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Sassarandainol: a new neolignan and anti-inflammatory constituents from the stem of Sassafras randaiense

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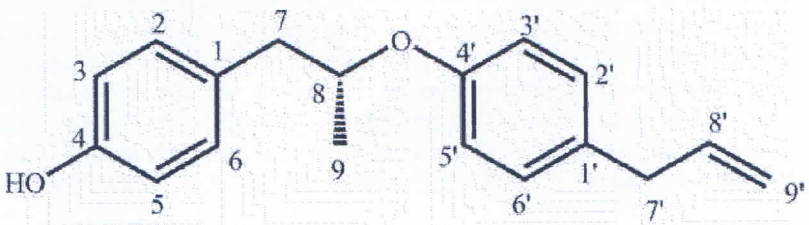

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A new neolignan, (R)-(2)-sassarandainol (1), together with 10 known compounds (2–11), was isolated from the stem of Sassafras randaiense. The structures were determined by spectroscopic techniques. Among these isolates, g-tocopherol (5), subamolide B (7) and b-sitosterone (9) exhibited moderate iNOS inhibitory activity on nitrite production induced (%) value of 30.51, 28.68 and 16.96, respectively.

Keywords: Sassafras randaiense; Lauraceae; stem; neolignan; (R)-(2)-sassarandainol; anti-inflammatory activity

1. Introduction

Over 60 species of Formosan Lauraceous plants have been screened for anti-inflammatory activity and the stem of Sassafras randaiense (Hay.) Rehd. (Lauraceae) was found to be active on this assay platform. The rationale of this study was thus to isolate the chemical constituents and to describe their anti-inflammatory activities. S. randaiense is a medium-sized deciduous tree, endemic to Taiwan, and grows in broad-leaved forests from 900 to 2400 m throughout the Island (Liao 1996). There are three species of Sassafras, S. albidum (Nuttall) Nees, S. tzumu (Hemsl.) Hemsl. and S. randaiense (Hayata) Rehder (Nie et al. 2007) in the world. S. albidum was famous for sassafras oil production from root bark which contained sesquiterpenes (Simić et al. 2004), alkaloids (Chowdhury et al. 1976), monoterpenoids (Hickey 1948; Simić et al. 2004), phenylpropanoids (Hickey 1948; Simić et al. 2004), monosaccharides (Schirmer 1912;

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Springer et al. 1965) and benzenoid (Simić et al. 2004). The second species, S. tzumu, contained different active constituents, including phenylpropanoids, lignans and steroid from the root (Zhong 1998) and 23 volatile oil constituents from the stem (Han & You 2012). The chemistry of Taiwan sassafras was not extensively studied, and only five biphenyl components, magnolol, isomagnolol, randaiol, randainal and randainol, and one steroid, b-sitosterol, were isolated from the root (Chen et al. 1983; El-Feraly et al. 1983; El-Feraly 1984). Among the isolates, magnolol and isomagnolol were shown to have antimicrobial activities (Wu et al. 1972; El-Feraly et al. 1983). Bioassay-guided fractionation of the active EtOAc-soluble fraction of the stem of this species led to the isolation of one new neolignan (1) and 10 known compounds (2–11) (Figure 1). The structures of these isolates were determined by spectroscopic analysis, and assessments of their in vitro anti-inflammatory activities were also evaluated.

