Tetrahedron Letters,Vol.30,No.29,pp 3785-3788,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Maxwell Pergamon Macmillan plc

KOBOPHENOL A, A UNIQUE TETRASTILBENE FROM CAREX KOBOMUGI OHWI (CYPERACEAE)

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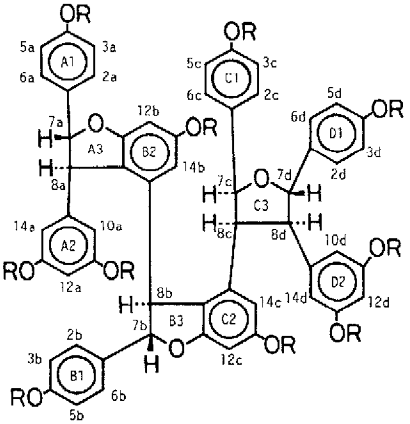
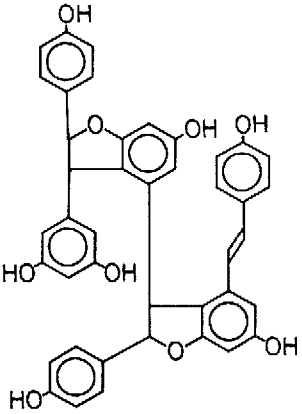
Abstract: An antimicrobial novel tetrastilbene named kobophenol A (**1**) has been isolated from Carex kobomugi Ohwi (Cyperaceae) together with previously known oligostilbenes, miyabenol C (**2**) and ε-viniferin (**3**). The structure of **1** has been determined on the basis of its spectroscopic and chemical properties.

There have been a number of oligostilbenes found in Dipterocarpaceae,1) Vitaceae,2) Cyperaceae3) and Gnetaceae,4) so far, most of which are composed of two to four resveratrol (3,5,4'-trihydroxystilbene) units, and some of them exhibit antimicrobial activity. Recently, we disclosed that some Carex spp. (Cyperaceae) were rich in a new type of oligostilbenes named miyabenols.5) We now report the isolation and structure elucidation of a novel tetrastilbene, kobophenol A (**1**), having a unique 2,3,4,5-tetraaryltetrahydrofuran skeleton, from subterranean parts of Carex kobomugi Ohwi.

Fresh roots and rhizome of C. kobomugi (100g) collected at the seashore of Atsutamura were extracted with methanol. The methanol extracts were fractionated by a series of solvent partitions and antibacterial activity against Staphylococcus aureus was



1 : R = H
4 : R = Me



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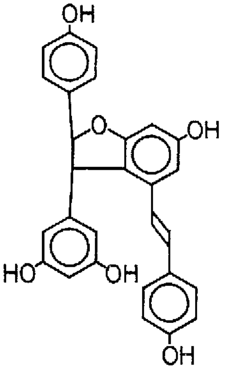

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concentrated into the ether soluble phenolic fraction. Chromatography of this fraction on Sephadex LH-20 with chloroform/methanol(1:1) followed by silica gel preparative TLC (chloroform/benzene/methanol(8:7:5)) gave a major active principle 1 (180mg) as a pale yellow oil ($[\alpha]_D^{20}$ +227°($\underline{c}$=0.17, methanol) along with miyabenol C^5) (**2**, 25mg) and ε-viniferin^2) (**3**, 27mg).

