# ABSOLUTE CONFIGURATION OF KUWANON L, A NATURAL DIELS-ALDER TYPE ADDUCT FROM THE MORUS ROOT BARK

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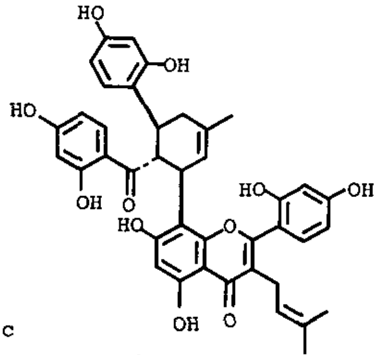
Abstract —— The absolute configuration of kuwanon L (**1**) isolated from the Morus root bark (Morus alba L., Moraceae) was determined as 2S, 3"R, 4"R, 5"S by circular dichroism (CD) spectroscopic study.

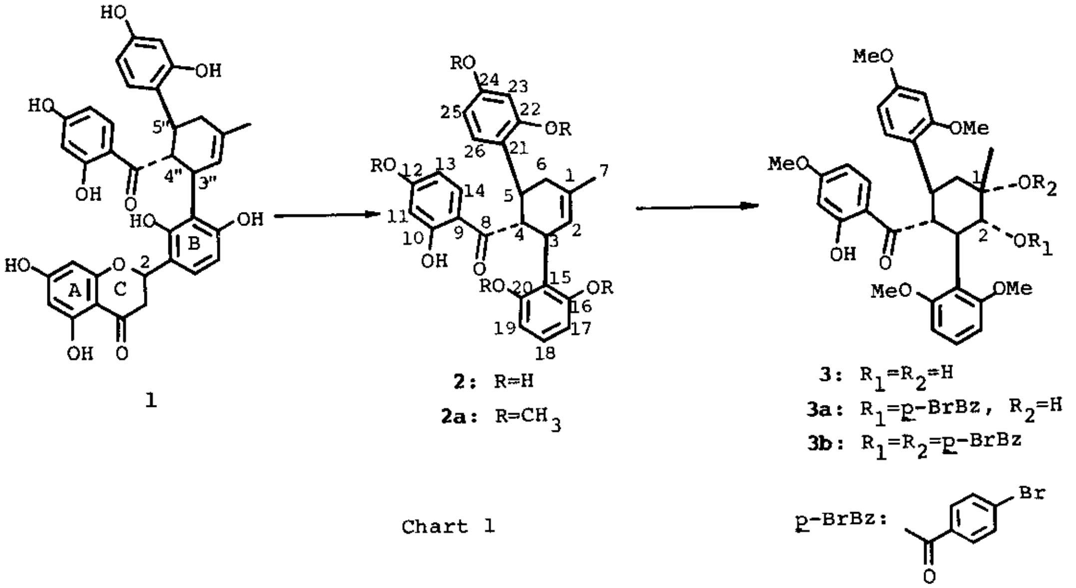
Previously, we reported the structure determination of various Diels-Alder type adducts isolated from the Morus root bark.[1] Some of them, such as kuwanon G[2], are the adducts of chalcones and dehydroprenylflavonoids. This type of adducts have three chiral centers on the methylcyclohexene ring, while the relative configuration of each substituents on the ring has been clarified by the nuclear magnetic resonance (nmr) spectroscopic studies.



kuwanon G

The plane structure of kuwanon L, one of the Diels-Alder type adducts, has been reported as formula **1** including the relative configuration on the methylcyclohexene ring.[3] In the course of our study, we attempt to determine the absolute structure of **1** by using the circular dichroism (CD) spectroscopic study.

Kuwanon L (**1**) possesses four chiral centers at C-2, C-3", C-4", and C-5" positions in the structure. In order to determine the absolute configurations of the three chiral centers on the methylcyclohexene ring, alkali degradation of **1** was carried out as follows: kuwanon L (**1**, $[\alpha]_D^{22}$ -227°, 1.5 g) was dissolved in 50% potassium hydroxide aqeous solution (20 ml), and the solution was refluxed for 1 h to afford compound A (**2**, 870 mg). The compound A (**2**) was obtained as colorless amorphous powder, $[\alpha]_D^{25}$ -208° (c=0.097, MeOH). The molecular ion peak was observed at m/z 448 in mass spectrum. In the $^1$H nmr spectrum of **2** (δ in acetone-$d_6$, 60°C), the proton signals on methylcyclohexene ring were observed at δ 1.70 (3H, s, C-1-$CH_3$), 2.19 (1H, dd, J=4 and 18 Hz, C-6-H), 2.50-2.80 (1H, m, C-6-H), 3.67 (1H, m, C-5-H),



