Knerachelins A and B, Antibacterial Phenylacylphenols from Knema furfuracea

Abdellatif Zahir, Akino Jossang, Bernard Bodo, Hamid A. Hadi, Hubert Schaller, and Thierry Sevenet

J. Nat. Prod., **1993**, 56 (9), 1634-1637• DOI: 10.1021/np50099a031 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 9, pp. 1634-1637, September 1993

KNERACHELINS A AND B, ANTIBACTERIAL PHENYLACYLPHENOLS FROM *KNEMA FURFURACEA*

ABDELLATIF ZAHIR, AKINO JOSSANG, BERNARD BODO,*

Laboratoire de Chimie, URA 401 CNRS, Muséum National d'Histoire Naturelle, 63, rue Buffon 75005, Paris, France

HAMID A. HADI, HUBERT SCHALLER,

Department of Chemistry, University of Malaya, Kuala Lumpur 0102, Malaysia

and THIERRY SEVENET

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France

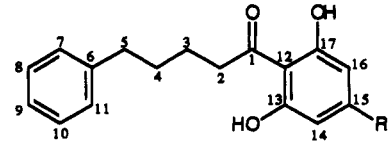
ABSTRACT.—Two new phenylacylphenols, knerachelins A [**1**] and B [**2**], with antibacterial activity have been isolated from the leaves of *Knema furfuracea* (Myristicaceae), and their structures were determined from spectral data (ir, ms, nmr).

The genus *Knema* (Myristicaceae), distributed in tropical Africa, Asia and Australasia, and used in traditional medicine, has been subjected to few phytochemical studies. In addition to lignans, a series of homologous anacardic acids was isolated from the seed oil of *Knema elegans* (1) and the stem bark of *Knema furfuracea* (Hk.f. et Th.) Warb. (2,3). Acyl phenol derivatives were identified as constituents of *Knema laurina, Knema tenuinervia* (4), and *Knema austrosiamensis* (5). Related compounds, malabaricones B and C, recently isolated from mace, the dried seed covers of nutmeg (*Myristica fragrans*), were shown to have antimicrobial activity (6).

Several parts of *K. furfuracea* are used in South Asian folk medicine. In this paper, we report the isolation from the leaves of *K. furfuracea* and the structure elucidation of two novel acylphenols, knerachelins A [**1**] and B[**2**].

RESULTS AND DISCUSSION

The EtOH extract of the leaves showed antibacterial activity against *Staphylococcus aureus*, with an MIC value of 19 μg/ml, and the CH_2Cl_2-soluble fraction of this extract showed a similar activity. Two bioactive components, isolated from this later fraction by Si gel cc monitored with the antibacterial bioassay against *S. aureus* and further recrystallized from EtOH/H_2O, were knerachelin A [**1**] and knerachelin B [**2**].



1 R=OMe
2 R=H

The molecular formula $C_{18}H_{20}O_4$ for knerachelin A was determined by hrms. The ^{13}C-nmr spectrum showed signals for one carbonyl carbon, 12 aromatic carbon atoms, one MeO, and four methylene groups. The ^{1}H-nmr spectrum displayed four methylene groups forming a short linear chain, one MeO group, seven aromatic protons, and the OH at δ 9.48 ppm (Table 1). From analysis of the nmr ^{1}H-^{1}H and ^{1}H-^{13}COSY spectra, the aromatic protons were distributed in two spin systems, one at δ 7.17 and 7.26 being characteristic of a monosubstituted phenyl, and a sharp singlet δ 5.95 belonging to a tetrasubstituted phenyl group.

The COLOC spectrum optimized for J_{CH}=7 Hz allowed the assembly of these systems. ^{1}H-^{13}C long range correlations were observed between the protons at δ 5.95 and the following carbon atoms: the quaternary carbon at δ 104.9 (C-12) and the oxygenated aromatic carbons at δ

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Knerachelins A [**1**] and B [**2**].

Position	Compound					
	1			**2**		
	δ ^{13}C	δ ^{1}H	*J* (Hz)	δ ^{13}C	δ ^{1}H	*J* (Hz)
1	206.4	—		208.2	—	
2	43.8	3.12 t	7.0	44.5	3.16 t	7.0
3	24.4	1.74 m		24.1	1.73 m	
4	31.4	1.74 m		31.0	1.73 m	
5	35.8	2.65 t	7.1	35.7	2.65 t	7.2
6	142.4	—		142.3	—	
7	128.3	7.17 m		128.3	7.18 m	
8	128.2	7.26 m		128.2	7.25 m	
9	125.6	7.17 m		125.6	7.17 m	
10	128.2	7.26 m		128.2	7.25 m	
11	128.3	7.17 m		128.3	7.18 m	
12	104.9	—		110.0	—	
13	163.4	—		161.3	—	
14	94.3	5.95 s		108.3	6.38 d	8.2
15	165.7	—		135.9	7.20 t	8.2
16	94.3	5.95 s		108.3	6.38 d	8.2
17	163.4	—		161.3	—	
OMe	55.4	3.75 s		—	—	
OH	—	9.48 s		—	9.86 s	

163.4 (C-13 and -17) and 165.7 (C-15). This later signal showed in addition a cross peak with the MeO protons. The quaternary carbon atom at δ 142.4 (C-6) was correlated with the methylene at δ 2.65, whereas the carbonyl carbon atom at δ 206.4 (C-1) gave a cross peak with the methylene protons at δ 3.12. From these observations, the structure **1** was proposed for knerachelin A.

