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NEOLIGNANS FROM *PIPER FUTOKADSURA*

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Key Word Index—*Piper futokadsura*; Piperaceae; neolignans; kadsurenone; kadsurin A; kadsurin B; structural determination; biological activity.

Abstract—The structures of three new neolignans, kadsurenone, kadsurin A and kadsurin B, isolated from *Piper futokadsura* were determined by chemical and spectral analysis and X-ray diffraction study. Their biological activities are reported.

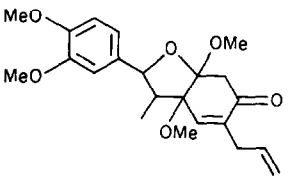
INTRODUCTION

Piper futokadsura is a medicinal plant that grows in Fu-Chien and Taiwan provinces, in the south east region of China. The stem part of this plant, haifengteng, is widely used in Chinease herbal medicinal prescriptions for the treatment of asthma and arthritic conditions. A number of natural lignans have been isolated from the leaf of *P. futokadsura* [1–4], and one of the components—piperenone (1) was reported to have insect anti-feeding activity [2]. In order to identify the active principle or ingredients in this plant that are responsible for its possible clinical effects, different extracts of haifengteng were evaluated in several *in vitro* receptor and enzymic assays in our laboratory. A fraction of the extracts were found to inhibit the binding of platelet activating factor (PAF) to a receptor site preparation from the rabbit platelet membrane [5]. PAF is a recently discovered lipid mediator of hypersensitivity and inflammation [6]. Studies have implicated PAF in such diseases as asthma, hypertension, cardiac anaphylaxis and arthritis [7, 8]. Using the PAF binding assay as a guide, three new neolignans were isolated from haifenteng extracts. Their structure assignments and biological activities and some simple chemical modifications are described below.

RESULTS AND DISCUSSION

The methylene chloride extract, which accounts for 2.3% of dry wt, of stems of *P. futokadsura* (haifengteng) was subjected to silica flash CC with a gradient solvent system of hexane and ethyl acetate increasing the amount of ethyl acetate stepwise. The fractions eluted were collected and tested in the PAF *in vitro* binding assay. Biologically active fractions were further purified on HPLC. Three new neolignans were isolated and identified kadsurenone (2), kadsurin A (3) and kadsurin B (4). Together, these three compounds account for all the PAF activity of the crude methylene chloride extract.

Kadsurenone (2), $C_{21}H_{24}O_5$, is a colourless crystalline compound, mp 62.5°. Being one of the major components, it accounts for 3.5% by weight of the methylene chloride extract. It is optically active, $[\alpha]_D^{22} + 3.2$. IR and UV spectra (ν_{max} 1671 cm^{-1}, λ_{max} 285 nm) characterize a dienone system. Mass spectrometry ($[M]^+$ m/z 356) and elemental analysis (Calc. C, 70.77; H, 6.79. Found: C, 70.40; H, 6.71) confirm the empirical formula assigned. The ^1H NMR spectrum (Table 1) revealed the presence of a 1,2,4-trisubstituted aromatic system, one aliphatic and two aromatic OMe groups, a CH_3CH group, an allyl moiety and three single proton resonances at δ5.24, δ5.89



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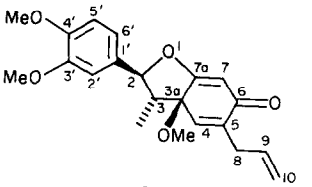

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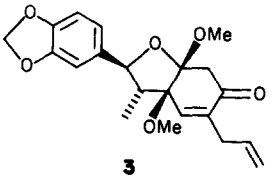

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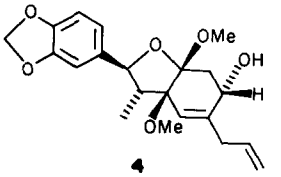

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Table 1. NMR parameters: kadsurenone (2) in $CDCl_3$

