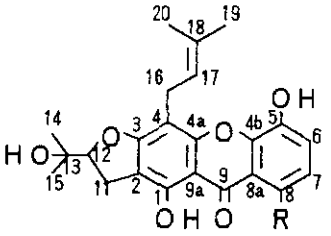
COMPONENTS OF THE ROOT BARK OF MORUS INSIGNIS BUR. 2. STRUCTURES OF FOUR NEW ISOPRENYLATED XANTHONES, MORUSIGNINS E, F, G, AND H[1,2]

Yoshio Hano, Tsuyoshi Okamoto, and Taro Nomura*
Faculty of Pharmaceutical Sciences, Toho University,
2-2-1, Miyama, Funabashi, Chiba 274, Japan

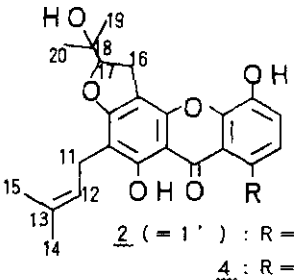
Abstract ——— Four new isoprenylated xanthones, morusignins E (**1**), F (**2**), G (**3**), and H (**4**) were isolated from the root bark of Morus insignis Bur. (Moraceae), collected in Paraguay. The structures of morusignins E – H were shown to be **1** – **4**, respectively, on the basis of spectroscopic data.

In the course of our studies on the constituents of the moraceous plants, we examined the constituents of Morus insignis Bur. collected in Paraguay and described the structures of four isoprenylated xanthones, morusignins A (**5**), B (**6**), C (**7**), and D (**8**).[3] This paper deals with the characterization of four new isoprenylated xanthones, morusignins E (**1**), F (**2**), G (**3**), and H (**4**).

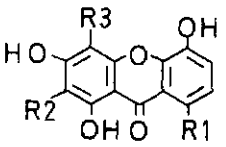
Morusignin E (**1**), yellow prisms, mp 196-203.5°C, $[\alpha]_D^{25}$+11°, $C_{23}H_{24}O_7$, gave a purple color with methanolic ferric chloride, and was negative to the Gibbs test. The uv spectrum of **1** resembled those of gartanin (**9**),[4,5] **5**,[3] and **6**[3] to indicate **1** to be a 1,3,5,8-tetraoxygenated xanthone derivative. The ^1H nmr spectrum showed the signals for the following protons: 1) protons in a 3,3-dimethylallyl (prenyl) group, δ 1.65, 1.83 (each 3H, br s), 3.46, 3.54 (each 1H, dd, J = 7 and 14 Hz), 5.34 (1H, m), 2) protons in a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring,[5] δ 1.29, 1.30 (each



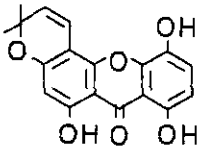
1 : R = OH
3 : R = H



2 (= 1') : R = OH
4 : R = H



5 : R1=OH, R2=H, R3=
6 : R1=OH, R2=, R3=H
9 : R1=OH, R2=R3=
12 : R1=R2=R3=H



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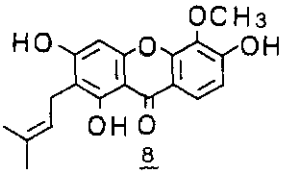

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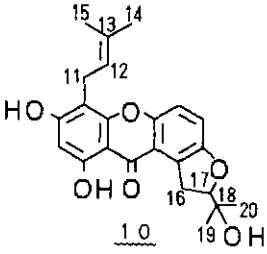

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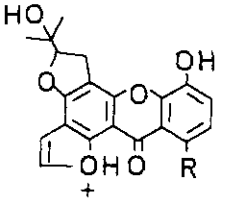

