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# Chemical constituents from *Tithonia diversifolia* and their chemotaxonomic significance

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ABSTRACT

Two new compounds 6″-O-β-D-apiofuranosyl-trichocarpin (15) and 1-heptade-4,6-diyne-3,10,16,17-tetraol-3-O-β-D-glucopyranoside (16), together with fourteen known compounds were isolated from *Tithonia diversifolia*. The chemotaxonomic significance of these compounds was discussed in the article.

## 1. Subject and source

*Tithonia diversifolia* (Hemsl.) A. Gray (Compositae: Heliantheae), known as Mexican sunflower, is native to Mexico and Central America (Chagas-Paula et al., 2011). Presently, *T. diversifolia* has been introduced into other countries for ornamental purposes or for their pharmacological action (Zhai et al., 2010). And *T. diversifolia* is widespread in southern parts of China. The aerial parts of *T. diversifolia* were collected from Mengzi, Yunnan province of China, in September 2008, and were authenticated by Prof. Wansheng Chen (Department of Pharmacy, Changzheng Hospital, Second Military Medical University). A voucher specimen (NO.TD20080819) was deposited in Department of Pharmacognosy, Second Military Medical University, Shanghai, P. R. China.

## 2. Previous work

Many classes of secondary metabolites have been isolated from the *Tithonia* species, including sesquiterpenoids and diterpenoids, as well as flavonoids. Further minor classes such as phytosterols, xanthanes, coumarins, ceramides, chromones, and chromenes have been also found (Chagas-Paula et al., 2012). The most studied species of the genus *Tithonia* is *T. diversifolia*, from which more than 150 compounds have been isolated. Previous phytochemical investigations on *T. diversifolia* have reported the presence of sesquiterpenes, including germacrane type sesquiterpenoids, eudesmane type sesquiterpenoids, guaiane type sesquiterpenoids, cadinane type sesquiterpenoids and xanthane type sesquiterpenoids (Baruah et al., 1979, Rüngeler et al., 1998, Kuroda et al., 2007; Kuo and Chen, 1998; Gu et al., 2002; Bordoloi et al., 1996; Kuo and Lin, 1999).

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Moreover, chromenes (Kuo and Lin, 1999), diterpenoids, quininic acids and three flavonoids (Kuroda et al., 2007) have also been isolated.

## 3. Present study

The air-dried aerial parts of *T. diversifolia* (22 kg) were powdered and extracted with 80% aqueous ethanol (Zhao et al., 2010). After removing the solvent under reduced pressure using a rotary evaporator at 60 °C, the resulting gum was dried to yield 2596 g of crude extract. A portion of the crude extract (2500 g) was suspended in distilled water and successively partitioned with petroleum ether (yield: 150 g), EtOAc (yield: 130 g), *n*-BuOH (yield: 160 g). The EtOAc extract (120 g) was chromatographed on silica gel column with the gradient petroleum ether/EtOAc (20:1, 10:1, 5:1, 3:1, 2:1, 1:1) as eluent. The eluate of petroleum ether/EtOAc (5:1) was purified by a Sephadex LH-20 to afforded compounds **1** (46 mg) and **2** (78 mg). The *n*-BuOH extract (160 g) was applied on silica column with gradient $CH_2Cl_2$/MeOH system (50:1, 20:1, 10:1, 5:1, 2:1, 1:1). The obtained fractions were combined on the basis of silica gel TLC and six fractions (Fr1–Fr6) were obtained. The Fr1 was subjected to silica gel column chromatography and eluted with $CH_2Cl_2$/MeOH (20:1, 15:1, 10:1) to afford compounds **3** (18 mg) and **4** (7 mg). Fr2 was retreated on silica gel column and eluated with $CH_2Cl_2$/MeOH (10:1) to yield compound **5** (11 mg). Fr3 was treated on silica gel column once more and eluted with $CH_2Cl_2$/MeOH (8:1) to yield compounds **6** (25 mg), **7** (100 mg) and 8 (10 mg). Fr4 was separated by reversed-phase silica gel (RP-18) with MeOH/$H_2O$ (1:9, 1:4, 2:3, 1:1, 1:0) and finally reapplied to a Sephadex LH-20 column using MeOH/$H_2O$ (1:1) to yield compounds **9** (32 mg), **10** (21 mg), **11** (12 mg), **12** (13 mg), **13** (28 mg) and **14** (80 mg). Compound **15** (18 mg) was isolated from Fr5 by RP-18 with MeOH/$H_2O$ (1:4), while compound **16** (7 mg) was purified from Fr6 by Sephadex LH-20 gel column with MeOH/$H_2O$ (1:1).

