# COMPONENTS OF THE ROOT BARK OF MORUS INSIGNIS BUR. 1. STRUCTURES OF FOUR NEW ISOPRENYLATED XANTHONES, MORUSIGNINS A, B, C, AND D[1]

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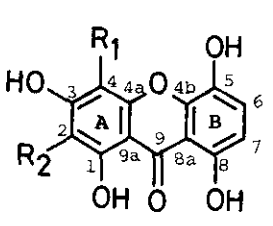
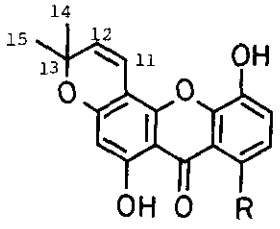
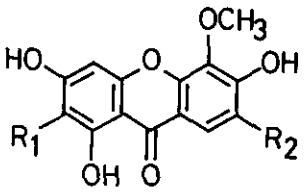
Abstract —— Four new isoprenylated xanthones, morusignins A (**1**), B (**2**), C (**3**), and D (**4**) were isolated from the root bark of Morus insignis Bur. (Moraceae), collected in Paraguay, along with three known isoprenylated xanthones, gartanin (**5**), garcinone B (**6**), and toxyloxanthone B (**7**). The structures of morusignins A – D were shown to be **1** – **4**, respectively on the basis of spectroscopic data.

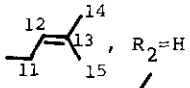
Previously we reported the structure determination of a series of isoprenylated phenolic compounds isolated from the moraceous plants.[2] Some of these compounds showed interesting biological activities such as hypotensive effect, anti-rhinoviral activity, inhibition of the formation of some prostanoids, anti-tumor promoting activity.[2] In the course of our studies on the constituents of the moraceous plants, we examined the constituents of Morus insignis Bur. collected in Paraguay. This paper deals with the characterization of four new isoprenylated xanthones, morusignins A (**1**), B (**2**), C (**3**), and D (**4**).

Morusignin A (**1**), yellow needles, mp 218-220°C, $C_{18}H_{16}O_6$, gave a purple color with methanolic ferric chloride, and was negative to the Gibbs test. The ir spectrum of **1** disclosed absorption bands for hydroxyl, conjugated carbonyl, and aromatic ring moieties. The uv spectrum of **1** resembled those of 1,3,5,8-tetraoxygenated xanthone derivatives,[3] and showed aluminum chloride-induced shift.[4] The $^1$H nmr spectrum showed the signals for the following protons: 1) protons in a 3,3-dimethylallyl (prenyl) group, $\delta$ 1.65, 1.84 (each 3H, br s), 3.54 (2H, br d, $J$=7 Hz), 5.34 (1H, m),







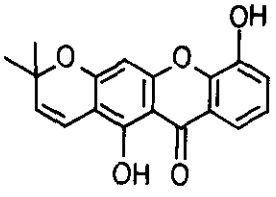
**1**: $R_1$=  , $R_2$=H

**2**: $R_1$=H, $R_2$= 

**5**: $R_1 = R_2$= 

**3**: R=OH

**9**: R=H

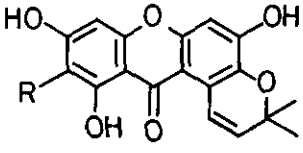


**10**

**4**: $R_1$=  , $R_2$=H

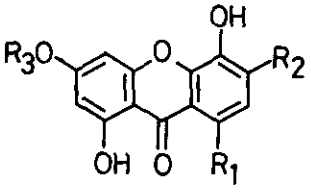
**11**: $R_1$=  , $R_2$= 

**12**: $R_1 = R_2$= 



**6**: R= 

**7**: R=H



**8**: $R_1$=OH, $R_2$=H, $R_3$=$CH_3$

**13**: $R_1$=$R_3$=H, $R_2$=OH

Table 1 $^{13}$C Nmr chemical shifts (ppm) of **1** – **5**, **8**, **9**, **10**, **12** and **13**

