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Flavonoids and Phenolic Compounds From the Parasitic Gymnosperm *Parasitaxus usta* Endemic to New Caledonia

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Abstract

Parasitaxus usta (Podocarpaceae) is the only parasitic gymnosperm and endemic to New Caledonia. In this survey, 11 flavonoids and 6 phenolic compounds were isolated from the aerial parts. As for flavonoids, six flavones, apigenin 7-*O*-glucoside (**1**), luteolin (**2**), luteolin 7-*O*-glucoside (**3**), chrysoeriol (**4**), chrysoeriol 7-*O*-glucoside (**5**) and tricetin 3′-*O*-glucoside (**6**), one *C*-glycosylflavone, orientin (**7**), one flavonol, quercetin 3-*O*-glucoside (**8**), one anthocyanin, cyanidin 3-*O*-glucoside (**9**), and two biflavones, isoginkgetin (**10**) and agathisflavone (**11**) were identified by UV, liquid chromatograph–mass spectra (LC-MS), acid hydrolysis, NMR and/or HPLC comparisons with authentic samples. On the other hand, six phenolic compounds were identified as 5-*O*-*E*-*p*-coumaroyl quinic acid (**12**), 5-*O*-*Z*-*p*-coumaroyl quinic acid (**13**), 5-*O*-*E*-*p*-coumaroyl quinic acid methyl ester (**14**), 5-*O*-*Z*-*p*-coumaroyl quinic acid methyl ester (**15**), *E*-caffeic acid methyl ester 3-*O*-β-glucopyranoside (**16**), and *Z*-caffeic acid methyl ester 3-*O*-β-glucopyranoside (**17**) by UV, LC-MS and NMR. Chemical components of *P usta* were reported in this survey for the first time. Their chemical characters were chemotaxonomically compared with those of other Podocarpaceae species.

Keywords

flavonoids, phenolic compounds, *Parasitaxus usta*, Podocarpaceae, parasitic gymnosperm

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Parasitaxus usta (Vieill.) de Laub. is the only parasitic gymnosperm in the world, and endemic to New Caledonia. It is a woody shrub or small tree, up to 1.5 m in height with deep wine red to purple scale leaves. The plant lacks roots and is always found attached to the roots of *Falcatifolium taxoides* (Brongn. & Gris) de Laub., which is also a member of Podocarpaceae[1,2]. Although some Podocarpaceae such as *Dacrycarpus*[3], *Podocarpus*[4-6] and *Phyllocladus*[7-9] have been reported for flavonoids, chemical compounds including flavonoids in *Parasitaxus* are not surveyed as far as we know. In this survey, flavonoids and related phenolic compounds were isolated and identified from *P. usta* for the first time.

Results

Eleven flavonoids (**1**-**11**) were isolated from *P. usta*. Compound **9** is anthocyanin and was identified as cyanidin 3-*O*-glucoside (chrysanthemin, Figure 1) by UV-vis spectra, liquid chromatograph–mass spectra (LC-MS), acid hydrolysis, and HPLC comparison with authentic sample from *Acer* spp. leaves[10]. Flavonoids **2** and **4** were flavone aglycones and identified as luteolin and chrysoeriol (Figure 1) by UV, LC-MS, and HPLC comparisons with authentic samples from the pubescence of *Glycine max* (L.) Merr.[11] and Extrasynthese (Genay). Of flavonoids **1**, **3** and **8**, the former two were flavone glycosides. On the other hand, **8** was flavonol glycoside which was shown by UV spectral survey. Finally, **1**, **3** and **8** were identified as apigenin 7-*O*-glucoside, luteolin 7-*O*-glucoside, and quercetin 3-*O*-glucoside (isoquercitrin) (Figure 1) by LC-MS, acid hydrolysis, and HPLC comparisons with authentic samples from

