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New biflavonoids from *Iresine diffusa*

Nuevos biflavonoides de *Iresine diffusa*

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Abstract

Three new methoxylated and unsymmetrical biflavonoids with: a flavone-flavanone structure, diffusanone A (1), flavonol-isoflavone, diffusanone B (2), 3-methoxylated-flavonol-flavone, diffusanone C (3) had been isolated from a dichloromethane fraction of *Iresine diffusa* Humb. & Bonpl. ex Willd. leaves, a plant with recognized medicinal properties. The structures of the new compounds were established based on extensive spectroscopic experiments of 1D and 2D NMR, IR, and mass spectrometry. This is the first report of biflavonoids in *Iresine* genus.

Keywords: *Iresine diffusa*, Amaranthaceae, biflavonoids, medicinal properties

Resumen

Tres nuevos asimétricos y metoxilados biflavonoides con estructuras de: flavona-flavanona, diffusanona A (1), flavonol-isoflavona. diffusanona B (2) y 3-metoxi-flavonol-flavona, diffusanone C (3) han sido aislados de una fracción de diclorometano obtenido de las hojas de *Iresine difussa* Humb. & Bonpl. ex Willd., una planta con reconocidas propiedades medicinales. Las estructuras de los nuevos compuestos fueron establecidas basadas en diversos experimentos de RMN en 1D y 2D, IR y espectrometría de masas. Este es el primer reporte de biflavonoides en el género *Iresine*.

Palabras clave: *Iresine diffusa*, Amaranthaceae, biflavonoids, propiedades medicinales

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Introduction

Iresine diffusa Humb. and Bonpl. ex Willd., belongs to the Amaranthaceae. Some species of *Iresine* are widely used in the traditional medicine of American countries for different purposes. *Iresine herbsti*, one of the more known species (Jaafar and Jaafar, 2021) had been pharmacologically validated with effects on the central nervous system (De Feo et al., 1996, Capasso and De Feo, 2002), and in Brazil is used as a wound-healing agent (Schmidth et al., 2009); in Peru, it is used in magical rituals to expel bad spirits (De Feo, 2003), the plant and their medicinal uses had been expanded in some Asia and Africa countries (Al-Fartosy and Abdulwahid, 2015, Mohmed, 2019). The antiviral activity of the Newcastle disease virus has been reported (Andleeb et al., 2020), as well as antibacterial activity (Chaudhuri and Sevanan, 2012), and treatment for anemia (Lee and Pei, 2008). Phytochemical studies demonstrated the presence of isoflavones (Valentová et al., 2011), and several classes of compounds were published from a GC/MS analysis (Ijioma et al., 2017).

Iresine diffusa, the target species of this study, is commonly used in Peru to treat hemorrhages, malaria, and ovary inflammations (Pabon et al., 2009), in Mexico the specie is used by the Mayas for the treatment of several diseases such as malaria, typhoid, and prostate cancer (Rios et al, 2005). Previous phytochemical studies of this plant have shown the isolation of steroids, glycosylated triterpenes and drimenes (Rios et al., 2005), guanidines alkaloids (Killian et al., 2020). In Venezuela, this plant is known as valerian, and it is recognized for sedative properties. Previous research in our group demonstrated the presence of methylenedioxy flavonoids including a coumaronochromone (Suárez et al., 2016), and the effect of one of these flavonoids on the cellular viability of *Leishmania mexicana* (De-Gouveia et al., 2017). As part of this continuing research on this specie, now three new unsymmetrical biflavonoids which have in common an ether linkage, and methoxyl and methylenedioxy groups in their structures, have been isolated from the dichloromethane fraction of *Iresine diffusa* leaves. The structural elucidations of the isolated flavonoids were established by spectroscopic and spectrometric methods. The dimeric compounds were identified as three different structures composed by flavone-flavanone named difussanone A (1), flavonol-isoflavone difussanone B (2) and flavonol-flavone difussanone C (3). Until now *I. diffusa* is the first specie in the genus to present biflavonoids.

