Communications to the Editor

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KUWANON J, A NEW DIELS-ALDER ADDUCT AND CHALCOMORACIN FROM CALLUS CULTURE OF MORUS ALBA L.

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From pigment-producing callus tissues induced from seedlings of Morus alba L., kuwanon J (1), a new Diels-Alder adduct of two prenylchalcone derivatives has been isolated along with chalcomoracin (2), β-sitosterol and stigmast-5-en-3β-ol-7-one.

KEYWORDS——Morus alba; Moraceae; tissue culture; kuwanon J; chalcomoracin; prenylchalcone; Diels-Alder adduct; phytoalexin; FD-mass spectra; ^{13}C NMR spectra

Recently, mulberry constituents have been examined in detail, mainly by the groups of Nomura, Masamune and Hikino.[1] In the course of their studies, several compounds have been isolated, such as kuwanon G,[2] having unique carbon skeletons regarded as Diels-Alder adducts of two prenylchalcone congeners. Moreover, a new phytoalexin chalcomoracin (2)[3] was obtained from mulberry leaves infected with Fusarium solani f. sp. mori.

On the other hand, during the past decade or so, a few reports concerning mulberry tissue culture have been published.[4] However, apart from the isolation of β-sitosterol[4c] and detection of yellow substances which have not yet been characterized at all,[4a,c] these works are mainly concerned with the response of the callus growth to the culture conditions.

As a part of studies on the production and biosynthesis of secondary metabolites in plant cell cultures, we attempted to obtain pigment-producing callus cultures of Morus alba L. Callus tissues induced from the seedlings were subcultured under a specified condition[5] and subjected to selection over six years, giving rise to cell strains having a high ability to produce pigment. The methanol extract of the callus tissues (75 g, fresh weight) was fractionated by column chromatography and preparative thin layer chromatography (prep TLC) on silica gel. Consequently, kuwanon J (1) (1.8 mg), a new chalcone derivative along with chalcomoracin (2) (3.5 mg). β-sitosterol (2.8 mg) and stigmast-5-en-3β-ol-7-one (0.2 mg) were isolated.[6] In addition to these, moracin C,[7] 3',5',6-trihydroxy-2-phenylbenzofuran,[8] kuwanon I (3)[9] and morachalcone A (4)[3] were also detected on TLC. Chalcomoracin (2) was obtained as a white powder, $[\alpha]_D^{17}$ + 193° (MeOH), field-desorption mass spectrum (FD-MS) m/z: 648 (M^+), 630 (M^+-H_2O), and identified with an authentic sample by comparison of ^1H NMR. β-Sitosterol and stigmast-5-en-3β-ol-7-one were identified with authentic samples by gas chromatography.

Kuwanon J (1), a yellow powder, $[\alpha]_D^{17}$ + 85° (MeOH), showed the molecular ion