11 : R = OH
13 : R = H

Fig. 1

Table 1 ^{13}C Nmr chemical shifts (ppm) of 1, 2, 3, 4, 9, 10, and 12

	1*	2*	3*	4*	9*	10*	12**
C-1	156.1	161.3	156.8	161.9	158.9	163.7	162.9
C-2	109.3	105.1	108.7	104.6	111.7	98.2	98.1
C-3	167.9	168.1	167.1	167.3	162.4	162.4	165.8
C-4	103.6	108.2	104.4	107.0	107.8	107.0	94.1
C-4a	155.6	151.2	155.4	151.2	153.7	156.1	157.3
C-4b	145.1	144.4	146.4	145.9	145.1	151.6	144.9
C-5	138.1	137.9	147.1	146.8	138.1	116.8	146.2
C-6	124.5	124.7	121.2	121.5	124.1	117.7	120.6
C-7	110.0	110.5	124.6	124.7	109.9	157.7	124.1
C-8	154.2	154.3	116.4	116.5	154.3	126.9	114.6
C-8a	108.3	107.4	122.2	122.3	108.4	118.3	121.0
C-9	185.8	185.4	181.9	181.5	186.0	182.9	180.2
C-9a	103.4	102.8	103.2	103.8	102.8	104.1	102.2
C-11	27.2	22.3	27.3	22.4	22.3	22.2	
C-12	92.8	122.5	92.6	122.7	123.0[a]	123.4	
C-13	71.6	132.2	71.6	132.0	132.9[b]	131.7	
C-14	25.2	25.9	25.1	25.9	25.9	25.9	
C-15	26.2	17.9	26.2	17.9	18.1[c]	18.0	
C-16	22.5	27.8	22.6	27.9	22.3	32.9	
C-17	122.8	92.8	123.0	92.6	122.8[a]	91.7	
C-18	132.3	71.6	132.1	71.6	132.8[b]	73.4	
C-19	25.9	25.1	25.9	25.0	25.9	25.5	
C-20	18.0	26.4	18.0	26.4	18.0[c]	26.0	

Solvent: *: acetone-d_6 **: DMSO-d_6

a-c: Assignments may be reversed.

Table 2 ^{1}H Nmr chemical shifts (ppm)

compound	$-CH_2-$ (on prenyl)	$-CH_2-$ (on furan ring)
1	3.46, 3.54 (16-H)	3.16, 3.23 (11-H)
2	3.22, 3.24 (11-H)	3.32, 3.38 (16-H)
3	3.49, 3.57 (16-H)	3.17, 3.24 (11-H)

measured in acetone-d_6

3H, s), 3.16 (1H, dd, J = 10 and 16 Hz), 3.23 (1H, dd, J = 8 and 16 Hz), 4.88 (1H, dd, J = 8 and 10 Hz), 3) ortho-coupled aromatic protons, δ 6.61, 7.29 (1H, d, J = 9 Hz), and 4) protons in two hydrogen-bonded hydroxyl groups, δ 11.33, 12.07 (each 1H, s). The ^{13}C nmr spectrum of **1** was analysed by comparing it with those of **9**[3] and cudraxanthone J (**10**)[6](Table 1). In the ^{13}C nmr studies, the chemical shifts of the carbon atoms in the B ring were of the similar values to those of the relevant carbon atoms of **9**. From the above results, **1** was suggested to be a 1,3,5,8-tetraoxygenated xanthone having a prenyl group and a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring in the A ring, and two possible structures (**1** and **1'**) were proposed. The long range selective ^{1}H decoupling (LSPD)[7] was carried out to discriminate the structures. When the signal at δ 12.07 (C-1-OH) was irradiated, the doublet of triplet signal at δ 156.1 (C-1, ^{2}J = 4 Hz, ^{3}J = 2 Hz) changed to triplet (^{3}J = 2 Hz). When the signal at δ 3.46 (C-16-H) was irradiated, the doublet of doublet signal at 155.6 (C-4a, ^{3}J = 3 and 4 Hz) changed to doublet (^{3}J = 4 Hz) and the multiplet signal at 167.9 (C-3) changed to the doublet of doublet of doublet signal (^{3}J = 2 and 4 Hz). These results indicate the prenyl group to be located at the C-4 position and the formula **1** was proposed for the structure of morusignin E.

Morusignin F (**2**), yellow prisms, mp 202-204°C, $[\alpha]_D^{25}$+11°, $C_{23}H_{24}O_7$, gave a brown color with methanolic ferric chloride, and was negative to the Gibbs test. The uv spectrum of **2** resembled that of **1** to indicate **2** to be a 1,3,5,8-tetraoxygenated xanthone derivative. The ^{1}H nmr spectrum showed the signals of the following protons: 1) protons in a prenyl group, 2) protons in a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring, 3) a pair of ortho-coupled aromatic protons, and 4) two protons of hydrogen-bonded hydroxyl groups. From the above results, **2** is a structural isomer of **1**. In the ^{13}C nmr spectrum of **2**, the chemical shifts of all the carbon atoms except those of the A ring carbons (C-1, 2, 4, and 4a) were similar to the shifts of the relevant carbons of **1** (Table 1). In the mass spectrum of **2**, the characteristic fragment ion at m/z 369 (**11**, $M^{+}-C_3H_7$) was observed.