2. Results and discussion

Compound 1 was isolated as a yellowish oil, with $[\alpha]_D^{23}$ 2 12.88(c = 0.37, $CHCl_3$). Its HR-ESI-MS spectrum showed a quasi-molecular $[M + Na]^+$ ion peak at m/z 291.13549, corresponding to the molecular formula $C_{18}H_{20}O_2$ with nine degrees of unsaturation. The UV spectrum showed absorptions at 206, 228 and 278 nm, and after addition of aqueous KOH, it gave a bathochromic shift indicating the presence of a phenolic moiety. The IR spectrum displayed absorptions of a hydroxy group (3387 cm^{-1}) and an aromatic ring (1613, 1506 cm^{-1}). The ^1H NMR spectrum (Table S1) showed signals due to one allyl group at δ 3.32 (d, J = 6.6 Hz, H-7′), 5.04 (dd, J = 9.8 and 1.8 Hz, H-9′b), 5.06 (dd, J = 16.9 and 1.8 Hz, H-9′a), 5.95 (ddt, J = 16.9, 9.8 and 6.6 Hz, H-8′), protons with two $AA'BB'$ systems at δ 6.75 (d, J = 8.6 Hz, H-3, H-5), 6.81 (d, J = 9.0 Hz, H-3′, H-5′), 7.08 (d, J = 9.0 Hz, H-2′, H-6′), 7.10 (d, J = 8.6 Hz, H-2, H-6),

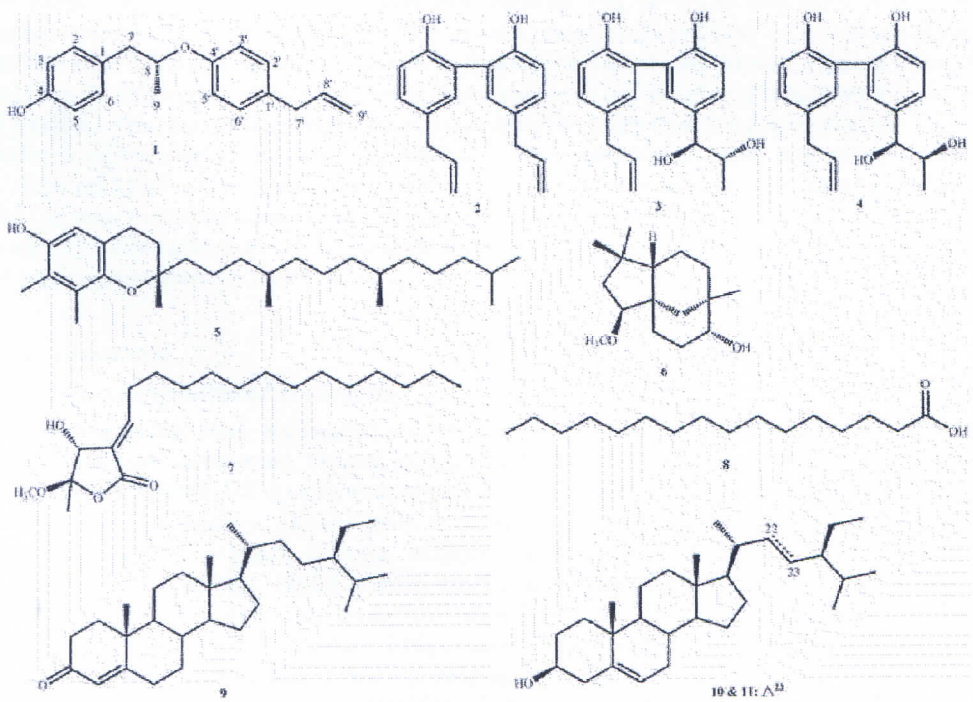

Figure 1. Structures of isolates from the stem of S. randaiense.

