Globrauneine A – F: Six new triterpenoid esters from the leaves of *Globimetula braunii*

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ABSTRACT

Phytochemical study was conducted on the leaves of *Globimetula braunii* which is a hemi parasitic plant belonging to the family Loranthaceae. Extraction was carried out using cold extraction method with increasing polarity of solvents i.e *n*-hexane, CH_2Cl_2 and MeOH. The components were separated by chromatographic technique and the structures of the compounds were elucidated by extensive spectroscopic analyses including MS, FTIR, 1D and 2D NMR, HRMS and chemical methods. Six new pentacyclic triterpenoid esters named as globrauneine A **(1)**, globrauneine B **(2)**, globrauneine C **(3)**, globrauneine D **(4)**, globrauneine E **(5)**, and globrauneine F **(6),** together with six known compounds **(7 – 12)** were successfully isolated from the leaves of *G. braunii* growing on *Piliostigma thonningii*. These results depict a substantial support to the chemotaxonomy of the genus *Globimetula*.

Keywords: *Globimetula braunii*, Loranthaceae, triterpenoid esters, globrauneine **A**-**F**

1. Introduction:

African continent is bestowed with the lengthiest, and perhaps rich biological and cultural diversity with marked regional differences in healing practices (Gurib-Fakim 2006). The documentation of therapeutic uses of African plants and conventional systems is becoming a persuasive need because of the prompt damage of the natural habitats of some of these plants due to anthropogenic actions and erosion of treasured traditional acquaintances (Gurib-Fakim 2013; Manach et al. 2004). Although the medicinal application of species from Loranthaceae have been rationalized based on the ethnomedicinal uses, isolation and identification of several bioactive compounds in the classes of flavonoids, phenols, glycosides, alkaloids and triterpenes (Deeni and Sadiq 2002; Fukunaga et al. 1989), there is paucity of report on the species of *Globimetula braunii*. *Globimetula braunii* (Loranthaceae) is a bushy parasitic plant found in a variety of host plants from Ghana to Nigeria and widely distributed across central tropical Africa (Burkill 1985). The plant is used in traditional medicine to treat cholera, asthma, hypertension, diabetes, cancer, gastrointestinal tract diseases, wound infections and as a blood purifier (Deeni and Sadiq 2002; Hussain and Karatela 1989; Obatomi et al. 1994; Obatomi et al. 1996). Aliyu et al. (2014) have reported the anticonvulsant activity of the ethyl acetate extract of *G. braunii* against pentylenetetrazole induced seizures in mice. Our previous studies revealed that the EtOAc and MeOH extracts of *G. braunii* possessed significant antioxidant activity and may have potential for the treatment of cancer and heart diseases (Ja'afar et al. 2017). As part of our ongoing phytochemical screening for biologically active compounds from this plant, further fractionation study was carried out on the *n*-hexane and CH_2Cl_2 extracts which resulted in the isolation of six new triterpenoid esters named globrauneine A – F **(1 - 6)**, together with six known compounds **(7 - 12)**. In this research, the isolation and structural elucidation of these compounds are

described.

2. Results and discussion:

The *n*-hexane (GBPTH) and dichloromethane (GBPTD) extracts (45 g) each, were fractionated using VLC on silica gel (600 g, 10 x 10 cm), eluted with *n*-hexane: EtOAc: MeOH, repeated column chromatography (CC) and preparative thin layer chromatography (PTLC). These purification processes led to the isolation of six new pentacyclic triterpenoids named globrauneine A **(1)**, globrauneine B **(2)**, globrauneine C **(3)**, globrauneine D **(4)**, globrauneine E **(5)**, and globrauneine F **(6)** together with lupeol **(7)** (Prachayasittukul et al. 2009), lupeol palmitate **(8)** (Appleton et al. 1971), lup-20(29)-en-3β,15α-diol **(9)** (Zhang et al. 2008), octacosanoic acid **(10)** (Mohamad et al. 2009), friedelin **(11)** (Akihisa et al. 1992) and β-sitosterol **(12)** (Moghaddam et al. 2007) (Figure 1). All the compounds **1**-**12** gave a positive Liebermann-Burchard test for triterpenoid.