Materials and Methods

GENERAL EXPERIMENTAL PROCEDURES

1D and 2D NMR experiments were performed in $CDCl_3$ on a Bruker AM-500 spectrometer operating at 500 MHz (^1H), 125 MHz (^{13}C). Chemical shifts (δ) were recorded in ppm with reference to the solvent signals. Electrospray mass spectrometry (ESI-MS) was performed using a QTRAP 3200 de Applied Biosystem. IR spectra were recorded on a Perkin-Elmer model 283. Column chromatography was performed on Silica gel 60H (230-400μ) from Merck, and precoated silica gel GF254 plates from (Merck) were used for TLC. Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotation was taken in a polarimeter ATAGO-Polax-2L.

Plant material

The leaves of *I. diffusa* were collected in San Diego de Los Altos, Miranda state, in June 2008, and authenticated by Dr. Stephen Tillett. A voucher specimen (MYF26730) has been deposited at the Herbarium Víctor Manuel Ovalles (MYF) of the Pharmacy Department of the Universidad Central de Venezuela.

Extraction and isolation

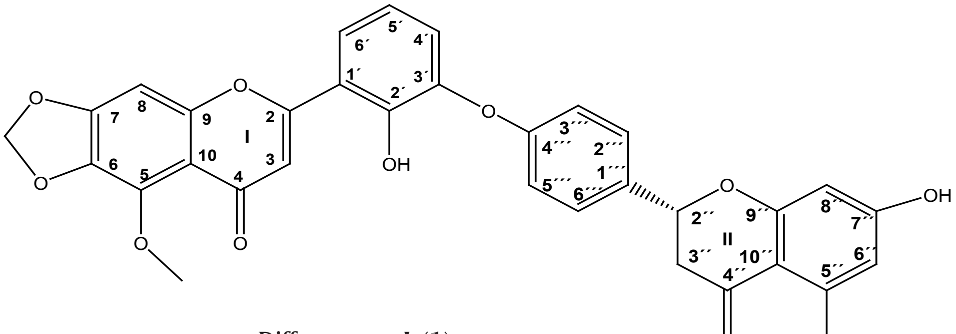
The air-dried and ground leaves of *I. diffusa* (350 g) were extracted by Soxhlet with methanol. The solvent was evaporated under reduced pressure to afford 12 g of crude. The obtained extract was dissolved in $MeOH:H_2O$ (50:50) and partitioned with n-hexane, dichloromethane, and ethyl acetate consecutively to give 1.06 g, 1.42 g, and 1.25 g respectively. The dichloromethane fraction (1.00 g) was chromatographed on a silica gel column eluted with a solvent system of $CHCl_3$/EtOAc in gradient to afford ten sub-fractions based on TLC analysis. The sub-fractions (F1 + F2), and (F3 + F4) gave pure single flavonoids previously reported by us. Sub-fractions F5 and F6 gave by precipitation two yellow solids, which after identification were characterized as the biflavonoids 1 and 2. The subfractions F7 and F10 were further combined and rechromatographed on a silica gel column eluting with $CHCl_3$/MeOH of increasing polarity, to yield the biflavonoids 2 and 3.

Results and Discussion

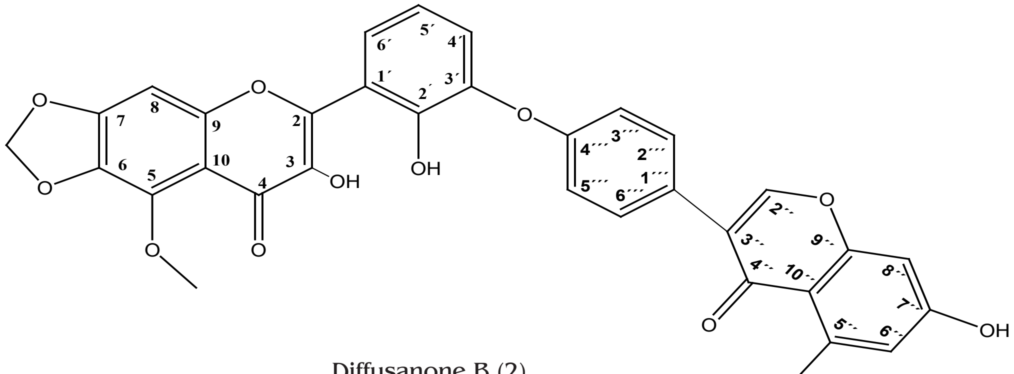
Chromatographic separation of the dichloromethane fraction of *I. diffusa* leaves has led to the isolation of three new biflavonoids (1-3) Figure 1.