| | **1*** | **8**** | **5*#** | **2*** | **3*** | **9*** | **10**** | **4*** | **13**** | **12***** |
|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 161.8 | 161.9 | 158.9 | 160.9 | 162.5 | 164.2 | 160.1 | 161.6 | 165.1 | 162.3 |
| C-2 | 98.9 | 97.3 | 111.7 | 112.1 | 100.0 | 99.7 | 103.9 | 111.6 | 97.9 | 109.1 |
| C-3 | 164.8 | 166.9 | 162.4 | 165.0 | 161.8 | 161.8 | 156.7 | 163.5 | 162.9 | 161.6 |
| C-4 | 108.1 | 92.8 | 107.8 | 94.7 | 103.2 | 102.2 | 94.8 | 94.4 | 94.0 | 94.3 |
| C-4a | 155.6 | 157.2 | 153.7 | 156.7 | 154.3 | 152.5 | 156.0 | 156.4 | 157.4 | 155.6 |
| C-4b | 145.1 | 143.3 | 145.1 | 144.6 | 144.6 | 146.1 | 144.9 | 151.5 | 146.1 | 147.9 |
| C-5 | 138.1 | 137.3 | 138.1 | 138.0 | 138.1 | 147.0 | 146.2 | 135.6 | 132.5 | 133.2 |
| C-6 | 124.7 | 123.7 | 124.7 | 124.4 | 125.5 | 122.0 | 120.7 | 156.9 | 151.9 | 152.6 |
| C-7 | 110.0 | 109.4 | 109.9 | 110.2 | 110.8 | 125.1 | 124.3 | 114.2 | 113.1 | 125.5 |
| C-8 | 154.2 | 151.8 | 154.3 | 154.2 | 152.7 | 116.5 | 114.4 | 122.0 | 115.9 | 120.7 |
| C-8a | 108.2 | 107.4 | 108.4 | 108.4 | 108.4 | 122.3 | 120.7 | 115.2 | 113.1 | 114.1 |
| C-9 | 185.8 | 183.9 | 186.0 | 185.6 | 185.9 | 181.9 | 180.5 | 180.9 | 179.7 | 180.3 |
| C-9a | 102.7 | 102.0 | 102.8 | 102.6 | 102.4 | 104.2 | 103.0 | 103.0 | 101.5 | 103.1 |
| C-11 | 22.1 | | 22.1a | 22.0 | 115.5 | 115.8 | 114.4 | 22.0 | | 21.5 |
| C-12 | 123.3 | | 123.0b | 123.0 | 128.5 | 128.2 | 128.4 | 123.3 | | 121.3 |
| C-13 | 131.9 | | 132.8c | 131.0 | 79.5 | 79.2 | 78.4 | 131.6 | | 136.0 |
| C-14 | 26.0 | | 25.9 | 25.9 | 28.5 | 28.5 | 28.0 | 25.9 | | 25.8 |
| C-15 | 18.0 | | 18.1d | 17.9 | 28.5 | 28.5 | 28.0 | 17.9 | | 18.0 |
| C-16 | | | 22.3a | | | | | | | 28.1 |
| C-17 | | | 122.8b | | | | | | | 121.0 |
| C-18 | | | 132.9c | | | | | | | 133.9 |
| C-19 | | | 25.9 | | | | | | | 25.8 |
| C-20 | | | 18.0d | | | | | | | 17.8 |
| $OCH_3$ | | — | | | | | | 61.7 | | 61.9 |

Solvent: *; acetone-$d_6$, **; DMSO-$d_6$, ***; $CDCl_3$ # Our data
a – d: Assignments may be interchanged.

2) an aromatic proton, δ 6.35 (1H, s), 3) ortho-coupled aromatic protons, δ 6.58, 7.29 (each 1H, d, J=9 Hz), and 4) two hydrogen-bonded hydroxyl groups, δ 11.27, 11.96 (each 1H, s). The $^{13}C$ nmr spectrum of **1** was analysed by comparing with those of 1,5,8-trihydroxy-3-methoxyxanthone[5] (**8**) and gartanin (**5**) (Table 1). In the $^{13}C$ nmr studies, **1** was suggested to be a 1,3,5,8-tetraoxygenated xanthone having a prenyl group in the A ring. To confirm the location of the prenyl group, the long-range selective $^{1}H$ decoupling (LSPD) was carried out.[6] When the signal at δ 11.27 was weakly irradiated, the doublet of doublet signal at δ 110.0 (C-7, $^{1}J$=163.6 Hz, $^{3}J$=7.3 Hz) changed to doublet ($^{1}J$=163.6 Hz). When the signal at δ 11.96 was weakly irradiated, the doublet of doublet signal at δ 98.9 (C-2, $^{1}J$=161.4 Hz, $^{3}J$=7.3 Hz) changed to doublet ($^{1}J$=161.4 Hz). These results indicate the prenyl group to be located at the C-4 position along with the oxygenated pattern of B ring. From the above results, the formula **1** was proposed for the structure of morusignin A.

