COMPONENTS OF THE ROOT BARK OF MORUS INSIGNIS BUR. 3. STRUCTURES OF THREE NEW ISOPRENYLATED XANTHONES MORUSIGNINS I, J, AND K AND AN ISOPRENYLATED FLAVONE MORUSIGNIN L.[1,2]

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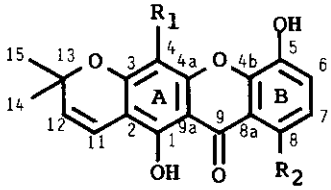
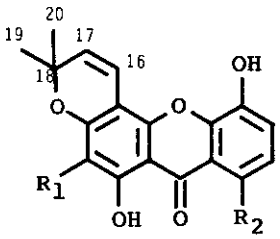
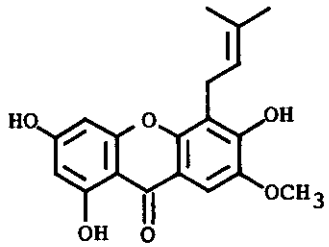
Abstract —— Three new isoprenylated xanthones, morusignins I (**1**), J (**2**), and K (**3**) as well as a new isoprenylated flavone, morusignin L (**4**) were isolated from the root bark of Morus insignis Bur. (Moraceae), collected in Paraguay. The structures of morusignins I - L were shown to be **1** - **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 reported the structures of eight isoprenylated xanthones, morusignins A - H.[3,4] This paper deals with the characterization of three new isoprenylated xanthones as well as an isoprenylated flavone.

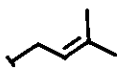
Morusignin I (**1**), yellow needles, mp 149-153 °C, $C_{23}H_{22}O_6$, gave a brown color with methanolic ferric chloride. The uv spectrum of **1** resembled with those of 1,3,5,8-tetraoxygenated xanthones.[5] The ^1H nmr spectrum







3

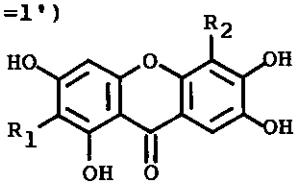
1 : R_1=  R_2=OH

(prenyl)

2 : R_1=prenyl R_2=OH (=1')

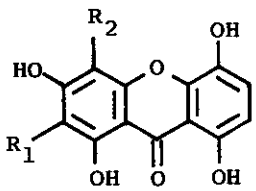
8 : R_1=R_2=H

9 : R_1=R_2=H



10 : R_1=R_2=H

11 : R_1=  R_2=prenyl



5 : R_1=R_2=prenyl

6 : R_1=H R_2=prenyl

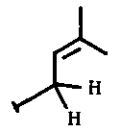
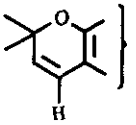
7 : R_1=prenyl R_2=H

Table 1 ^{13}C Nmr chemical shifts (ppm) of 1, 2, 3, 5, 10, and 11

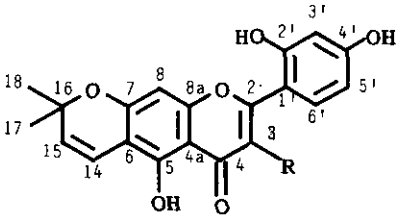
C	1*	2*	5*	3*	10**	11*		1*	2*	5*	3*	11*
C-1	156.0	160.4	158.9	164.5	162.7	163.4	C-11	115.8	21.7	22.3	23.0	41.6
C-2	108.4	110.4	111.7	98.8	97.7	115.2	C-12	129.0	122.8	123.0	122.3	29.0
C-3	160.0	160.1	162.4	165.8	164.7	164.1	C-13	79.4	131.9	132.9	132.6	29.0
C-4	105.4	102.1	107.8	94.6	93.6	95.1	C-14	28.6	18.1	18.1	18.1	150.8
C-4a	155.1	150.9	153.7	158.9	157.4	156.5	C-15	28.6	25.9	25.9	25.9	109.0
C-4b	145.0	144.6	145.1	151.5	151.0	150.4	C-16	21.9	115.9	22.3		23.0
C-5	138.2	138.0	138.1	116.5	102.7	116.3	C-17	123.1	128.2	122.8		122.4
C-6	125.1	125.2	125.1	152.0	154.0	151.4	C-18	132.1	79.4	132.8		132.3
C-7	110.3	110.5	109.9	146.1	143.8	142.7	C-19	18.1	28.4	18.0		18.1
C-8	154.2	154.3	154.3	103.1	108.2	106.6	C-20	25.9	28.4	25.9		25.9
C-8a	109.0	108.4	108.4	113.2	111.9	113.4	OCH_3				56.7	
C-9	186.1	185.9	186.0	180.6	178.9	180.9						
C-9a	103.0	102.7	102.8	103.0	101.7	104.1						

