The New Schizandrin-type Lignans, Kadsurin and Kadsurarin

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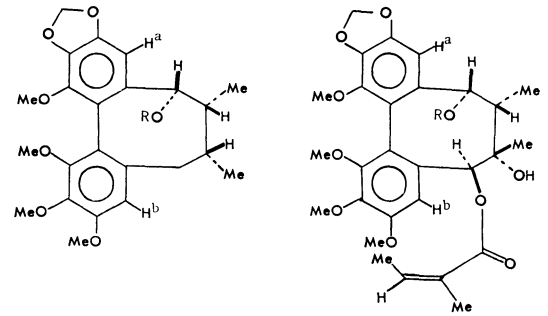
Two new lignans, kadsurin and kadsurarin, were isolated from *Kadsura japonica* Dunal, and their structures were established. The stereostructures of the schizandrin-type lignans including their conformations were elucidated by measurements of intramolecular nuclear Overhauser effects.[1)]

The decoction of the stems of *Kadsura japonica* Dunal ("Binnan Kadsura" in Japanese) is used in Taiwan as a remedy for snake-bites and also as an antipyretic, antispasmodic and anodyne by the local people.[2)] In the course of our search for physiologically active substances of this plant, we isolated two new schizadrin-type lignans, named kadsurin (**1**) and kadsurarin (**2**). In this paper we wish to describe the isolation and structures of kadsurin and kadsurarin. The conformation of these new lignans with a cyclooctadiene system, which can adopt two possible conformers, is discussed.

The dried stems of *Kadsura japonica* Dunal were pluverized, and then extracted with a large amount of hexane. The extracts were directly chromatographed on silica gel (Kieselgel, E. Merck, Darmstadt) and eluted with hexane–EtOAc (4 : 1) to give colorless needles of kadsurin (**1**), mp 157—158 °C, in a 0.0015% yield. Further elution with hexane–EtOAc (2 : 1) afforded colorless needles of kadsurarin (**2**), mp 255—256 °C, in a 0.005% yield.

Structure of Kadsurin. Kadsurin (**1**) has a molecular formula $C_{25}H_{30}O_8$, and can be regarded as a biphenyl-type compound with two aromatic protons (δ 6.48 and 6.60) on the basis of its spectral data [ν_{max} 1615, 1600, 1585, and 1495 cm^{-1}; λ_{max} 278, 254, and 230 nm (ε, 3500, 11300, and 26300, respectively)]. The UV spectrum of this lignan in particular is almost superimposable on that of schizandrin (**3**),[3)] indicating that kadsurin is a schizandrin-type lignan. From the NMR spectral data (see the Table), kadsurin has four methoxyl groups (δ 3.65, 3.83, 3.89, and 3.92) and one methylenedioxy group (δ 5.99). All of them should be attached to the two aromatic rings, although their accurate positions are still uncertain. The presence of a partial structure [■–CH(OAc)–CH(Me)–CH(Me)–CH_2–■] can be confirmed by analysis of the NMR spectrum of kadsurin with aid of double resonance experiments: irradiation at the center of δ 1.92—2.20 (2H, complex) caused each signal at δ 0.95, 1.08, 2.67, and 5.67 to collapse to a sharp singlet. The presence of the AcO–$\underset{|}{C}$H– grouping is also confirmed by its mass and NMR spectra [*m/e* 398(M^+—AcOH); δ 1.60 and 5.67] together with the following chemical evidence. Reduction of Kadsurin (**1**) with $LiAlH_4$ (room temp, 6 h) readily afforded the corresponding hydroxy compound (**4**) (mp 134—135 °C; $C_{23}H_{28}O_7$; ν_{max} 3560 cm^{-1}) in high yields, although **1** resisted base-catalyzed hydrolysis (0.5 M KOH in MeOH–dioxane) in contrast to kadsurarin (**2**). In the NMR spectrum of **4**, the sharp singlet at δ 1.60 in **1** was not observed, but the signal at δ 5.67 in **1** shifted to δ 4.64 in a higher magnetic field. The structure including the position of each functional group and conformation of kadsurin (**1**) was clarified by measurement of intramolecular nuclear Overhauser effects (NOE), as follows.