Morusignin B (**2**), yellow needles, mp 259-261°C, $C_{18}H_{16}O_6$, gave a brown color with methanolic ferric chloride, and was positive 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 due to protons of a prenyl, an aromatic, a pair of ortho-coupled aromatic, and two hydrogen-bonded hydroxyl protons. In the $^{13}C$ nmr spectrum of **2**, the chemical shift values of all the carbon atoms except those of the C-2 and C-4 positions were similar to the values of the relevant carbon atoms of **1** (Table 1). From the above results, the formula **2** was proposed for the structure of morusignin B.

Morusignin C (**3**), yellow needles, mp 218°C, $C_{18}H_{14}O_6$, gave a dark green color with methanolic ferric chloride, and was negative to the Gibbs test. The uv spectrum of **3** was indicative of the 1,3,5,8-tetraoxygenated xanthone structure.[3] The $^{1}H$ nmr spectrum showed the signals due to protons of 2,2-dimethylpyran ring, a long-range coupled aromatic, a pair of ortho-coupled aromatic, and two hydrogen-bonded hydroxyl protons. The $^{13}C$ nmr spectrum of **3** was analysed by comparing with the spectra of **1**, **2**, 6-deoxyisojacareubin[7] (**9**), and 6-deoxyjacareubin[8] (**10**) (Table 1). The $^{13}C$ nmr studies indicate that **3** is a 1,3,5,8-tetraoxygenated xanthone derivative having an angular type 2,2-dimethylpyran ring in the A ring. From the above results, the formula **3** was proposed for the structure of morusignin C.

Morusignin D (**4**), yellow needles, mp 226-228°C, $C_{19}H_{18}O_6$, gave a greenish brown color with methanolic ferric chloride, and was positive to the Gibbs test. The uv spectrum of **4** resembled those of cudraxanthones E[7b] (**11**) and F[7b] (**12**) suggesting

a 1,3,5,6-tetraoxygenated xanthone derivative. The $^{1}H$ nmr spectrum showed the signals for the following protons: 1) protons in a prenyl group, 2) methoxyl protons 3) an aromatic proton, 4) ortho-coupled aromatic protons, and 5) proton in a hydrogen-bonded hydroxyl group. The $^{13}C$ nmr spectrum of **4** was analysed by comparing with the spectra of 1,3,5,6-tetrahydroxyxanthone[5] (**13**) and cudraxanthone F[7b] (**12**) (Table 1). In the $^{13}C$ nmr spectrum of **4**, the chemical shift values of the carbon atoms in the A ring were similar to the values of the relevant carbon atoms of **12**. These results indicate that **4** is a 1,3,5,6-tetraoxygenated xanthone derivative having a prenyl group at C-2 position. The chemical shift value of the methoxyl carbon atom ( $\delta$ 61.7) of **4** indicates the methoxyl group to be di-ortho-substituted.[9] From the above results, the formula **4** was proposed for the structure of morusignin D.

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.[10,11] The instruments used are described in our previous paper.[10]

Plant Materials

The bark of Morus insignis Bur. (Moraceae) was collected in the suburbs of Encarnacion city, Itapua prefecture, Paraguay, in February 1989, and identified by Prof. I. Basualdo, Faculty of Chemistry, Asuncion National University. The sample was deposited in the Herbarium of Toho University.

Isolation of Morusignins A (**1**), B (**2**), C (**3**), and D (**4**) from the Root Bark of M. insignis Bur.

The dried root bark of Morus insignis Bur. (3.4 Kg) was extracted with n-hexane (10 l) at room temperature for 3 days, and such was repeated two more times. The residue was extracted successively with benzene (10 l x 3) and acetone (10 l x 3) as described above. Evaporation of the n-hexane, benzene, and acetone solutions to dryness yielded 170 g, 54 g, and 70 g of the residue, respectively. The n-hexane extract (35 g) was chromatographed on silica gel (300 g) with n-hexane containing increasing amount of ethyl acetate as an eluent (fractions 1-120), each fraction (eluted volume of 300 ml) being monitored by tlc. The fractions eluted with n-hexane containing 10% ethyl acetate (frs. 29-47) were combined, and the mixture was evaporated to give a residue (95 mg), which was fractionated by preparative tlc (silica gel, solvent system, n-hexane:acetone=5:1, benzene:ethyl acetate=30:1) followed by crystallization from n-hexane-acetone to give morusignin C (**3**, 20 mg). The fractions eluted with n-hexane containing 10% ethyl acetate (frs. 25-26) gave the residue (1.3 g), which was extracted with methanol. The methanol extract (1.0 g) was rechromatographed on silica gel (100 g) with chloroform containing increasing amount of ethyl acetate as an eluent (frs. 1'-37'), each fraction (100 ml) being monitored by tlc. The fraction eluted with chloroform (frs. 3'-4', 0.1 g in total) was fractionated by gel filtration (Sephadex LH-20, solvent system, methanol) to give the fractions (frs. 1"-10", 10 ml each). The fraction (frs. 1"-7", 60 mg in total) was purified by preparative hplc (solvent, n-hexane:ethyl acetate=12:1, column, Senshu Pak SSC-Silica 4251-N, 1 cm ø x 25 cm, detector, uv 280 nm) followed by crystallization from benzene to give gartanin[12] (**5**, mp 180-181°C, 21 mg). The fraction eluted with n-hexane containing 14% ethyl acetate (frs. 48-55) gave the residue (234 mg), which was extracted with methanol. The methanol extract (158 mg) was purified