suggesting the presence of two 1,4-disubstituted benzene rings, one methyl group at δ 1.27 (d, J = 6.4 Hz, H-9), one methylene group at δ 2.73 (dd, J = 13.8 and 6.5 Hz, H-7b), 3.00 (dd, J = 13.8 and 5.9 Hz, H-7a) and one oxymethine proton at δ 4.47 (m, H-8). The ^{13}C NMR spectrum (Table S1) showed the presence of 18 carbons, which were categorised into 4 quaternary, 1 methyl, 3 methylenes and 10 methines. NOESY spectrum (Figure S3) showed correlations between H-8 and H-2, H-6, H-3′indicating the connection of two phenyl propanoid moieties via an oxygen atom. HMBC correlations (Figure S4) of H-7/C-2 (δ_C 130.6), C-6 (δ_C 130.6), C-8 (δ_C 75.0), C-9 (δ_C 19.3), H-8/C-1 (δ_C 130.4), C-7 (δ_C 41.7), C-4′(δ_C 156.2), H-7′/C-2′(δ_C 129.5), C-6′(δ_C 129.5), C-8′(δ_C 137.8), C-9′(δ_C 115.4) suggested a planar structure as 1 and excluded the possible structure to be isomagnolol (El-Feraly 1984). Compound 1 showed a levorotatory optical activity with $[\alpha]_D^{23}$ 2 12.88(c = 0.37, $CHCl_3$), and after comparing to the R-isomers of (4-hydroxy-3-methoxy-1′allyl-3′,5′dimethoxy)-8-O-4′neolignan with $[\alpha]_D^{20}$ 2 7.08 (c = 0.2, $CHCl_3$) (Hiroyuki et al. 1995), the configuration at C-8 of 1 was proposed to be R-form. According to the above evidence, the structure of 1 was elucidated as (R)-4-(2-(4-allylphenoxy) propyl)phenol, which was further confirmed by DEPT, HSQC, COSY (Figure S5), NOESY and HMBC experiments, namely (R)-(2)-sassarandainol.

The known isolates (Figure 1) were readily identified by comparison of physical and spectroscopic data (UV, IR, ^1H NMR, ^{13}C NMR, $[a]_D$ and MS) with corresponding literature values, and these included three neolignans, magnolol (2) (Chen et al. 1983), erythro-7′hydroxy-strebluslignanol (3) (Li et al. 2012) and threo-7′hydroxy strebluslignanol (4) (Li et al. 2012), one benzopyran, g-tocopherol (5) (Baxter et al. 1943), one sesquiterpenoid, 2b-methoxyclovan-9a-ol (6) (Musharraf et al. 2011), one butanolide, subamolide B (7) (Chen et al. 2007), one fatty acid, palmitic acid (8) (Barbosa et al. 2012), three steroids, b-sitosterone (9) (Cambie et al. 1991) and a mixture of b-sitosterol (10) and stigmasterol (11) (Kojima et al. 1990).

Nitrite production induced (%) and cell viability (%) of iNOS inhibitory activity of compounds 2–11 were obtained at the concentration range of 20 mM, and the results are shown in Table S2. The most active compounds 5, 7 and 9 had nitrite production induced value of 30.51%, 28.68% and 16.96%, respectively. The increase in cell viability of compounds 3–5 and 7 indicates these compounds might have some activities other than lipopolysaccharide (LPS)-induced nitrite production, which is worthy to be explored by other evaluation models. Compound 9 showed strongest activity among the tested compounds but with slight toxic effect. We consider that compounds 5 and 7 increased cell viability might be a result of reversing LPS toxicity, indicating, besides inhibiting nitric formation, they can contribute to against the process of inflammation through multiple ways.

3. Experimental

3.1. General experimental procedures

All melting points were determined on a Yanaco micromelting apparatus (Yanaco, Kyoto, Japan) and were uncorrected. Optical rotations were measured on a Jasco P-2000 polarimeter (Jasco, Kyoto, Japan), UV spectra were obtained with a Jasco-V-530 UV/vis spectrophotometer (Jasco) and IR spectra (KBr) were acquired with a Mattson Genesis IITM FTIR spectrophotometer (Mattson Genesis, Mattson, Germany). 1D (^1H, ^{13}C, DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectra were recorded on a Varian Germini-2000 spectrometer (Varian, Inc., Vacuum Technologies, Lexington, MA, USA) operated at 200 MHz (^1H) and 50 MHz (^{13}C), Varian Unityplus-400 spectrometer (Varian, Inc., Vacuum Technologies, Lexington, MA, USA) operated at 400 MHz (^1H) and 100 MHz (^{13}C), Varian Mercuryplus-400 spectrometer (Varian, Inc., Vacuum Technologies, Lexington, MA, USA) operated at 400 MHz (^1H) and 100 MHz (^{13}C) and Varian VNMRS-600 spectrometer (Varian,