This result supports that a prenyl group is located at the C-2 position.[8] From the above results, the formula **2** was proposed for the structure of morusignin F.

Morusignin G (**3**), yellow prisms, mp 203-206°C, $[\alpha]_D^{25}$-4.0°, $C_{23}H_{24}O_6$, gave a brown color with methanolic ferric chloride and was positive to the Gibbs test. The uv spectrum of **3** resembled those of 1,3,5-trioxygenated xanthone derivatives.[9] The ^1H nmr spectrum showed the signals for the following protons: 1) protons in a prenyl group, δ 1.65, 1.84 (each 3H, br d, J = 1 Hz), 3.49, 3.57 (each 1H, dd, J = 7 and 14 Hz), 5.37 (1H, m), 2) protons in a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring, δ 1.29 (6H, s), 3.17 (1H, dd, J = 10 and 16 Hz), 3.24 (1H, dd, J = 8 and 16 Hz), 4.86 (1H, dd, J = 8 and 10 Hz), 3) ABC type aromatic protons, δ 7.26 (1H, t, J = 8 Hz), 7.36 (1H, dd, J = 2 and 8 Hz), 7.68 (1H, dd, J = 2 and 8 Hz), and 4) a proton in a hydrogen-bonded hydroxyl group, δ 13.11 (1H, s). The ^{13}C nmr spectrum of **3** was analysed by comparing it with those of **1**, **2**, and 1,3,5-trihydroxyxanthone (**12**).[10] The chemical shifts of the carbon atoms in the A ring were similar to the shifts of the relevant atoms in **1** while of the B ring atoms to the shifts of the relevant atoms in **12**. Comparative study of the ^1H nmr spectra of **3** with **1** and **2** showed that the chemical shifts of the methylene proton signals in the prenyl group and in the dihydrofuran ring of **3** were more similar to those of the relevant methylene proton signals of **1** than those of the relevant signals of **2** (Table 2). From the above results, the formula **3** was proposed for the structure of morusignin G.

Morusignin H (**4**), yellow prisms, mp 234-239°C, $[\alpha]_D^{25}$+5.7°, $C_{23}H_{24}O_6$, gave a brown color with methanolic ferric chloride and was positive to the Gibbs test. The uv spectrum of **4** resembled those of 1,3,5-trioxygenated xanthone derivatives.[9] The ^1H nmr spectrum showed the signals of the following protons: 1) protons in a prenyl group, 2) protons in a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring, 3) ABC type aromatic protons, and 4) a proton of a hydrogen-bonded hydroxyl group. In the ^{13}C

nmr studies, the chemical shifts of the carbon atoms except those of the A ring carbons (C-1, 2, 4, and 4a) were similar to the shifts of the relevant carbons of **3** (Table 1). The mass spectrum of **4** showed the fragment ion at m/z 353 (**13**, $M^+-C_3H_7$) to suggest the presence of the prenyl group at the C-2 position.[8] From the above results, the formula **4** was proposed for the structure of morusignin H.

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder. The general procedures followed as described in our previous papers.[11,12] The instruments used are described in our previous paper.[11]

Isolation of Morusignins E (**1**), F (**2**), G (**3**), and H (**4**) from the Root Bark of M. insignis Bur.

