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THE 7,7″-β-DIGLUCOSIDE OF (2*S*,3*R*)-CHAMAEJASMIN FROM *ORMOCARPUM KIRKII**

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Key Word Index—*Ormocarpum kirkii*; Fabaceae; root bark; ormocarpin; biflavanones; circular dichroism.

Abstract—From root bark of *Ormocarpum kirkii* a new biflavanone diglucoside, named ormocarpin, was isolated. Its structure and absolute configuration, assigned by chemical and spectroscopic methods, corresponds to 7,7″-β-diglucoside of (2*S*,3*R*)-chamaejasmin.

INTRODUCTION

Some species of the genus *Ormocarpum* (Fabaceae), *O. flavum*, *O. muricatum*, *O. trachycarpum*, *O. trichocarpum* and *O. kirkii*, are utilized in African traditional medicine [2] but hitherto the sole phytochemical mention in the literature has been the identification of rotenone in *O. glabrum* [3] in agreement with the large occurrence of isoflavonoids in Fabaceae. The *Ormocarpum* species examined here, *O. kirkii*, collected in Kwale District, South of Mombasa, is indigenous to Kenya and traditionally has magical properties. The ash of the plant is used for allergic diseases and to reduce oedemas, the roots are used for rheumatism and stomach troubles, whereas the crushed leaves are used for headache.

RESULTS AND DISCUSSION

The methanolic extract of the root bark was submitted to counter-current distribution (CCD) and a crystalline substance, **1**, mp 230–232°, $[\alpha]_D^{20}$ +103 (MeOH–H_2O, 2:1), UV λ_{max}^{MeOH} nm: 225, 289, 335 (sh) (log ε 4.39, 4.24, 3.43), was isolated as the main constituent. NMR spectra of **1**, as well as those of its derivatives **2**–**7**, account for only half of the molecule, $C_{42}H_{42}O_{20}$ (FAB mass spectrum: *m/z* 866, $[M]^+$, and 433) which is therefore symmetrical. For each unit the ^1H NMR spectrum shows a *peri* disubstituted aromatic ring (4H, δ6.73 and 6.86, *d*, *J* = 8 Hz), two *meta* aromatic protons (δ6.13 and 6.24, *d*, *J* = 2 Hz), two vicinal protons at δ2.79 and 5.94 (*d*, *J* = 12 Hz) and finally a monose (anomeric hydrogen at δ4.75, *d*, *J* = 7 Hz) whose ^{13}C NMR signals are typical of those of a β-glucopyranoside (Tables 1 and 2).

By methylation with diazomethane, **1** (named ormocarpin) gave the corresponding methyl derivative, **2**, $C_{46}H_{50}O_{20}$ (δ3.76 and 3.86, four OMe), mp 210–212°. Whereas by acetylation with pyridine and acetic anhydride **1** gave a dodecaacetyl derivative, **3**, $C_{66}H_{66}O_{32}$ (δ2.02, 2.05, 2.06, 2.07, 2.33, 2.37, 12 OAc), mp 146–148°.

By hydrolysis with β-glucosidase, ormocarpin gave D-glucose and an optically active aglucone, **4**, mp 149–151°, FAB mass spectrum: *m/z* 542, $[M]^+$, $C_{30}H_{22}O_{10}$, $[\alpha]_D^{20}$ +163 (MeOH). The latter, which gave a hexamethyl derivative, **5**, by methylation (mp 117–119°, $[\alpha]_D^{20}$ = +88.7, MeOH, δ3.76, 3.84 and 3.86, six OMe), was identified, except for the rotatory power, as chamaejasmin, a C-3/C-3″ biflavanone made up of two units of naringenin with *trans–trans* identical geometry at C-2/C-3 and C-2″/C-3″ (*J* = 12 Hz) [4]. Chamaejasmin was first isolated from *Stellera chamaejasme* (Thymelaeaceae) as a racemate, probably originating by isomerization (due to the use of NaOH in the extraction) of the co-occurring diastereoisomers, neochamaejasmin A (*cis–cis* identical) or of neochamaejasmin B (*cis–trans*) [5], or by racemization of optically active chamaejasmin. Natural chamaejasmin occurring in *S. chamaejasme* is, however, a mixture of laevo- and dextro-rotatory forms in the ratio 17:9 [6] whereas no optical information is given for chamaejasmin, first isolated from a member of the Fabaceae, *Diphysa robinioides* [7].