by preparative tlc (benzene:ethyl acetate=4:1, n-hexane:ethyl acetate=5:1) followed by crystallization from n-hexane-acetone to give garcinone B[13] (**6**, mp 189-190°C, 46 mg). The fraction eluted with n-hexane containing 25% ethyl acetate (frs. 90-94, 54 mg in total) was purified by preparative tlc (n-hexane:acetone=3:1) followed by crystallization from n-hexane-acetone to give morusignin D (**4**, 16 mg). The benzene extract (54 g) of the root bark was chromatographed on silica gel (300 g) with n-hexane containing increasing amount of ethyl acetate as an eluent (frs. 1-145, 300 ml each). The fraction eluted with n-hexane containing 14% ethyl acetate (frs. 34-38, 4.9 g in total) was rechromatographed on silica gel (150 g) with n-hexane-chloroform (frs. 1'-79', 300 ml each). The fraction eluted with chloroform (frs. 50'-79', 2.1 g in total) was crystallized from n-hexane-acetone to give morusignin A (**1**, 1.1 g). The fraction eluted with n-hexane containing 14% ethyl acetate (frs. 41-43, 3.5 g in total) was rechromatographed on silica gel (150 g) with n-hexane-acetone as an eluent (frs. 1'-37', 300 ml each). The fraction eluted with n-hexane containing 14% acetone (frs. 18'-23', 14 mg in total) was crystallized from n-hexane-acetone to give toxyloxanthone B[14] (**7**, mp > 300°C, 8 mg). The fraction eluted with n-hexane containing 14% ethyl acetate (frs. 66-80, 1.7 g in total) was rechromatographed on silica gel (100 g) with n-hexane-acetone as an eluent (frs. 1'-62', 100 ml each). The fraction eluted with n-hexane containing 17% acetone (frs. 23'-30', 100 mg in total) was crystallized from n-hexane-acetone to give morusignin B (**2**, 60 mg). The identification of the known compounds (**5** — **7**) was carried out by comparing the physical and spectral data of these compounds with the relevant data.

Morusignin A (**1**)

*Compound* **1** *was recrystallized from* n-hexane-acetone to give yellow needles, mp 218-220°C. $FeCl_3$ test; positive (purple). Gibbs test; negative. EI-Ms: m/z (rel. int.) 328 ($M^+$, 30), 313 (13), 285 (2), 273 (100), 260 (6). High-resolution ms (HR-ms): m/z 328.0977 ($C_{18}H_{16}O_6$ requires 328.0947), m/z 273.0305 ($C_{14}H_9O_6$ requires 273.0400). Uv $\lambda_{max}^{EtOH}$ nm (log ε): 205 (4.05), 224 (3.98), 256 (4.05), 280 (3.87), 303 (3.35), 349 (3.97). Uv $\lambda_{max}^{EtOH+AlCl_3}$ : 205 (4.19), 270 (3.95), 291 (3.97), 385 (3.96). Ir $\nu_{max}^{KBr}$ $cm^{-1}$ : 3380, 1660, 1630, 1615, 1590, 1575, 1520, 1490. $^1H$ Nmr (acetone-$d_6$): δ 1.65, 1.84 (each 3H, br s, C-13-$CH_3$), 3.54 (2H, br d, J≈7 Hz, C-11-H x 2), 5.34 (1H, m, C-12-H), 6.35 (1H, s, C-2-H), 6.58 (1H, d, J=9 Hz, C-7-H), 7.29 (1H, d, J=9 Hz, C-6-H), 11.27 (1H, s, C-8-OH), 11.96 (1H, s, C-1-OH).

