A Novel Podophyllotoxin Lignan from *Justicia heterocarpa*

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Chromatographic separation of the extract of *Justicia heterocarpa* T. ANDERS. afforded, in addition to known fatty acids, terpenoids and steroids, a new podophyllotoxin lignan. Structures were elucidated by spectroscopic methods, and the structure of the new lignan was confirmed by single crystal X-ray diffraction studies, which have shown that there is a H-bonding stabilized dimer.

Key words *Justicia heterocarpa*; Acanthaceae; podophyllotoxin lignan; X-ray analysis

The lignan family of natural products includes compounds with important antineoplastic and antiviral properties[1] such as podophyllotoxin, which is included in a wide variety of cancer chemotherapy protocols and was used as a precursor for the semi-synthesis of anticancer therapeutics.[2—4]

Typical chemical constituents of the genus *Justicia* are lignans, especially those of the arylnaphthalene and arylnaphthalide types.[5—9] In addition, some coumarins[10,11] and amine derivatives[12,13] were isolated from *Justicia* species.

To the best of our knowledge, the species *J. heterocarpa* was not investigated previously regarding its natural product content. In this article, we report the isolation of a new lignan related to podophyllotoxin, in addition to several known compounds.

Results and Discussion

The extract of *J. heterocarpa* was separated, as described in the experimental part, to give twelve natural products. Nine of them were identified by the GC/MS technique. These are, the fatty acids palmitic, linoleic, oleic and stearic acids[14] as well as the terpenoids hexahydrofarnesylacetone (**1**), farnesylacetone (**2**), phytol, (4*R*,8*R*,12*R*)-4-hydroxy-4,8,12-tetramethylheptadecanoic acid lactone (**3**)[14] and farnesyl acetate (**4**).[15] The remaining three compounds were identified by ^{1}H-NMR. These are β-sitosterol and stigmasterol,[14] in addition to the new lignan (**5**). The structures of the known compounds were confirmed by comparing their spectra with authentic ones.

MS of the lignan (**5**) showed a highly abundant $[M]^+$ (87.7%) at *m/z* 398, as well as M+1 (24.23% of $[M]^+$) and M+2 (4.24% of $[M]^+$), in good agreement with $C_{21}H_{18}O_8$ (Calcd M+1: 23.1% and M+2: 4.27%). IR spectrum showed absorption bands due to OH (3467.5 cm^{-1}), γ-lactone (1772.7 cm^{-1}) and benzene rings (1620.9, 1484.4 cm^{-1}).

^{1}H-NMR spectrum of (**5**) (Table 1) showed the presence of two benzene rings having two *para* protons in each (δ 6.66 s, 6.52 s; δ 6.62 s, 6.21 s). The 4-proton multiplet at δ 5.89 ppm was assigned to two methylenedioxy groups (O–CH_2–O) on the basis of C, H COSY (correlation spectroscopy) correlation, with two C signals at δ 101.04 and 101.33 ppm; both are triplets (from DEPT, distortionless enhancement by polarization transfer). The proton spectrum also showed the presence of a 1H-multiplet at δ 2.82 proved by COSY to be coupled with four protons: a lactonic methylene (δ 3.90, 4.57) and a benzylic methylene (δ 2.58, 3.34).

The molecular formula $C_{21}H_{18}O_8$ revealed the presence of thirteen degrees of unsaturation. Thus, a hexacyclic compound including two benzene rings, a γ-lactone ring and two methylenedioxy rings led to a podophyllotoxin lignan type.[4]

The hydroxyl group detected by the IR spectrum was confirmed by the presence of a singlet in the ^{1}H-NMR at δ 4.18 ppm, which showed no correlation with any carbon signals by the hetero correlation COSY spectrum. This OH group was located on C-8, based on the multiplicity of H-7 (singlet) and H-8′ being coupled with only four protons.

Finally, the ^{1}H-NMR spectrum showed a methoxyl group singlet at δ 3.88, which was located at C-2 to fulfill the *para* coupling of the aromatic protons.

