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FLAVONOID *O*-GLYCOSIDES FROM THE LEAVES OF *MORINDA MORINDOIDES*

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Key Word Index—*Morinda morindoides*; Rubiaceae; flavonoid *O*-glycosides; chrysoeriol 7-neohesperidoside.

Abstract—Eight known flavonoids: quercetin, quercetin 7,4′-dimethylether, luteolin 7-glucoside, apigenin 7-glucoside, quercetin 3-rhamnoside, kaempferol 3-rhamnoside, quercetin 3-rutinoside, kaempferol 3-rutinoside, and a new glycoside, chrysoeriol 7-neohesperidoside, were isolated from the leaves of *Morinda morindoides*. Their structures were established by the combined use of chemical and spectroscopic methods.

INTRODUCTION

Morinda morindoides (Baker) Milne Redh. (Rubiaceae) is one of the most popular medicinal plants used in Zairese traditional medicine. The decoction of its leaves is used against various diseases such as scabies, gonorrhoea, malaria, amoebiasis, haemorrhoids and worms [1, 2].

The genus *Morinda* is known for its many anthraquinone constituents [3], but very little is known about the occurrence of other polyphenolic compounds in this genus. In previous studies, quercetin and kaempferol 3-rutinosides, acacetin 7-glucopyranoside and apigenin 5,7-dimethylether 4′-galactoside were isolated from the leaves and flowers of *M. tinctoria* Roxb. [4] and the flowers of *M. citrifolia* L. [5], respectively. In this paper, we report the isolation and structural elucidation of nine flavonoids, including two aglycones and seven glycosides, one of which is new natural product.

RESULTS AND DISCUSSION

From the ethyl acetate and *n*-butanol fractions, eight known flavonoids were isolated by column chromatography and preparative TLC on silica gel. They were identified by spectroscopic methods (UV; positive ion FAB-MS; ^{1}H and ^{13}C NMR) as quercetin (**1**), quercetin 7,4′-dimethylether (**2**), luteolin 7-glucoside (**3**), apigenin 7-glucoside (**4**), quercetin 3-rhamnoside (**5**), kaempferol 3-rhamnoside (**6**), quercetin 3-rutinoside (**7**) and kaempferol 3-rutinoside (**8**) by comparison with literature data [6–9]. These compounds were isolated for the first time from *M. morindoides*.

Compound **9** responded positively to Shinoda's and Neu's reagents. Its FAB mass spectrum recorded in the positive ion mode showed a molecular ion at m/z 609 $[M + H]^+$, corresponding to a molecular formula of $C_{22}H_{32}O_{15}$. Other important peaks occurred at m/z 463 $[M - 146 + H]^+$ (loss of deoxyhexose moiety) and at m/z 301 $[M - 162 - 146 + H]^+$. The latter fragment ion was attributed to the protonated aglycone formed by loss of the disaccharide moiety. The ^{13}C NMR spectrum indicated a flavone glycoside with a 3′-methoxyl-4′-hydroxyl substitution pattern in the B-ring and a free 5-hydroxyl (C-4 carbonyl resonance at 182.0 ppm), leaving only the 7-hydroxyl group as the possible glycosylation site. The ^{13}C NMR data of the aglycone were in agreement with a 7-substituted chrysoeriol moiety. The ^{13}C NMR peaks of the glycoside part could be assigned to an α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl or neohesperidosyl moiety [6–10]. This was supported by the ^{1}H NMR spectrum showing two anomeric signals at δ5.24 (1H, *d*, J = 5.0 Hz, glucosyl H-1) and δ5.14 (1H, *br s*, rhamnosyl H-1). The UV spectrum recorded in methanol was in agreement with a flavone moiety. After adding sodium hydroxide 2 M, no new band was observed between 320 and 335 nm, whereas band II was not shifted by adding NaOAc. This was in agreement with a substitution of the 7-hydroxyl group [9]. Therefore, **9** was identified as chrysoeriol 7-*O*-neohesperidoside. Chrysoeriol 7-(2″-*O*-β-D-allopyranosyl)-β-D-glucopyranoside has been reported before from *Sideritis grandiflora* [11]. The spectroscopic data for this compound are in good

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agreement with those obtained for **9**, except for the C-1′ resonance which occurs at δ121.2 for **9** but at δ127.6 in the compound described in the literature. However, the latter value is rather unusual as compared with chrysoeriol (C-1′ at δ121.7) [10].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or in CD_3OD at room temp. on a JOEL FX 200 MHz or a Varian Unity 400 MHz instrument. The chemical shifts are reported in δ values (ppm) with TMS as the int. standard. FAB-Mass spectra were recorded in the positive ion mode on a Fisons VG 70 SEQ instrument using glycerol or thioglycerol as the liquid matrix.

Plant material. Leaves of *M. morindoides* were collected in Kinshasa (Zaire) and the plant was identified by Mr Breyne of the Institut National d'Etudes et de Recherches Agronomiques (INERA) of the University of Kinshasa, where a voucher specimen is deposited.

Extraction and isolation. Dried and powdered leaves (500 g) were defatted by Soxhlet extraction with *n*-hexane. The dried residue was macerated and percolated exhaustively with 80% MeOH. The MeOH extract was concd to dryness under red. press. (123.13 g) and 50 g of the dried extract dissolved in hot H_2O and filtered after 24 hr. The filtrate was extracted successively with $CHCl_3$, EtOAc and *n*-BuOH.