Morusignin B (**2**)

Compound **2** was recrystallized from n-hexane-acetone to give yellow needles, mp 259-261°C. $FeCl_3$ test; positive (brown). Gibbs test; positive. EI-Ms: m/z (rel. int.) 328 ($M^+$, 88), 313 (42), 285 (77), 273 (100). HR-Ms: m/z 328.0981 ($C_{18}H_{16}O_6$ requires 328.0947), m/z 285.0414 ($C_{15}H_9O_6$ requires 285.0400). Uv $\lambda_{max}^{EtOH}$ nm (log ε): 202 (3.92), 228 (3.76), 242 (3.77), 255 (3.76), 282 (3.73), 317 (3.41), 341 (3.49). Uv $\lambda_{max}^{EtOH+AlCl_3}$ : 205 (3.99), 240 (3.80), 263 (3.73), 298 (3.80), 335 (3.45), 369 (3.47), 407 (3.01). Ir $\nu_{max}^{KBr}$ $cm^{-1}$ : 3480, 1660, 1635, 1615, 1595, 1585, 1505, 1465. $^1H$ Nmr (acetone-$d_6$): δ 1.66, 1.79 (each 3H, br s, C-13-$CH_3$), 3.36 (2H, br d, J=7 Hz, C-11-H x 2), 5.34 (1H, m, C-12-H), 6.56 (1H, s, C-4-H), 6.62 (1H, d, J=9 Hz, C-7-H), 7.27 (1H, d, J=9 Hz, C-6-H), 11.24 (1H, s, C-8-OH), 12.27 (1H, s, C-1-OH).

Morusignin C (**3**)

Compound **3** was recrystallized from n-hexane-acetone to give yellow needles, mp 218°C. $FeCl_3$ test; positive (dark green). Gibbs test; negative. EI-Ms: m/z (rel. int.) 326 ($M^+$, 19), 311 (100), 295 (6). HR-Ms: m/z 326.0839 ($C_{18}H_{14}O_6$ requires 326.0791), m/z 311.0559 ($C_{17}H_{11}O_6$ requires 311.0556). Uv $\lambda_{max}^{EtOH}$ nm (log ε): 203 (4.23), 221 (4.24), 266 (4.54), 305 (3.73), 363 (4.00). Uv $\lambda_{max}^{EtOH+AlCl_3}$ : 220 (4.44), 282 (4.62), 403 (4.05). Ir $\nu_{max}^{KBr}$ $cm^{-1}$ : 3400, 1665, 1640, 1630, 1605, 1580, 1485, 1465. $^1H$ Nmr (acetone-$d_6$): δ 1.49 (6H, s, C-13-$CH_3$ x 2), 5.78 (1H, d, J=10 Hz, C-12-H), 6.19 (1H, d, J=

0.7 Hz, C-2-H), 6.65 (1H, d, J=9 Hz, C-7-H), 7.01 (1H, dd, J=0.7 and 10 Hz, C-11-H), 7.32 (1H, d, J=9 Hz, C-6-H), 11.17 (1H, s, C-8-OH), 12.10 (1H, s, C-1-OH).

Morusignin D (**4**)

Compound **4** was recrystallized from n-hexane-acetone to give yellow needles, mp 226-228°C. $FeCl_3$ test; positive (greenish brown). Gibbs test; positive. EI-Ms: m/z (rel. int.) 342 ($M^+$, 54), 327 (24), 299 (62), 287 (100). HR-Ms: m/z 342.1107 ($C_{19}H_{18}O_6$ requires 342.1104), m/z 299.0552 ($C_{16}H_{11}O_6$ requires 299.0556). Uv $\lambda_{max}^{EtOH}$ nm (log ε): 203 (4.15), 246 (4.36), 281 (sh 3.69), 321 (4.08). Uv $\lambda_{max}^{EtOH+AlCl_3}$ : no shift. Ir $\nu_{max}^{KBr}$ $cm^{-1}$ : 3440, 1645, 1610, 1520, 1450. $^1H$ Nmr (acetone-$d_6$): δ 1.65, 1.79 (each 3H, br s, C-13-$CH_3$), 3.37 (2H, br d, J=7 Hz, C-11-H x 2), 3.98 (3H, s, C-5-$OCH_3$), 5.29 (1H, m, C-12-H), 6.57 (1H, s, C-4-H), 7.00 (1H, d, J=9 Hz, C-7-H), 7.84 (1H, d, J=9 Hz, C-8-H), 13.37 (1H, s, C-1-OH).

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