The uncommon position of the OH group at C-8 and the

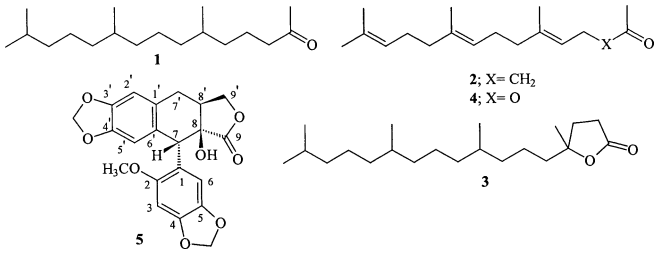

Table 1. ^{1}H- and ^{13}C-NMR Data of Lignan (**5**) ($CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C)

Atom #	δ ^{1}H	Multiplicity	*J* (Hz)	δ ^{13}C	Multiplicity
1	—	—	—	118.13	s
2	—	—	—	141.72	s
3	6.66	s	—	108.42	d
4	—	—	—	152.76	s
5	—	—	—	146.97	s
6	6.52	s	—	109.62	d
7	4.90	s	—	43.18	d
8	—	—	—	77.22	s
9	—	—	—	178.19	s
1′	—	—	—	127.95	s
2′	6.21	s	—	109.70	d
3′	—	—	—	147.22	s
4′	—	—	—	147.22	s
5′	6.62	s	—	94.97	d
6′	—	—	—	128.77	s
7′	2.58,	dd	16.6, 2.9	31.42	t
	3.34	dd	16.6, 7.9		
8′	2.82	m	—	39.86	d
9′	3.90,	dd	8.0, 8.0	72.14	t
	4.57	dd	8.0, 5.0		
2× O–CH_2–O–	5.89	m	—	101.04, 101.32	t
OCH_3	3.88	s	—	56.84	q
OH	4.18	s	—	—	—

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Table 2. Crystal Data and Structure Refinement

Empirical formula	$C_{21}H_{18}O_8$
Formula weight	398.35
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a=24.412(9) Å α=90°
	b=9.243(3) Å β=90°
	c=7.920(3) Å γ=90°
Volume	1787.1(11) Å^3
Z	4
Density (calculated)	1.481 Mg/m^3
Absorption coefficient	0.115 mm^{-1}
F(000)	832
Crystal size	0.42×0.30×0.13 mm^3
Theta range for data collection	1.67 to 24.98°
Index ranges	$-2\leqq h\leqq 28$, $-3\leqq k\leqq 10$, $0\leqq l\leqq 9$
Reflections collected	1882
Independent reflections	1828 [R(int)=0.0060]
Completeness to theta=24.98°	99.8%
Max. and min. transmission	0.9858 and 0.9534
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1828/0/264
Goodness-of-fit on F^2	1.080
Final R indices [I>2sigma(I)]	$R1$=0.0662, w$R2$=0.1309
R indices (all data)	$R1$=0.1427, w$R2$=0.1592
Absolute structure parameter	−5(4)
Largest diff. peak and hole	0.287 and −0.272 e·Å^{-3}

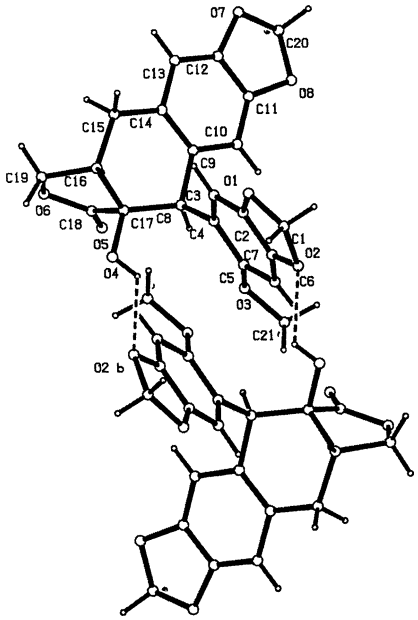

Fig. 1. Computer-Generated Perspective Drawing of Lignan **5**

OCH_3 group at C-2 was confirmed, as well as the overall structure, by single crystal X-ray analysis (Tables 2—4), which indicated an orthorhombic crystal system. A computer-generated perspective drawing of (**5**) revealed the presence of this lignan as a H-bonding stabilized dimer (Fig. 1).