1

**2**: R=H

**2a**: R=$CH_3$

**3**: $R_1=R_2=H$

**3a**: $R_1$=p-BrBz, $R_2=H$

**3b**: $R_1=R_2$=p-BrBz

p-BrBz:

Chart 1

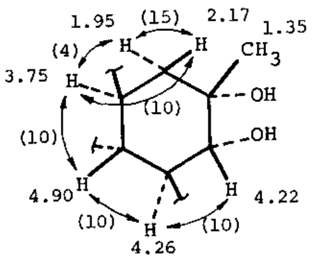


Fig. 1 $^1H$ nmr spectrum of 3, δ in $CDCl_3$ at 55°C

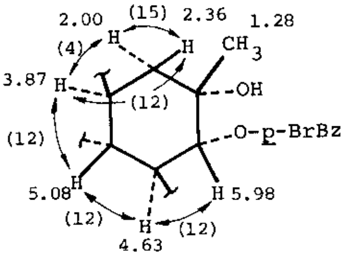


Fig. 2 $^1H$ nmr spectrum of 3a, δ in $CDCl_3$ at 55°C

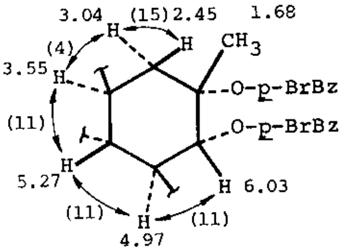


Fig. 3 $^1H$ nmr spectrum of 3b, δ in $CD_3OD$

Table 1 $^{13}C$ nmr Chemical shifts (ppm) of 2 and 3

| | 2$^{a)}$ | 3$^{b)}$ |
|---|---|---|
| C-1 | 133.7 | 71.1 |
| C-2 | 124.9 | 74.6 |
| C-3 | 37.6 | 39.7 |
| C-4 | 46.7 | 47.1 |
| C-5 | 37.6 | 39.7 |
| C-6 | 38.6 | 42.3 |
| C-7 | 23.3 | 27.6 |
| C-8 | 208.8 | 205.9 |
| C-9 | 116.2 | 114.4 |
| C-10 | 163.9 | 164.1 |
| C-11 | 102.3 | 98.3 |
| C-12 | 165.3 | 164.5 |
| C-13 | 107.1 | 104.4 |
| C-14 | 133.6 | 130.8 |
| C-15 | 115.5 | 113.9 |
| C-16 | 155.8 | 158.5 |
| C-17 | 108.1 | 103.5 |
| C-18 | 129.5 | 129.4 |
| C-19 | 107.2 | 103.3 |
| C-20 | 157.3 | 158.7 |
| C-21 | 121.6 | 122.5 |
| C-22 | 155.8 | 157.6 |
| C-23 | 103.4 | 99.4 |
| C-24 | 156.5 | 158.2 |
| C-25 | 107.0 | 105.8 |
| C-26 | 127.5 | 127.9 |
| $OCH_3$ | | 54.8 x2<br>54.9<br>55.0<br>56.3 |