Compound 1 was isolated as a yellow amorphous powder, mp 197-201 °C. The preliminary analysis of its NMR spectra suggested a biflavonoid structure or a mixture of two different flavonoids. The ESI-MS m/z 583 (M+H) clearly confirmed a biflavonoid compound type, which molecular formula was determined as $C_{32}H_{22}O_{11}$ corroborated with the ^{13}C NMR analysis. The IR spectrum exhibited the more significant signals, carbonyl absorption bands at 1648, 1655, and 1620 cm^{-1}; typical absorption of the methylenedioxy group was also evident between 935-920 cm^{-1}. The ^{13}C NMR spectrum analyzed with the help of the DEPT-135 experiment showed that from the total 32 carbons of the molecule, 4 are sp3 and 28 are sp2 including two carbonyls. The ^{13}C NMR resonances showed two different carbonyl systems at δ_C 195.7 and 183.1 ppm, these chemical shifts suggested that the biflavonoid is made up of a flavanone and flavone units. The signals at 183.1, 105.3, and 163.1 in ^{13}C NMR, are part of an α-β unsaturated carbonyl system, which clearly identified the flavone unit. An ABC system with resonances at δ_H 7.45 (d, $J = 8.10$ Hz), 7.42, and 7.92 as multiplet was observed in the ^1H spectra. The ^1H and ^{13}C NMR also revealed the signal of one methoxyl group (δ_H 4.17, δ_c 61.3 ppm) and among this, the presence of a methylenedioxy moiety in 1 was corroborated by the resonances at (δ_H 6.04, δc 102.5 ppm).

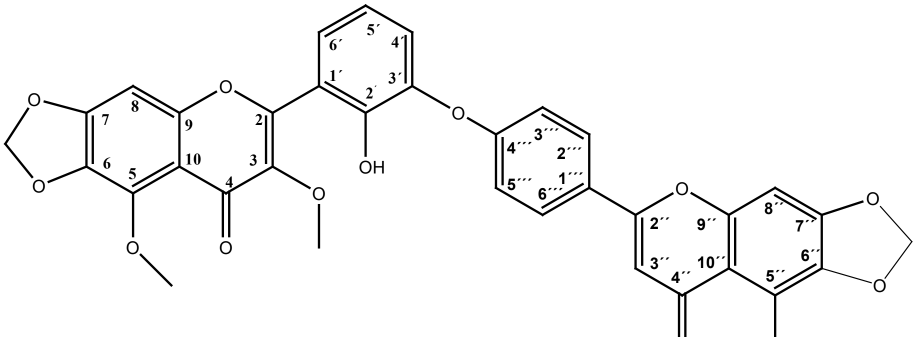
The flavanone unit (II) showed characteristic ^{13}C signals at 195.7, 43.3, and 79.2 ppm supported by the resonances observed in ^1H NMR at δ 5.42 (1H, dd, J = 12.8, 2.9 Hz) corresponding to the oxygenated methine at 2 positions, and methylene with δ 3.17 (1H, dd, J=12.8, 15.6 Hz) and 2.83 (1H, dd, J =15.6, 2.9 Hz) signals corresponding at the protons in



Diffusanone A (1)



Diffusanone B (2)



Diffusanone C (3)

Figure 1. Structures of diffusanones A, B and C

the position 3 of the flavanone (Table I). The ring B of the flavanone unit was identified as a p-substituted benzene system evidenced by a set of two ortho-coupled doublets at δ_H 7.19 (d, J=8.6 Hz), and 7.55 (d, J=8.6 Hz) each integrating for two protons. The ^{1}H-NMR spectrum (in $CDCl_3$) showed also relevant signals, a low-frequency broad singlet at 13.01 ppm corresponding to a chelated hydroxyl group at the C-5 position in a flavonoid moiety and a signal for OH (brs) at 11.96 ppm.