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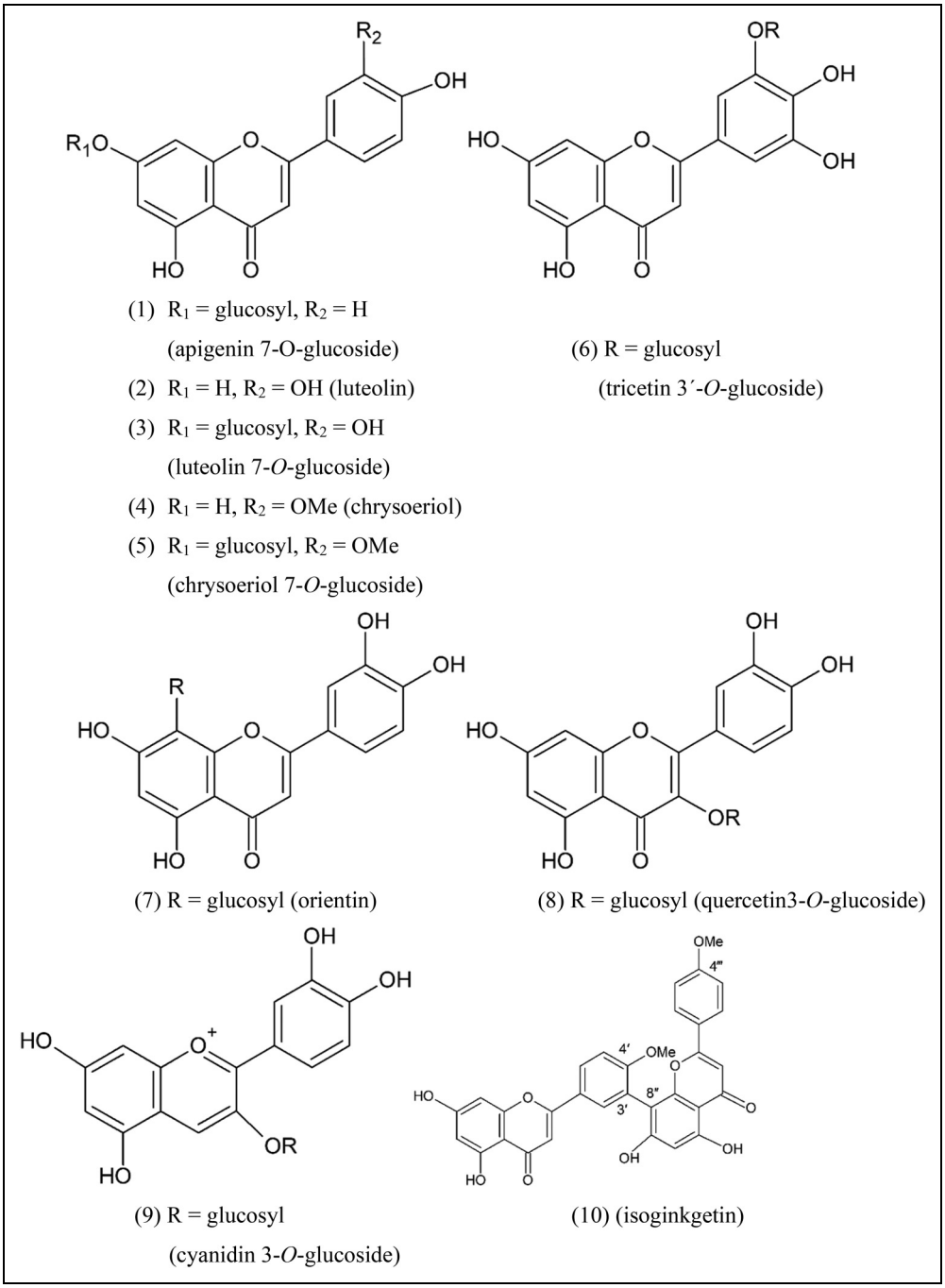

Figure 1. Chemical structures of flavonoids and phenolic compounds isolated from *Parasitaxus usta*. (continued)

Extrasynthese, the leaves of *Schmalhausenia nidulans* Petrak (Asteraceae)[12], and the fronds of *Cyrtomium* spp. (Dryopteridaceae)[13], respectively. Flavonoid **7** was unhydrolyzable, showing that the compound is *C*-glycosylflavone. Finally, **7** was identified as orientin (Figure 1) by HPLC and TLC comparison with authentic sample from the leaves of *Barringtonia asiatica* (L.) Kurz. (Lecythidaceae)[14]. Flavonoids **5** and **6** were flavone glycosides and characterized as chrysoeriol 7-*O*-glucoside and tricetin 3′-*O*-glucoside (Figure 1) by UV spectral survey according to Mabry et al.[15], LC-MS and acid hydrolysis. Flavonoids **10** and **11** were presumed as biflavones by the behavior of HPLC. The molecular ion peaks of **10** showed m/z 567 $[M+H]^+$ and 565 $[M-H]^-$, suggesting that it is tetrahydroxy-dimethoxybiflavone. In NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC, and HMBC. The ^{1}H NMR spectrum of **10** showed 10 aromatic proton signals, H-6′, H-2′, H-2‴,6‴, H-5′, H-3, H-8, H-3″, H-3‴,5‴, H-6″, and H-6, together with 2 methoxyl proton signals. Of their proton signals, two