solvent: a) acetone-$d_6$ b) $CDCl_3$

4.49 (1H, br d, J=10 Hz, C-3-H), 4.78 (1H, t, J=10 Hz, C-4-H), and 5.41 (1H, br s, C-2-H). In the aromatic region, two ABX and a $A_2B$ type signals were observed as follows: δ 5.98 (1H, d, J=2 Hz), 6.01 (1H, dd, J=2 and 9 Hz), 6.88 (1H, d, J=9 Hz); δ 6.11 (1H, dd, J=2 and 9 Hz), 6.22 (1H, d, J=2 Hz), 7.56 (1H, d, J=9 Hz); δ 6.17-6.21 (2H, br), 6.66 (1H, t, J=8 Hz). A singlet signal of a hydrogen-bonded hydroxyl group at C-10-OH was observed at δ 12.90 ppm, and the corresponding proton signals for the A and C rings of **1** were not observed in the $^1H$ nmr spectrum of **2**. Moreover, the coupling constants and coupling patterns of the proton signals on the methylcyclohexene ring of **2** were similar to those of the relevant protons of **1**.[4] From the above results, the structure of compound A was represented by the formula **2**, and the compound **2** maintained the same conformation of methylcyclohexene ring of kuwanon L (**1**). Methylation of **2** with dimethyl sulfate afforded a pentamethyl ether (**2a**). The pentamethyl ether (**2a**) was obtained as colorless needles, mp 160 °C, $[\alpha]_D^{23}$ -222° (c=0.095, EtOH), and was positive to methanolic ferric chloride test. Its mass spectrum showed the molecular ion peak at m/z 518. In the $^1H$ nmr spectrum of **2a** (δ in $CDCl_3$), five methoxyl signals and a hydrogen-bonded hydroxyl signal were observed at δ 3.56, 3.78, 3.88 (each 3H, s), 3.69 (6H, s), and 13.08 ppm, respectively. The compound **2a** was treated with osmium tetroxide in ether-35% hydrogen peroxide (1 : 1)[5] to afford a cis-diol **3**. The compound **3** was obtained as colorless needles, mp 115-117 °C, $[\alpha]_D^{24}$ +30° (c=0.11, EtOH), and was positive to methanolic ferric chloride test. The molecular ion peak was observed at m/z 552 in mass spectrum. In the $^1H$ nmr spectrum of **3** (δ in $CDCl_3$, 55°C), five methoxyl signals [δ 3.64, 3.67, 3.75, 3.79 and 3.89 (each 3H, s)], two ABX type signals due to 2-hydroxy-4-methoxybenzoyl and 2,4-dimethoxyphenyl groups, and the signals of 2,6-dimethoxyphenyl group were observed as follows: δ 6.03 (1H, d, J=2 Hz), 6.07 (1H, dd, J=2 and 8 Hz), 7.43 (1H, d, J=8 Hz); δ 6.22 (1H, d, J=2 Hz), 6.24 (1H, dd, J=2 and 8 Hz), 7.00 (1H, d, J=8 Hz); δ 6.30, 6.39 (each 1H, d, J=8 Hz), 6.94 (1H, t, J=8 Hz). The proton signals of the methylcyclohexane-diol ring are shown in Fig. 1. In the $^{13}C$ nmr spectrum of **3**, two sp$^3$ carbon signals bearing the oxygen function were observed at δ 71.1 and 74.6 ppm assignable to C-1 and C-2 positions respectively in the methylcyclohexane ring (Table 1). Furthermore, as shown in Fig. 1, a doublet signal at δ 4.22 (J=10 Hz) was assignable to C-2-H bearing a hydroxyl group. From the above spectral data, the structure of cis-diol was represented by formula **3**. The solution of **3**, p-bromobenzoic anhydride, and 4-dimethylaminopyridine as a catalyst in tetrahydrofuran was refluxed for 10.5 h. After the reaction mixture was treated as usual, the product was purified by preparative thin-layer chromatography to afford a mono-p-bromobenzoate (**3a**, 35% yield) and a di-p-bromobenzoate (**3b**, 1.2% yield) (Chart 1). Mono-p-bromobenzoate (**3a**) was obtained as colorless amorphous powder, $[\alpha]_D^{25}$ +99° (c=0.10, EtOH), and was positive to methanolic ferric chloride test. The molecular ion peak was shown at m/z 734 in the mass spectrum. In the $^1$H nmr spectrum of **3a** ( δ in CDCl$_3$) 55°C), characteristic $A_2B_2$ type signals of a p-bromobenzoyl group were observed at δ 7.44 (2H, d, J=9 Hz) and 7.62 (2H, d, J=9 Hz). The signals of the methylcyclohexane ring moiety were shown in Fig. 2. As shown in Fig. 2, a doublet signal at δ 5.98 ppm (J=12 Hz) assignable to C-2-H was observed in lower field than that of **3** remarkably indicating the p-bromobenzoyl ester attached to C-2 position. From these results, the formula **3a** was proposed for the structure of mono-p-bromobenzoate. Di-p-bromobenzoate (**3b**) was obtained as colorless amorphous powder, $[\alpha]_D^{24}$ +83° (c=0.10, MeOH), and was positive to methanolic ferric chloride test. In its mass spectrum, the molecular ion peak was shown at m/z 918. In the $^1$H nmr spectrum of **3b** (δ in CD$_3$OD), two $A_2B_2$ type signals due to two p-bromobenzoyl groups were observed as follows: δ 7.53 (2H, d, J=9 Hz), 7.65 (2H, d, J=9 Hz); δ 7.86 (2H, d, J=9 Hz), 8.28 (2H, d, J=9 Hz). The proton signals of the methylcyclohexane ring moiety are shown in Fig. 3. The coupling pattern of each proton on the ring was the same as in **3** and **3a**. A doublet signal at δ 6.03 ppm (J=11 Hz) and a double doublet signal at δ 3.04 ppm (J=4 and 15 Hz), which were assigned to C-2-H and one of C-6-H, respectively, appeared in lower field than the corresponding signals of **3**. Moreover, a singlet signal of a hydrogen-bonded hydroxyl group (C-10-OH) was observed at δ 12.80 ppm in the $^1$H nmr spectrum (CDCl$_3$) of 3b. These findings proved the two p-bromobenzoyl groups attached to the C-1 and C-2 positions, respectively. From the above spectral data, the structure of di-p-bromobenzoate was represented by **3b**. The CD spectra of kuwanon L (**1**),[6] compound A (**2**),[7] mono-p-bromobenzoate (**3a**),[8] and di-p-bromobenzoate (**3b**)[9] are shown in Fig. 4 as well as the ultra violet (uv) spectra of each compounds. Comparing the CD spectrum of **3b** with that of **3a**, the exciton coupling between two p-bromobenzoyl chromophores ( $\lambda_{max}$ ca. 245 nm) was clearly recognized in CD curve of **3b**.[10] Furthermore, the differential CD curve of **3b** and **3a** ($\Delta\varepsilon_{3b} - \Delta\varepsilon_{3a}$) emphasized the exciton coupling between two p-bromobenzoyl ones more clearly (Fig. 5). Taking into account the $^1$H nmr spectrum of **3b**, particularly in the methylcyclohexane ring, the conformation of the ring was described as **A** or B which are enantiomers each other (Fig. 6). In these