Table 3. Bond Lengths [Å] and Angles [°]

C(1)–O(1)	1.412(10)	O(1)–C(1)–O(2)	109.4(7)
C(1)–O(2)	1.419(10)	C(7)–C(2)–C(3)	120.9(8)
C(2)–C(7)	1.352(11)	C(7)–C(2)–O(1)	108.7(7)
C(2)–C(3)	1.359(10)	C(3)–C(2)–O(1)	130.4(8)
C(2)–O(1)	1.393(9)	C(2)–C(3)–C(4)	120.0(7)
C(3)–C(4)	1.362(10)	C(3)–C(4)–C(5)	119.1(7)
C(4)–C(5)	1.395(10)	C(3)–C(4)–C(8)	122.4(7)
C(4)–C(8)	1.503(10)	C(5)–C(4)–C(8)	118.5(7)
C(5)–O(3)	1.363(9)	O(3)–C(5)–C(4)	117.6(7)
C(5)–C(6)	1.410(11)	O(3)–C(5)–C(6)	121.4(7)
C(6)–C(7)	1.357(11)	C(4)–C(5)–C(6)	120.8(8)
C(7)–O(2)	1.392(9)	C(7)–C(6)–C(5)	116.6(7)
C(8)–C(17)	1.529(11)	C(2)–C(7)–C(6)	122.6(8)
C(8)–C(9)	1.543(10)	C(2)–C(7)–O(2)	111.6(7)
C(9)–C(14)	1.368(10)	C(6)–C(7)–O(2)	125.9(7)
C(9)–C(10)	1.388(10)	C(4)–C(8)–C(17)	114.3(7)
C(10)–C(11)	1.366(10)	C(4)–C(8)–C(9)	112.1(6)
C(11)–C(12)	1.354(11)	C(17)–C(8)–C(9)	110.3(6)
C(11)–O(8)	1.380(10)	C(14)–C(9)–C(10)	121.6(7)
C(12)–C(13)	1.356(12)	C(14)–C(9)–C(8)	121.6(7)
C(12)–O(7)	1.380(9)	C(10)–C(9)–C(8)	116.8(7)
C(13)–C(14)	1.434(10)	C(11)–C(10)–C(9)	117.8(8)
C(14)–C(15)	1.464(12)	C(12)–C(11)–C(10)	121.8(8)
C(15)–C(16)	1.565(11)	C(12)–C(11)–O(8)	109.7(7)
C(16)–C(19)	1.493(11)	C(10)–C(11)–O(8)	128.5(8)
C(16)–C(17)	1.512(10)	C(11)–C(12)–C(13)	122.1(8)
C(17)–O(4)	1.433(9)	C(11)–C(12)–O(7)	110.9(8)
C(17)–C(18)	1.510(11)	C(13)–C(12)–O(7)	126.9(9)
C(18)–O(5)	1.200(9)	C(12)–C(13)–C(14)	117.5(8)
C(18)–O(6)	1.340(10)	C(9)–C(14)–C(13)	119.1(8)
C(20)–O(8)	1.425(11)	C(9)–C(14)–C(15)	124.0(8)
C(20)–O(7)	1.441(12)	C(13)–C(14)–C(15)	116.8(8)
C(21)–O(3)	1.411(9)	C(14)–C(15)–C(16)	118.1(8)
C(19)–O(6)	1.461(10)	C(19)–C(16)–C(17)	101.0(7)
O(4)–H(4)	0.8200	C(19)–C(16)–C(15)	110.0(8)
		C(17)–C(16)–C(15)	112.0(7)
		O(4)–C(17)–C(18)	106.0(6)
		O(4)–C(17)–C(16)	106.1(6)
		C(18)–C(17)–C(16)	101.2(7)
		O(4)–C(17)–C(8)	111.3(7)
		C(18)–C(17)–C(8)	113.4(7)
		C(16)–C(17)–C(8)	117.8(6)
		O(5)–C(18)–O(6)	120.3(8)
		O(5)–C(18)–C(17)	130.2(8)
		O(6)–C(18)–C(17)	109.3(7)
		O(8)–C(20)–O(7)	107.6(8)
		O(6)–C(19)–C(16)	104.7(7)
		C(2)–O(1)–C(1)	106.0(7)
		C(7)–O(2)–C(1)	104.2(6)
		C(5)–O(3)–C(21)	118.9(7)
		C(17)–O(4)–H(4)	109.5
		C(18)–O(6)–C(19)	109.1(7)
		C(12)–O(7)–C(20)	102.7(7)
		C(11)–O(8)–C(20)	103.9(8)

Table 4. Hydrogen Bonds

D–H···A	d (D–H) (Å)	d (H···A) (Å)	d (D···A) (Å)	∠(DHA) (°)
O(4)–H(4)···O(2)$^{a)}$	0.82	2.35	2.864 (8)	121.5

Symmetry transformations used to generate equivalent atoms: a) $-x+2$, $y-1/2$, $-z+1/2$.

