Novel skeleton sesquiterpenoids isolated from guava leaves

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ABSTRACT
A chemical investigation of the plant \textit{Psidium guajava} L., collected in Guangdong province, afforded two novel skeleton sesquiterpenoids 1 and 2. Compound 2 also known as isocaryolan-9-one was a new natural product. The structure of the novel compound 1 was determined as guavacid A by various spectroscopic methods. A possible biosynthetic pathway for 1 and 2 was proposed.

1. Introduction

\textit{Psidium guajava} L. (Myrtaceae), commonly known as guava, is an important food crop and medicinal plant in tropical and subtropical countries. It is widely used like food and folk medicine around the world, and different parts of the plant have been reported to have many pharmacological properties including anti-diabetes, anti-inflammatory, anti-fungal, anti-hypertension and anti-diarrhoea activities (Gutierrez et al. 2008). Previous investigations on the guava leaves have led to the isolation of several triterpenoids, flavonoids, tannins and carotenoids. Some similar trans-feruloyloxy triterpenoid compounds had also been identified from the roots of \textit{Ampelopsis japonica} (Mi et al. 2014). Recently, unusual meroterpenoids and benzophenone glycosides were reported in the guava leaves and intrigued great interest.
because of its obvious bioactivities such as anti-tumour (Yang et al. 2007; Fu et al. 2010; Shao et al. 2010; Gao et al. 2012; Shao et al. 2012; Ukwueze et al. 2015). To find potentially hypoglycaemic secondary metabolites from *P. guajava* L., this study was undertaken to investigate the chemical constituents of the guava leaves collected from south of China. One novel skeleton sesquiterpenoid 1 together with a new natural product 2 was obtained as a result. Compound 2 also known as isocaryolan-9-one could be synthesised by the allylic alcohol treated with TCNE at room temperature (Colado et al. 1998; Racero et al. 2000). Interestingly, the isocaryolane skeleton of 2 exists in the psiguadials B as a sesquiterpenoid moiety (Shao et al. 2010). In this study, the novel structure of compound 1 was elucidated by the combination of EI-MS, ESI-MS, 1D and 2D NMR techniques including 1H NMR, 13C NMR, HSQC, HMBC, NOeSY. Furthermore, the possible biosynthetic pathway of 1 and 2 was also proposed.

### 2. Results and discussion

Compound 1 was obtained as colourless needle-like crystals in PE-EtOAC. The molecular mass was deduced to be 236 amu according to molecular ion peak at *m/z* 236 ([M] +) in its El mass spectrum and the giving quasi-molecular ion peak at *m/z* 235 ([M–H]−) in its ESI mass spectrum. Analysis of the 1H NMR data revealed three methyls at δh 1.01, 1.01, 0.95 (each 3H, s). Connecting with the HSQC, four quaternary C-atoms including one C=O group (δc 184.5, 52.6, 41.9, 33.5), two CH groups (δc 46.0, 48.9), six CH2 groups (δc 48.9, 45.4, 37.2, 34.3, 29.5, 25.8) and three Me groups (δc 30.5, 22.0, 20.3) were observed in its 13C NMR spectrum. The above spectral evidence revealed that the molecular formula of 1 was C15H24O2 (Ω = 4) suggesting a tricycle sesquiterpenoid.

The 1H and 13C NMR signals (Table S1) were assigned based on the 1H-1H COSY, HSQC and HMBC experiments. The observed signals in its 1H NMR spectrum at δh 1.80 (1H, dd, *J* = 13.0, 6.0 Hz, H-7α), 2.17 (1H, dd, *J* = 14.0, 8.5 Hz, H-9α) and 1.95 (1H, dd, *J* = 13.0, 2.5 Hz, H-11β) suggested that these three methylenes (C-7, 9 and 11) were connected with quaternary carbons, respectively. The 1H-1H COSY correlations (Figure S6) between H-7α and H-6, H-9α and H-10 revealed the presence of two spin systems (C-6/C-7 and C-9/C-10). A series of HMBC (Figure S6) cross-peaks from H-9 to C-7, C-8, C-11 and C-12; from H-11 to C-7, C-8, C-9 and C-12; from H-7 to C-8, C-11, C-12 were observed. According to these findings, the structural unit of 1a (Figure S6) was constructed as a consequence.