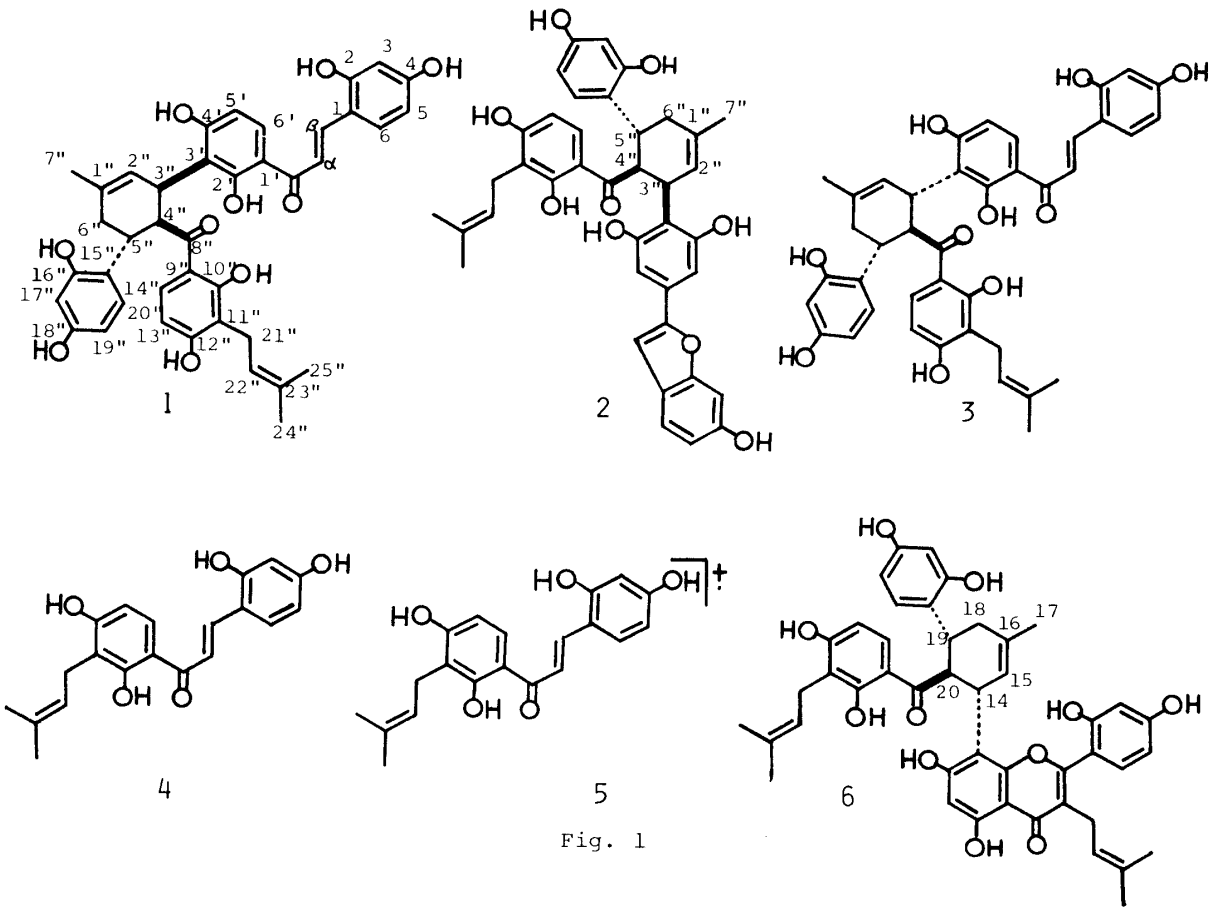


Fig. 1

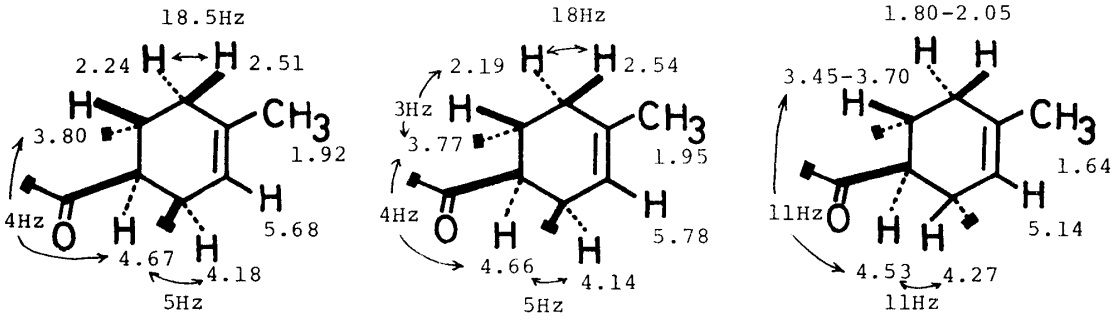
Table 1 ^{13}C NMR Chemical Shifts

Compound	1	3	4	2	6
C-1	113.2	113.5	113.3		
C-2	159.0	159.0	159.2		
C-3	102.5	103.0	102.4		
C-4	161.4	161.4	161.5		
C-5	107.9	108.1	108.0		
C-6	130.0	129.9	130.4		
C-α	116.2	116.2	115.6		
C-β	139.4	139.8	139.7		
C=O	191.5	192.0	191.9		
C-1'	112.2	112.8	112.7		
C-2'	164.8	164.5	163.4		
C-3'	115.5	116.2	114.4		
C-4'	163.0	162.2	161.9		
C-5'	107.5	107.6	107.2		
C-6'	130.0	128.6	129.6		
C-1"	132.2	131.8		133.2	132.9
C-2"	121.7	124.3		121.6	123.4
C-3"	33.0	(39.4 br)[c]		33.1	(39.8)[c]
C-4"	46.6	44.8		46.9	45.7
C-5"	33.0	(39.4 br)[c]		33.9	(39.8)[c]
C-6"	35.8	(38.2 br)[c]		33.9	(39.2)[c]
C-7"	23.3	22.5		23.2	22.9
solvent	a	b	a	a	a