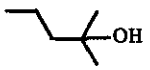
solvent: *; acetone-d_6 **; dmso-d_6

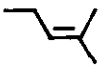
Table 2 ^{1}H Nmr chemical shifts (ppm)

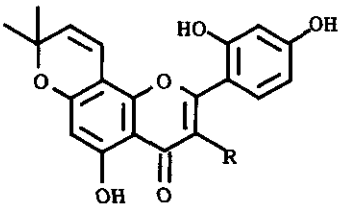
	1	2	6	7		1	2	8	9
	3.54	3.35	3.54	3.36		6.70	7.05	6.67	7.06

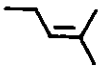
measured in acetone-d_6



4 : R= 

14 : R= 



12 : R= 

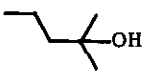
13 : R= 

Table 3 ^{13}C Nmr chemical shifts (ppm) of 4, 12, and 14

	4	12	14		4	12	14
C-2	162.5	162.7	162.8	C-9	21.1	24.6	24.6
C-3	123.0	121.8	121.7	C-10	43.1	122.5	122.5
C-4	183.5	183.2	183.2	C-11	70.2	132.3	132.2
C-4a	105.6	101.6	105.6	C-12	(28.7)*	17.7	17.7
C-5	160.1	162.4	160.0	C-13	(28.7)*	25.8	25.8
C-6	105.7	99.7	105.7	C-14	116.0	115.5	115.9
C-7	158.5	159.9	158.1	C-15	129.1	127.9	129.1
C-8	95.2	105.6	95.2	C-16	78.6	78.6	78.6
C-8a	157.2	153.3	157.2	C-17	28.4	28.3	28.3
C-1'	112.5	112.6	112.7	C-18	28.4	28.3	28.3
C-2'	157.4	157.4	157.3				
C-3'	104.0	103.9	103.8				
C-4'	161.5	161.6	161.6				
C-5'	108.1	108.1	108.0				
C-6'	132.1	132.2	132.2				

solvent: acetone-d_6 ()*; dmso-d_6

showed the signals for the following protons: 1) protons in a 3,3-dimethylallyl (prenyl) group, δ 1.65, 1.86 (each 3H, br s), 3.54 (2H, br d, J = 7 Hz), 5.29 (1H, m), 2) protons in a 2,2-dimethylpyran ring, δ 1.51 (6H, s), 5.79, 6.70 (each 1H, d, J = 10 Hz), 3) ortho-coupled aromatic protons, δ 6.64, 7.33 (each 1H, d, J = 9 Hz), and 4) protons in two hydrogen-bonded hydroxyl groups, δ 11.23, 12.32 (each 1H, s). The ^{13}C nmr spectrum of **1** was analysed by comparing it with those of gartanin (**5**)[6] and 1,3,5,8-tetraoxygenated xanthones[3,4] (Table 1). In the ^{13}C nmr studies, **1** was suggested to be 1,3,5,8-tetraoxygenated xanthone having a prenyl group and a 2,2-dimethylpyran ring in the A ring, and two possible structures (**1** and **1'**) were proposed. Discrimination between the structures was carried out on the following results. Comparative study of the ^{1}H nmr spectrum of **1** with those of morusignins A (**6**)[3] and B (**7**)[3] showed that the chemical shift of the methylene proton signal in the prenyl group of **1** was more similar to the shift of the methylene proton signal of **6** than that of the relevant signal of **7** (Table 2). Furthermore the chemical shift of the olefinic proton signal at C-11 position was more similar to that of the relevant proton signal of 6-deoxyjacareubin (**8**)[7] than that of 6-deoxyisojacareubin (**9**)[8] (Table 2). From the above results, formula (**1**) was proposed for the structure of morusignin I.

Morusignin J (**2**), yellow prisms, mp 181-186 °C, $C_{23}H_{22}O_6$, gave a brown color with methanolic ferric chloride. The uv spectrum of **2** resembled with that of **1**. The ^{1}H nmr spectrum showed the signals for the following protons: 1) protons in a prenyl group, δ 1.67, 1.82 (each 3H, br s), 3.35 (2H, br d, J = 7 Hz), 5.24 (1H, m), 2) protons in a 2,2-dimethylpyran ring, δ 1.52 (6H, s), 5.80, 7.05 (each 1H, d, J = 10 Hz), 3) ortho-coupled aromatic protons, δ 6.66, 7.33 (each 1H, d, J = 9 Hz), and 4) protons in two hydrogen-bonded hydroxyl groups, δ 11.25, 12.46 (each 1H, s). The ^{13}C nmr spectrum of **2** was analysed by comparing with that 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). These results suggest that **2** is a structural isomer of **1**. In the ^{1}H nmr spectrum of **2**, the chemical shifts of the methylene proton signal and the olefinic proton signal at C-11 position were similar to the relevant signals of **7** and **9**, respectively (Table 2). From the above results, formula (**2**) was proposed for the structure of morusignin J.