H	δ
6′	7.02 *d*, 2
2′	6.90 *dd*, 8.5, 2
3′	6.86 *d*, 8.5
4	6.22 *t*, 1.5
7	5.89 *S*
9	5.85 *m*
2	5.24 *S**
10	5.12 *dt*, 17, 2
	5.11 *dt*, 13, 1.5
ArOMe	3.90
ArOMe	3.89
8	3.15 *d**, 8
OMe	3.04
3	2.69 *qd*, 7, 1.5
Me	1.12 *d*, 7

*Long-range splittings not described.

and δ6.22. Irradiating the allylic methylene collapsed the weakly split δ6.22 signal to a singlet strongly suggesting the sequence HC=C–CH_2CH=CH_2. Irradiating the δ5.24 peak indicated the presence of very weak couplings with the methine quartet at δ3.15 and with the aromatic protons at δ7.02 and δ6.09, thus establishing the fragment AR–CH(X)–CH(Me) where X was most probably oxygen on chemical shift grounds.

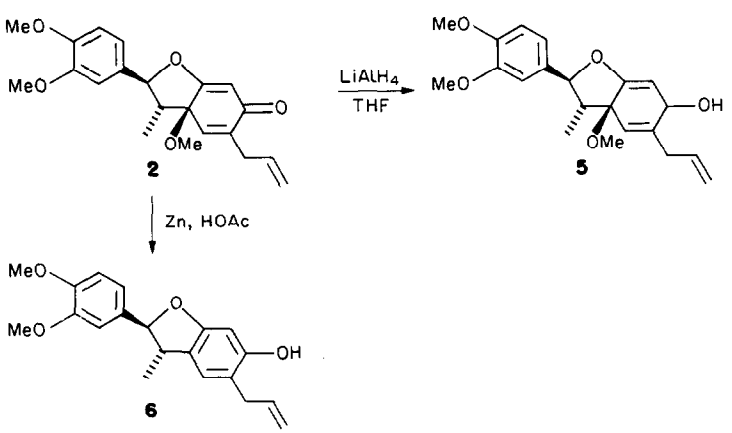
Irradiating the methoxy protons at δ3.04 resulted in a 30% NOE (Nuclear Overhauser Effect) on the methine proton at the C-3 position which strongly suggests that the methine proton at C-3 is *cis* with respect to the neighboring methoxy group at C-3a. This also varifies the assignment of the methoxy group to C-3 and the allyl group to the C-5 position. Furthermore, since irradiating the methoxy group had no effect on the methyl group at C-3, a *trans* relationship between the C-3 methyl and the C-3a methoxy group is indicated. Results of the NOE study coupled with a single X-ray diffraction of the close analogue kadsurin B (4) confirmed the relative stereochemical assignment of kadsurenone as (2*S*,3*S*,3a*S*)-5-allyl -3a -methoxy-2- (3′,4′-dimethoxyphenyl) -3-methyl-2,3,3a,6-tetrahydro-6-oxobenzofuran (2). The structure of kadsurenone (2) is similar to that of mirandin A; the only difference being that 2 has a proton at the 5′-position instead of a methoxy group as in mirandin A [9, 10].

On zinc reduction, 2 was converted to an aromatic derivative 6 (λ_{max}^{MeOH} 285 nm, ε7230). The IR spectrum showed no carbonyl absorption and a new hydroxy absorption at 3605 cm^{-1}. Reduction of 2 with $LiAlH_4$ yielded the derivative 5, whose IR absorption showed no carbonyl absorption. The mass spectrum showed a peak at *m/z* of 358 $[M]^+$.

Kadsurin A (3), $C_{21}H_{24}O_6$, is a very minor component which constitutes 0.1 % of the methylene chloride extract. It is a colourless oil and is optically active ($[\alpha]_D^{22}$ −10.1). IR (ν_{max} 1656 cm^{-1}) and UV (λ_{max} 286 nm) indicate an α,β-unsaturated ketone. Mass spectrometry ($[M]^+$ *m/z* 372) and elemental analysis (Calc. C, 74.48; H, 7.45. Found: C, 74.29; H, 7.38) verified the empirical formula assignment of $C_{21}H_{24}O_6$. In the NMR spectrum, the distinctive methylene dioxy protons at δ5.60 and two aliphatic methoxy signals at δ3.40 and δ3.52 led to the final assignment as (2*S*,3*S*,3a*S*,7a*R*)-5-allyl-3a,7a-dimethoxy-2-(3′,4′-methylenedioxy)-3-methyl-2,3,3a,6,7,7a-hexahydro-6-oxobenzofuran (3).

