Sesquiterpenes and Lignans from *Tephrosia vogelii*

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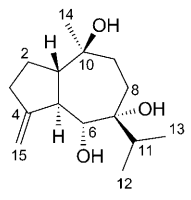
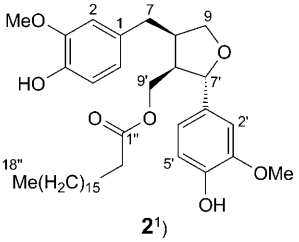
A new guaiane sesquiterpene, (1β,6α,10α)-guai-4(15)-ene-6,7,10-triol (**1**), and a new lignan, (+)-lariciresinol 9′-stearate (**2**), were isolated from the aerial parts of a leguminosae plant, *Tephrosia vogelii*, together with three known compounds, teclenone B, (1β,7*R**)-opposit-4(15)-ene-1,7-diol, and pinoresinol. Their structures were elucidated on the basis of spectral data.

Introduction. – *Tephrosia vogelii* HOOK f., a perennial leguminous shrub native to tropical Africa, is known as a source of the rotenoids including rotenone and deguelin [1][2]. It was introduced into agriculture in the southern area of Guangdong Province, P. R. China, in 1980s, where its aerial parts have been used for the commercial production of rotenoids [3]. The general process for the preparation of rotenoids from this plant yields, besides rotenoids, a large amount of nonrotenoid fractions. Currently, the rotenoids are applied to pesticides, while the nonrotenoid fractions are discarded as undesirable substances, which results not only in an adverse impact on the environment but also in the waste of a resource. Therefore, it is important to investigate the nonrotenoid constituents of *T. vogelii* so that they can be utilized as valuable compounds rather than just be discarded as waste. However, the nonrotenoid constituents of this plant are almost unknown. Herein, we report the isolation and structure elucidation of a new sesquiterpene, (1β,6α,10α)-guai-4(15)-ene-6,7,10-triol[1]) (**1**), and of a new lignan, (+)-lariciresinol 9′-stearate[1]) (**2**), from this plant cultivated in South China.



1[1])



2[1])

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – The EtOH extract of the aerial parts of *T. vogelii* was partitioned with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$-soluble portion was separated by a combination of silica gel, *ODS*, and *Sephadex LH-20* column chromatography and semi-prep. HPLC to afford two new compounds, **1** and **2**, together with two known sesquiterpenes, teclenone B [4], (1*β*,7*R**)-opposit-4(15)-ene-1,7-diol [5], and a known lignan, pinoresinol [6][7]. The structures of the known compounds were determined by interpretation of their spectroscopic data as well as by comparison with reported data.

Compound **1** was obtained as a colorless gum. Its molecular formula was determined to be $C_{15}H_{26}O_3$ by the combined analysis of its HR-ESI-MS, ^{13}C-NMR, and DEPT data. The ^{1}H-NMR spectrum (see *Exper. Part*) exhibited signals for 23 nonexchangeable H-atoms, including two *d* at δ(H) 0.86 (J = 7.0 Hz, Me(12)) and 0.92 (J = 7.0 Hz, Me(13)) for two secondary Me groups, a *s* at δ(H) 1.10 (Me(14)) for a tertiary Me group, a *d* at δ(H) 3.25 (J = 10.0, H−C(6)) for an O−CH group, and two broad *s* at δ(H) 4.78 (H_a−C(15)) and 5.04 (H_b−C(15)) for an exocyclic olefinic CH_2 group. The ^{13}C-NMR and DEPT spectra indicated the presence of three Me groups (δ(C) 16.5 (C(13)), 17.8 (C(12)), and 25.6 (C(14))), five CH_2 groups including one olefinic (δ(C) 109.0 (C(15))), four CH groups including one oxygenated (δ(C) 74.4 (C(6))), and three quaternary C-atoms, of which two were oxygenated (δ(C) 75.0 (C(7)) and 73.2 (C(10))) and one was olefinic (δ(C) 154.3 (C(4))). The ^{1}H,^{1}H-COSY in combination with the HMQC spectrum revealed the partial structures shown by the bold lines in *Fig. 1*. In the HMBC spectrum (*Fig. 1*), correlations observed from H−C(1), H−C(5), CH_2(8), CH_2(9), and Me(14) to C(10), and from H−C(5), H−C(6), CH_2(8), CH_2(9), H−C(11), Me(12), and Me(13) to C(7) indicated the connectivity of C(10) to C(1) and C(9), and of C(7) to C(6) and C(8), forming a seven-membered ring (*B*) with a Me group (C(14)) and an OH group attached at C(10) and an ^{i}Pr group and an OH group attached at C(7). HMBC Cross-peaks were also observed between the olefinic CH_2 H-atoms (H_a−C(15) and H_b−C(15)) and C(4), C(3), and C(5), showing the connection of C(4) to C(3) and C(5), forming a five-membered ring (*A*) with a CH_2 (C(15)) attached at C(4) *via* a C=C bond. Thus, the planar structure of **1** was derived as guai-4(15)-ene-6,7,10-triol.