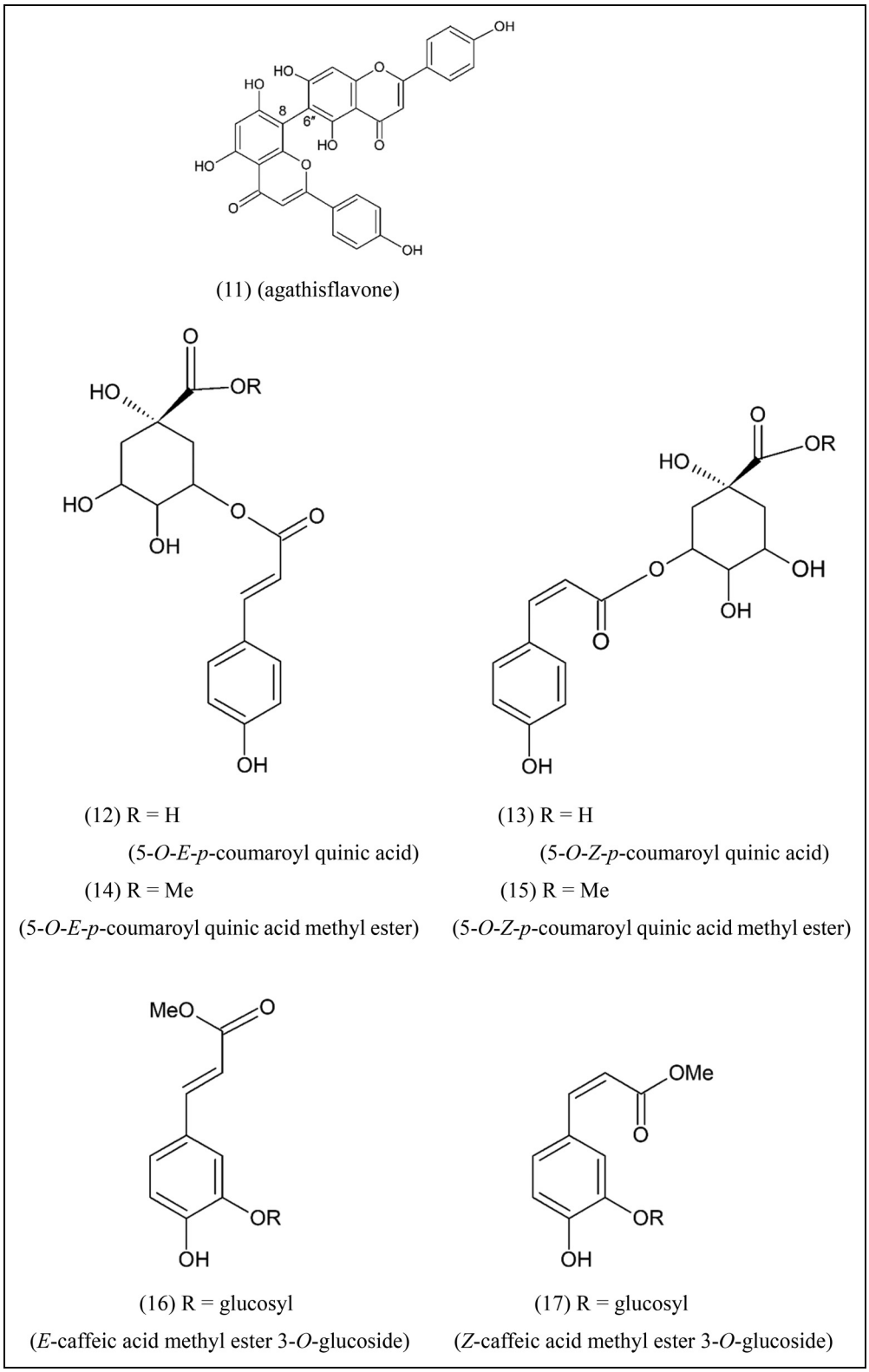

Figure 1. Continued.

methoxyl proton signals at δ_H 3.87 and 3.82 correlated with C-4‴ at δ_C 166.1 and C-4′ at δ_C 161.5, showing the attachment of methoxyl groups to 4′- and 4‴-positions of the biflavone. On the other hand, the proton signals of H-3′ and H-8″ were missing. From the results described above, **10** was identified as acacetin-(3′→8)-acacetin (isoginkgetin, Figure 1).