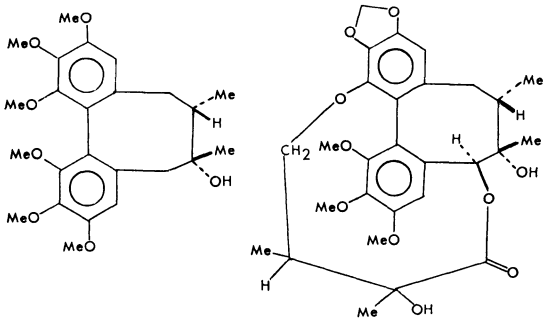
Low intensity irradiation at δ 5.67 (AcO–$\underset{|}{C}$H–) caused a 21% increase in the integrated intensity of one of the aromatic protons (H^a), no enhancement of the signal intensity of the remaining proton (H^b) being observed. This indicates that the distance between H^a and the proton, which is attached to the carbon atom bearing the acetoxyl group, is short. It should be noted that the signal intensity of H^a was not increased by irradiation at the frequencies corresponding to the absorbance of each methoxyl group, indicating that none of them



1 R=Ac
4 R=H

2 R=Ac
5 R=H

(a)



3

6

(b)

Fig. 1.

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Table. NMR spectra of kadsurin (**1**) and kadsurarin (**2**)

1	2
0.95 (3H, d, J=7.0 Hz)	
1.08 (3H, d, J=7.0 Hz)	1.23 (3H, d, J=6.8 Hz)
	1.36 (3H, s)
	1.40 (3H, fine spritted s)
1.60 (3H, s)	1.60 (3H, s)
	1.85 (3H, dq, J=7.5, 1.5 Hz)
	2.10 (1H, br s, OH)
1.92—2.20 (2H, complex)	2.16 (1H, q, J=6.8 Hz)
2.67 (2H, d, J=4.2 Hz)	
3.65 (3H, s)	3.64 (3H, s)
3.83 (3H, s)	3.75 (3H, s)
3.89 (3H, s)	3.90 (3H, s)
3.92 (3H, s)	3.95 (3H, s)
5.67 (1H, br s)	5.68 (2H, br s)
5.99 (2H, br s)	5.90 (2H, s)
	6.00 (1H, br q, J=7.5 Hz)
6.48 (1H, s)	6.43 (1H, s)
6.60 (1H, s)	6.60 (1H, s)

can occupy the position adjacent to H^a. Thus, the remaining methylenedioxy group should be located at the position adjacent to H^a, as shown in [A]. In the next step, the measurements of NOE were focused on the signal (δ 6.60) corresponding to the aromatic proton H^b. Low intensity irradiation at δ 3.92, 2.67, and 0.95 caused 11, 13, and 9.6% enhancement, respectively, of the signal intensity of H^b. Therefore, one of four methoxyl groups (δ 3.92) should be located at a position adjacent to H^b, and one of two secondary methyl groups (δ 0.95) and H^b should be close to each other. The configuration of the remaining secondary methyl group was clarified by the following NOE measurements. Low intensity irradiation at δ 1.08 caused a 12% increase of the signal intensity of the proton attached to the carbon atom bearing the acetoxyl group (δ 5.67), no interaction between the secondary methyl group (δ 1.08) and H^a (δ 6.48) being detected. The undetermined secondary methyl group (δ 1.08) should be placed in such a space as depicted in [A]. This is also confirmed by the appearance of the NMR singlet at δ 5.67 with J value ($\approx$0 Hz), indicating that the dihedral angle between the two vicinal methine protons is almost 90°. Thus, the results can be explained only by the conformation [A] for kadsurin (**1**), and not by the other conformation [B].

Structure of Kadsurarin. Kadsurarin (**2**), whose molecular formula is $C_{30}H_{36}O_{11}$, has the same schizandrin-type chromophore as that of kadsurin (**1**) on the basis of its spectral data [ν_{max} 1620, 1585 sh and 1500 cm^{-1}; λ_{max} 280, 255, and 231 nm (ε, 3200, 10600, and 30000, respectively); δ 6.43 and 6.60]. Further analysis of its NMR spectrum indicates that kadsurarin (**2**) has the same carbon skeleton as that of **1** except for some functional groups.