Morusignin K (**3**), yellow needles, mp 251-253 °C, gave a greenish brown color with methanolic ferric chloride and was positive to the Gibbs test. The uv spectrum of **3** resembled with those of 1,3,6,7-tetraoxygenated xanthones.[5] The ^{1}H nmr spectrum showed the signals for the following protons: 1) protons in a prenyl group, δ 1.66, 1.89 (each 3H, br s), 3.63 (2H, br d, J = 7 Hz), 5.31 (1H, m), 2) meta-coupled aromatic protons, δ6.35, 6.47 (each 1H, d, J = 2 Hz), 3) an aromatic proton, δ 7.45 (1H, s), 4) methoxyl protons, δ 3.99 (3H, s), and 5) proton in a hydrogen-bonded hydroxyl group, δ 13.20 (1H, s). The ^{13}C nmr spectrum of **3** was analysed by comparing it with those of 1,3,6,7-tetraoxygenated xanthone (**10**)[9] and cudraxanthone L (**11**)[10] (Table 1). In the ^{13}C nmr studies, **3** was suggested to be 1,3,6,7-tetraoxygenated xanthone having a prenyl group at the C-5 position. In the NOE experiment of **3**, irradiation at the methoxyl signal (δ 3.99) increased the intensity of the aromatic proton at δ 7.45 (21 %). Thus, the location of the methoxyl group was supported to be C-7 position. From the above results, formula (**3**) was proposed for the structure of morusignin K.

Morusignin L (**4**), yellow prisms, mp 202-204 °C, $C_{25}H_{26}O_{7}$, gave a green color with methanolic ferric chloride and was positive to the magnesium-hydrochloric acid test. The uv spectrum of **4** resembled with that of morusin (**12**).[11] The ^{1}H nmr spectrum showed the signals for the following protons: 1) protons in a 2,2-dimethylpyran ring, δ 1.45 (6H, s), 5.75 (1H, d, J = 10Hz), 6.69 (1H, dd, J = 0.7 and 10 Hz), 2) protons in a

3-hydroxy-3-methylbutyl group, δ 1.07 (6H, s), 1.62, 2.48 (each 2H, m), 3) an ABX type aromatic protons, δ 6.52 (1H, dd, J = 2 and 8 Hz), 6.56 (1H, d, J = 2 Hz), 7.23 (1H, d, J = 8 Hz), 4) a doublet aromatic proton, δ 6.27 (1H, d, J = 0.7 Hz), and 5) proton in a hydrogen-bonded hydroxyl group, δ 13.61 (1H, s). The chemical shifts and coupling patterns of the above spectrum resembled with those of oxydihydromorusin (**13**).[12] These results suggest that **4** is a structural isomer of **13**. The ^{13}C nmr spectrum of **4** was analysed by comparing with those of **12** and cudraflavone B (**14**)[13] (Table 3). In the ^{13}C nmr spectrum of **4**, the chemical shifts of all the carbon atoms except those of a 3-hydroxy-3-methylbutyl group and the C-3 position were good agreement with those of **14**. From the above results, formula (**4**) was proposed for the structure of morusignin L.

EXPERIMENTAL

Abbreviations: sh = shoulder. The general procedures followed as described in our previous paper.[14]

Isolation of Morusignins I (**1**), J (**2**), K (**3**), and L (**4**) from the Root Bark of M. insignis Bur.