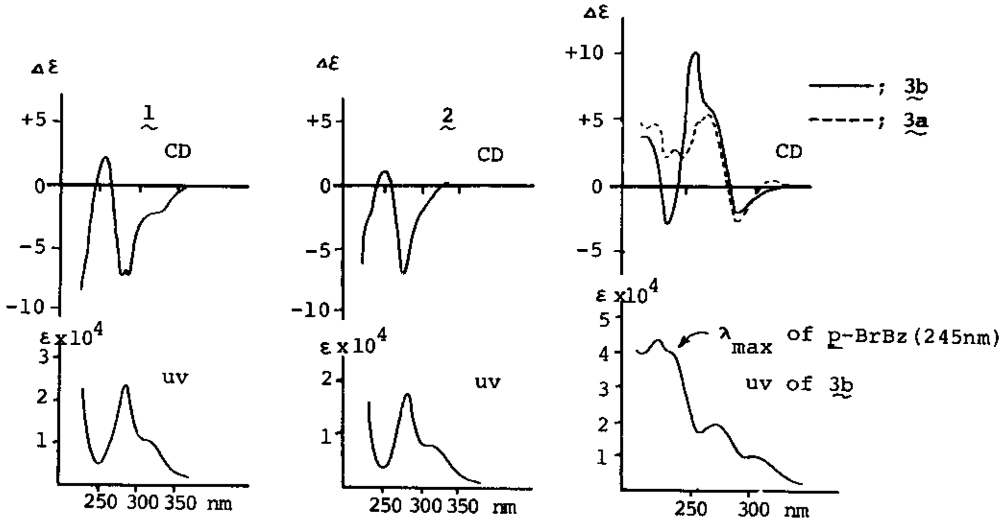


Fig. 4 The CD spectra of 1, 2, 3a and 3b

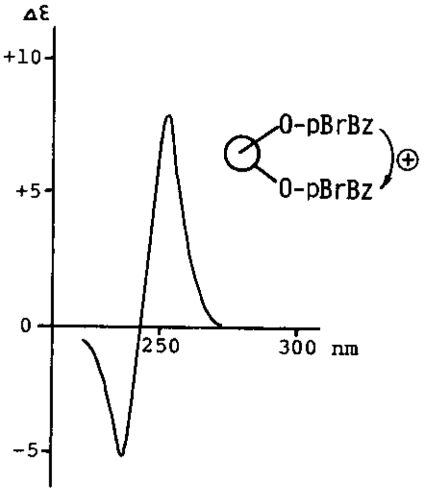


Fig. 5 Differential spectrum of 3a and 3b ($\Delta\varepsilon_{3b} - \Delta\varepsilon_{3a}$)

