STRUCTURE OF MULBERROFURAN K, AN OPTICALLY ACTIVE NATURAL DIELS-ALDER TYPE ADDUCT FROM CHINESE CRUDE DRUG "SANG-BAI-PI" (MORUS ROOT BARK)

Yoshio Hano,[a] Toshio Fukai,[a] Hideaki Kohno,[a] Kazuhiro Hirakura,[a] Taro Nomura,*[,a] and Jun Uzawa[b]

a) Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

b) The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

Abstract ———From the methanol extract of the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi"), the root bark of Morus sp. (Moraceae), a 2-arylbenzofuran derivative was isolated and named mulberrofuran K. Its structure was shown to be **1** on the basis of the spectral and chemical evidence. Mulberrofuran K is optically active and regarded biogenetically as a variation of a Diels-Alder type adduct of a chalcone derivative and a dehydroprenyl-2-arylbenzofuran derivative.

In the previous papers,[1] we reported the structure determination of a series of natural Diels-Alder type adducts isolated from the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi") imported from the People's Republic of China. In this paper, the structure determination of mulberrofuran K (**1**) isolated from the crude drug, is described.

The methanol extract of the crude drug was dissolved in ethyl acetate. The ethyl acetate extract was fractionated sequentially by the silica-gel column chromatography and preparative thin layer chromatography, resulting in the isolation of **1** (5×10^{-4} % yield from the crude drug).

Mulberrofuran K (**1**), obtained as colorless needles, mp 176 °C (decomp.), $[\alpha]_D^{21}$ + 425° (c = 0.024, MeOH), $FeCl_3$ test (negative), gave the FD-MS which showed the molecular ion peak at m/z 628. Treatment of **1** with dimethyl sulfate and potassium carbonate in acetone effected exhaustive methylation to give the tetramethyl ether (**1a**) as an amorphous powder. The molecular formula of **1a** was determined to be $C_{43}H_{40}O_8$ by the high-resolution mass

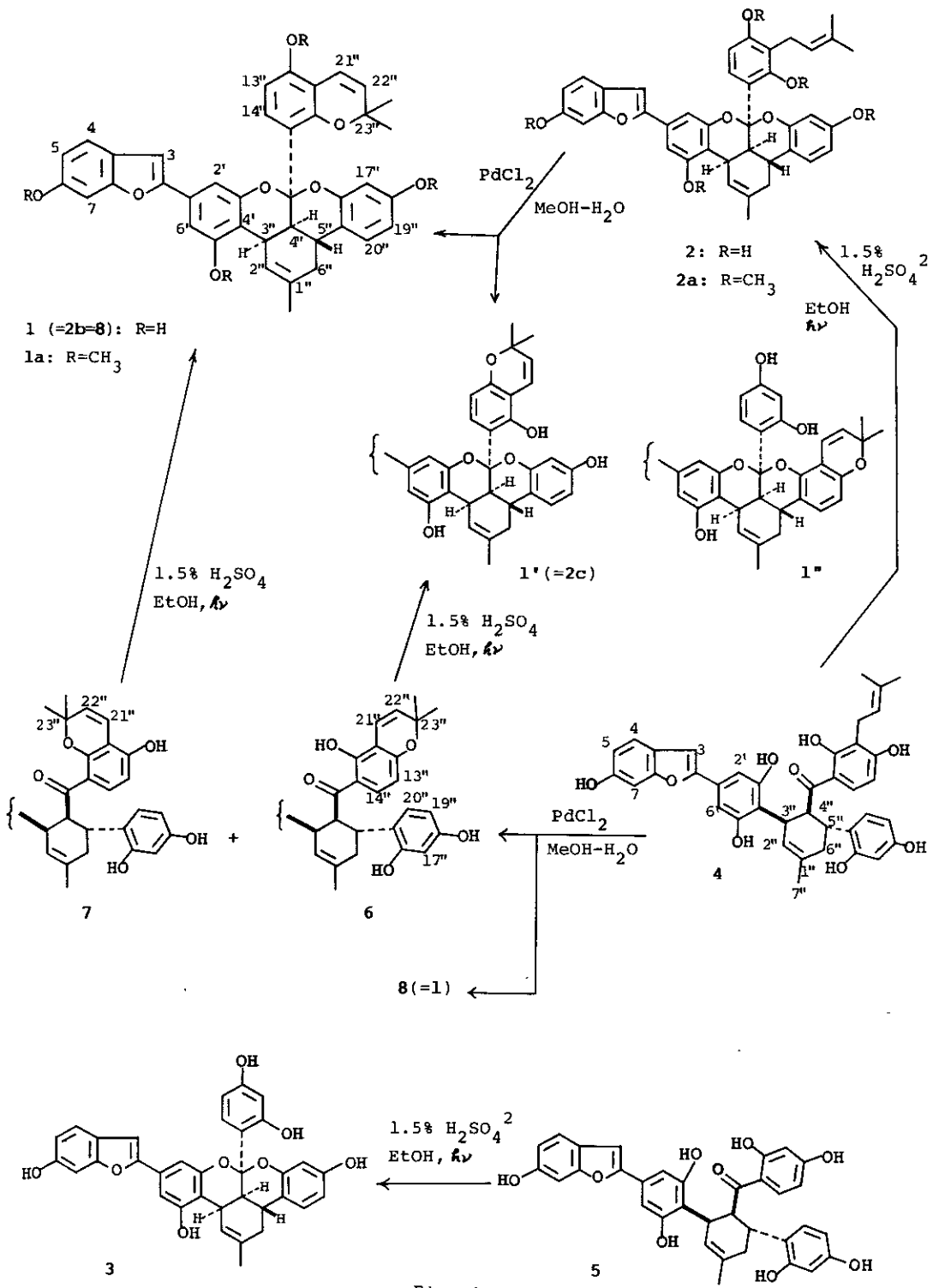
1 (=2b=8): R=H
1a: R=CH3
2: R=H
2a: R=CH3
PdCl2
MeOH-H2O
1.5% H2SO4
EtOH, hν
1'(=2c)
1"
4
7
6
8(=1)
3
5

