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A PHENOLIC GLYCOSIDE AND TRITERPENOIDS FROM *STAUNTONIA HEXAPHYLLA*

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Key Word Index—*Stauntonia hexaphylla*; Lardizabalaceae; staunoside F; phenolic glycoside; 3-hydroxy-30-norolean-12,20(29)-dien-28-oic acid; nortriterpenoids.

Abstract—From the whole plant of *Stauntonia hexaphylla*, a new phenolic glycoside, staunoside F, was isolated along with six triterpenoids: 3-hydroxy-30-norolean-12,20(29)-dien-28-oic acid, oleanolic acid, hederagenin, lupeol, betulonic acid and betulinic acid. The phenolic glycoside was characterized by 1D- and 2D-NMR techniques. The biogenetic relationship of 30-nortriterpenoids with an exocyclic double bond at C-20(29) is briefly discussed.

INTRODUCTION

The whole plants of *Stauntonia hexaphylla* Decne have been used in Chinese folk medicine as an analgesic, sedative, diuretic, etc. [1]. Previously, we reported the isolation and characterization of 12 triterpenoid glycosides including the sodium salt of a triterpenoid glycoside [2–4] and three bisepoxylignan glycosides [5]. In our further search for potential bioactive components, a new phenolic glycoside, called staunoside F (**1**), and six triterpenoids including a 30-nortriterpenoid have been isolated. The present paper deals with their isolation and structural elucidation on the basis of spectroscopic and chemical methods and the direct elucidation of the glycosidic linkage of **1** by 2D-NMR techniques. Also, the biogenetic relationship of 30-nortriterpenoids with an exocyclic C-20(29) double bond is briefly discussed.

RESULTS AND DISCUSSION

An aqueous ethanolic extract of the whole plant of *S. hexaphylla* was separated into ethyl acetate-soluble, *n*-butanol-soluble and water-soluble fractions, as described in ref. [2]. The *n*-butanol- and ethyl acetate-soluble fractions were separated by a combination of column chromatography on Sephadex LH-20, normal- and reversed-phase silica gel to give a highly polar glycoside **1** and six known triterpenoids **2**–**7**.

Compound **1** exhibited prominent quasi-molecular ion peaks in the positive ion FAB mass spectrum at *m/z* 635 $[M+Na]^+$ and 613 $[M+H]^+$ indicating a relative molecular mass of 612. Its IR showed a conjugated carboxylic ester group (1710 cm^{-1}), a carboxylic group (1690 cm^{-1}) and aromatic rings (1600, 1550 and 1510 cm^{-1}). The ^1H NMR showed two methoxy groups [δ 3.75 (3H, *s*) and 3.77 (3H, *s*)], two anomeric protons [δ 6.62 and 5.53 (*d*, $J = 8.5$ Hz)], eleven protons at δ 3.90–5.10 and furthermore two ABX-type aromatic proton systems [8.22 (*dd*, $J = 8.5$, 1.5 Hz), 8.18 (*d*, $J = 1.5$ Hz), 7.81 (*dd*, $J = 8.5$, 1.5 Hz), 7.78 (*d*, $J = 1.5$ Hz), 7.39 (*d*, $J = 8.1$ Hz), 7.15 (*d*, $J = 8.1$ Hz)]. The ^{13}C NMR spectrum (Table 1) showed two anomeric sugar carbons at δ 99.81 and 109.86. Thus, the presence of two sugar moieties and two aromatic rings (12 carbons), one carbonyl (δ 166.69) and one carboxyl (δ 172.96) carbon was deduced. The ^1H and ^{13}C long-range shift correlation indicated a correlation of the carbonyl carbon at δ 166.69 (C-7′) with the aromatic proton at δ 7.81 (H-6′) and 7.78 (H-2′) through a three-bond coupling, indicating that the carbonyl carbon was connected to the C-1′ position. All these observations suggested, that for each aromatic ring in **1** there were two possible substitution patterns [Fig. 1(a, b)]. The ^1H-^1H ROESY spectrum (Fig. 2) showed correlation cross-peaks between the anomeric proton H-1″) of one sugar and an aromatic proton at δ 7.39 (*d*, $J = 8.1$ Hz) and between the methoxyl protons at δ 3.77 (*s*) and one aromatic proton at δ 8.18 (*d*, $J = 1.5$ Hz). This indicated that the sugar moiety was linked to the *para*-position of the carbonyl group next to an aromatic ring (B-ring) through its C-1″ position and the methoxy group was connected to the *meta*-position of