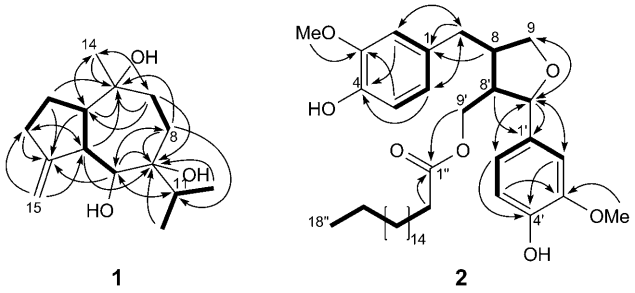


Fig. 1. *^{1}H,^{1}H-COSY* (bold line) *and key HMBC* (arrow) *data of* **1** *and* **2**

The relative configuration of **1** was determined from the NOE interactions in the NOESY plot (*Fig. 2*). The presence of the mutual NOE correlations H−C(6)/H−C(1),

H−C(6)/H_β−C(8), H−C(1)/H_β−C(8), Me(12)/H−C(6), Me(13)/H−C(6), Me(12)/CH_2(8), Me(13)/CH_2(8), H−C(5)/Me(14), and Me(14)/H_α−C(9) and the absence of the correlations Me(14)/H_β−C(9), Me(12)/H−C(1), Me(12)/CH_2(9), Me(13)/H−C(1), Me(13)/CH_2(9), and Me(14)/H−C(1) as well as the proton coupling constant, $J(5,6) = 10.0$ Hz, in the ^{1}H-NMR spectrum indicated that the fusion between rings *A* and *B* was *trans*, and the ring *B* was in a chair-like conformation with C(1), C(5), C(7), and C(8) lying in the same plane, while C(9) and C(10) were oriented below and C(6) above this plane (*Fig. 2*). These correlations further indicated that OH−C(6) was α-oriented and in equatorial position, and OH−C(7) and Me−C(10) were also α-oriented but in axial positions. Therefore, the structure of **1** was established as (1β,6α,10α)-guai-4(15)-ene-6,7,10-triol. When the modified *Mosher* method [8] was applied to establish the absolute configuration, **1** failed to react with (αR)- or (αS)-α-methoxy-α-(trifluoromethyl)benzeneacetic acid (MTPA) in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) and catalytic *N*,*N*-dimethylpyridin-4-amine (DMAP) [9], presumably due to the hindrance of the vicinal ^{i}Pr group at C(7). This was in accordance with the attributed relative configuration of **1** as shown in *Fig. 2*.

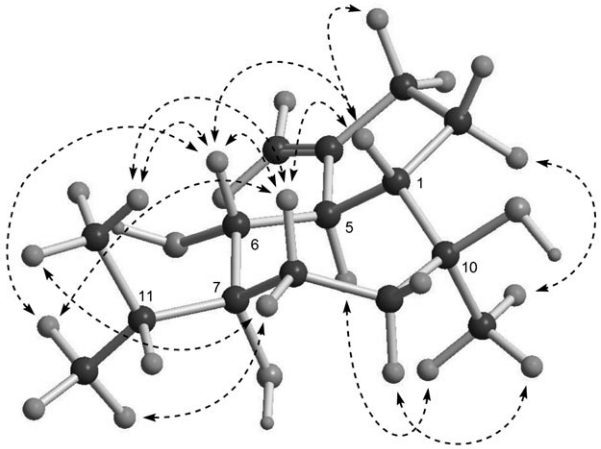


Fig. 2. *Relative configuration and significant NOESY interactions of* **1**

Compound **2** was obtained as a pale yellow powder. Its molecular formula was established as $C_{38}H_{58}O_7$ from the HR-ESI-MS ion peak at *m*/*z* 649.4080 ($[M + Na]^+$). In the ^{1}H-NMR spectrum (see *Exper. Part*), the presence of a *t* at δ(H) 0.83 ($J = 6.8$ Hz, Me(18″)) for a Me group, a *t* at δ(H) 2.19 ($J = 7.2$ Hz, CH_2(2″)) assignable to a CH_2 group vicinal to a C=O group, and a number of *m*s with great intensity overlapping in the region δ(H) 1.10–1.30 indicated that **2** had a fatty acid moiety in the molecule. This was supported by the presence of C-atom signals at δ(C) 172.8 for an ester C=O group and δ(C) 13.9 for a Me group, and partially overlapping peaks from δ(C) 22.1 to 33.5 for a number of CH_2 in the ^{13}C-NMR and DEPT spectra. After having attributed the signals due to the fatty acid moiety, the remaining NMR signals were very similar to those of (+)-lariciresinol (=(2*S*,3*R*,4*R*)-tetrahydro-2-(4-hydroxy-3-methoxyphenyl)-4-[(4-hy-