Cross-peaks from C (4)-Me to C-3, C-4 and C-5 in its HMBC spectrum and carefully comparing the five carbons (C-3, C-4, C-5, C-14 and C-15) chemical shift values with those of 2 (isocaryolan-9-one) led to establish the trans-fusion four-member ring moiety 1b (Figure S6). Additionally, the significant HMBC correlations from C (1)-Me to C-1, C-2, C-10 and C-11, from H-9 to C-1, from H-11 to C-1 confirmed the two structural units (1a/1b) should be connected at C-1.

The relative configuration of compound 1 was elucidated by analysis of its NOeSY data. In the NOeSY spectrum, key correlations (Figure S11) between H-5 (δh 1.55) and H-15 (δh 1.01)/H-11β (δh 1.95) suggested that these protons were cofacial and assigned as β-orientation. On this basis, an unambiguous structure of compound 1 was showed in Figure 1.

Bicyclocaryophyllene-type (-)-caryophyllene oxide intermediate 3 have been isolated as a major product from the leaves of *P. guajava* L. (Meckes 1996). By opening the ether ring, 3 could be transformed into intermediate 4 which was also reported in the guava leaves (Ekundayo 1991). Under acidic condition and acting in Markovnikov fashion, 4 can produce...
intermediate 6 which would further be transformed into intermediate 7 by an alkyl migration model. Then, 1 and 2 can be generated from 7 and 6 by oxidation, respectively. Therefore, a plausible biosynthetic pathway for compounds 1–2 could be proposed through this route (Scheme 1).
3. Experimental

3.1. Plant material

The leaves of *P. guajava* L. were collected in September 2012 in Maoming, Guangdong prov-ince, P.R. China, and identified by associate professor Bo-Ru Liao. A voucher specimen (No. 2012-9) was deposited in the key Laboratory of Natural Active Products Research Center at South China Agricultural University, Guangzhou, P.R. China.

3.2. Instruments and reagents

Column Chromatography (CC): silica gel H (200–300 mesh; Qingdao Haiyang Chemical Co., Ltd.). TLC: normal-phase silica gel GF254 on plates; visualisation under UV light (254 and 365 nm) and spraying with 0.5% vanillin/H$_2$SO$_4$ followed by heating at 105 °C for 5–10 min. M.p.: X-4 numeral melting-point instrument (Beijing Tech Instrument Co., Ltd.). Optical rotations: WZZ-2B polarimeter (cell length 1.0 dm, Shanghai Precision Instruments Co., Ltd.). NMR (CDCl$_3$): Bruker Avance-500/600 spectrometer; at 500/600 (1H) and 125/150 (13C) MHz; residual solvent peaks as internal standard; δ in ppm and J in Hz. ESI-MS: Finnigan LCQ Deca mass spectrometer (Thermo Quest). GC-MS: Agilent 7890A GC and Agilent 5975C inert MSD.

3.3. Extraction and Isolation

The air-dried leaves of *P. Guajava* L. (9.0 kg) were extracted with 95% EtOH three times. After the filtrate was concentrated at 60 °C under the low pressure, the EtOH extract was successively extracted by petroleum ether and EtOAc. The EtOAc extract (243 g) was then subjected to column chromatography over silica gel and eluted with a gradient of petroleum ether-EtOAc (100:1, 50:1, 20:1, 10:1, 5:1, 1:1, 1:0) and finally EtOAc-MeOH (4:1). On the basis of the differences in composition indicated by TLC visualised by spraying 0.5% vanillin–H$_2$SO$_4$ followed by heating at 105 °C, twenty crude fractions (A-T) were collected. Fraction E (7.3 g) eluted by petroleum ether-EtOAc (50:1) was further separated into ten fractions (E1–E10). E9 (0.4 g) was purified via extensive silica gel column chromatography with petroleum ether-EtOAc (3:1) to afford compound 1 (6.9 mg). Compound 2 (12.1 mg) was obtained by repeated chromatography over silica gel eluting with petroleum ether-EtOAc (30:1) from E2 (0.7 g).

