
	55.29	55.18	55.23

TABLE 3. Atomic Coordinates for Non-hydrogen Atoms of Madurensin B [**2**] (with ESDs in parentheses).

Atom	*x*	*y*	*z*	B(A^2)[a]
C-1	0.3061(3)	0.2232(6)	0.1659(2)	4.51(7)
C-2	0.3064(2)	0.0645(5)	0.1193(1)	3.81(6)
C-3	0.2156(2)	0.0307(5)	0.0786(1)	3.47(6)
C-4	0.1190(2)	0.1516(5)	0.0838(1)	3.34(5)
C-5	0.1195(2)	0.3076(5)	0.1299(2)	4.34(7)
C-6	0.2128(3)	0.3448(6)	0.1706(2)	4.85(7)
C-7	0.0163(2)	0.1077(4)	0.0420(1)	3.19(5)
C-8	0.0180(2)	0.1224(4)	−0.0351(1)	3.21(5)
C-9	0.1208(2)	0.1980(5)	−0.0705(1)	3.24(5)
C-10	0.1694(2)	0.3833(5)	−0.0520(2)	3.81(6)
C-11	0.2584(2)	0.4587(5)	−0.0873(2)	4.18(6)
C-12	0.3007(2)	0.3526(5)	−0.1406(1)	4.06(6)
C-13	0.2500(2)	0.1697(5)	−0.1588(1)	4.00(6)
C-14	0.1605(2)	0.0914(5)	−0.1236(1)	3.77(6)
O-15	0.4005(2)	0.2382(5)	0.2047(1)	6.19(6)
C-16	0.4035(3)	0.4070(8)	0.2498(2)	9.0 (1)
C-17	0.4024(2)	−0.0507(4)	0.1192(1)	5.36(5)
C-18	0.4078(3)	−0.2099(7)	0.0726(3)	8.8 (1)
O-19	0.2993(2)	0.6443(4)	−0.0660(1)	6.23(6)
C-20	0.3896(3)	0.7325(6)	−0.1024(2)	6.65(9)
O-21	0.2806(2)	0.0543(4)	−0.2117(1)	5.61(5)
C-22	0.3793(3)	0.1116(7)	−0.2458(2)	5.28(8)

[a]Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: (4/3)* [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab(cos gamma)*B(1,2) + ac(cos beta)* B(1,3) + bc(cos alpha)*B(2,3)].

PLANT MATERIAL.—The leaves and stems of *C. madurensis* were collected in November–December 1978, from Karnool, Andhra Pradesh, India (2), and a voucher specimen No. 110601 of the same is preserved in the Herbarium of the Institute.

EXTRACTION.—The air-dried plant material (5 kg) was extracted with 95% EtOH and fractionated as reported earlier (2,3).

CHROMATOGRAPHY OF $CHCl_3$-SOLUBLE FRACTION.—The $CHCl_3$-soluble fraction (40 g) was chromatographed on a column of Si gel (1.5 kg) and eluted with a C_6H_6/MeOH gradient as reported earlier (3). Fractions 42–67 eluted with C_6H_6-MeOH (99:1) were combined and rechromatographed using C_6H_6-MeOH (98:2) as eluent. Preparative tlc of this fraction yielded madurensin A [**1**] (13 mg), madurensin B [**2**] (130 mg), and madurensin C [**3**] (19 mg) on crystallization with MeOH as microcrystalline powders.

MADURENSIN A [**1**].—Mp 139–141°, λ max 280 nm; ν max (KBr) 2900, 1600, 1520, 1285, 1240, 1205, 1030 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; cims (CH_4/NH_4Cl) *m/z* $[M+Cl]^-$ 635, 637, $[M+H]^+$ 601; eims *m/z* 300 (base peak).

MADURENSIN B [**2**].—Mp 116°, λ max 280 nm, ν max (KBr) 2900, 1590, 1500, 1450, 1230, 1028 cm^{-1}, ^1H nmr see Table 1; ^{13}C nmr see Table 2; cims (CH_4/NH_4Cl) *m/z* $[M+H]^+$ 601, eims *m/z* 300 (base ion).

MADURENSIN C [**3**].—Mp 110–111°; λ max 280 nm; ν max 2900, 1590, 1510, 1460, 1305, 1230 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; cims *m/z* $[M+H]^+$ 601, $[M+Cl]^-$ 635, 637.

X-RAY STUDIES.—A rod-shaped crystal of madurensin B (dimensions 0.2 × 0.25 × 0.49 mm) obtained by slow evaporation from C_6H_6 was used for data collection on an automated Enraf-Nonius CAD 4 diffractometer.