Compound **1** was obtained as an amorphous powder from GBPTH with R_f value of 0.80 in *n*-hexane: EtOAc (4:1), $[\alpha]_D^{25}$ = -46.8^o (*c* = 0.03, acetone) and m.p 71-72^{o}C. The HR-NSIMS of compound **1** established its molecular formula as $C_{50}H_{88}O_3$ $[M + NH_4]^+$ at *m/z* 754.7074 (calc. 754.7072) implying seven degrees of unsaturation. The IR absorption at 1730 cm^{-1} could be assigned to an ester carbonyl and confirmed by the carbon signal at δ 173.7 in the ^{13}C NMR spectrum. The ^{13}C NMR and DEPT spectra indicated the presence of 50 carbons, among them eight methyl carbons at δ 7.9 (C-27), 16.2 (C-24), 16.5 (C-26), 19.0 (C-28), 18.3 (C-25), 27.9 (C-23), 19.3 (C-30) and the terminal methyl carbon of fatty acid side chain at δ 14.1 (C-20'). Twenty-eight methylene carbons were identified between δ 23.7-109.0 (among them 18 multiple fatty acid methylene groups and one olefinic group), seven sp^3 methines among them two oxygenated carbons at δ 80.5 (C-3) and δ 69.7 (C-15) and seven quaternary carbons. The

^{1}H NMR data of compound **1** suggested that it was a lupane-type triterpene with a long-chain ester functionality (Lie et al. 2015; Maza et al. 2016; Ogechukwu et al. 2011). The ^{1}H NMR data and HMQC spectra displayed signals attributable to six methyl signals at δ 0.86 (H-28), 0.89 (H-25), 0.90 (H-24), 0.91 (H-23), 0.98 (H-27) and 1.15 (H-26); one vinylic methyl at δ 1.71 (H-30); a pair of singlets at δ 4.70 (1H, s, H-29b) and δ 4.61 (1H, s, H-29a) indicative of terminal isopropylene moiety and a terminal methyl of fatty acid chain signal at δ 0.88 (H-20'). The assessment of the chemical shift of the two doublet of doublets oxymethine proton at δ 4.18 (1H, dd, *J*= 11.2 Hz and 4.8 Hz, H-15) and δ 4.47 (1H, dd, *J*= 10.8 Hz and 5.6 Hz, H-3) indicated the ester moiety to be in position C-3, whereas the hydroxyl group at position C-15. These were confirmed by the ^{1}H-^{1}H COSY which showed a correlation between H-3 at δ 4.47 with H-2 at δ 1.63 and between H-15 at δ 4.18 with H-16a/b at δ 1.44/1.82, respectively. In addition, a strong HMBC correlation between the oxymethine proton H-3 (δ 4.47) and the carbonyl ester carbon, C-1' (δ 173.7) was observed. Other HMBC correlations were observed between H-30 (δ 1.71) and C-20 (δ 150.3), and between C-29 (δ 109.6) and C-18 (δ 48.1), respectively. In the NOESY Spectrum, H-3 showed NOE correlations with H-1, H-2, and H-5; H-15 displayed NOE correlations with H-6, H-26, and H-28; H-19 showed NOE correlation with H-13, and H-29 exhibited NOE correlations with H-19 and H-30. Therefore, the OH-15 was determined to be in an α-orientation and the structure of compound **1** was established as 15α-hydroxyl-lup-20(29)-ene-3β-*O*-eicosanoate and was given a trivial name globrauneine A (Figure 1).

The HRAPCI signals of compound **2** showed the molecular ion peak $[M+H]^+$ at *m/z* 765.7118 in agreement with the molecular formula $C_{52}H_{92}O_3$ (calc. 765.7119), indicating seven degrees of unsaturation. The NMR spectrum of compound **2** were almost similar to those of **1**. The structural analysis of the ester moiety of **2** also displayed

noticeable similarities to those observed in **1**. They differed only by the addition of two methylene groups (28 amu) in the long chain ester functionality. Therefore, the structure of compound **2** was established as 15α-hydroxyl-lup-20(29)-ene-3β-*O*-docosanoate and was given a trivial name globrauneine B (Figure 1).