	1	3
C-8"	207.5	208.7
C-9"	114.2	114.1
C-10"	162.0	161.8
C-11"	113.6	114.4
C-12"	161.3	161.4
C-13"	105.9	106.2
C-14"	130.2	129.9
C-15"	122.4	120.7
C-16"	155.3	156.0
C-17"	102.3	103.0
C-18"	155.8	155.7
C-19"	106.5	106.2
C-20"	132.2	129.5
C-21"	21.1	21.1
C-22"	122.4	122.5
C-23"	130.2	129.9
C-24"	25.3	25.2
C-25"	17.5	17.5
	a	b

a: in DMSO-d_6 at 20°C, b: in DMSO-d_6 at 90°C, c: in CD_3OD at 35°C

peak at m/z 678 in its FD-MS. The ^{13}C NMR spectrum of 1 revealed the presence of the following forty carbons: fourteen aliphatic carbons (CH_3- x 3, $-CH_2$- x 2, CH x 3, $>C=C\langle^{H}$ x 2, $^{H}>C=C\langle_{H}$ x 1), twenty four aromatic carbons (CH x 10, C x 6, C-O- x 8) and two carbonyl carbons (Table 1). From these data the composition of kuwanon J was considered to be $C_{40}H_{38}O_{10}$. This substance (1), giving a brown color with methanolic ferric chloride, was negative to both the magnesium-hydrochloric acid test and sodium borohydride test.[10] The compound (1) showed the following spectra: UV λ_{max}^{EtOH} nm (log ε): 264 (4.03, sh), 298 (4.30), 390 (4.47); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 266 (4.04, sh), 301 (4.30), 392 (4.44), 460 (3.68, infl). IR ν_{max}^{KBr} cm^{-1}: 3350, 1640 (sh), 1630 (sh), 1625 (sh), 1615 (sh), 1605. The UV spectrum of 1, being similar to that of kuwanon I (3), did not show any aluminum chloride-induced shift.[11] The ^1H NMR spectrum of 1 indicated signals assignable to two hydrogen-bonded hydroxyl groups at δ 12.86 (1H, s) and 14.28 (1H, s). These data suggest that 1 comprises a chalcone structure and that a prenyl group is present ortho to each of the two hydrogen-bonded hydroxyl groups. In the FD-MS, the fragment ions of 1 appeared at m/z 661[9] and 500,[12] while in the EI-MS, they appeared at m/z 340 (5), 205,[12] 178,[9] 123[9] and 110.[9,12] These data also suggested that kuwanon J (1) is a Diels-Alder adduct such as kuwanon I (3), which is regarded as a cycloaddition product of a chalcone and a dehydroprenylchalcone derivative. The ^1H NMR spectrum of 1 (270 MHz, 35°C, acetone-d_6) showed signals of a γ,γ-dimethylallyl group at δ 1.58, 1.70 (each 3H, s), 3.27 (2H, d, J=7 Hz) and 5.17 (1H, br t, J=7 Hz) and the following signals of the protons excluding those on the cyclohexene ring: double doublets, δ 6.31 (J=2.5 and 8.5 Hz, C-19" H) and 6.43 (J=2.5 and 9 Hz, C-5 H); ortho coupled doublets, δ 6.37 (J=9 Hz, C-5' H), 6.45 (J=9 Hz, C-13" H), 6.98 (J=8.5 Hz, C-20" H), 7.63 (J=9 Hz, C-6 H), 7.84 (J=9 Hz, C-6' H) and 8.36 (J=9 Hz, C-14" H); meta coupled doublets, δ 6.49 (J=2.5 Hz, C-3 H or C-17" H), 6.52 (J=2.5 Hz, C-3 H or C-17" H); doublets of vinyl protons on the trans double bond, δ 7.71 (J=15.5 Hz, C-α H) and 8.14 (J=15.5 Hz, C-β H). These spectral data suggest that 1 comprises two 1,2,4-trisubstituted- and two 1,2,3,4-tetrasubstituted benzene rings and a trans double bond structure. Comparisons of the ^{13}C NMR spectrum of 1 (DMSO-d_6) with those of kuwanon I (3) and morachalcone A (4) revealed that the chemical shifts of the carbon atoms, except those of the cyclohexene ring, are similar to the chemical shifts of the relevant carbon atoms of 3 and 4 (Table 1). These facts suggest that ku-