The assignment of ^{13}C NMR and ^{1}H resonances in the dimeric structure were confirmed by HMQC and HMBC experiments (Figure 2). The methoxyl and methylenedioxy groups were placed at the 5, and 6,7 positions of the ring A in the flavone unit. The HMBC and HMQC correlations were consistent with this placement. Finally, an exhaustive analysis of NMR data, considering the quaternary and oxygenated carbons, and comparison with literature data (Ariyasena et al., 2004,

Table I. ^{13}C, ^{1}H NMR data of the compounds (1-3) in $CDCl_3$

	Compound					
	1		**2**		**3**	
Position	δ_C(ppm)	δ_H(ppm) m(J Hz)	δ_C(ppm)	δ_H(ppm) m(J Hz)	δ_C(ppm)	δ_H(ppm) m(J Hz)
2	163.1		151.7		153.9	
3	105.2	6.83 (s)	136.2		141.4	
4	183.1		173.6		174.2	
5	152.1		153.9		153.3	
6	138.3		135.8		134.8	
7	153.2		152.4		153.2	
8	93.5	6.57 (s)	93.9	6.78(s)	93.1	
9	156.6		154.8		154.8	
10	112,2		112.7		112.9	
1′	123.3		123.4		125.9	
2′	164.1		163.7		161.2	
3′	138.9		142.6		141.2	
4′	128.9	7.43(d, 7.6)	125.2	7.37 (m)	130.5	7.43 (m)
5′	126.3	7.92 (dd, 7.6, 2.0)	125.1	7.37(m)	131.2	
6′	126.1	7.45 (d, 8.1)	111.1	7.06(d, 2.7)	129.0	
2′′	79.2	5.42 (dd, 12.8, 2.9)	153.5	7.89(s)	154.0	
3′′	43.3	2.83 (dd, 15.6, 2.9) 3.17 (dd, 12.8, 15.6)	125.7		108.1	
4′′	195.7		178.7		178.2	
5′′	164.7		149.4		153.9	
6′′	96.7	6.05 (s)	121.9	8.16(d,6.8)	134.6	
7′′	156.7		156.7		153.5	
8′′	95.4	6.04 (s)	92.9	6.68(s)	92.9	
9′′	153.3		141.5		155.2	
10′′	103.2		99.5		112.6	
1′′′	138.3		112.8		131.4	
2′′′	120.1	7.55 (d, 8.6)	119.5	7.06 (d,6.9)	128.6	
3′′′	130.5	7.19 (d,8.6)	130.5	7.20 (d,6.9)	128.2	
4′′′	164.4		163.7		161.2	
5′′′	130.3	7.19 (d,8.6)	130.0	7.20 (d,6.9)	128.2	
6′′′	119.9	7.55 (d,7.6)	120.7	7.06 (d,6.9)	128.6	
OCH_2-O	102.5	6.04 (s)	102.3	6.10 (s)	102.1	
OCH_3	61.3	4.17 (s)	61.4	4.15, 4.20	61.1 61.2	

de Oliveira et al., 2002) suggested that the linkage between the flavanone/flavone units is made up of ether between I-C-3´and II-C-4´´´, which was confirmed by heteronuclear multiple bond connectivity experiment (HMBC) (Figure 2). A comparison with the literature on flavanones related to the values of the diaxial coupling between H-2 and H-3 (J =12.8 Hz) suggested that the configuration of C-2 is S, with an optical