Fig. 1

spectrum (M^+ = 684.2748), and hence **1** could be formulated as $C_{39}H_{32}O_8$. The compound (**1**) showed the following spectra; ir ν_{max}^{KBr} cm^{-1} : 3400 (br), 1620, 1600, 1570 (sh), 1430; uv λ_{max}^{EtOH} nm (log ϵ): 225 (4.72), 286 (4.29), 306 (infl. 4.45), 320 (4.56), 334 (4.47); uv $\lambda_{max}^{EtOH+AlCl_3}$: 225 (4.72), 286 (4.29), 306 (infl. 4.45), 320 (4.56), 334 (4.47). The ir spectrum of **1** showed the absence of carbonyl function. The uv spectrum was similar to those of mulberrofurans F[2] (**2**) and G[2] (=albanol A[3], **3**), and suggested that **1** is one of the 4'-substituted 6,3',5'-trioxygenated 2-arylbenzofuran derivatives. This suggestion was supported through a comparative examination of the ^1H nmr spectrum of **1** (400 MHz, acetone-d_6) with those of **2**, **3**, chalcomoracin (**4**),[4] and mulberrofuran C (**5**).[5] The chemical shifts and coupling constants (Hz) of the 2-arylbenzofuran moiety are shown as follows: δ 6.81 (1H, dd, J=2 and 8, C-5-H), 6.94 and 6.95 (each 1H, d, J=2, C-2' and -6'-H), 6.97 (1H, br d, J=2, C-7-H), 7.03 (1H, d, J=1, C-3-H), 7.40 (1H, d, J=8, C-4-H). As the chemical shift values and the coupling constants of the protons of the 2-arylbenzofuran moiety was similar to those of the relevant protons of **2**[2] and **3**,[2] it is suggested that **1** has the same substituted pattern on the moiety. The presence of the following moieties was also supported by the comparative examination of the ^1H nmr spectrum of **1** with those of **2** and **3**. The signals of protons in a 5-oxygenated 2,2-dimethylchromene moiety were observed at δ 1.34, 1.35 (each 3H, s, C-23"-CH_3 x 2), 5.66 (1H, d, J=10, C-22"-H), 6.26 (1H, d, J=9, C-13"-H), 6.68 (1H, d, J=10, C-21"-H), 7.05 (1H, d, J=9, C-14"-H), and those of the aromatic protons in a 2,4-dioxygenated phenyl moiety at 6.37 (1H, d, J=2, C-17"-H), 6.50 (1H, dd, J=2 and 8, C-19"-H), 7.13 (1H, d, J=8, C-20"-H). The presence of trisubstituted methylcyclohexane ring was also supported by the examination of the ^1H nmr spectrum of **1a** with the aid of sequential decoupling experiments, and the deduced structure is shown in Fig. 2 along with chemical shifts (ppm) and the coupling constants (Hz). The chemical shift values and the coupling constants of the protons on the ring of **1a** were similar to those of the relevant protons of **2a**.[2] From above results, three possible structures (**1**, **1'**, and **1"**) were suggested. The structure (**1"**) was excluded from the following results. The treatment of mulberrofuran F (**2**, 39 mg) with palladium chloride[6] (11 mg) in 95 % methanol aqueous solution (5 ml) gave the reaction products, which were purified by preparative TLC (benzene : ethyl acetate = 2 : 1, silica gel) to give the compound (**2b**, 6 mg) and (**2c**, 13 mg). The ir spectrum of **2b** was in agreement with that of **1**. In order to corroborate the structure, **1** was derived from chalcomoracin

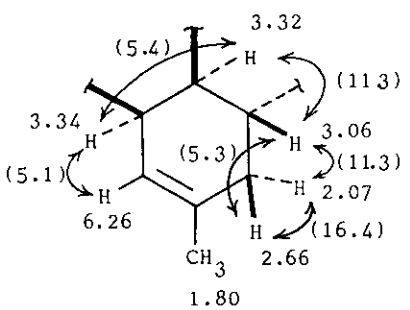

Fig. 2 **1a** in $CDCl_3$

(4) as described bellow. A mixture of **4** (108 mg) and palladium chloride (27 mg) in 95 % methanol aqueous solution (5 ml) was kept at room temperature for 4 h. The products were purified by preparative TLC (benzene : ethyl acetate = 1 : 1, silica gel) to give the compounds **6** (7 mg), **7** (4 mg), and **8** (4 mg).