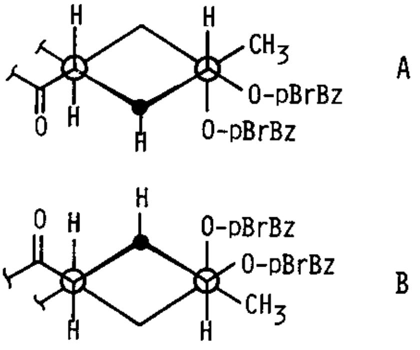


Fig. 6 Two conformations A and B in 3b

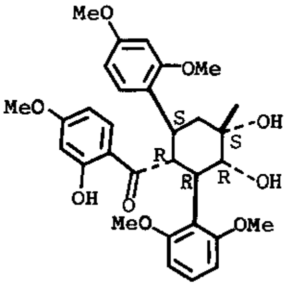


Fig. 7 Absolute configuration of 3

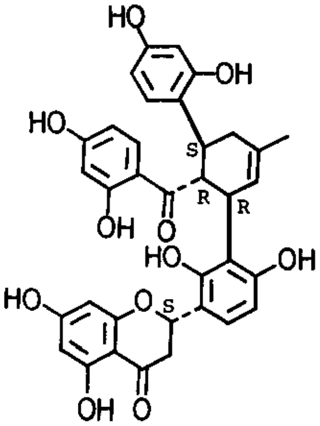


Fig. 8 Absolute configuration of kuwanon L (1)

enantiomers, a positive Cotton effect ascribed to the exciton coupling between two p-bromobenzoyl chromophores should be expected in the A-conformation.[10] Consequently, the absolute configuration on the methylcyclohexane-1,2-diol ring of **3** is illustrated as shown in Fig. 7, and therefore that of methylcyclohexene ring of kuwanon L (**1**) is established to be 3"R, 4"R, 5"S (Fig. 8). On the other hand, the CD spectrum of **1** compared with that of **2**, the trough at 283 nm in CD curve of **2** is fair agreement with one of two troughs in **1**, and hence another trough at 290 nm in **1** would be due to the chromophore in flavanone skeleton. Considering the report by Gaffield[11] connected with the CD spectra of flavanones, **1** has 2S configuration from the CD curve.

In all respects, the absolute configuration of kuwanon L (**1**) is established to be 2S, 3"R, 4"R, 5"S as illustrated in Fig. 8. The determination of the absolute configuration of the chiral centers on the methylcyclohexene ring of **1** would be significant for elucidating the absolute stereochemistry of other Diels-Alder type adducts from the Morus root bark.

## ACKNOWLEDGEMENT

We wish to thank Dr. H. Ishii, Faculty of Pharmaceutical Sciences, Chiba University, for the CD spectral measurement.

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4. The proton signals of methylcyclohexene ring of **1** were described as follows: (δ in dmso-$d_6$ at 120°C) δ 1.72 (3H, s, C-1"-$CH_3$), 2.11 (1H, br d, J=16 Hz,

C-6-H), 3.55 (1H, m, C-5"-H), 4.37 (1H, br d, J=11 Hz, C-3"-H), 4.67 (1H, t, J=11 Hz) C-4"-H), 5.32 (1H, br s, C-2"-H).

5. J. F. Eastham, G. B. Miles, and C. A. Krauth, J. Am. Chem. Soc., 1958, **81**, 3155.
6. The CD spectrum of **1** showed following data: $\Delta\varepsilon_{nm}$; $\Delta\varepsilon_{330}$ -1.9, $\Delta\varepsilon_{290}$ -7.0, $\Delta\varepsilon_{283}$ -7.3, $\Delta\varepsilon_{253}$ +2.3 ($c=4.5 \times 10^{-5}$, EtOH). The uv data: see ref. 3.
7. The CD and uv spectra of **2** showed the following data: CD $\Delta\varepsilon_{nm}$; $\Delta\varepsilon_{335}$ +0.3, $\Delta\varepsilon_{283}$ -7.3, $\Delta\varepsilon_{253}$ +2.3 ($c=4.69 \times 10^{-5}$, EtOH). uv $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 225 (infl. 4.21), 280 (4.28), 320 (sh 3.95).
8. The CD and uv spectra of **3a** showed the following data: CD $\Delta\varepsilon_{nm}$; $\Delta\varepsilon_{286}$ -2.7, $\Delta\varepsilon_{266}$ +5.5, $\Delta\varepsilon_{240}$ +2.7, $\Delta\varepsilon_{222}$ +4.5 ($c=3.40 \times 10^{-5}$, MeOH). uv $\lambda_{max}^{EtOH}$ nm (log$\varepsilon$): 238 (4.36), 244 (sh 4.18), 275 (4.12), 316 (3.72).
9. The CD and uv spectra of **3b** showed the following data: CD $\Delta\varepsilon_{nm}$; $\Delta\varepsilon_{287}$ -2.0, $\Delta\varepsilon_{265}$ +5.8, $\Delta\varepsilon_{253}$ +11.1, $\Delta\varepsilon_{235}$-3.3 ($c=3.27 \times 10^{-5}$, MeOH). uv $\lambda_{max}^{MeOH}$ nm (log$\varepsilon$): 237 (4.62), 245 (sh 4.57), 277 (4.27), 319 (3.87).
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Received, 21st September, 1987