Inc., Vacuum Technologies, Lexington, MA, USA) operated at 600 MHz (^{1}H) and 150 MHz (^{13}C). Low-resolution mass spectra were obtained with POLARIS Q Thermo Finnigan (Thermo Fisher Scientific, Chicago, IL, USA). Water ZQ 4000 (Waters, Milford, MA, USA) and VG Quattro GC/MS/MS/DS (Waters) mass spectrometers. HR-ESI-MS were recorded on a Bruker APEX II mass spectrometer (Bruker, Karlsruhe, Germany). Silica gel (70–230 and 230–400 mesh; Silicycle, Quebec, Canada) was used for column chromatography (CC), and silica gel 60 F254 (Merck, Darmstadt, Germany) and RP-18 F254S (Merck) were used for TLC and preparative TLC, respectively, visualised with $Ce_2(SO_4)_3$ aq solution. Futher purification was performed by medium-performance liquid chromatography (MPLC) (ceramic pump: VSP-3050; EYELA, Kyoto, Japan).

3.2. Plant material

The stem of S. randaiense was collected at the Ilan County, in June 2012, and identified by one of the authors (I.-S.C.). A voucher specimen (Chen 6437) has been deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Taiwan.

3.3. Extraction and isolation

The dried stem of S. randaiense (1.5 kg) was extracted with MeOH (10 L × 3) at room temperature for 9 days. The MeOH extract (170 g) was partitioned with EtOAc and H_2O, which afforded EtOAc-soluble (87 g) and H_2O-soluble (83 g) layers. The EtOAc-soluble layer showed anti-inflammatory activity (Table S3). The EtOAc-soluble fraction (87 g) was subjected to a silica gel CC, eluted with a gradient of *n*-hexane/acetone (20:1), to produce 22 fractions (A1–A22). Fraction A7 (931 mg) was subjected to silica gel CC, eluting with *n*-hexane/acetone (20:1), to yield 5 (2.9 mg) and 9 (2.9 mg). Fraction A10 (1.15 g) was washed with MeOH to yield a mixture of 10 and 11 (571 mg) after recrystallisation from $CHCl_3$/MeOH. Fraction A12 (521 mg) was subjected to silica gel CC, eluting with *n*-hexane/EtOAc (5:1), to give nine fractions (A12-1–A12-9), of which fractions A12-2 (112 mg), A12-5 (135 mg) and A12-8 (57.7 mg) contained 8 (10.0 mg), 7 (3.9 mg) and 6 (14.5 mg), respectively. Fraction A12-3 (137 mg) was separated over a RP-C_{18} column, eluting with acetone/H_2O (3:1), to afford seven fractions (A12-3-1–A12-3-7). Fraction A12-3-2 (25.5 mg) was purified by MPLC (CH_2Cl_2/acetone, 80:1) to give six fractions (A12-3-2-1–A12-3-2-6). Fraction A12-3-2-4 (11.7 mg) was further purified by preparative TLC with MeOH/H_2O (2:1) to obtain 1 (7.4 mg, R_f 0.5). Fraction A13 (18.6 g) was subjected to a silica gel CC, eluted with of *n*-hexane/acetone (7:1), to produce 11 fractions (A13-1–A13-11), of which fraction A13-9 (1.40 g) contained 2 (23.7 mg), 3 (1.5 mg) and 4 (10.7 mg), respectively.

3.4. Anti-inflammatory activity

3.4.1. Anti-iNOS activity assay

RAW 264.7 cells (a transformed murine macrophage cell line), obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan), were maintained by once-weekly passage in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal calf serum and penicillin–streptomycin.