The dried root bark (3.4 Kg) of M. insignis, collected in the suburb of Encarnacion City, Itapua Prefecture, Paraguay, in February 1989, was extracted with n-hexane (1 l) at room temperature for 3 days, and such was repeated two more times. Evaporation of the n-hexane solution yielded 170 g of the residue.[3] The n-hexane extract (135 g)[3] was chromatographed on silica gel (1 Kg) with n-hexane containing increasing amount of ethyl acetate as an eluent (fractions 1-9, eluted volume of 1 l each). The fraction eluted with n-hexane containing 25% ethyl acetate (fr. 7, 2.9 g) was rechromatographed on silica gel (100 g) with n-hexane containing increasing amount of acetone as an eluent (frs. 1'-29'), each fraction (200 ml) being monitored by tlc. The fraction eluted with n-hexane containing 20% acetone (frs. 15'-16', 253 mg) was fractionated by preparative tlc (silica gel, chloroform : acetone = 10 : 1, chloroform : acetone = 30 : 1), and then by preparative hplc (n-hexane : ethyl acetate = 2 : 1, column, Senshu Pak SSC-Silica 4251-N, 1 cmϕ x 25 cm, detector, uv 280 nm) to give morusignins E (**1**, 14 mg) and G (**3**, 10 mg). The fraction eluted with n-hexane containing 50% ethyl acetate (fr. 9, 950 mg) was fractionated by preparative tlc (n-hexane : acetone = 2 : 1), and then by preparative hplc (n-hexane : ethyl acetate = 1 : 1, above-described conditions) to give morusignins F (**2**, 84 mg) and H (**4**, 16 mg).

Morusignin E (**1**)

Compound **1** was recrystallized from n-hexane-ether to give yellow prisms, mp 196-203.5°C. $FeCl_3$ test; positive (purple). Gibbs test; negative. $[\alpha]_D^{25}$ +11° (c = 0.09, acetone). EI-Ms: m/z (rel. int.) 412 (M^+, 66%), 397 (7), 379 (9), 357 (100), 353 (10). HR-Ms: m/z 412.1525 $(M)^+$ ($C_{23}H_{24}O_7$ requires 412.1522), m/z 357.0977 ($C_{19}H_{17}O_7$ requires 357.0975). Ir ν_{max}^{KBr} cm^{-1}: 3420, 1660, 1635, 1610, 1565, 1480, 1420. Uv λ_{max}^{EtOH} nm (log ε): 206 (4.59), 226 (4.55), 243 (sh 4.46), 257 (4.53), 286 (4.46), 344 (4.29), 390 (sh 3.80). Uv $\lambda_{max}^{EtOH+AlCl_3}$: 208 (4.65), 222 (4.56), 261 (4.44), 300 (4.42), 344 (4.18), 374 (4.14). Uv $\lambda_{max}^{EtOH+AcONa}$: no shift. ^1H Nmr (acetone-d_6, 400 MHz): δ 1.29, 1.30 (each 3H, s,

C-13-CH_3), 1.65, 1.83 (each 3H, br s, C-18-CH_3), 3.16 (1H, dd, J = 10 and 16 Hz, C-11-H), 3.23 (1H, dd, J = 8 and 16 Hz, C-11-H), 3.46, 3.54 (each 1H, dd, J = 7 and 14 Hz, C-16-H), 4.88 (1H, dd, J = 8 and 10 Hz, C-12-H), 5.34 (1H, m, C-17-H), 6.61 (1H, d, J = 9 Hz, C-7-H), 7.29 (1H, d, J = 9 Hz, C-6-H), 11.33 (1H, s, C-8-OH), 12.07 (1H, s, C-1-OH).

Morusignin F (2)

Compound **2** was crystallized from n-hexane-ether to give yellow prisms, mp 202-204 °C. $FeCl_3$ test; positive (brown). Gibbs test; negative. $[\alpha]_D^{25}$ +11° (c = 0.23, acetone). EI-Ms: m/z (rel. int.) 412 (M^+, 100%), 397 (33), 379 (14), 369 (38), 357 (70), 59 (17). HR-Ms: m/z 412.1533 $(M)^+$ ($C_{23}H_{24}O_7$ requires 412.1522), 369.0967 ($C_{20}H_{19}O_7$ requires 369.0974). Ir ν_{max}^{KBr} cm^{-1} : 3500, 3440, 1655 (sh), 1635, 1570, 1490, 1460. Uv λ_{max}^{EtOH} nm (log ε): 204 (4.27), 228 (4.25), 240 (4.20), 256 (4.22), 284 (4.11), 352 (4.02). Uv $\lambda_{max}^{EtOH+AlCl_3}$: 205 (4.37), 227 (4.24), 270 (4.20), 301 (4.15), 341 (3.75), 392 (3.95), 469 (3.46). Uv $\lambda_{max}^{EtOH+AcONa}$: no shift. ^1H Nmr (acetone-d_6, 400 MHz): δ 1.29 (6H, s, C-18-CH_3 x 2), 1.66, 1.78 (each 3H, br s, C-13-CH_3), 3.24, 3.32 (each 1H, dd, J = 8 and 14 Hz, C-11-H), 3.32 (1H, dd, J = 10 and 16 Hz, C-16-H), 3.38 (1H, dd, J = 8 and 16 Hz, C-16-H), 4.90 (1H, dd, J = 8 and 10 Hz, C-17-H), 5.28 (1H, m, C-12-H), 6.61 (1H, d, J = 9 Hz, C-7-H), 7.26 (1H, d, J = 9 Hz, C-6-H), 11.33 (1H, s, C-8-OH), 12.46 (1H, s, C-1-OH).