Compound 1 (guavacid A): Colourless needle-like crystals in PE-EtOAc. The visualised colour was red by spraying 0.5% vanillin–H$_2$SO$_4$ followed by heating at 105 °C. M.p. 168–169 °C; [α]$_D$ = -11.8° (c = 0.06, CHCl$_3$). $^1$H NMR (CDCl$_3$, 500 MHz) δ: 1.62 (1H, m, H-2), 1.36 (1H, m, H-3β), 1.58 (1H, m, H-3α), 1.55 (1H, m, H-5), 1.36 (1H, m, H-6α), 1.66 (1H, m, H-6β), 1.66 (1H, m, H-7β), 1.80 (1H, dd, J = 13.0, 6.0 Hz, H-7α), 1.70 (1H, m, H-9β), 2.17 (1H, dd, J = 14.0, 8.5 Hz, H-9α), 1.46 (1H, m, H-10β), 1.55 (1H, m, H-10α), 1.62 (1H, m, H-11α), 1.95 (1H, dd, J = 13.0, 2.5 Hz, H-11β), 0.95 (3H, s, H-13), 1.01 (6H, s, H-14, 15). $^{13}$C-NMR (CDCl$_3$, 125 MHz) δ: 41.9 (C-1), 46.0 (C-2), 37.2 (C-3), 33.5 (C-4), 48.9 (C-5), 25.8 (C-6), 34.3 (C-7), 52.6 (C-8), 29.5 (C-9), 45.4 (C-10), 48.9 (C-11), 184.5 (C-12), 22.0 (C-13), 20.3 (C-14), 30.5 (C-15). El-MS, m/z ($I_{rel}$, %): 236.1 (2.4), 207.1 (1.7), 191.1 (5.8), 180.1 (100), 151.0 (12.4), 135.1 (83.5), 93.0 (55.8), 41.1 (73.9).
Compound 2 (isocaryolan-9-one): Colourless oil-like material in PE-EtOAc. The visualised colour was yellow by spraying 0.5% vanillin–H₂SO₄ followed by heating at 105 °C. [α]D = -27.3° (c = 0.09, CHCl₃). ¹H NMR (CDCl₃, 600 MHz) δ: 1.65 (1H, m, H-2), 1.48 (1H, m, H-3β), 1.56 (1H, m, H-3α), 1.62 (1H, m, H-5), 1.34 (1H, m, H-6α), 1.46 (1H, m, H-6β)), 1.17 (1H, m, H-7α), 2.28 (1H, m, H-7β), 2.51 (1H, m, H-8), 2.45 (2H, m, H-10), 1.64 (1H, m, H-11α), 1.72 (1H, m, H-11β), 1.74 (1H, m, H-12α), 2.04 (1H, dd, J = 14.4, 8.4 Hz, H-12β), 0.90 (3H, s, H-13), 0.94 (3H, s, H-14), 0.98 (3H, s, H-15). ¹³C-NMR (CDCl₃, 150 MHz) δ: 31.7(C-1), 42.1(C-2), 35.9(C-3), 32.9(C-4), 46.3(C-5), 29.8(C-7), 44.9(C-8), 215.8(C-9), 36.7(C-10), 36.7(C-11), 42.0(C-12), 26.0(C-13), 21.8(C-14), 30.5(C-15). El-MS, m/z (Irel, %): 220 (31.3), 164.1 (100), 146.1 (95.3), 123.1 (55.3), 95.1 (94.5), 41.1 (81.5).

4. Conclusions

To the best of our knowledge, compound 1 was a novel compound. Although the isocaryolane skeleton of compound 2 has been obtained by rearrangement of (-)-trans-caryophyllene with several electrophilic reagents (Colado et al. 1998), both the sesquiterpenoid skeletons of 1 and 2 were reported for the first time from the natural products.

Supplementary material

The NMR and MS spectra of the two compounds are available online as supplementary material.

Disclosure statement

No potential conflict of interest was reported by the authors.

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