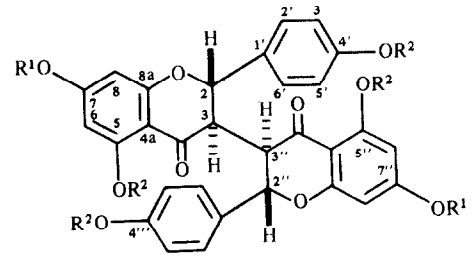
From the same *S. chamaejasme* the diastereoisomer with *trans–trans* opposite geometry at C-2/C-3 and C-2″/C-3″ (*meso* form made of two specular units), named isochamaejasmin [8], and the optically active 4′,4‴-dimethyl chamaejasmin, named chamaejasmenin A, [9], have been isolated. From another Thymelaeacea, *Wikstroemia sikokiana*, 4′-methylneochamaejasmin A (named sikokianin A), 4′- (or 4‴) methylneochamaejasmin B (sikokianin B) [10] and 4′,4‴-dimethylneochamaejasmin A (named chamaejasmenin B) [11] have been isolated.

In order to establish the identical attachment position of the two glucose units of ormocarpin in the molecule of (+)-chamaejasmin, tetramethylormocarpin, **2**, was hydrolysed and the tetramethylchamaejasmin obtained, **6**, mp 285–287°, $C_{34}H_{30}O_{10}$ (δ3.73 and 3.77, 4 OMe) was acetylated with pyridine and acetic anhydride to give the corresponding diacetyl derivative **7**, $C_{30}H_{16}O_4$ $(OMe)_4$ $(OAc)_2$, mp 124–127°.

By comparison of the ^1H NMR spectra (Table 1) of **7**, **6** and **5** a remarkable downfield shift was observed for H-6 and H-8 in **7** (6.29 and 6.31 against 6.01 and 6.06 in **5** and 5.83 and 6.02 in **6**) whereas the chemical shifts of the B

*Part 20 in the series, 'Research on African Medicinal Plants'. For Part 19 see ref [1].

	R^1	R^2	R^3
1	Gl	H	H
2	Gl	Me	H
3	Gl	Ac	Ac
4	H	H	
5	Me	Me	
6	H	Me	
7	Ac	Me	



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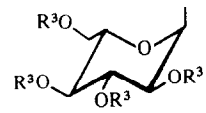

ring protons are practically identical. Positions 5 and 5″ for the glucose units in ormocarpin could be discarded as the resonance of C-4 in **1** (197.7 ppm) is much higher than the typical one of the aromatic carbonyl group, not engaged in hydrogen bonding, observed in **2**, **3**, **5** and **6** (188.5, 189.1, 190.0 and 188.5, respectively). The sole attachment positions assignable for the glucose units in **1** are therefore 7 and 7″ in agreement with the aforementioned downfield shift of the *ortho* hydrogens observed in **7** and caused by the acetyl groups. This assignment is also confirmed by the well known downfield shift of H-6 and H-8 due to glucosylation in **7** observed in **1** (δ6.13 and 6.24) and **2** (δ6.15 and 6.26) with respect to **4** (δ5.78 and 5.88).

Ormocarpin is therefore the 7,7″-diglucoside of (+)-chamaejasmin. The β-glucosidic linkage is confirmed by the behaviour with β-glucosidase and by the value of the coupling constant of the anomeric hydrogen ($J = 7$ Hz). In order to establish the absolute configuration at C-2 and C-3 in the two identical units of the ormocarpin and its derivatives, the circular dichroism curve of **2** was registered. It exhibits two strong Cotton effects, at 323 nm ($[\Theta] = +21 \times 10^3$) corresponding to $n \rightarrow \pi^*$ transition and at 275 nm ($[\Theta] = -52 \times 10^3$) corresponding to $\pi \rightarrow \pi^*$ transition for the carbonyl groups. The positive sign of the former predicts the configuration 2*S*,3*R* for 2,3-diequatorial substituents ($J = 12$ Hz) on the basis of the octant rule, modified for aryl ketones [12]. The negative