The compound (**6**), amorphous powder, gave a brown color with methanolic ferric chloride, and gave the FD-MS spectrum which showed the molecular ion peak at m/z 646, and showed the following spectra: ir ν_{max}^{KBr} cm^{-1} : 3400 (br), 1630 (sh), 1610, 1570 (sh), 1430; uv λ_{max}^{EtOH} nm (log ε): 254 (sh 4.14), 278 (4.47), 306 (infl. 4.29), 321 (4.63), 335 (4.53). In the ^1H nmr spectrum (400 MHz, acetone-d_6) of **6**, the proton signal of the hydrogen-bonded hydroxyl group was observed at δ 12.98, and other signals as follows: δ 1.37, 1.40 (each 3H, s, C-23"-CH_3), 1.94 (3H, br s, C-1"-CH_3), 2.23 (1H, m, C-6"-H), 2.52 (1H, dd, J=5 and 16, C-6"-H), 3.77 (1H, m, C-5"-H), 4.16 (1H, m, C-3"-H), 4.64 (1H, t, J=4, C-4"-H), 5.65 (1H, d, J=10, C-22"-H), 5.78 (1H, s, C-2"-H), 6.30 (1H, dd, J=2 and 8, C-19"-H), 6.27 (1H, d, J=9, C-13"-H), 6.49 (1H, d, J=2, C-17"-H), 6.59 (1H, d, J=10, C-21"-H), 6.77 (2H, s, C-2' and C-6'-H), 6.78 (1H, dd, J=2 and 8, C-5-H), 6.92 (1H, d, J=2, C-7-H), 6.93 (1H, s, C-3-H), 6.98 (1H, d, J=8, C-20"-H), 7.36 (1H, d, J=8, C-4-H), 8.44 (1H, d, J=9, C-14"-H), 8.11, 8.16, 8.51, 8.54, 8.83 (each 1H, br s, OH).

The compound (**7**), amorphous powder, $FeCl_3$ test (negative), gave the FD-MS which showed the molecular ion peak at m/z 646, and showed the following spectra: ir ν_{max}^{KBr} cm^{-1} : 3400 (br), 1650 (sh), 1620, 1600 (sh), 1580, 1430; uv λ_{max}^{EtOH} nm (log ε) : 285 (4.49), 293 (sh 4.48), 300 (infl. 4.51), 321 (4.63), 335 (4.55). The ^1H nmr spectrum (200 MHz, acetone-d_6) of **7** showed the absence of the proton signal of the hydrogen-bonded hydroxyl group and the signals as follows: δ 1.31, 1.35 (each 3H, s, C-23"-CH_3), 1.90 (3H, br s, C-1"-CH_3), 2.44 (1H, dd, J=5 and 16, C-6"-H), $\sim$ 2.30 (1H, m, C-6"-H, overlapping with solvent), 3.84 (1H, m, C-5"-H), 4.17 (1H, m, C-3"-H), 4.75 (1H, br t, C-4"-H), 5.66 (1H, d, J=10, C-22"-H), 5.77 (1H, br s, C-2"-H), 6.30 (1H, dd, J=2 and 8, C-19"-H), 6.39 (1H, d, J=9, C-13"-H), 6.40 (1H, d, J=2, C-17"-H), 6.66 (1H,d, J=10, C-21"-H), 6.81 (1H, d, J=8, C-20"-H), 6.83 (2H, s, C-2' and C-6'-H), 6.97 (1H, s, C-3-H), 6.98 (1H, dd, J=2 and 8, C-5-H), 7.00 (1H, d, J=2, C-7-H), 7.41 (1H, d, J=8, C-4-H), 8.47 (1H, d, J=9, C-14"-H), 8.06, 8.14, 8.22, 8.46, 8.51, 8.58 (each 1H, br s, OH).

The compound (**8**) was obtained as amorphous powder, $[\alpha]_D^{23}$ +455° (c = 0.011, MeOH), $FeCl_3$ test (negative), and identical with mulberrofuran K (**1**) by comparing the ^1H nmr and ir spectra of **8** with those of **1**.

The compound (**1**) was also derived from **7** by the following reaction.[2] A solution of **7** (4 mg) in ethanol containing 1.5 % sulfuric acid was externally irradiated in a glass vessel with 100 W high-pressure mercury lamp for 24 h. The product was purified by preparative

TLC to give 1 (2 mg), the ^{1}H nmr and ir spectra of which were identical with those of mulberrofuran K.

On the other hand, the compound (2c) was derived from 6 as follows: a solution of 6 (17 mg) in ethanol solution containing 1.5 % sulfuric acid was irradiated as described above to give amorphous powder (5 mg), the ir spectrum of which was identical with that of 2c. From above results, we propose the formula (1) for the structure of mulberrofuran K.

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