Compound 1 displayed an M^+ ion at $\underline{m/z}$ 924 by FD-MS giving the molecular formula $C_{56}H_{44}O_{13}$, which corresponds to a tetrastilbene, from ^{13}C- and ^{1}H-NMR data (see Table).^6) The UV spectrum of 1 showed an absorption maximum at 285 nm (ε=10800, methanol), but not a characteristic absorption band due to a conjugated stilbene system around 320 nm. The ^{1}H-NMR spectrum indicated the presence of two sets of two benzylic protons coupled to each other (δ 3.48(d,$\underline{J}$=3.5)-5.00(d,$\underline{J}$=3.5) and 4.31(d,$\underline{J}$=1.8)-5.52(brs)), each one of which was bonded to a carbon bearing oxygen, and four successive benzylic protons (δ 5.03(d,$\underline{J}$=4.2)-3.29(dd, $\underline{J}$=5.9,4.2)-3.09(dd, $\underline{J}$=10.6,5.9)-5.15(d,$\underline{J}$=10.6)), two of which were also attached to carbons adjacent to oxygen, as well as of four 4-hydroxyphenyl groups, two 3,5-dihydroxyphenyl groups, and two 1,2,3,5-tetrasubstituted benzene rings. Exhaustive methylation of 1 with Me_2SO_4/K_2CO_3 yielded a decamethyl-derivative (**4**, M^+ 1064) whose IR and ^{1}H-NMR spectra no longer had absorptions assignable to hydroxyl functions, and hence, **1** should have ten phenolic hydroxyl groups, leaving three ether links. Two ether links are attributable to 2,3-diaryl-2,3-dihydrobenzofuran rings as shown in miyabenols and the remaining ether oxygen must be contained in a 2,3,4,5-tetraaryltetrahydrofuran ring which accounts for an isolated four successive benzylic methines in the ^{1}H-NMR.

Table. NMR Data of Kobophenol A (**1**)*

	^{13}C				^{1}H			
	a	b	c	d	a	b	c	d
1	135.2	133.5	131.8	134.3	--	--	--	--
2,6	126.6	127.1	127.4	128.6	7.34 d(8.4)	6.20 d(8.4)	6.40 d(8.4)	7.07 d(8.4)
3,5	116.3	115.7	115.2	115.8	6.88 d(8.4)	6.49 d(8.4)	6.59 d(8.4)	6.76 d(8.4)
4	157.9	157.2	156.0	157.6	--	--	--	--
7	92.3	93.3	84.8	85.1	5.52 brs**	5.00 d(3.5)	5.03 d(4.2)	5.15 d(10.6)
8	58.0	52.1	52.3	62.0	4.31 d(1.8)	3.48 d(3.5)	3.29 dd(5.9,4.2)	3.09 dd(10.6,5.9)
9	147.4	144.5	136.2	139.2	--	--	--	--
10	106.6	119.8	124.2	109.1	6.02 brs	--	--	5.80 d(2.2)
11	159.4	161.8	160.8	158.1	--	--	--	--
12	101.9	96.1	95.7	103.3	6.02 brs	6.51 d(1.8)	5.99 d(2.0)	6.08 t(2.2)
13	159.4	160.7	158.6	158.1	--	--	--	--
14	106.6	108.2	110.2	109.1	6.02 brs	5.95 d(1.8)	6.41 d(2.0)	5.80 d(2.2)

*Assignments of signals were established by HH-, CH- and long range CH-COSY experiments.
**Coupling constants could not be clearly given because of the presence of allylic coupling with H-2(6)a.

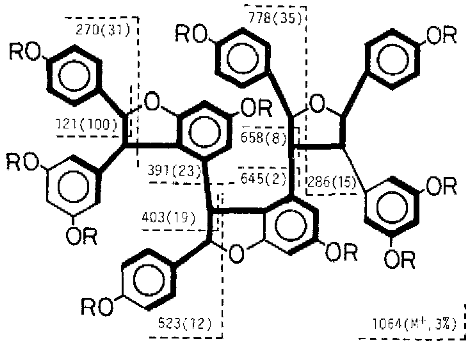

Fig.1. MS Fragmentation Pattern of **4** (R=Me)
Thickened connectivities were established by the HH-, CH-, and long range CH-COSY of **1** (R=H).