Spectral data for knerachelin B [**2**] were similar to those of **1**, suggesting a closely related structure. Its hrms was in agreement with the molecular formula $C_{17}H_{18}O_3$. The ^{1}H- and ^{13}C-nmr spectra differed only from those of knerachelin A by the absence of the MeO group and hence indicated a trisubstituted symmetrical phenolic ring with a three-proton system forming a triplet (1H) and a doublet (2H) with a large vicinal mutual coupling (8.2 Hz). The structure was corroborated by the COLOC spectrum optimized for J_{CH}=7 Hz, which displayed correlations between the quaternary carbon atom at δ 110.0 (C-12), 161.3 (C-13 and C-17), and the aromatic protons at δ 6.38. The aromatic carbon at δ 161.3 was correlated with the proton at δ 7.20 (H-15).

The antibacterial activities (MIC) of **1** and **2** against various Gram-positive and Gram-negative bacteria examined by the agar dilution method are shown in Table 2. Both compounds are active against Gram-positive bacteria, showing for various strains of *S. aureus* an MIC value of 8 μg/ml for **1** and 4 μg/ml for **2**. With *Streptococcus pneumoniae*, the MIC value of 8 μg/ml was observed for both compounds.

Knerachelins A [**1**] and B [**2**] are structurally related to antibiotic 5-alkylresorcinols such as those isolated from *Hakea trifurcata* (7) and also to malabaricones B and C (6). The 5-alkylresorcinols of *H. trifurcata* were found to promote ΦX174DNA strand scission in the presence of Cu(II) (7). Knerachelins A and B were tested for their ability to cleave form I DNA according to the experimental procedure described by

TABLE 2. Antibacterial Spectra (MIC, μg/ml) of Knerachelins A [**1**] and B [**2**].

Test organism	Compound		
	1	**2**	Crude Extract
Staphylococcus aureus IP 8203	8	4	
Staphylococcus aureus 209 P	8	4	19
Staphylococcus aureus ATCC 25923	8	4	
Staphylococcus aureus V. de G. 26109	8	4	
Staphylococcus aureus Duc	8	4	
Staphylococcus aureus Duchaussoy	8	4	
Streptococcus A Dig 7	8	4	
Streptococcus pneumoniae 4314-03	8	8	
Streptococcus pneumoniae 6254.013	8	8	
Streptococcus pneumoniae 6176-23F	8	8	
Enterococcus faecalis ATCC 29212	32	16	
Enterococcus faecium ATCC 19581	32	16	
Escherichia coli NIHJ-JC2	>128	>128	
Escherichia coli V 2019	>128	>128	
Salmonella typhimurium IPL	>128	>128	
Klebsiella pneumoniae IP 7824 (Caroli)	>128	>128	
Enterobacter aerogenes HM12	>128	>128	
Serratia marcescens Guilpin (759)	>128	>128	
Pseudomonas aeruginosa IP A 237	>128	>128	

Scannel *et al.* (7). However, with treatment of 100 ng of ΦX174 DNA with knerachelin A [**1**] (48 μg) or knerachelin B [**2**] (24 μg) in the presence of $CuSO_4$ (120 μM), no DNA cleavage was observed. Knerachelins A and B do not appear to exert their antibacterial activity through the same mechanism at 5-alkylresorcinols. This may be explained by the different disposition of the OH groups on the phenyl ring with respect to the acyl chain.

EXPERIMENTAL

GENERAL METHODS.—Nmr spectra (^1H 300.13 MHz and ^{13}C 75.47 MHz) were performed on an AC 300 Bruker Spectrometer. Ei and cims were obtained with a Nermag Sidar V 3.0 mass spectrometer, and the hrms were obtained on a V.G. Analytical ZAB-HF mass spectrometer at the Centre d'Analyses du CNRS, Lyon. The ir spectra were recorded in KBr discs on a Perkin-Elmer 881 ir spectrophotometer.

PLANT MATERIAL.—The leaves of *K. furfuracea* were collected in October 1990 at Bukit Besi (Terengganu, Malaysia). A voucher specimen is on deposit (KL 3964) in the National Herbarium (Laboratoire de Phanérogamie, MNHN, Paris).

ISOLATION OF COMPOUNDS.—The dried and ground leaves (0.37 kg) were extracted with EtOH, and the solvent was evaporated under reduced pressure to yield a crude extract (19.5 g) which was fractioned by dissolution in CH_2Cl_2. The soluble fraction (11 g) was chromatographed on Si gel with CH_2Cl_2 and then CH_2Cl_2-MeOH (9:1) as eluent. Fractions 6–9 yielded crude **1** (3.8 g), which was crystallized in EtOH/H_2O. Fraction 5 (0.6 g) was rechromatographed under the same conditions to yield **2** (0.1 g), which was similarly crystallized.