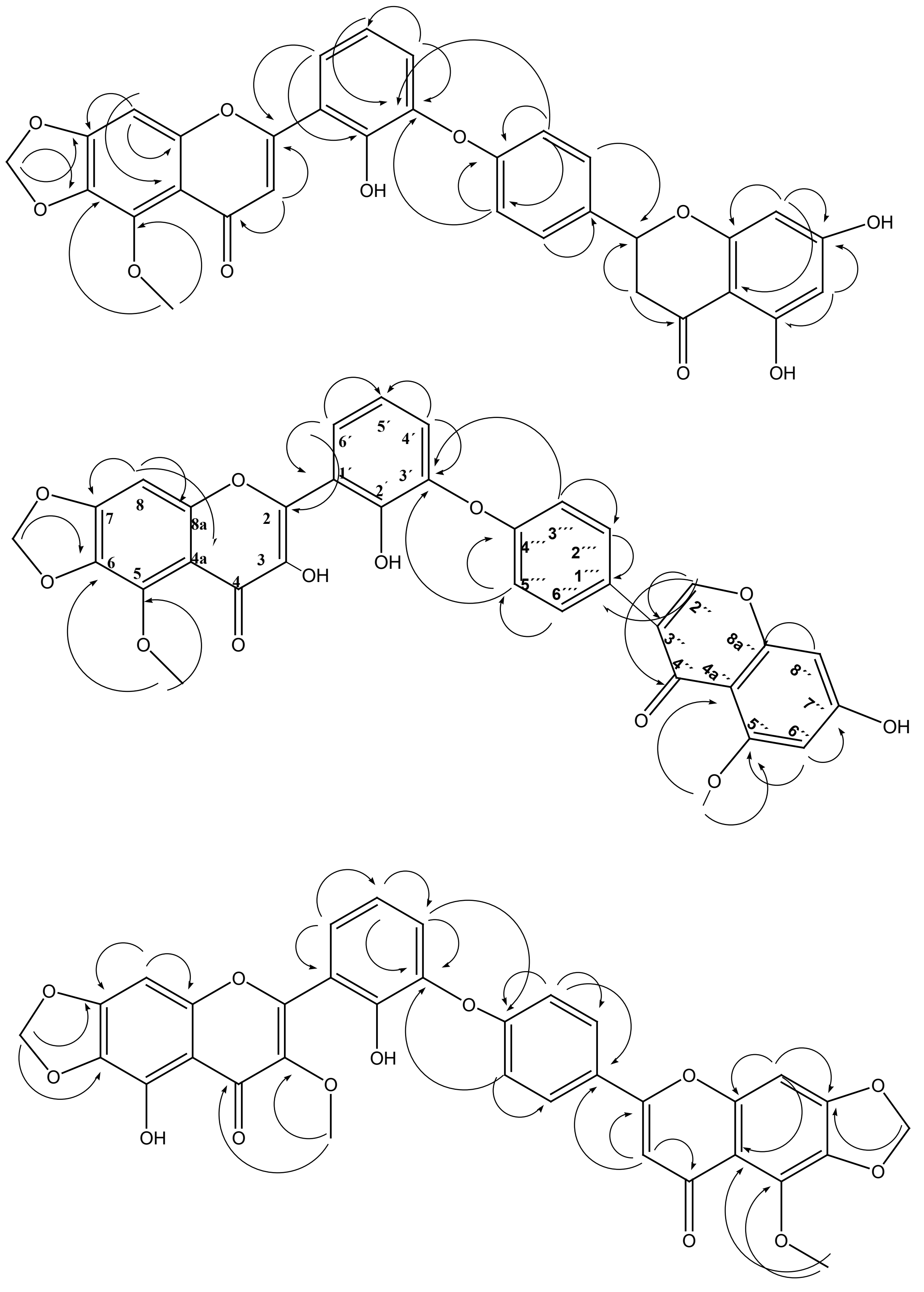


Figure 2. Selected HMBC correlations for 1, 2 and 3

rotation of (-19.5°) (Liu et al., 2018, Kang et al., 2000). A search in the literature data of the characterized compound showed, first that the methylenedioxy group is not very common in reported biflavonoids, and that the molecular weight and formula assigned to compound 1 did not lead to a known structure. Biflavonoid 1 was given the name diffusanone-A.