The EtOAc fraction (2 g) was subjected to CC on polyamide MN-SC6, and eluted with a gradient of MeOH–H_2O with increasing polarity. Several fractions of 10 ml were collected and analysed by TLC on silica gel $60F_{254}$ Merck (layer thickness 0.2 mm) in BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer) (solvent system 1). Flavonoid spots were detected with UV (254 and 366 nm) before and after spraying with Neu's reagent (1% diphenylboric acid ethanolamine complex in MeOH). Fractions producing positive spots were combined in 2 fractions A (0.554 g) and B (1.07 g) according to their chromatographic pattern. These fractions were further chromatographed separatively on a silica gel 60 Merck (230–400 mesh ASTM) column with EtOAc–HOAc–H_2O (8:1:1) followed by prep. TLC on the same sorbent (layer thickness 1 mm) in solvent system 1. This resulted in the isolation of **1** (15.6 mg) and **2** (5.4 mg) from fraction A; **3** (6.3 mg), **4** (25.8 mg), **5** (10.5 mg) and **6** (14.1 mg) from fraction B; after final clean up of each compound on Sephadex LH-20 with MeOH.

CC of the *n*-BuOH fraction 93.5 g) on microcrystalline cellulose (AVICEL^R) using $CHCl_3$–MeOH–Me_2CO–H_2O (100:50:45:5) as a solvent system yielded several fractions of 10 ml which were analysed as described above. These were combined in 2 fractions C (1.54 g) and D (0.672 g) according to their chromatographic pattern, separated by CC on polyamide NM-SC6, eluted with a gradient of MeOH–H_2O with increasing polarity, followed by prep. TLC in solvent system 1. This led to the isolation of **7** (34.5 mg), **8** (15.2 mg), **9** (84 mg) from fractions C and D, respectively which were purified as described above.

Table 1. ^{13}C NMR spectral data of **9** (CD_3OD, 50 MHz)

	Aglycone			Sugars			
C		C					
2	164.2[a]	10	105.5	7-*O*-inner-Glc		terminal-Rha	
3	103.6	1′	121.2	1′	99.4	1	100.5
4	182.0	2′	110.3	2′	77.2[b]	2	69.7[a]
5	161.1	3′	151.2	3′	76.3[b]	3	70.4[c]
6	98.0	4′	148.1	4′	70.4[c]	4	71.9
7	162.6[a]	5′	115.9	5′	77.2[b]	5	68.4
8	94.7	6′	120.5	6′	60.6	6	18.2
9	157.0	OMe	56.2				

[a–c]Assignments may be interchanged.
Rha, rhamnosyl; Glc, glucosyl.

Acid hydrolysis. Acid hydrolysis of flavonoid glycosides was performed with 2 N HCl–MeOH (1:1) (10 ml) of 2 mg of each glycoside by heating under reflux for 2 hr, diluting with H_2O and then extracting with EtOAc. The EtOAc phase was dried and evapd to yield the aglycone, and the aqueous layer was neutralized with $BaCO_3$, and evapd to yield the sugar fraction. Aglycones were identified in the EtOAc fraction by co-chromatography on silica gel TLC using BAW and 15% HOAc as solvents with reference samples, except for chrysoeriol which was not available. Sugars were identified by Co-PC in *n*-BuOH–pyridine–H_2O (6:3:1) with authentic sugars and detected with β-naphthol–H_2SO_4 reagent prepared by mixing 10.5 ml of 15% ethanolic solution of 1-naphthol, 6.7 ml 97% H_2SO_4, 40.5 ml EtOH and 4 ml H_2O.

Chrysoeriol 7-O-neohesperidoside (**9**). A yellow powder, mp 129°. R_f values on silica gel TLC in BAW 0.42; on cellulose TLC in 15% HOAc 0.23 and in BAW 0.43. UV λ_{max}^{MeOH} 253, 267, 345; + NaOH 245, 262, 305, 390; + $AlCl_3$ 272, 300, 353, 389; + $AlCl_3$/HCl 276, 303, 352, 390; + NaOAc 250, 266, 349; + H_3BO_3 250, 266, 346 nm. IR ν_{max}^{KBr} cm^{-1}: 3600–3400, 1655, 1610, 1590, 1490, 1370–1325, 1260, 1040, 990, 820, 810, 760, 680–620. FAB-MS positive ion mode (probe), *m/z* 609 $[M + H]^+$, 463 $[M$ − rhamnosyl + $H]^+$, 301 $[M$ = rhamnosyl − glucosyl + $H]^+$. ^{1}H NMR (DMSO-d_6): δ7.58 (2H, *m*, H-2′, H-6′), 7.0 (1H, *br s*, H-8), 6.94 (1H, *m*, H-5′), 6.83 (1H, *br s*, H-6), 6.38 (1H, *s*, H-3), 5.24 (1H, *d*, *J* = 5.0 Hz, H-1‴(Glc)), 5.14 (1H, s, H-1⁗(Rha)), 3.90 (–OMe *s*, 3H), 1.21 (3H, *d*, *J* = 5.0 Hz, H-6⁗(Rha)). ^{13}C NMR (Table 1). Complete hydrolysis of **9** with 2 N HCl–MeOH (1:1) yielded chrysoeriol, glucose and rhamnose.

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