The molecular ion peaks of **11** showed *m/z* 539 $[M+H]^+$ and 537 $[M-H]^-$, showing it is hexahydroxybiflavone. In ^1H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC, and HMBC. The ^1H NMR spectrum of **11** showed eight aromatic proton signals, H-2′,6′, H-2‴,6‴, H-3, H-3″, H-3′,5′, H-3‴,5‴, H-8″, and H-6. Moreover, the proton signals of H-8 and H-6″ were missing. Thus, **11** was identified as apigenin-(6→8)-apigenin (agathisflavone, Figure 1).

Six phenolic compounds (**12**-**17**) were isolated from *P. usta*. The molecular ion peaks of **12** and **13** showed *m/z* 339 $[M+H]^+$ and 337 $[M-H]^-$, showing the attachment of 1 mol *p*-coumaric acid to quinic acid. In ^1H NMR, four proton signals corresponding to H-2,6, H-3,5, H-α, and H-β of *p*-coumaric acid occurred. Moreover, four proton signals corresponding to H-2,6, H-3, H-4, and H-5 of quinic acid were recognized. In HMBC, H-5 proton signal of quinic acid correlated with COOH carbon signal of *p*-coumaric acid. Although proton and carbon signals of **12** and **13** were essentially the same, the coupling constants of H-α and H-β of *p*-coumaric acid were different, i.e. J = 15.6 Hz and 15.6 Hz in **12**, and J = 13.2 Hz and 13.2 Hz in **13**, showing that **12** and **13** are *E*- and *Z*-forms, respectively. Thus, **12** and **13** were identified as 5-*O*-*E*-*p*-coumaroyl quinic acid and 5-*O*-*Z*-*p*-coumaroyl quinic acid (Figure 1), respectively. ^1H and ^{13}C NMR data of **14** and **15** were essentially the same with those of **12** and **13**, except for the presence of methoxyl proton and carbon signals. In HMBC, since methoxyl proton signal correlated with COOH carbon signals of quinic acid, it was shown that methoxyl group is attached to the carboxyl group of quinic acid. Thus, **14** and **15** were identified as 5-*O*-*E*-*p*-coumaroyl quinic acid methyl ester and 5-*O*-*Z*-*p*-coumaroyl quinic acid methyl ester (Figure 1), respectively. The molecular ion peaks of **16** and **17** showed *m/z* 357 $[M+H]^+$ and 355 $[M-H]^-$, showing the attachment of 1 mol hexose to dihydroxy-monomethoxy-cinnamic acid. In ^1H NMR, five proton signals corresponding to H-2, H-5, H-6, H-α, and H-β of caffeic acid occurred, together with a methoxyl proton signal. In HMBC, anomeric proton signal correlated with the C-3 carbon signal of caffeic acid. On the other hand, a methoxyl proton signal correlated with COOH carbon signal of caffeic acid. Moreover, coupling constants of H-α and H-β of **16** were J = 15.6 Hz, showing the caffeic acid is *E*-form. Thus, **16** was identified as *E*-caffeic acid methyl ester 3-*O*-β-glucopyranoside (Figure 1). On the other hand, since coupling constants of H-α and H-β of **17** were J = 12.6 Hz, caffeic acid is *Z*-form. From the results described above, **17** was identified as *Z*-caffeic acid methyl ester 3-*O*-β-glucopyranoside (Figure 1).

Discussion

In this survey, six flavones (**1**-**6**), one *C*-glycosylflavone (**7**), one flavonol (**8**), one anthocyanin (**9**), two biflavones (**10** and **11**), and six phenolic compounds (**12**-**17**) were isolated from *P. usta* for the first time. Many flavonoids have been reported from Podocarpaceae species such as *Dacrycarpus*[3], *Podocarpus*[5,6] and *Phyllocladus*[9]. Of the flavonoids which were isolated in this survey, biflavones were widespread reported in gymnosperms[16]. However, isoginkgetin (**10**) and agathisflavone (**11**) are not found in Podocarpaceae as far as we know. Compounds **1**, **3**, **7** to **9** are common flavonoids and have been reported from some Podocarpaceae species, e.g. **1** and **3** from *Prumnopitys* spp.[5], **7** to **9** from *Podocarpus* spp.[4,5], *Dacrycarpus dacrydioides*[3,5], and *Prumnopitys* spp.[5]. Although **6** is a comparatively rare flavonoid, it has been found in *Podocarpus totara*[5]. Flavonoids **2**, **4,** and **5** are also common compounds. However, they were reported from Podocarpaceous species for the first time. Six phenolic compounds (**12**-**17**) which were isolated in this survey are not reported from the Podocarpaceous plants.