Kadsurin B (4), $C_{21}H_{26}O_6$, forms colourless crystals mp 101–102°. It is also optically active ($[\alpha]_D^{22}$ −18.5). Mass spectrometry (*m/z* 374) and elemental analysis result (Calc. C, 67.34; H, 6.95. Found: C, 67.52; H, 6.88) agreed with the empirical formula assignment. Absorption at 3500 cm^{-1} in the IR spectrum and lack of carbonyl absorption indicated a hydroxy group at the C-6 position instead of a ketone functionality as in kadsurin A. NMR data [δ0.9 (*d*, 3H, *J* = 7 Hz), 3.25 (*s*, 3H), 3.52 (*s*, 3H), 6.0 (*s*, 2H)] revealed the close relationship of this compound to kadsurin A (3). A single crystal X-ray diffraction analysis (Fig. 1) identified the structure of kadsurin B as (2*S*,3*S*,3a*S*,6*S*,7a*R*)-5-allyl-3a,7a-dimethoxy-2-(3′,4′-methylenedioxy)-3-methyl-2,3,3a,6,7,7a-hexahydro-6-benzofuranol (5).

In the PAF receptor binding assay kadsurenone (2) is the most potent neolignan in this group (Table 2). It inhibits in a specific and competitive manner the binding of ^{3}H-PAF (1 nM) to its receptor site on isolated rabbit platelet plasma membranes with an IC_{50} of 1.7×10^{-7} M.



Scheme 1. Products of reduction of kadsurenone (2).

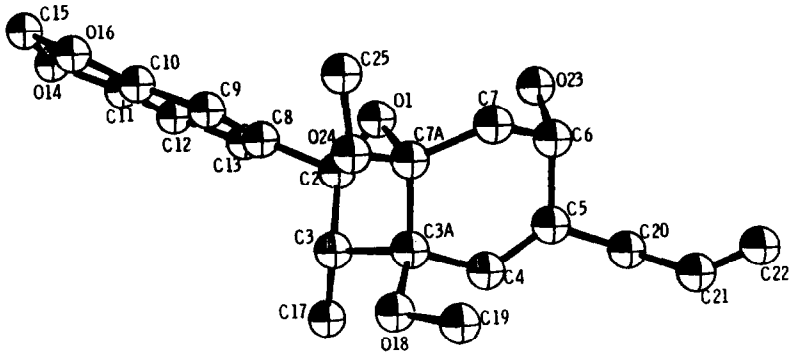


Fig. 1. Computer generated perspective drawing of **4** from X-ray co-ordinates showing the relative stereochemistry.

For comparison, the inhibitory activities of three other neolignans in haifengteng, piperenone, kadsurin A and B, as well as other chemically modified derivatives, are listed in Table 2.

EXPERIMENTAL

The dried plant material was collected from Taiwan. One kilogram of sliced stems of *P. futokadsura* Sieb. et Zucc. was ground and soaked in 10 l. of CH_2Cl_2 for 72 hr at room temp. The extract was filtered and concd under red. pres. to yield 23 g of a dark green semi-solid with a very distinctive pepper-like aroma. A flash column (10 × 75 cm) packed with 300 g of silica (Kieselgel 60) was equilibrated with hexane–EtOAc (20:1). The crude CH_2Cl_2 extract, which was dissolved in 10 ml of warm CH_2Cl_2 was loaded onto the column and eluted at 5 psi of N_2 with 500 ml hexane–EtOAc (20:1), 1 l. hexane–EtOAc (10:1); 1 l. hexane–EtOAc (5:1) and finally 1 l. hexane–EtOAc (1:1). Fractions of 100 ml were collected and concd. Samples from each fraction were tested in the PAF *in vitro* binding assay. Active fractions were combined to give 2.3 g of a light green semi-solid. This fraction was further purified by prep. HPLC using silica columns and eluted with hexane–EtOAc (3:1). Three compounds active in the PAF assay were obtained. They are kadsurenone (1.14 g), kadsurin A (0.231 g) and kadsurin B (0.247 g).