Comparing the NMR spectra of **1** and **2** (see the Table), we see that the former has two secondary methyl groups (δ 0.95 and 1.08) and one methylene group

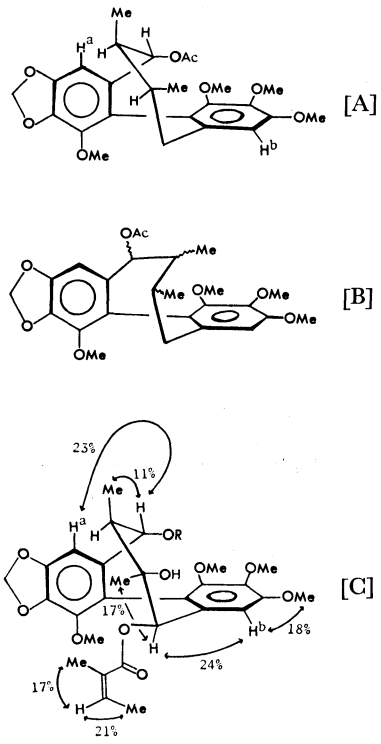

Fig. 2.

(δ 2.67), while **2** has one secondary methyl group (δ 1.27) and no methylene group. However, the latter has a tertiary methyl group (δ 1.36) on the carbon atom having a hydroxyl group, and one proton (δ 5.68)[4] attached to the carbon atom bearing an oxygen atom constituting a part of the angelate (δ 1.40, 1.85, and 6.00).

Kadsurin (**1**) is quite stable under basic conditions. However, when treated with 0.5 M KOH in MeOH–dioxane (room temp, 4.5 h), kadsurarin (**2**) was selectively converted into deacetyl kadsurarin (**5**), mp 227—228 °C ($C_{28}H_{34}O_{10}$), in high yields. In the NMR spectrum of **5**, a new singlet was observed at δ 4.84 (1H) instead of disappearance of the sharp singlet at δ 1.60 (3H) in **2** together with decrease in the integrated intensity at δ 5.68 in **2**. The easy and selective hydrolysis of the acetoxyl group in **2** is attributable to a proximity effect of the tertiary hydroxyl group, whose configuration is based on the NOE measurements of **5**. As shown in [C], the results are essentially similar to those of kadsurin (**1**) except for the following points. No interaction between the tertiary methyl group (δ 1.32) and H^b was detected. However, low intensity irradiation at δ 1.32 caused a 17% enhancement of the integrated intensity of the singlet at δ 5.66. This indicates that the configuration of the tertiary methyl group in **2** clearly differs from that of the corresponding secondary methyl group in **1**. As the first example, the stereostructures of schizandrin-type lignans were established. The stereostructure of schizandrin is still undetermined,[3] but may be similar to that of kadsurarin (**2**), as shown in **3**.

Quite recently, Ikeya *et al.* reported the isolation of gomicin D (**6**), whose stereostructure was also elucidated by means of an X-ray crystallographic analysis of the

corresponding dibromide.[5] The stereostructure of gomicin D is identical with that of kadsurarin (**2**), suggesting that the former can be produced from deacetoxykadsurin in the plant.

Experimental

All the mps are uncorrected. IR spectra were recorded on a JASCO IR-S spectrophotometer. UV spectra were taken on a Perkin-Elmer 202 spectrophotometer using MeOH as a solvent. NMR spectra were recorded on a Varian HA-100 NMR spectrometer using $CDCl_3$ as a solvent. The chemical shifts are given in ppm relative to the internal TMS, only prominent signals being cited (d, doublet; m, multiplet; q, quartet; s, singlet; t, triplet). Mass spectra were obtained on a Hitachi RMU-6D mass spectrometer, operating with an ionization energy of 70 eV. Optical rotations were measured on a JASCO ORD/UV-5 spectrometer using $CHCl_3$ as a solvent.