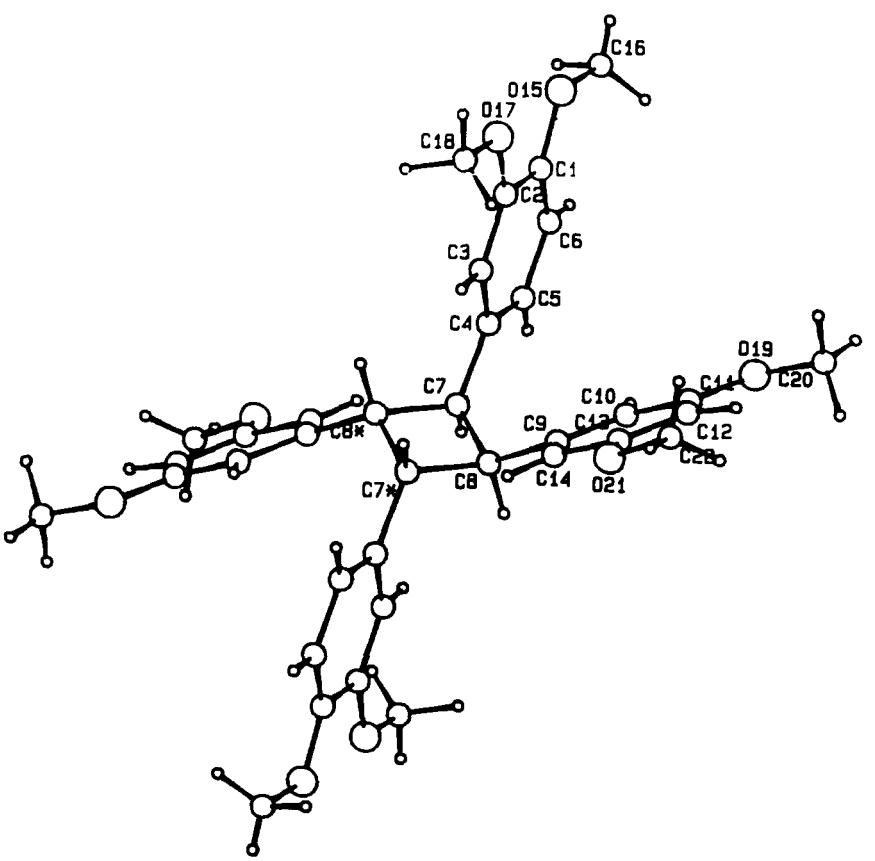

FIGURE 3. PLUTO diagram of madurensin B [**2**].

X-ray crystal data for madurensin B [**2**].—$C_{36}H_{40}O_8$; MW 600.72; $a = 11.971(2)$ Å, $b = 6.516(3)$ Å, $c = 20.305(3)$ Å, $\beta = 91.20(2)°$, $V = 1583(1)$ Å^3, $Z = 2$; $D = 1.262$ g·cm^{-1}; monoclinic $P2_1/n$ space group was assigned from systematic extinctions.

Monochromatized MoK_α radiation [μ(MoK_α) = .826 cm^{-1}] and $\theta/2\theta$ scan mode were used during data collection. In θ range 0–26°, 4126 reflections were collected; 2317 of these with $I > 2\sigma(I)$ were observed. Intensities were corrected by Lorentz, polarization, and PSI-scan based absorption factors.

The structure was solved by direct methods and refined by full-matrix least-squares procedure to $R = 0.040$ and $R_w = 0.059$ [$w = 1/\sigma(F)^2$], with hydrogens found from difference maps, and refined. All calculations were done on a Microvax II computer using commercial programs included in the Enraf-Nonius SDP System. Atomic coordinates for **2** are provided in Table 3.[3]

The molecular conformation of **2** with the numbering scheme adopted during X-ray structure determination is shown in Figure 3. The molecule is centrosymmetric with center of symmetry located in the middle of the four-membered ring; thus it has to be a meso form. The four-membered central ring is perfectly planar with intra-ring torsion angles values below 0.035°. The C-4–C-7–C-8–C-9 torsion angle of −4.9(4)° shows that substituted benzene rings in the symmetry-independent part of the molecule have cis configuration. The angles between least-squares plane of the former and 1,2- and 1,3-methoxy substituted rings are 100.8(1) and 42.1(1)°, respectively. Molecules are hydrophobic and are packed in a crystal lattice due to van der Waals interactions only.

X-ray crystal data for madurensin C [**3**].—$a = 6.10(6)$ Å, $b = 5.85(4)$ Å, $c = 44.2(1)$ Å, $\alpha = 85.1(6)°$, $\beta = 87.3(6)°$, $\gamma = 86.0(7)°$, $V = 1566$ Å^3, $Z = 2$, triclinic system.

The unit cell parameters obtained on madurensin C corresponded to the same molecular volume in the crystal lattice as for madurensin B. This molecular volume is consistent with a tetra-aryl cyclobutane of type S_1 or S_2.

ACKNOWLEDGMENTS

We are grateful to Stevens Institute of Technology for Visiting Scientist fellowships (to H.S.G. and Z.U.L.) and instrumental facilities. Thanks are due to Dr. Om Prakash for ^{1}H- and ^{13}C-nmr measurements and to Dr. K.P. Madhusudanan and Dr. B. Pramanik for mass spectra. We thank Director B.N. Dhawan of C.D.R.I. and Dean F. Boesch of Stevens for their interest and encouragement.

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Received 27 April 1990

[3]Atomic coordinates for compound **2** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.