Compound **3** has a molecular formula $C_{50}H_{88}O_3$ established from the molecular ion peak $[M+H]^+$ at *m/z* 737.6811 (calc. 737.6806) in the HRAPCI spectrum displaying similar molecular formula with compound **1**. In fact, the assessment of the NMR spectral data of this compound to those of compounds **1** and **2** showed a correlation between them, indicating that compound **3** is also a lupane-type triterpene with a long chain ester functionality at position C-3. The difference can be deduced from the ^{1}H NMR data that revealed the presence of a doublet of doublets oxymethine protons at δ 3.83 (1H, dd, *J*= 11.2 Hz and 5.2 Hz) assignable to position C-7 instead of δ 4.18 (dd, *J*= 11.2 Hz and 4.8 Hz) position C-15 as in compounds **1** and **2**. On the other hand, the ^{1}H-^{1}H coupling between H-7 (δ 3.83) and H-6 (δ 1.53/1.70) was further confirmed by the COSY spectrum. Compound **3** also differed in the ^{13}C NMR spectrum with compounds **1** and **2** by the absence of the oxymethine carbon at position C-15 (δ 69.6) as observed in compounds **1** and **2**. In its place, there was a slight shift to downfield due to change in position of the oxymethine carbon to position C-7 (δ 74.4). The relative configuration of **3** was established in the NOESY spectrum. H-3 showed NOE correlations with H-2 and H-23; H-7 exhibited NOE correlations with H-6, H-24, and H-26; H-16 with H-26 and H-28; H-19 correlated with H-13, H-21 and H-28, and H-29 displayed NOE correlation with H-19 and H-30. Thus, the naturally occurring triterpenoid was identified as 7α-hydroxyl-lup-20(29)-ene-3β-*O*-eicosanoate and was given a trivial name globrauneine C (Figure 1). Lie et al. 2015 and Maza et al. 2016 isolated 7β-hydroxyl-lup-20(29)-ene-3β-hexadecanoate and globimetulin C from *S. parasitica* and *G. dinklagei*, respectively.

Compound **4** was obtained as an amorphous powder from GBPTH, $[\alpha]_D^{25}$ = -2.4^o (*c* = 0.03, acetone), m.p 49-50^{o}C and R_f value of 0.39 in *n*-hexane: EtOAc (4:1). The molecular formula was found to be $C_{54}H_{95}O_3$ based on the HR-NSIMS value ($[M+H]^+$ at *m/z* 791.7272 (calc. 791.7276). Valence bond calculations revealed eight degrees of unsaturation. The FTIR absorption bands at 1641 cm^{-1} could be assigned to the olefinic C=C and 1174 cm^{-1} due to the C-O-C stretching. The ^{1}H NMR data indicated the presence of olefinic protons at δ 4.69 (1H, s, H-29b) and δ 4.61 (1H, s, H-29a) of an isopropenyl moiety. The evidence of ester linkage was provided by the presence of oxymethine signal at δ 4.47 (1H, dd, *J* = 11.2 Hz, 4.4 Hz, H-3) attached to carbon at δ 80.2 (C-3). Six singlets at δ 0.85 (H-28), 0.86 (H-25), 0.90 (H-24), 0.92 (H-23), 1.01 (H-27) and 1.10 (H-26) corresponding to six methyls, a multiplet at δ 0.88 (H-24') corresponding to the terminal methyl of fatty acid chain and methylenes $(>CH_2)_n$ of fatty acid at δ 1.25-1.29 (H-4'-H-23') were observed. The ^{13}C NMR and DEPT spectra showed the presence of 54 carbons comprising of eight methyls, thirty-one methylenes, six methines, two oxymethines and seven quaternary carbons. The signal at δ 173.7 of a carbonyl of an ester group was confirmed by the FTIR absorption band at 1732 cm^{-1}. The olefinic carbons of the oxymethylene double bond appeared at δ 150.3 (C-20) and 109.7 (C-29), long aliphatic fatty acid side chain carbons at δ 29.3-29.6 (C-4' - C-23') and the terminal carbon at C-24' (δ 14.1). The presence of two doublet of doublet oxymethine protons at δ 4.18 (dd, *J*= 11.2 Hz, 4.8 Hz, H-15)/ δ 67.9 (C-15) and δ 3.82 (dd, *J* = 11.2 Hz, 4.8 Hz, H-7)/ δ 72.5 (C-7) hinted the hydroxyl proton to be at position C-15 and C-7, respectively. These were confirmed by the ^{1}H-^{1}H COSY which showed a correlation between H-7 (δ 3.82) with H-6 (δ 1.74) and H-15 (δ 4.18) with H-16 (δ 1.46). The position of the ester linkage was supported by strong HMBC correlation between H-3 (δ 4.47) of the lupeol moiety and the carbonyl ester C-1' (δ 173.7). Additionally, the oxymethine protons, H-15 (δ 4.18)

and H-7 (δ 3.82) showed strong HMBC connectivity with C-27 (δ 8.3) and C-26 (δ 10.9) respectively. Positive NOESY experiment confirmed the spatial arrangements or configurations. H-3 showed NOE correlations with H-2 and H-23; H-7 with H-24 and H-26; H-15 correlated with H-26 and H-28, and H-29 exhibited NOE correlations with H-19 and H-30. This revealed that the 7,15-epoxy ring is in α-orientation and the ester group at C-3 in β-orientation. Compound **4** was established as a new lupeol type of triterpenoid with a long chain ester moiety having a tetrahydrofuran ring. Compound **4** was elucidated as 7α,15α-epoxy-lup-20(29)-ene-3β-*O*-tetracosanoate and was given a trivial name globrauneine D (Figure 1). Maza et al. (2016) reported the isolation of globimetulin A and B from the leaves of *G. dinklagei* growing on *Manihot esculenta* (Cassava).