Experimental

General Melting points were measured on a Bock Monoscop hot plate-type melting point apparatus, and are uncorrected; GC/MS spectra were taken on a QP-7000 Shimadzu, with fused silica capillary column (30 m× 0.25 mm ID), film (5% phenyl, 95% methylsilicon) thickness 0.25 μm, and the output of an IBM computer with software Class 500 and NIST library for comparison; NMR spectra were recorded on Bruker FT-400; IR spectra were taken on a Nicolet Magenta 550 FT IR spectrometer.

The Plant Material *Justicia heterocarpa* T. ANDERS. (family Acanthaceae) was collected at Um Essabayya Island about 80 km south Konfoza City in the Red Sea in February 2001, and identified by Prof. Dr. A. Faied,

Prof. of Plant Taxonomy, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. A voucher specimen was deposited at the Herbarium of King Abdulaziz University.

Processing of Plant Material The fresh aerial parts of *J. heterocarpa* (200 g) were soaked at room temperature in MeOH for 24 h. Evaporation under reduced pressure gave a crude extract (5.4 g) which was defatted by cold MeOH, giving a defatted extract (2.8 g).

Separation of the Constituents The defatted extract was separated by silica gel CC into five fractions, Jh1—Jh3, Jh4a and Jh4b.

Fraction Jh1 (350 mg, eluted by pet.ether/ether 9 : 1) was separated by GC/MS into palmitic acid at t_R 10.79 min (27.3%), linoleic acid at t_R 12.54 min (36.4%), oleic acid at t_R 12.59 min (13.6%) and stearic acid at t_R 12.73 min (9.1%).

Fraction Jh2 (620 mg, eluted by pet.ether/ether 3 : 1) afforded, by GC/MS, hexahydrofarnesylacetone (**1**) at t_R 9.43 min (9.1%), farnesylacetone (**2**) at t_R 10.21 min (4.5%), palmitic acid at t_R 10.76 min (13.6%), phytol at t_R 12.18 min (12.7%), oleic acid at t_R 12.53 min (31.8%) and stearic acid at t_R 12.73 min (4.5%), 4,8,12,16-tetramethylheptadecan-4-olide (**3**) at t_R 14.40 min (9.5%) and (*Z*,*E*)-farnesyl acetate (**4**) at t_R 14.64 min (4.6%).

Fraction Jh3 (200 mg, eluted by pet.ether/ether 1 : 1) gave, by GC/MS, palmitic acid at t_R 10.79 min (36.4%), linoleic acid at t_R 12.53 min (45.5%) and stearic acid at t_R 12.73 min (9.1%).

Fraction Jh4a (360 mg, eluted by ether) contained β-sitosterol and stigmasterol (4 : 1).

Fraction Jh4b (eluted by ether after Jh4a) precipitated crystals of lignan (**5**) (900 mg).

Lignan (**5**): Colorless crystals; mp 215 °C; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3467.5 (OH), 3090.0, 2966.3, 2903.6 (stretching CH_3, CH_2, CH), 1772.7 (γ-lactone), 1620.9 (C=C), 1484.4 (benzene rings), 1226.3, 1189.0, 1034.9, 927.9, 513.1; ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1; MS *m*/*z* (rel. int. %): 398.370 $[M^+]$ (87.7) (Calcd for $C_{21}H_{18}O_8$: 398.370), 399 $[M+1]^+$ (24.2% of M^+), 400 $[M+2]^+$ (4.2% of M^+), 380 $[M-H_2O]^+$ (44.0), 297 (15.0), 267 (100.0), 237 (15.8), 225 (17.2), 152 (15.0), 134 (21.2), 104 (38.0), 91 (24.0); $[\alpha]_D^{22}$ +75.0° ($CHCl_3$; *c*=0.33).

Crystal Structure Determination Data were collected at 293 K on an Enraf-Nonius CAD4 diffractometer using Mo$K\alpha$ radiation (λ 0.71069 Å). All data were corrected for Lorentz and polarization effects.

The structure was solved by a direct method with SHELXS-97 then refined anisotropically (non-hydrogen atoms) by full matrix least squares on F^2 using the SHELXL-97. The H-atoms were calculated geometrically and refined with a riding model. Further details are given in Table 2. Bond lengths and angles are given in Tables 3 and 4.

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