It has been shown that *P. usta* correlated with the genera *Lagarostrobus* and *Manoao* by chloroplast *trn*L-F intron/spacer and nuclear rDNA ITS2 sequences[17].On the other hand, it was shown by the single-copy nuclear gene that *Parasitaxus* correlates with *Phyllocladus tricomanoides*[18]. Although the flavonoids of *Lagarostrobus* and *Manoao* species are not reported as far as we know, those of *P. trichomanoides* have been isolated and some catechins such as (+)-catechin and (−)-epicatechin have been identified[7,8,19]. However, catechins were apparently not found in *Parasitaxus* in this survey. As a result, the chemical composition of *P. usta* have the chemical characters of Podocarpaceae species, e.g. the presence of common flavones, flavonol, *C*-glycosylflavone, anthocyanin, and especially biflavones. However, two biflavones, isoginkgetin (**10**) and agathisflavone (**11**), and phenolic compounds **12** to **17** are not reported from the family as far as we know. Thus, it was suggested that the chemical characters of *Parasitaxus* is chemotaxonomically unique in Podocarpaceae.

Materials and Methods

Plant Materials

P. usta was collected in Mt. Dzumac (22°01′50.94″S, 166° 28′03.42″E, 900 m alt.), New Caledonia in January 11, 1998 by the authors (H. Tobe and T. Jaffré), and deposited in the herbarium of New Caledonian Herbarium at Nouméa (NOU).

General

Analytical HPLC was performed with Shimadzu HPLC systems using Inertsil ODS-4 column (I.D. 6.0 × 150 mm, GL Sciences, Tokyo) at a flow rate of 1.0 mL/min. Detection wavelength was 350 (flavonoids and phenolic compounds) and 530 nm (anthocyanin). Eluent was $MeCN/H_2O/H_3PO_4$ = 20:80:0.2). LC-MS was performed on a Shimadzu HPLC/UV-vis/ESI-MS system using Inertsil ODS-4 column (I.D. 2.1 × 100 mm), flow rate of 0.2 mL/min, detection wavelength of 350 and 530 nm, electrospray ionization (ESI^+) 4.5 kV and ESI^- 3.5 kV, 25°C, and elution with $MeCN/H_2O$/HCOOH (20:75:5 for flavonoids except for biflavones and phenolic compounds or 65:35:5 for

biflavones). NMR spectra (^{1}H and ^{13}C NMR, ^{1}H-^{1}H correlation spectroscopy, ^{1}H-^{1}H total COSY, heteronuclear quantum correlation, and HMBC) were recorded on a Bruker AV-600 in DMSO-d_6 at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR). Preparative paper chromatography (prep. PC) was performed with solvent systems, BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase), BEW (*n*-BuOH/EtOH/H_2O = 4:1:2.2), and then 15%HOAc. Acid hydrolysis was performed in 12% hydrochloric acid at a temperature of 100°C for 30 min. After shaking with diethyl ether, flavonoid aglycones were migrated to the organic layer, and sugars and *C*-glycosylflavone were left in the aqueous layer. Preparative HPLC was performed with Shimadzu HPLC systems using Inertsil ODS-4 column (I.D. 10 × 250 mm), at a flow rate of 3.0 mL/min, detection wavelength of 350 nm, and the elution with MeCN/H_2O/HCOOH (20:75:5 for phenolic compounds or 50:45:6 for biflavones).

Extraction and Isolation

Dried aerial parts (25.0 g) of *P. usta* were extracted with MeOH/HCOOH (92:8). The concentrated extracts were applied to prep. PC. Isolated flavonoid glycosides **1**, **3**, **5** to **9** were purified by Sephadex LH-20 column chromatography using solvent systems, 70% MeOH (**1**, **3**, **5** to **8**) and MeOH/H_2O/HCOOH (70:25:5) (**9**). Other compounds were applied to preparative HPLC. Compounds **3** (3.2 mg), **10** (1.2 mg), **11** (1.3 mg), **12** (1.7 mg), **13** (1.7 mg), **14** (14.8 mg), **15** (3.6 mg), and **16** (2.6 mg) were obtained as pale yellow or white powders.

Identification of the Compounds

Flavonoids and phenolic compounds were identified by UV-vis spectral survey according to Mabry et al.[15], LC-MS, characterization of acid hydrolysates, ^{1}H and ^{13}C NMR, and/or HPLC comparisons with authentic samples. UV, LC-MS and NMR data are shown in online Supplemental Material.

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Declaration of Conflicting Interests

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Supplemental Material

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