droxy-3-methoxyphenyl)methyl]furan-3-methanol) [10][11], except that the signals of $CH_2(9')$ (δ(H) 4.25 and 4.06 (each *dd*, $J = 11.2$, 7.2 Hz, 1 H) and C(9′) (δ(C) 62.0) of **2** were downfield-shifted. The relative molecular mass of **2**, 626, was 266 units more than that of lariciresinol, indicating that the fatty acid moiety was a stearoyl group. The presence of correlations between $CH_2(9')$ and C(1″) (δ(C) 172.8) in the HMBC plot (*Fig. 1*) indicated that the stearoyloxy group was linked to C(9′). The configuration of **2** should be the same as that of (+)-lariciresinol, as deduced from its positive optical rotation and negative *Cotton* effects at 232 and 289 nm in the CD spectrum [12]. Thus, the structure of **2** was determined as (+)-lariciresinol 9′-stearate.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2, 100–200 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), *Develosil ODS* (10 μm; *Nomura Chemical Co. Ltd.*, Japan), and *Sephadex LH-20* (*Pharmacia Biotech AB*, Uppsala, Sweden). Prep. HPLC: *Shimazu-LC-6A* pump, *Shimazu-RID-10A* refractive-index detector, and *XTerra-prep-MS-C_{18}* column (10 μm, 19 × 300 mm). Optical rotations: *Perkin-Elmer 341* polarimeter. CD Spectrum: *Jasco-810* spectropolarimeter; λ ([θ]) in nm. UV Spectra: *Perkin-Elmer Lambda-25* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. NMR Spectra: *Bruker-DRX-400* instrument; at 400 (^{1}H) or 100 MHz (^{13}C); in $CDCl_3$ or (D_6)DMSO; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *MDS-SCIEX-API-2000* LC/MS/MS instrument. HR-ESI-MS: *API-QSTAR*-TOF mass spectrometer.

Plant Material. The aerial parts of *T. vogelii* were collected at the garden for pesticidal plants, South China Agricultural University, Guangzhou, China, in June, 2006. The voucher specimens were deposited with the Key Laboratory of Natural Pesticides and Chemical Biology, Ministry of Education, South China Agriculture University, Guangzhou, China.

Extraction and Isolation. The powdered dry stems and leaves of *T. vogelii* (2.5 kg) were extracted with 90% EtOH (4 ×) at r.t. After evaporation of the solvent, the extract was suspended in H_2O and then partitioned sequentially with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$-soluble part (94.6 g) was subjected to CC (SiO_2, petroleum ether/acetone 19:1 → 4:1): *Frs. I–VIII*. *Fr. IV* was further subjected to CC (SiO_2, $CHCl_3$/MeOH 9:1 → 7:3): *Frs. IV-1–IV-7*. *Fr. IV-2* was separated by CC (*ODS*, 70% MeOH/H_2O) followed by purification by prep. HPLC (60% MeOH/H_2O) to afford **1** (35 mg), teclenone B (52 mg), and (1β,7*R**)-opposit-4(15)-ene-1,7-diol (17 mg). By the same method, *Fr. IV-5* afforded **2** (21 mg), and *Fr. IV-7* gave pinoresinol (66 mg).