The structures of isolated compounds (Fig. 1) were elucidated by analysis of their spectral data (including IR, NMR and HRESIMS) and by comparison with the literature. The compounds identified include a new phenolic glycoside 6″-O-β-D-apiofuranosyl-trichocarpin (**15**) and a new polyyne glucoside 1-heptade-4,6-diyne-3,10,16,17-tetraol-3-O-β-D-glucopyranoside (**16**), together with fourteen known compounds 3-indolecarboxylic acid (**1**) (Feng et al., 2007), protocatechuic acid (**2**) (Shen et al., 2009), phloroglucinol trimethyl ether (**3**), 2-mercaptobenzothiazole (**4**), uracil (**5**) (Hu et al., 2008), pinoresinol (**6**) (Duan et al., 2002), methyl 3,5-dicaffeoyl quinate (**7**) (An et al., 2008), 2-hydroxy-5-acetylbenzoic acid (**8**), arbutin (**9**) (Sun et al., 2006), 3-(4-hydroxyphenyl)-3-oxopropyl-β-D-glucopyranoside (**10**) (Shen et al., 1999), vanilloloside (**11**) (Ida et al., 1994), harman-3-carboxylic acid (**12**) (Cardoso et al., 2004), (−)-isolariciresinol-3α-O-β-D-glucopyranoside (**13**) (Latté et al., 2008), 3,5-dicaffeoyl quinate (**14**) (An et al., 2008).

Compound **15** obtained as pale yellow powder, the molecular formula of it was $C_{25}H_{30}O_{13}$ based on HR-ESIMS *m/z* 561.1560 $[M + Na]^+$ (calc 561.1579). The $^1$H-NMR spectrum of **15** (Table 1) displayed one set of aromatic proton signals at $\delta$ 7.27 (1H, d, $J = 7.2$ Hz, H-4′), 7.31 (2H, dd, $J = 7.2, 7.2$ Hz, H-3′, 5′) and 7.47 (2H, d, $J = 7.2$ Hz, H-2′, 6′), as well as an aromatic ABX system $\delta$ 7.66 (1H, d, $J = 3.0$ Hz, H-6), 7.85 (1H, d, $J = 8.4$ Hz, H-3) and 7.42 (1H, dd, $J = 3.0, 8.4$ Hz, H-4). The $^{13}$C-NMR spectrum of compound **15** (Table 1) showed 25 signals, assigned as four oxymethylene, seven oxymethines, eight methines, one carbonyl carbon, five quaternary carbons. The chemical shifts and coupling constants of these signals, in combination with the observed HMBC and $^1$H-$^1$H COSY correlations, also suggested the presence of one monosubstituted aromatic ring and one trisubstituted aromatic ring. Furthermore, a methine the HMBC correlations from H-2′ and H-6′ to methylene ($\delta$ 66.8, C-7′), from H-6 to carbonyl carbon ($\delta$ 166.7, C-7) as well as from H-7′a ($\delta$ 5.33, d, $J = 12.0$ Hz) and H-7′b ($\delta$ 5.38, d, $J = 12.0$ Hz) to C-7 indicated the presence of a benzoic acid benzyloate moiety. Besides above signals, a glucose could be assigned at $\delta$ 105.1, 75.1, 77.4. 71.4, 78.0, 68.9, $\delta$ 4.10–5.33 and an apiose at 111.1, 77.7, 80.3, 74.9, 65.3, $\delta$ 4.09–5.77, with HSQC, $^1$H-$^1$H COSY and HMBC correlations. The HMBC correlations from H-1‴ ($\delta$ 5.77, d, $J = 2.4$ Hz) to C-6″ ($\delta$ 68.9) identified an apiofuranosyl (1→6) glucopyranosyl linkage, while the glucose moiety was located at the C-2 of the benzoyl, which was confirmed by the HMBC correlations from H-1″ ($\delta$ 5.33, d, $J = 7.2$ Hz) to C-2 ($\delta$ 151.2). The β-anomeric configuration for the glucose was determined from large coupling constant value of H-1‴ ($\delta$ 5.33, d, $J = 7.2$ Hz), also, the β-anomeric configuration for the apiose was indicated from the anomeric signal of H-1‴ ($\delta$ 5.77, d, $J = 2.4$ Hz). The glucose and apiose in compound **15** were tentatively assigned the D-configuration because of naturally occurring. Thus, the structure of compound **15** was established as 6″-O-β-D-apiofuranosyl-trichocarpin.