For compound **5**, the studies of the ASAP-TOF-MS provided the evidence of the absolute molecular weight which showed the molecular ion peak $[M+H]^+$ at *m/z* 735.6655 (calc. 735.6647) in agreement with the molecular formula $C_{50}H_{86}O_3$ corresponding to double bond equivalent of eight. The ^1H and ^{13}C NMR data of compound **5** displayed similar profile with that of compound **4** suggesting a lupane-type triterpene with a long-chain ester functionality having six rings in its structure including a tetrahydrofuran moiety (Maza et al. 2016). This led to the assertion of the possible structure of compound **5** to be deficient of four methylene groups (56 amu) when compared with compound **4**. The correlations between ^1H and ^{13}C established by COSY, HMBC and NOESY spectra enabled the assessment of the missing methylene groups to be part of the long aliphatic chain in compound **4**. Thus, compound **5** was elucidated as 7α,15α-epoxy-lup-20(29)-ene-3β-*O*-eicosanoate and was given a trivial name Globrauneine E.

Compound **6** was isolated as a colorless amorphous solid from GBPTH with $[\alpha]_D^{25}$ = +18.03^o (*c* = 0.03, acetone), m.p 103-104^{o}C and R_f 0.15 in *n*-hexane: EtOAc (4:1). Its molecular formula $C_{54}H_{95}O_4$ containing eight degrees of unsaturation indicated by HR-

NSIMS spectra showed molecular ion peak $[M+H]^+$ *m/z* 807.7226 (calc: 807.7225). The ^1H and ^{13}C NMR data of compound **6** were in accordance with those of compounds **4** and **5** with the exception that the signals for doublet oxymethylene group were detected at δ 4.14 (*J*= 5.2 Hz, d, H-30) in the ^1H NMR which correlated with the carbon at δ 65.1 (C-30) in the HMQC spectrum. Direct ^1H-^{13}C correlations in the HMQC spectrum of **6** and DEPT spectra showed correlations between the olefinic protons at δ 4.99 (1H, s, H-29b)/δ 4.93 (1H, s, H-29a) with carbon at δ 107.3 (C-20) and between the methine proton at δ 2.26 (1H, m, H-19) with carbon at δ 43.2 (C-19), while the quaternary carbon (C-20) was observed at δ 154.3. These signals showed noticeable differences to those observed in compound **4** which showed comparable signals at δ 4.69 (1H, s, H-29b)/δ 4.61 (1H, s, H-29a)/109.7, δ 2.37 (1H, m, H19)/ δ 47.5 (C-19) and the quaternary carbon (C-20) at δ 150.3. The assignment was further supported by the oxymethylene protons that cross-correlated with the olefinic methylene protons in the ^1H-^1H COSY and by the oxymethine proton signal at δ 4.48 (dd, *J* = 11.2Hz, 4.8 Hz, H-3) that cross-correlated with the carbonyl ester carbon C-1' at δ 173.7 in the HMBC spectrum. The relative configuration of compound **6** was established in the NOESY experiment. H-3 showed NOE correlation with H-23; H-7 with H-26; H-15 correlated with H-26 and H-28; H-29 exhibited NOE correlations with H-22; and H-30 displayed NOE correlation with H-22. Therefore, the 7,15-epoxy ring are in α- orientation and the ester group at C-3 in β-orientation. Thus, the structure of compound **6** was established as 7α,15α-epoxy-lup-20(29)-ene-30-hydroxyl-3β-*O*-tetracosanoate and is given a trivial name globrauneine F (Figure 1).

3. Conclusion

Analysis carried out on the leaves of *G. braunii* resulted in the isolation of six new pentacyclic triterpenes of the lupane series (compounds **1**-**6**). The structures were

elucidated by extensive spectroscopic analysis. Compounds **4**, **5** and **6** represent a subtype of compounds with a unique tetrahydrofuran ring. Their lupeol skeleton was found to be similar with globimetulin A and globimetulin B isolated from *G. dinklagei* (Maza et al. 2016) and might depict a substantial support to the chemotaxonomy of the genus *Globimetula* from other species of Loranthaceae.

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Conflict of Interest:

We have no conflict of interest.

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