(1β,6α,10α)-Guai-4(15)-ene-6,7,10-triol (= rel-*(3a*R,*4*R,*5*R,*8*S,*8a*S*)-Decahydro-8-methyl-3-methylene-5-(1-methylethyl)azulene-4,5,8-triol*; **1**). Colorless gum. $[\alpha]_D^{20} = +20.0$ ($c = 0.25$, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): 5.04 (br. *s*, H_b–C(15)); 4.78 (br. *s*, H_a–C(15)); 3.25 (*d*, $J = 10.0$, H–C(6)); 2.31 (br. *dd*, $J = 14.0$, 6.4, H_α–C(3)); 2.25 (*m*, H_β–C(3)); 2.23 (*dd*, $J = 10.0$, 8.8, H–C(5)); 2.13 (*m*, H–C(11)); 2.10 (*ddd*, $J = 14.0$, 10.8, 2.0, H_α–C(9)); 1.93 (*m*, H–C(1)); 1.86 (*m*, H_β–C(2)); 1.67 (br. *dd*, $J = 15.2$, 11.2, H_β–C(8)); 1.57 (*ddd*, $J = 15.2$, 6.8, 6.8, H_α–C(8)); 1.43–1.50 (*m*, H_α–C(2), H_β–C(9)); 1.10 (*s*, Me(14)); 0.92 (*d*, $J = 7.0$, Me(13)); 0.86 (*d*, $J = 7.0$, Me(12)). ^{13}C-NMR (100 MHz, $CDCl_3$): 154.3 (C(4)); 109.0 (C(15)); 75.0 (C(7)); 74.4 (C(6)); 73.2 (C(10)); 51.0 (C(1)); 46.4 (C(5)); 35.8 (C(9)); 34.9 (C(3)); 34.8 (C(11)); 27.3 (C(2)); 25.6 (C(14)); 21.4 (C(8)); 17.8 (C(12)); 16.5 (C(13)). ESI-MS (pos.): 531 ($[2M + Na]^+$), 293 ($[M + K]^+$), 277 ($[M + Na]^+$). HR-ESI-MS: 277.1773 ($[M + Na]^+$, $C_{15}H_{26}NaO_3^+$; calc. 277.1780).

(+)-Lariciresinol 9′-Stearate (= *Octadecanoic Acid {(2*S,*3*R,*4*R*)-Tetrahydro-2-(4-hydroxy-3-methoxyphenyl)-4-[(4-hydroxy-3-methoxyphenyl)methyl]furan-3-yl}methyl Ester*; **2**). Pale yellow amorphous

powder. $[\alpha]_D^{20} = +8.1$ ($c = 1.0$, MeOH). CD ($c = 1 \cdot 10^{-4}$ M, MeOH): 232 (−10362), 289 (−1980). UV (MeOH): 220 (4.37), 277 (4.00). ^{1}H-NMR (400 MHz, (D_6)DMSO): 6.82 (*d*, *J* = 1.6, H−C(2′)); 6.74 (*d*, *J* = 1.6, H−C(2)); 6.70 (*d*, *J* = 8.0, H−C(5′)); 6.67 (*dd*, *J* = 8.0, 1.6, H−C(6′)); 6.66 (*d*, *J* = 8.0, H−C(5)); 6.55 (*dd*, *J* = 8.0, 1.6, H−C(6)); 4.61 (*d*, *J* = 6.8, H−C(7′)); 4.25 (*dd*, *J* = 11.2, 7.2, H_a−C(9′)); 4.06 (*dd*, *J* = 11.2, 7.2, H_b−C(9′)); 3.89 (*dd*, *J* = 8.2, 6.6, 1 H−C(9)); 3.73 (*s*, MeO−C(3), MeO−C(3′)); 3.56 (*dd*, *J* = 8.2, 6.6, 1 H−C(9)); 2.72 (*dd*, *J* = 13.4, 4.6, H_a−C(7)); 2.65 (*m*, H−C(8)); 2.47 (*m*, H_b−C(7)); 2.41 (*m*, H−C(8′)); 2.19 (*t*, *J* = 7.2, CH_2(2″)); 1.45 (*m*, CH_2(3″)); 1.10–1.30 (*m*, 28 H, CH_2(4″) to CH_2(17″)); 0.83 (*t*, *J* = 6.8, Me(18″)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 172.8 (C(1″)); 147.4 (C(3), C(3′)); 145.8 (C(4′)); 144.7 (C(4)); 133.5 (C(1′)); 131.1 (C(1)); 120.5 (C(6)); 118.5 (C(6′)); 115.3 (C(5)); 115.0 (C(5′)); 112.7 (C(2)); 110.1 (C(2′)); 82.3 (C(7′)); 71.8 (C(9)); 62.0 (C(9′)); 55.5 (*Me*O−C(3), *Me*O−C(3′)); 48.6 (C(8′)); 42.0 (C(8)); 33.5 (C(2″)); 31.3 (C(16″)); 32.4 (C(7)); 29.0–28.5 (C(4″) to C(15″)); 24.4 (C(3″)); 22.1 (C(17″)); 13.9 (C(18″)). ESI-MS (pos.): 1275 ($[2M + Na]^+$), 649 ($[M + Na]^+$). HR-ESI-MS: 649.4080 ($[M + Na]^+$, $C_{38}H_{58}NaO_7^+$; calc. 649.4080).

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