The nitrite measurement was based on our published technique (Ting et al. 2014). Cell aliquots (2×10^5 cells/well) were grown on 24-well plates for 24 h. To assess the effects on LPS-induced NO production, the compounds and a positive control, aminoguanidine (a selective iNOS inhibitor; 100 mM), or the vehicle, DMSO (0.1%), were added in the absence or presence of LPS (200 ng/mL) to the cells for another 24 h. The culture supernatant was subsequently

collected and assayed for nitrite as a reflection of NO production. Briefly, an aliquot of supernatant was mixed with an equal volume of Griess reagent (prepared by adding 1 part 0.1% naphthylethylenediamine dihydrochloride to 1 part 1% sulphanilamide in 5% H_3PO_4) and incubated at room temperature for 10 min. The absorbance at 550 nm was measured with a microplate spectrophotometer (Bio-Tek Instrument, Inc., Winooski, VT, USA). Fresh medium was used as the blank. The results are expressed as the percentage of inhibition calculated relative to the cells treated with vehicle and LPS.

3.4.2. Cell viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to measure cytotoxicity, as previously described (Wang et al. 2013). After the culture supernatant was removed for NO measurement as described above, a solution of 0.5 mg/mL MTT in DMEM was added to each well containing RAW 264.7 cells. The plates were incubated at 378C in a humidified atmosphere with 5% CO_2 for 3 h. Following incubation and removal of the medium from the wells by aspiration, 100 mL of DMSO was added to each well to dissolve the formazan crystals, and read spectrophotometrically at wavelength of 570 nm against the blank prepared from cell-free wells. The absorbance for cultures treated with LPS plus vehicle was considered to indicate 100% cell viability.

3.5. (R)-(2)-Sassarandainol (1)

Yellowish oil; $[\alpha]_D^{23}$ 2 12.88 (c = 0.37, $CHCl_3$); UV (MeOH) λ_{max} (log 1) 206 (4.16), 228 (4.25), 278 (3.50) nm; UV (MeOH + KOH) λ_{max} (log 1) 213 (4.59), 241 (4.14), 286 (3.50) nm; IR υ_{max} (KBr) 3387(OH), 1613, 1506 (aromatic ring) $cm^{2\,1}$; ESI-MS m/z 291 $[M + Na]^+$; HR-ESI-MS m/z 291.13549 $[M + Na]^+$ (calcd for $C_{18}H_{20}NaO_2$, 291.13555. ^1H NMR δ ($CDCl_3$, 400 MHz): 1.27 (3H, d, J = 6.4 Hz, H-9), 2.73 (1H, dd, J = 13.8 and 6.5 Hz, H-7b), 3.00 (1H, dd, J = 13.8 and 5.9 Hz, H-7a), 3.32 (2H, d, J = 6.6 Hz, H-7′), 4.47 (1H, m, H-8), 5.04 (1H, dd, J = 9.8 and 1.8 Hz, H-9′b), 5.06 (1H, dd, J = 16.9 and 1.8 Hz, H-9′a), 5.95 (1H, ddt, J = 16.9, 9.8 and 6.6 Hz, H-8′), 6.75 (2H, d, J = 8.6 Hz, H-3, H-5), 6.81 (2H, d, J = 9.0 Hz, H-3′, H-5′), 7.08 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.10 (2H, d, J = 8.6 Hz, H-2, H-6); ^{13}C NMR δ ($CDCl_3$, 100 MHz): 19.3 (C-9), 39.3 (C-7′), 41.7 (C-7), 75.0 (C-8), 115.1 (C-3, C-5), 115.4 (C-9′), 116.0 (C-3′, C-5′), 129.5 (C-2′, C-6′), 130.4 (C-1), 130.6 (C-2, C-6), 132.2 (C-1′), 137.8 (C-8′), 154.0 (C-4), 156.2 (C-4′).

4. Conclusions

The chemistry of the stem from S. randaiense was not studied previously. Investigation of the EtOAc-soluble layer of the stem led to the isolation of 11 compounds, including one new neolignan: (R)-(2)-sassarandainol (1). Among these isolates, g-tocopherol (5), subamolide B (7) and b-sitosterone (9) exhibited moderate iNOS inhibitory activity on nitrite production induced (%) value of 30.51, 28.68 and 16.96, respectively. This study added eight compounds, 1 and 3–9 as additional components of S. randaiense.

Supplementary material

Supplementary material relating to this paper is available online, alongside Tables S1–S3 and Figures S1–S11.

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