Morusignin G (3)

Compound **3** was crystallized from n-hexane-acetone to give yellow prisms, mp 203-206 °C. $FeCl_3$ test; positive (brown). Gibbs test; positive. $[\alpha]_D^{25}$ -4.0°(c = 0.10, EtOH). EI-Ms: m/z (rel. int.) 396 (M^+, 100%), 381 (32), 363 (28), 337 (25). HR-Ms: m/z 396.1618 $(M)^+$ ($C_{23}H_{24}O_6$ requires 396.1573), 337.1049 ($C_{20}H_{17}O_5$ requires 337.1076). Ir ν_{max}^{KBr} cm^{-1} : 3400, 1660, 1620, 1590, 1565, 1500, 1465. Uv λ_{max}^{EtOH} nm (log ε): 202 (4.40), 223 (4.47), 246 (4.55), 323 (4.28). Uv $\lambda_{max}^{EtOH+AlCl_3}$: 205 (4.48), 226 (4.51), 247 (4.41), 284 (4.45), 350 (4.37), 418 (3.63). ^1H Nmr (acetone-d_6, 400 MHz): δ 1.29 (6H, s, C-13-CH_3 x 2), 1.65, 1.84 (each 3H, br d, J =1 Hz, C-18-CH_3), 3.17 (1H, dd, J = 10 and 16 Hz, C-11-H), 3.24 (1H, dd, J = 8 and 16 Hz, C-11-H), 3.49, 3.57 (each 1H, dd, J = 7 and 14 Hz, C-16-H), 4.86 (1H, dd, J = 8 and 10 Hz, C-12-H), 5.37 (1H, m, C-17-H), 7.26 (1H, t, J = 8 Hz, C-7-H), 7.36 (1H, dd, J = 2 and 8 Hz, C-6-H), 7.68 (1H, dd, J = 2 and 8 Hz, C-8-H), 13.11 (1H, s, C-1-OH).

Morusignin H (4)

Compound **4** was crystallized from n-hexane-acetone to give yellow prisms, mp 234-239 °C. $FeCl_3$ test; positive (brown). Gibbs test; positive. $[\alpha]_D^{25}$ +5.7° (c = 0.11, EtOH). EI-Ms: m/z (rel. int.) 396 (M^+, 83%), 381 (29), 363 (9), 353 (34), 341 (100), 337 (4). HR-Ms: m/z 396.1609 $(M)^+$ ($C_{23}H_{24}O_6$ requires 396.1573), 353.1025 ($C_{20}H_{17}O_6$ requires 353.1025). Ir ν_{max}^{KBr} cm^{-1} : 3380, 1650, 1610, 1575, 1495, 1450, 1440. Uv λ_{max}^{EtOH} nm (log ε): 204 (4.46), 221 (4.50), 244 (4.65), 259 (4.45), 325 (4.36). Uv $\lambda_{max}^{EtOH+AlCl_3}$: 205 (4.49), 244 (4.61), 265 (4.39), 328 (4.30). ^1H Nmr (acetone-d_6, 400 MHz): δ 1.29, 1.30 (each 3H, s, C-18-CH_3), 1.66, 1.79 (each 3H, br s, C-13-CH_3), 3.27, 3.35 (each 1H, dd, J = 7 and 14 Hz, C-11-H), 3.35 (1H, dd, J = 10 and 16 Hz, C-16-H), 3.41 (1H, dd, J = 8 and 16 Hz, C-16-H), 4.88 (1H, dd, J = 8 and 10 Hz, C-17-H), 5.30 (1H, m, C-12-H), 7.26 (1H, t, J

= 8 Hz, C-7-H), 7.33 (1H, dd, J = 2 and 8 Hz, C-6-H), 7.69 (1H, dd, J = 2 and 8 Hz, C-8-H), 13.50 (1H, s, C-1-OH).

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