Table 1. ^{1}H NMR spectral data of compounds **1**–**7**

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	5.94 *d* (12)	5.95	5.95	5.76	6.08	5.76	6.00
3	2.79 *d* (12)	2.69	2.57	2.75	2.67	2.40	2.67
6 and	6.13	6.15	6.34	5.78	6.01	5.83	6.29
8	6.24 *d* (2)	6.26	6.45	5.88	6.06	6.02	6.31
2′,6′ and	6.73	6.88	7.02	6.67	6.85	6.85	6.85
3′,5′	6.86 *d* (8)	7.05		6.85	7.08	6.92	7.05
Glucose							
1	4.75 *d* (7)	4.90	5.1	—	—	—	—
2–5	3.8–4.0	3.8–4.0	5.1–5.3*	—	—	—	—
H_2-6	3.45 3.65 *m* (12)	3.5	4.2	—	—	—	—
MeO	—	3.76 3.86	—	—	3.76 3.84 3.86	3.73 3.77	3.85 3.90
AcO	—	—		2.02 2.07 2.05 2.33 2.06 2.37	—	—	2.26

Compounds **1**, **2** and **4** in $CDCl_3 + CD_3OD$; **3**, **5** and **7** in $CDCl_3$; **6** in $CDCl_3$ + DMSO-d_6 (δ values). *J* (Hz) in parentheses.

*H-5 at 3.85, *m*.

Table 2. ^{13}C NMR spectral data of compounds **1–3**, **5** and **6**

C	1	2	3	5	6
2	84.1	82.5	83.1	83.3	82.3
3	50.1	51.3	51.8	52.1	51.5
4	197.7	188.5	189.1	190.0	188.5
4a	104.0	105.0	108.0	106.6	104.4
5	166.4[a]	163.8[a]	163.7[a]	164.8[a]	164.1[a]
6	96.3[b]	95.5[b]	106.5	93.1[b]	95.5[b]
7	163.2[a]	163.4[a]	162.2[a]	165.6[a]	163.7[a]
8	97.7[b]	93.5[b]	102.4	93.3[b]	93.0[b]
8a	159.2	161.2	158.0	162.6	162.1
1′	130.1	128.6[c]	134.7	129.7[c]	129.0[c]
2′, 6′	126.9	128.5[c]	128.6	129.2[c]	128.5[c]
3′, 5′	116.2	113.5	122.2	114.0	113.4
4′	163.7	159.0	150.3	160.1	159.5
OMe	—	54.2	—	55.3, 55.4	55.1
		54.6		55.9	54.8
Glucose					
1	100.4	98.9	98.0		
2	73.4	76.9	70.9		
3	77.8	79.4	72.4		
4	70.3	69.0	68.2		
5	77.0	77.5	72.6		
6	61.4	60.9	61.8		
Me–CO	—	—	20.4, 21.0		
Me–CO	—	—	169.5, 169.7		
			170.5, 170.7		

[a–c]Assignments may be interchanged in the same column.
Compounds **1** and **6** in DMSO-d_6; **3** and **5** in $CDCl_3$ and **2** in DMSO-d_6 + $CDCl_3$.

sign of the second Cotton effect agrees with the same assignment [13].

The structure and stereochemistry of ormocarpin is thus represented by formula **1**. Chamaejasmin isolated from *S. chamaejasme* [6] as a partial racemate (68% of laevo-rotatory form) is mainly the 2*R*,3*S* enantiomer.

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for CCD. ^1H and ^{13}C NMR, 400 MHz and 100 MHz, respectively, TMS as int. std.

Material. Root bark of *O. kirkii* was collected in District Kwale, south coast of Mombasa, *ca* 600 km from Nairobi. A voucher specimen (N. 277) has been deposited with the Herbarium of the University of Nairobi by Joy Adamson in 1971.