The dried root bark (3.4 Kg) of M. insignis Bur., collected in the suburb of Encarnacion City, Itapua Prefecture, Paraguay, in February 1989, was extracted with n-hexane, benzene, and acetone, successively. The procedures are described in the previous papers.[3,4] Evaporation of the n-hexane, benzene, and acetone solution to dryness yielded 170 g, 54 g, and 70 g of the residue, respectively. The n-hexane extract (135 g) was chromatographed on silica gel (3 Kg) with n-hexane containing increasing amount of ethyl acetate as an eluent (fractions 1 - 9, eluted volume of 1 l each).[4] The fraction (fr. 5, eluent, n-hexane : ethyl acetate = 86 : 14, 7.1 g) was rechromatographed on silica gel (100 g) with n-hexane-acetone as an eluent. The fraction (n-hexane : acetone = 86 : 14, 0.2 g) was fractionated by preparative hplc (solvent, n-hexane : ethyl acetate = 6 : 1, column, Senshu Pak SSC-Silica 4251-N, 1 cm ϕ x 25 cm, detector, uv 280 nm) to give morusignin J (**2**, 3 mg). The benzene extract (54 g) was chromatographed on silica gel (300 g) with n-hexane-ethyl acetate as an eluent (frs. 1' - 145', eluted volume 300 ml each). The fraction (frs. 14' - 39', n-hexane : ethyl acetate = 86 : 14, 31.4 g) was rechromatographed on silica gel (300 g) with n-hexane-acetone as an eluent. The fraction (n-hexane : acetone = 95 : 5, 1.5 g) was rechromatographed on silica gel (100 g) with benzene-acetone as an eluent. The fraction (benzene, 0.85 g) was fractionated by preparative hplc (n-hexane : ethyl acetate = 9 :

1, above-described conditions) to give morusignin I (**1**, 7 mg). The fraction (n-hexane : acetone = 9 : 1, 4.7 g) was rechromatographed on silica gel (100 g) with benzene-acetone as an eluent. The fraction (benzene : acetone = 9 : 1, 1.9 g) was fractionated by column chromatography (silica gel, n-hexane-ethyl acetate) and preparative tlc (silica gel, solvent system, chloroform : acetone = 15 : 1), successively, to give morusignin K (**3**, 8 mg). The fraction (frs. 103'-125', n-hexane : ethyl acetate = 3 : 1, 2 g) was rechromatographed on silica gel (50 g) with benzene-acetone as an eluent. The fraction (benzene : acetone = 91 : 9, 0.1 g) was fractionated by preparative tlc (n-hexane : acetone = 2 : 1) to give morusignin L (**4**, 15 mg).

Morusignin I (**1**)

Compound **1** was crystallized from n-hexane-acetone to give yellow needles, mp 149-153 °C. $FeCl_3$ test; positive (brown). EI-Ms: m/z (rel. int.) 394 (M^+, 28%), 379 (100), 351 (9), 339 (23), 323 (7). HR-Ms: m/z 394.1423 (M^+, $C_{23}H_{22}O_6$ requires 394.1416). Ir ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1645, 1635, 1610, 1590. Uv λ_{max}^{EtOH} nm (log ε): 224 (3.56), 272 (3.56), 300 (3.86), 350 (3.31), 405 (2.85).

Morusignin J (**2**)

Compound **2** was crystallized from n-hexane-acetone to give yellow prisms, mp 181-186 °C. $FeCl_3$ test; positive (brown). EI-Ms: m/z (rel. int.) 394 (M^+, 42), 379 (100), 351 (18), 339 (19), 323 (27). HR-Ms: m/z 394.1423 (M^+, $C_{23}H_{22}O_6$ requires 394.1416). Ir ν_{max}^{KBr} cm^{-1}: 3430, 1655, 1625, 1575, 1495, 1465, 1450, 1420. Uv λ_{max}^{EtOH} nm (log ε): 229 (4.23), 260 (4.45), 271 (4.43), 310 (sh 3.60), 366 (3.98).

Morusignin K (**3**)

Compound **3** was crystallized from n-hexane-acetone to give yellow needles, mp 251-253 °C. $FeCl_3$ test; positive (greenish brown). Gibbs test; positive. EI-Ms: m/z (rel. int.) 342 (M^+, 100), 327 (15), 313 (6), 299 (5), 287 (100), 272 (11), 257 (19). HR-Ms: m/z 342.1104 ($C_{19}H_{18}O_6$ requires 342.1106). Ir ν_{max}^{KBr} cm^{-1}: 3260, 1645, 1605, 1580, 1510 sh, 1470. Uv λ_{max}^{EtOH} nm (log ε): 211 (4.32), 240 (4.37), 255 (4.43), 277 (3.84), 316 (4.14), 360 (4.02).

Morusignin L (**4**)

Compound **4** was crystallized from chloroform-acetone to give yellow prisms, mp 202-204 °C. $FeCl_3$ test; positive (green). Mg-HCl test; positive (orange). EI-Ms: m/z (rel. int.) 438 (M^+, 47), 423 (52), 420 (19), 405 (100), 203 (30). HR-Ms: m/z 438.1703 (M^+, $C_{25}H_{26}O_7$ requires 438.1679). Ir ν_{max}^{KBr} cm^{-1}: 3400, 3240, 1665, 1625, 1585, 1570, 1545, 1510, 1475, 1465. Uv λ_{max}^{EtOH} nm (log ε): 205 (4.46), 278 (4.40), 327 (3.97).

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