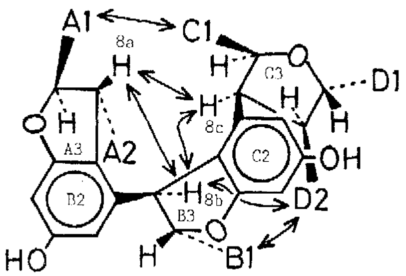

Fig.2. Relative Stereostructure of 1
A1=B1=C1=D1= 4-hydroxyphenyl
A2=D2= 3,5-dihydroxyphenyl

These data suggest **1** to be a novel resveratrol tetramer similar to miyabenols. The plane structure was deduced principally from HH-, CH- and long range CH-COSY experiments, e.g. $^{3}\underline{J}_{CH}$ cross peaks between H-7a/C-2(6)a, H-8a/C-10(14)a, H-7b/C-2(6)b, H-2(6)c/C-7c, and H-7d/C-2(6)d, and confirmed by the mass fragmentation pattern of **4** (Fig.1). Co-existence of **1** with miyabenol C (**2**) and ε-viniferin (**3**) in the same plant also supports this structure from biogenetic grounds.

The relative stereochemistry of **1** has been assigned from results of NOESY experiments. On the ring A3, NOE interactions were clearly observed between H-7a/10(14)a and H-8a/2(6)a. The same relation was obtained on the ring B3, that is, NOEs between H-7b/14b and H-8b/2(6)b. These results strongly suggested the relative configurations of the two aryls on both rings were trans.$^{7)}$ On the ring C3, NOEs between H-7c/8c, H-7c/8d, and H-8c/8d indicated cis-relationship of these protons, and NOEs on H-8d/2(6)d, H-7c/2(6)d, and H-7d/10(14)d revealed that H-7d was oriented trans to H-8d. The relative stereochemical relationship among the rings A3, B3, and C3 is more complicated and the NOESY experiments are again informative to determine it. The presence of NOEs between H-8a/8b, H-8a/8c, and H-8b/8c indicates the spatial relationship of the rings A3, B3 and C3 as depicted in Fig. 2. Although this configuration of **1** accounts for NOE interactions between H-8b/10(14)d as well as between protons on the rings A1 and C1, and between B1 and D2, the relative orientation of the ring C3 to the ring C2 is somewhat ambiguous at this stage.

Compound **1** showed moderate antibacterial activity against *Staphylococcus aureus*.$^{8)}$ It is noteworthy that kobophenol A has a unique 2,3,4,5-tetraaryltetrahydrofuran skeleton which is unprecedented in nature. This partial structure has only been seen in a synthetic product,9) whereas a number of 2,5-diaryl-3,4-dimethyltetrahydrofuranoids have been isolated as lignans.10) Studies on the absolute configuration and biological role of **1** are now in progress.

Acknowledgments: We thank Ms. Yumiko Misu, Center for Instrumental Analysis, Hokkaido University, and Mr. Kenji Watanabe of our Faculty for NMR and MS mesurements, respectively.

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6) FD- and EI-MS were determined with a JEOL 01SG-2 instrument. NMR spectra were recorded on JEOL FX-270 (270MHz for ^{1}H and 67.5MHz for ^{13}C) and Bruker AM-500 (125MHz for ^{13}C) instruments using acetone-d_6 as a solvent.
7) The NOE difference spectra of **1** exhibited weak NOEs on H-7a/8a and H-7b/8b in spite of their trans configurations. Two trans-oriented pseudoequatorial vicinal protons on a dihydrobenzofuran ring can locate proximally enough, ca. 2.7Å from molecular models, to cause dipole interaction as observed in lophyrochalcone. cf. R. G. Tih, B. L. Sondengam, M. T. Martin and B. Bodo, Tetrahedron Lett., **30**, 1807 (1989).
8) Tested microbes were kindly delivered by Prof. S. Takao, Hokkaido University. Diameters of inhibitory zones by the paper disk method using 8mm disks are as follows (mm/μg disk): **1**:12/500, 9/50; **2**:13/500, 11/50; **3**:16/100, 15/50.
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(Received in Japan 16 May 1989)