Knerachelin A [**1**].—$C_{18}H_{20}O_4$: colorless needles; mp 101–102° (EtOH/H_2O); ir (KBr) ν max cm^{-1} 3280, 2943, 2864, 1647, 1593, 1527, 1460, 1429, 1396, 1374, 1334, 1305, 1250, 1226, 1206, 1166, 1074, 1053, 984, 902, 818, 728, 696; eims *m/z* (rel. int.) $[M]^+$ 300 (10), 282 (15), 253 (1), 195 (7), 182 (9), 167 (100), 153 (2), 140 (23), 95 (3), 91 (15), 77 (4), 69 (3), 65 (5), 55 (5); hrms $[M]^+$ 300.1362 (calcd for $C_{18}H_{20}O_4$, 300.1362); cims (NH_3) *m/z* $[M+NH_4]^+$ 318, $[M+H]^+$ 301.

Knerachelin B [**2**].—$C_{17}H_{18}O_3$: colorless needles; mp 107–108° (EtOH/H_2O); ir (KBr) ν max cm^{-1} 3300, 2945, 2860, 1628, 1599, 1510, 1455, 1382, 1341, 1239, 1204, 1183, 1040, 985, 920, 900, 799, 749, 724, 699; eims *m/z* (rel. int.) $[M]^+$ 270 (9), 252 (16), 164 (14), 152 (9), 137 (100), 91 (55), 81 (23), 65 (20), 55 (21); hrms $[M]^+$ 270.1256 (calcd for $C_{17}H_{18}O_3$, 270.1256); cims (NH_3) *m/z* $[M+NH_4]^+$ 288, $[M+H]^+$ 271.

ANTIBACTERIAL ACTIVITY.—The isolation of

knerachelins A [**1**] and B [**2**] was monitored by antibacterial activity against *Staphylococcus aureus* (strain 209 P; ATCC 6538) by the agar diffusion test using 6 mm diameter pits. The samples were dissolved in DMSO, and inhibitions were measured after 24 h of incubation at 37°. MIC values were determined by microplate titration in agar medium (8).

DNA CLEAVAGE ACTIVITY.—To reaction mixtures (15 μl final volume, pH 7.5) containing 50 mM sodium cacodylate and 100 ng of ΦX174 RF I DNA, were added the examined samples: 48 μg knerachelin A [**1**] and $CuSO_4$ 120 μM; 24 μg knerachelin B [**2**] and $CuSO_4$ 120 μM; $CuSO_4$ 160 μM; $CuSO_4$ 200 μM. The reaction mixtures were incubated at 25° for 1 h and then stopped by the addition of 1 μl of EDTA 200 mM. Two μl of a 0.15% bromophenol blue/75% glycerol solution was added to each reaction mixture. Each sample was analyzed on a 0.8% agarose gel by electrophoresis carried out at 80 volts for 1.5 h in 44.5 mM Tris-borate buffer, pH 8.0. The DNA was stained by 100 ml of a 0.5 μg/ml aqueous solution of propidium iodide. Only the experiment with $CuSO_4$ 200 μM showed the presence of relaxed circular duplex DNA (Form II DNA) due to partial cleavage.

ACKNOWLEDGEMENTS

This work was conducted under the framework of the collaborative program between CNRS and the University of Malaya (Fitolab, Kuala Lumpur). We gratefully acknowledge M.F. Rémy for the extraction of plant material, Dr. J.-P. Brouard (MNHN) for the ms, Dr. J. Favre-Bonvin (Service central d'analyse due CNRS, Lyon) for the hrms, Mrs. C. Servy for the preliminary antibacterial bioassays and Rhône-Poulenc Rorer (Antibiotic Department) for the larger screening.

LITERATURE CITED

1. G.F. Spencer, L.W. Tjarks, and R. Kleiman, *J. Nat. Prod.*, **43**, 724 (1980).
2. M.M.M. Pinto, A. Kijjoa, I.-O. Monanondra, A.B. Gutierrez, and W. Herz, *Phytochemistry*, **29**, 1985 (1990).
3. M.M.M. Pinto and A. Kijjoa, *Quim. Nova*, **13** 243, (1990).
4. A. Kijjoa, M.-J.T.G. Gonzalez, M.M.M. Pinto, I.-O. Monanondra, and W. Herz, *Planta Med.*, **57**, 575 (1991).
5. M.-J.T.G. Gonzalez, M.M.M. Pinto, A. Kijjoa, C. Anantachoke, and W. Herz, *Phytochemistry*, **32**, 433 (1993).
6. K.Y. Orabi, J.S. Mossa, and F.S. El-Feraly, *J. Nat. Prod.*, **54**, 856 (1991).
7. R.T. Scannel, J.R. Barr, V.S. Murty, K.S. Reddy, and S.M. Hecht, *J. Am. Chem. Soc.*, **110**, 3650 (1988).
8. D.A. Vanden Berghe and A.J. Vlietinck, in: "Methods in Plant Biochemistry." Ed. by K. Hostettmann, Academic Press, New York, London, 1991, Vol. 6, pp. 55–57.

Received 17 February 1993