Compound **16** was obtained as white power, whose molecular formula $C_{23}H_{36}O_9$ was inferred from the positive-ion HRESIMS (*m/z* 479.2279 $[M + Na]^+$), indicating six degrees of unsaturation. IR also gave an absorption band at 2254 cm$^{-1}$ suggesting it was an acetylenic derivative. The $^1$H NMR spectrum (Table 1) showed the presence of three olefinic proton signals at $\delta$ 5.24 (ddd, $J = 1.2, 2.4, 10.8$ Hz, H-1a), 5.48 (ddd, $J = 1.2, 10.8, 17.4$ Hz, H-1b) and 5.94 (ddd, $J = 5.4, 1.8, 17.4$ Hz, H-2), which were coupled to the $^{13}$C NMR resonances at $\delta$ 118.2 (C-1) and 135.9 (C-2) in the HMQC spectrum, respectively. Furthermore, an oxymethine $\delta$ 69.1 (C-3), which was coupled to the $^1$H NMR resonances at $\delta$ 5.26 in the HMQC, was linked to C-2 on the basis of $^1$H-$^1$H COSY (H-2/H-3). By long-range HMBC correlations from H-3 to the acetylenic carbons at C-4 ($\delta$ 72.8), C-5 ($\delta$ 65.2), C-6 ($\delta$ 73.5) and from $H_2$-8 ($\delta$ 2.42) to C-7 ($\delta$ 82.7), C-6, C-5, a conjugated diyne was deduced between C-3 and C-8 ($\delta$ 16.2). The connection of C-8 to C-10 was deduced from the $^1$H-$^1$H COSY correlations $H_2$-8 with $H_2$-9 ($\delta$ 1.58 and 1.69), $H_2$-9 with H-10 ($\delta$ 3.62), coupled with the HMBC correlations of $H_2$-8 with C-9 ($\delta$ 36.8) and C-10 ($\delta$ 71.0), H-9 ($\delta$ 1.58) with C-8 and C-10, H-10 with C-8. As similarly, the connection of C-16 to C-17 was deduced by the $^1$H-$^1$H COSY correlation H-16 ($\delta$ 3.58) with H-17 ($\delta$ 3.47). The $^1$H NMR spectrum of **16** exhibited a sugar moiety with an anomeric proton at $\delta$ 4.60 (d, $J = 7.8$); The $^{13}$C NMR data of **16** (Table 1) also revealed the presence of a glucose moiety: five oxygenated methine carbons at $\delta$ 101.1, 74.9, 78.0, 71.7, 78.2 and one methylene carbon at $\delta$ 62.8. The location of the glucosylmoiety was concluded to be C-3 by the HMBC correlation