Compound 2 was obtained as a yellowish solid mp 193-196 °C. The molecular formula of 2 was determined as $C_{34}H_{22}O_{13}$, based on its ESI-MS data (m/z 639 [M + H]) and ^{13}C NMR. Both the ^1H and ^{13}C NMR of 2 gave common signals with 1 such as the methoxyl and methylenedioxy groups, but differences were also evident between these substances in the carbonyls' chemical shifts (183.1, 195.7) in 1 vs (173.7, 178.7) in 2. The clear presence of an isoflavone as part of the dimeric structure was demonstrated by the signals at (δ_H 7.82 (s), δ_C 153.5 ppm), further established by HMBC and HMQC correlations. Two singlets at δ_H 4.15 and 4.20 ppm which correlated in the HMQC with the carbons at δ_C 61.4 ppm indicated that two methoxyl groups were present in the biflavonoid skeleton. The ^1H NMR spectrum also revealed a para-substituted aromatic system as part of the isoflavone unit, as was confirmed by strong correlations between the doublets of doublets integrating for 2 at δ 7.06 in the ^1H NMR and the C-2 (153.5 ppm) evidenced in the HMBC. Close analysis of the remaining spectral data suggested that the second part of the biflavonoid 2 was a flavonol. Correlations observed in the HMBC spectrum between the signal at δ_H 7.20 (H-3´´, H-5´´) and 163.7, revealed a substitution at C-3´ of the flavonol moiety. Moreover, additional correlations in the HMBC spectrum (Figure 2), proved the ether linkage between the flavonol and the isoflavone monomers, similar to the difussanone A. Thus, a literature survey revealed that this is the first isolation of compound 2 from natural sources, and by analogy with the previously described compound 2 was named diffusanone B.

Compound 3 was isolated as a pale yellow amorphous solid, mp.148-150°C, and possessed a molecular formula of $C_{35}H_{24}O_{13}$ determined from its ESI-MS data m/z 653 [M+H], suggesting again a flavonoid dimeric structure. Close examination of NMR spectra of 3 indicated that is a dimeric structure composed of two asymmetric units, a 3-methoxylated-flavonol and a flavanone. The ^{13}C and DEPT experiments were consistent with the calculated formula. The ^{13}C NMR spectrum of 3 displayed the signals of two different α,β unsaturated carbonyl groups at δ 174.2 and 178.2 ppm, which suggested that the dimer was composed of a flavonol and flavanone units. Two sets of orto coupled protons at δ_H 7.92 (d, J = 8.8 Hz) and 8.24 (d, 8.8 Hz), identified the para-substituted phenyl ring, also characterized in the compounds 1 and 2. The ^1H NMR spectrum showed three signals for methoxy groups at δ 3.81, 4.06, and 4.08 ppm, integrating for three protons each one, this fact and the lack of chelated hydroxyl group suggested that both C-5 and C-5´´ are methoxylated. The signal for the methylenedioxy group was also integrated for four protons indicating that both units of the biflavonoid have this moiety (Agrawal, 1989; Harbone et al., 1975). The key long-range correlation between δ 7.43 ppm (H-4´) and 141.2 (C-3´); and between 7.92 (H-3´´´, H-5´´´) with C-3´, required an ether linkage between C-3´ and C-4´´´ such as was established for compounds 1 and 2 and, corroborated by the presence of two non-hydrogenated carbons at δ

141.2, and 161.2 ppm. Accordingly, the structure of 3 was established as shown, and this biflavonoid was given the name diffusanone C.

Conclusions

Three new biflavonoids (1-3) were isolated from *Iresine diffusa* in this study. These dimers composed of flavone-flavanone, flavonol-isoflavone, and methoxy-flavonol-flavone showed all their linkage through an ether function. The flavonoids especially the isoflavones are known in the Amaranthaceae, and *Iresine* genus, but so far this is the first report of biflavonoids in *Iresine*.

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