Isolation of Kadsurin and Kadsurarin. The dried material (2 kg) of the stems of the plant *Kadsura japonica* Dunal was pulverized and extracted with hexane (8 l) by refluxing for 3 h. The hot mixtures were filtered from the undissolved residue which was extracted again in the same way using hexane (8 l). The combined filtrates were evaporated to dryness under reduced pressure. The resulting dark green substance (20 g) was directly chromatographed on silica gel (Kieselgel, E. Merck, Darmstadt) (800 g), and eluted with hexane–EtOAc (4 : 1) to give crude crystals which were repeatedly recrystallized from EtOH to afford colorless needles (30 mg) of kadsurin (**1**); mp 157—158 °C; $[\alpha]_D^{25}=-39°$ ($c=0.13$); ν_{max} (Nujol) 1735, 1615, 1600, 1585, and 1495 cm^{-1}; *m/e* 458 (M^+) and 398 (M^+-60) ($m^*=346$) (Found: C, 65.33; H, 6.66%. Calcd for $C_{25}H_{30}O_8$: C, 65.49; H, 6.60 %). Further elution with hexane–EtOAc (2 : 1) afforded crude crystals, which were recrystallized from EtOAc to give colorless needles (100 mg) of kadsurarin (**2**); mp (dec) 255—256 °C (in a sealed tube); $[\alpha]_D^{25}=-65°$ ($c=0.10$); ν_{max} (Nujol) 3550, 1735, 1720 sh, 1643 sh, 1620 1600 1585 sh, and 1500 cm^{-1}; *m/e* 572 (M^+), 512 (M^+-60) ($m^*=458$), and 412 (Found: C, 63.49; H, 6.28%. Calcd for $C_{30}H_{36}O_{11}$: C, 62.95; H, 6.30%).

Reduction of Kadsurin with $LiAlH_4$. To a solution of kadsurin (20 mg) in tetrahydrofuran (2 ml) was added $LiAlH_4$ (10 mg) at room temperature for 6 h with stirring. After decomposition of excess reagent with ice water, the reaction mixture was diluted with 5% HCl aq solution, and then extracted with EtOAc. The extract was washed with water and then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave a white solid (17 mg), which was recrystallized from MeOH to afford colorless needles of deacetylkadsurin (**4**); mp 134—135 °C; ν_{max} (Nujol) 3560, 1620, 1595, 1575, and 1495 cm^{-1}; δ 0.95 (3H, d, $J=7.0$ Hz), 1.17(3H, d, $J=7.0$ Hz), 1.45 (1H, br s, OH), 1.95 (1H, q, $J=7.0$ Hz), 2.05(1H, m), 2.65(2H, d, $J=4.5$ Hz), 3.68(3H, s), 3.84 (3H, s), 3.89(3H, s), 3.90(3H, s), 4.64(1H, br.s), 5.98 (2H, s), 6.34(1H, s), and 6.60(1H, s); *m/e* 416 (M^+) and 398 (Found: *m/e* 416.1829. Calcd for $C_{23}H_{28}O_7$: *m/e* 416.1835).

Hydrolysis of Kadsurain with Methanolic KOH. To a solution of kadsurarin (23 mg) in a mixed solvent of dioxane (1 ml) and MeOH (0.5 ml) was added, with stirring, 0.5 M methanolic KOH (1 ml) at room temperature. The resulting solution was stirred at room temperature for 4.5 h, and then diluted with a large amount of water to give crystalline precipitates, which were washed with water and then dried under reduced pressure. Recrystallization from hexane–EtOAc afforded colorless needles (16 mg) of deacetylkadsurarin (**5**); mp 227—228 °C; ν_{max} (Nujol) 3500 br, 1715, 1645 sh, 1620, 1585 sh, and 1500 cm^{-1}; δ 1.32 (3H, s), 1.36 (3H, d, $J=7.2$ Hz), 1.39(3H, fine splitted s), 1.73(2H, br s, OH), 1.85 (3H, dq, $J=7.5$, 1.7 Hz), 1.98(1H, q, $J=7.2$ Hz), 3.73(6H, s), 3.87(3H, s), 3.90(3H, s), 4.84(1H, s), 5.66(1H, s), 5.88 (1H, d, $J=1.2$ Hz), 5.92(1H, d, $J=1.2$ Hz), 5.99(1H, qq, $J=7.5$, 1.5 Hz), 6.28(1H, s), and 6.76(1H, s), *m/e* 530 (M^+), 512 and 412 (Found: *m/e* 530.2146. Calcd for $C_{28}H_{34}O_{10}$: *m/e* 530.2152).

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†† The plant was collected at Chiayi, Taiwan.