1 (in acetone-d_6) 2 (in acetone-d_6) 6 (in DMSO-d_6)

Fig. 2. ^1H NMR Chemical Shifts and Coupling Constants of Cyclohexene Ring Protons of Kuwanon J (1), Chalcomoracin (2) and Kuwanon H (6).

wanon J (1) is an isomer of 3 regarding the location of the substituents on the cyclohexene ring or the relative configuration of the substituents. The location of the 2,4-dihydroxyphenyl and 2,4-dihydroxybenzoyl moieties and the relative configuration of the substituents of the cyclohexene ring of 1 were determined by comparing the ^{13}C and ^{1}H NMR spectra of 1 with those of chalcomoracin (2) and kuwanon H (6).[12] Substance 1 resembled 2 more than 6 in the chemical shifts of the relevant carbon atoms of the methylcyclohexene ring (Table 1) and also in the chemical shifts and coupling constants of the relevant protons of the methylcyclohexene ring (Fig. 2). On the basis of these findings, we propose formula 1 for the structure of kuwanon J.

It is noteworthy that two stereoisomers 1 and 3 along with 4 coexist[13] in M. alba callus tissues, suggesting a biosynthetic process analogous to the cycloaddition reaction of the trans chalcone and 3-methyl-1-phenyl-1,3-butadiene.[2b] Occurrence of chalcomoracin (2), a phytoalexin of mulberry leaves, as a major phenolic component of the callus tissues is also notable.

REFERENCES AND NOTES

1) T. Nomura and T. Fukai, Heterocycles, 15, 1531 (1981), and references cited therein.
2) a) T. Nomura and T. Fukai, Chem. Pharm. Bull., 28, 2548 (1980); b) T. Nomura, T. Fukai, T. Narita, S. Terada, J. Uzawa, Y. Iitaka, M. Takasugi, S. Ishikawa, S. Nagao and T. Masamune, Tetrahedron Letters, 22, 2195 (1981).
3) M. Takasugi, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, Chemistry Letters, 1980, 1573.
4) a) K. Ohyama, Japan Agricultural Research Quarterly, 5, 30 (1970); b) R. Narasimhan, B. Dhruva, S. V. Paranjpe, D. D. Kulkarni, A. F. Mascarenhas and S. B. David, Proc. Indian Acad. Sci., 71 B, 204 (1970); c) D. D. Kulkarni, D. D. Ghugale and R. Narasimhan, Indian J. Exp. Biol., 8, 347 (1970); d) H. Seki, M. Takeda, K. Tsutsumi and Y. Ushiki, Nippon Sanshigaku Zasshi, 40, 81 (1971).
5) S. Ueda, K. Inoue, Y. Shiobara, I. Kimura and H. Inouye, Planta medica, 40, 168 (1980).
6) These substances were also detected on TLC of the methanol extract of M. alba suspension cultures. The culture medium was the one[5] from which agar was deleted.
7) M. Takasugi, S. Nagao, S. Ueno, T. Masamune, A. Shirata and K. Takahashi, Chemistry Letters, 1978, 1239.
8) V. H. Deshpande, R. Srinivasan and A. V. Rama Rao, Indian J. Chem., 13, 453 (1975).
9) T. Nomura, T. Fukai, J. Matsumoto, A. Imashimizu, S. Terada and M. Hama, Planta medica, in press.
10) R. M. Horowitz, J. Org. Chem., 22, 1733 (1957).
11) E. A. Sherif, R. K. Gupta and M. Krishnamurti, Tetrahedron Letters, 21, 641 (1980).
12) T. Nomura, T. Fukai and T. Narita, Heterocycles, 14, 1943 (1980).
13) Meanwhile, the co-occurrence of 1, 3, and 4 in M. bombycis and M. alba plants was noticed. T. Nomura, unpublished data.

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