X-ray diffraction analysis of kadsurin B (**4**). Suitable crystals for X-ray diffraction studies formed with space group symmetry of $P2_1$ with $a = 7.030(1)$ Å, $b = 6.514(1)$ Å, $c = 21.318(2)$ Å, and $\beta = 98.38(1)$ Å for $Z = 2$. Of the 1446 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 1413 were observed ($I > 3\sigma I$). The structure was solved with a multi-solution tangent formula approach and difference Fourier analysis and refined using full-matrix least-squares techniques [9]. Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\Sigma w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.047. Tables I, II and III containing the final fractional coordinates, temperature parameters, bond distances, and bond angles have been deposited with the Cambridge Crystallographic Data Centre, Cambridge, England. The cyclohexene ring and tetrahydrofuran ring of **4** are *cis* fused with OMe groups at the bridgehead positions. The cyclohexene ring had a sofa conformation with C-7 0.57 Å from the plane of the remainder of the ring atoms while the tetrahydrofuran has an envelope conformation with C-3a 0.60 Å from the least squares plane of the remaining four atoms.

Table 2. Inhibition of binding of platelet activity factor by some neolignans

Compound	Concentration	% Inhibition
Piperenone (**1**)	2.58×10^{-6} M	25
Kadsurenone (**2**)	3.0×10^{-6} M	95
	3.0×10^{-7} M	56
	3.0×10^{-8} M	19
Kadsurin A (**3**)	5.0×10^{-5} M	80
	5.0×10^{-6} M	44
	2.7×10^{-8} M	22
Kadsurin B (**4**)	3.0×10^{-6} M	22
5	5.0×10^{-6} M	24
6	5.0×10^{-6} M	26
	1.0×10^{-6} M	0

Reduction of kadsurenone (**2**). Kadsurenone (**2**) (15 mg) was dissolved in 0.5 ml of dry Et_2O and stirred at 0° under N_2 atmosphere. $LiAlH_4$ (1.5 mg) was added and the reaction mixture stirred at 0° for 10 min. H_2O (0.5 ml) was added and the mixture extracted with Et_2O (2 × 5 ml). The combined Et_2O extracts were dried ($MgSO_4$) and concd to a colourless oil. Purified by prep. TLC, a new compound was obtained which was identified by NMR, IR and mass spectra as 2α-(3,4-dimethoxyphenyl-2β, 3α-dihydro-3a,α-methoxy-3β-methyl-5-allyl-6-tetrahydrobenzofuranol (**5**).

Compound **2** (40 mg) was dissolved in 3 ml of HOAc. Freshly prepared Zn powder (300 mg) was added portionwise and the heterogeneous mixture stirred at ambient temp for 3 hr. HOAc was removed under red. pres. and the residue extracted with Et_2O (2 × 10 ml). The Et_2O extracts were concd to give a colourless oil. Purified by prep. TLC using hexane–EtOAc (2:1) R_f 0.25), the reduced product (23 mg, 65% yield) was identified by NMR, IR and mass spectra as 2α-(3,4-dimethoxyphenyl)-2β,3α-dihydro-3β-methyl-5-allyl-6-benzofuranol (**6**).

Inhibition of PAF receptor binding. The inhibition of ^{3}H-PAF (New England Nuclear, Boston, Mass.) binding to isolated rabbit platelet plasma membranes was carried out by a filtration technique to separate the free and bound ^{3}H-PAF. The percent inhibition of PAF receptor binding by the compound was expressed as

$$\% \text{ Inhibition} = \frac{\text{Total binding} - \text{Total binding with compound}}{\text{Total binding} - \text{Total binding with excess unlabeled PAF}} \times 100\%.$$

Detailed procedures for platelet plasma membrane isolation and receptor binding inhibition has been described earlier [12].

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