Extraction and separation. Root bark (250 g) was ground and then extracted with MeOH (× 3). The residue of the combined elutions (20.5 g) was submitted in portions to CCD with H_2O–EtOAc–*n*-BuOH (10:9:1) and an unitary fraction (Kr = 1.5, 3.9 g) was obtained.

Ormocarpin (**1**). Crystallized from EtOH and EtOAc, mp 230–232°; $[\alpha]_D^{20}$ = +103 (MeOH–H_2O, 2:1, *c* 0.3). UV λ_{max}^{MeOH} nm: 225, 289, 335 (sh) (log ε 4.39, 4.24, 3.43). FABMS: *m/z* 866, $[M]^+$, and 433. Found C, 57.98; H, 4.64; calcd for $C_{42}H_{42}O_{20}$ C, 58.20; H, 4.88%).

Tetramethylormocarpin (**2**). Compound **1** dissolved in MeOH was methylated with CH_2N_2–Et_2O. After two days solvents were evapd and the residue purified by CCD with H_2O–EtOAc–*n*-BuOH (10:9:1), Kr = 0.43. The Me derivative **2**, crystallized from EtOH and EtOAc, mp 210–212°; $[\alpha]_D^{20}$ +32.9 (MeOH; *c* 0.4); UV λ_{max}^{MeOH} nm: 225, 285, 320 (sh) (log ε 4.37, 4.19, 3.82). CD (MeOH): Cotton effect at 323 nm ($[\Theta] = +21 \times 10^3$) and at 275 nm ($[\Theta] = -52 \times 10^3$). Found C, 59.67; H, 5.26; calcd for $C_{46}H_{50}O_{20}$ C, 59.86; H, 5.46%).

Dodecacetylormocarpin (**3**). Compound **1** was acetylated with pyridine–$(Ac)_2O$ (1:1) overnight. After evapn of reagents *in vacuo*, the residue was purified by CCD with H_2O–EtOH–Me_2CO–EtOAc–cyclohexane (7:3:4:1:10), Kr = 1.0. Crystals from *n*-hexane, mp 146–148°.

Hydrolysis of ormocarpin (**1**). β-Glucosidase (10 mg) was added to a soln of **1** (450 mg) in H_2O (200 ml) and acetate buffer pH 5.5 (50 ml). The soln, covered by toluene, was allowed to stand at 35°. After 6 days some drops of HOAc were added to the turbid soln which was extracted with EtOAc. The aq. soln was then extracted with *n*-BuOH and percolated through a column of Dowex 50 W (H^+). In the residue, glucose was identified by TLC on silica gel (H_2O–MeOH–HOAc–CH_2ClCH_2Cl, 2:3:5:10) and through its β-pentacetate by comparison with an authentic specimen.

The residue of the EtOAc soln was purified by CCD with H_2O–EtOH–Me_2CO–EtOAc–cyclohexane (14:6:8:7:13), Kr = 0.8. The aglucone **4** crystallized from EtOAc and *n*-hexane, mp 149–151°; $[\alpha]_D^{20}$ +163 (MeOH; *c* 0.8); FABMS: *m/z* 542, $[M]^+$, $C_{30}H_{22}O_{10}$. ^1H NMR data are reported in Table 1. For racemic chamaejasmin see ref. [6].

Hexamethylchamaejasmin (**5**). Chamaejasmin (**4**) dissolved in MeOH was methylated with CH_2N_2–Et_2O. After two days solvents were evapd and the residue purified by CCD with H_2O–EtOH–Me_2CO–EtOAc–cyclohexane (7:3:4:1:9), Kr = 2; mp 117–119° from *n*-hexane; $[\alpha]_D^{20}$ +88.7 (MeOH, *c* 0.5); FABMS: *m/z* 626, $[M]^+$, $C_{36}H_{34}O_{10}$, and 313.

Hydrolysis of tetramethylormocarpin: 4',4''',5,5''-*tetramethylchamaejasmin* (**6**). Cellulase (200 mg) was added to a suspension of tetramethylormocarpin (300 mg) in H_2O (200 ml) and Pi buffer pH 4.5 (100 ml) which was allowed to stand under stirring for 3 days at 36°. The turbid soln was then extracted with EtOAc and the residue of the organic phase purified by CCD with H_2O–EtOH–Me_2CO–EtOAc–cyclohexane (16:6:8:7:13), Kr = 0.58. Crystals from *n*-hexane, mp 285–287°.

7,7''-*Diacetyl*-4',4''',5,5''-*tetramethylchamaejasmin* (**7**). Obtained by acetylation of **6** as reported for **3**. Crystals from *n*-hexane, mp 124–127°.

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REFERENCES

1. Rasoanaivo, P., Ratsimamanga- Urveg, S., Messana, I., De Vicente, Y. and Galeffi, C. (1990) *Phytochemistry* (in press).
2. Kokwaro, J. O. (1976) *Medicinal Plants in East Africa*, p. 141. East African Literature Bureau, Nairobi.
3. Jones, A. H. (1942) *U.S. Dept. Agr. Bur. Entomol. Plant Quarantine E-571*, p. 14.
4. Hwang, Wen-Kuei and Chang, Chen-Chieh (1979) *K'O Hsueh T'ung Pao* **24**; 24; *Chem. Abst.* **90**, 135086 m.
5. Niwa, M., Tatematsu, H., Liu, Guo-Quan and Hirata, Y. (1984) *Chem. Letters* 539.
6. Niwa, M., Otsuji, S., Tatematsu, H., Liu, Guo-Quan, Chen, Xiang-Fang and Hirata, Y. (1986) *Chem. Pharm. Bull.* **34**, 3249.
7. Castro, O. and Valverde, V. (1985) *Phytochemistry* **24**, 367.
8. Niwa, M., Chen, Xiang-Fang, Liu, Guo-Quan, Tatematsu, H. and Hirata, Y. (1984) *Chem. Letters* 1587.
9. Liu, Guo-Quan, Tatematsu, H., Kurokawa, M., Niwa, M. and Hirata, Y. (1984) *Chem. Pharm. Bull* **32**, 362.
10. Niwa, M., Jiang, Pei-Fen and Hirata, Y. (1986) *Chem. Pharm. Bull.* **34**, 3631.
11. Niwa, M., Jiang, Pei-Fen and Hirata, Y. (1987) *Chem. Pharm. Bull.* **35**, 108.
12. Snatzke, G. (1965) *Tetrahedron* **21**, 413.
13. Gaffield, W. (1970) *Tetrahedron* **26**, 4093.

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FLAVONOIDS OF *BRICKELLIA LONGIFOLIA*

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Key Word Index—*Brickellia longifolia*; Asteraceae; Eupatorieae; sulphated 6-methoxyflavonols; FABMS.

Abstract—Three 6-methoxyflavonoid sulphates and one glycoside were isolated from *Brickellia longifolia*. These include the first report of 6-methoxykaempferol 7,4'-dimethyl ether 3-sulphate and the known compounds: 6-methoxykaempferol 7-methyl ether and quercetagetin 6,7,4'-trimethyl ether 3-sulphate and quercetagetin 6,7-dimethyl ether 3-galactoside.

INTRODUCTION

Brickellia Ell. (Asteraceae, Eupatorieae, Alomiinae) is a large genus of 100 species distributed in xeric regions of the southwestern United States and northern Mexico [1]. In our previous chemosystematic investigations of the subtribe Alomiinae, we reported that most members of the New World genus *Brickellia* yielded 6-methoxyflavones, flavonol aglycones, glycosides and sulphates [2–4]. However, non 6-methoxylated flavonols have been detected in some *Brickellia species* [5] as well as in other genera of the Alomiinae [6, 7]. *Brickellia vernicosa* and *B. diffusa* are unique because they accumulate both types of compounds [8, 9]. As part of our continuing biochemical systematic studies of this genus, we report here four flavonoids from *B. longifolia*, namely, the previously unreported 3-O-sulphate of 6-methoxykaempferol 7,4'-dimethyl ether (**1**) and three known flavonoids: 6-methoxykaempferol 7-methyl ether 3-sulphate (**2**), quercetagetin 6,7,4'-trimethyl ether 3-sulphate (**3**) and quercetagetin 6,7-dimethyl ether 3-galactoside (**4**).