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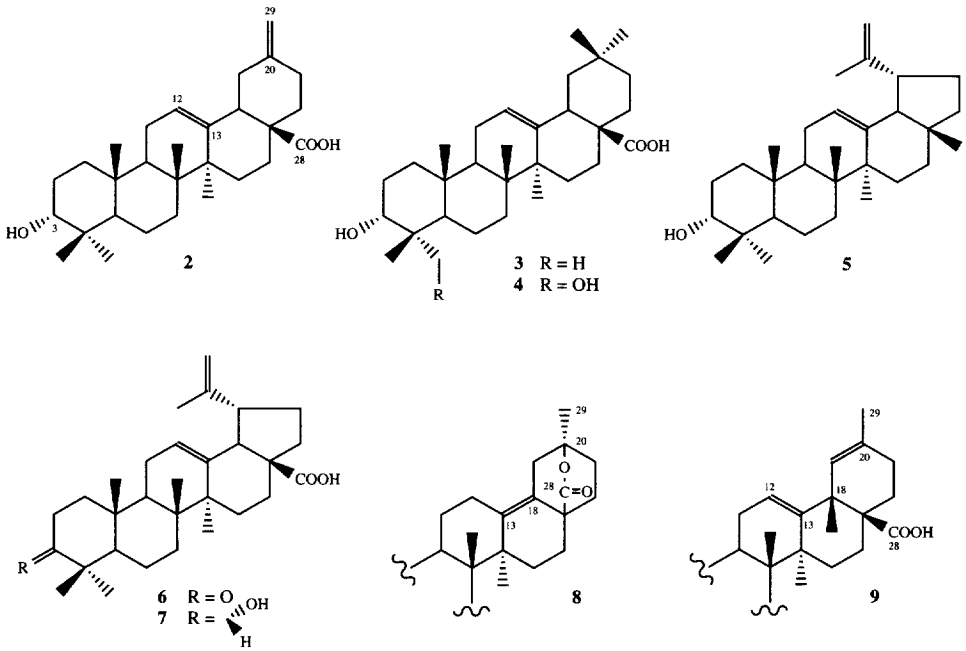

Structure 1.

the carbonyl group. Further, the correlation cross-peaks between the methoxyl proton at δ 3.75 (*s*) and one aromatic proton at δ 7.78 (*d*, J = 1.5 Hz) in ring A, showed that ring B was linked to the *para*-position of the phenolic ester bond. These details revealed the substitution patterns of rings A and B as depicted in Fig. 1(a). In the ^{1}H NMR spectrum of the sugar moiety, a pair of vicinal methine protons (δ 6.62 and 4.85, each 1 H, *br s*) and two isolated methylene groups [δ 5.06, 4.51 (2H, *ABq*, J = 9.7 Hz) and 4.92 (2H, *ABq*, J = 11.5 Hz)] suggested a pentose branched at C-3‴. A ^{1}H-^{13}C-COSY spectrum (HMBC) permitted the assignments of the carbons of the pentose residue (Table 1, Fig. 2), and the data matched those previously reported for apiose [6–9]. The correlation cross-peaks between the branching methylene protons (H-5‴) and one methine proton (H-2‴) as well as between the branching methylene protons (H-5‴b) in the ROESY spectrum, the magnitude of $J_{1'''2'''}$ in the ^{1}H NMR and the ^{13}C NMR data confirmed, that this sugar was β-D-apiose. The remaining sugar carbon signals were appropriate for a 2-linked D-glucosyl unit, which was stated by comparison of ^{13}C NMR data with those of methyl-D-glucopyranoside [10] and the reported values [9]. The linkage of the terminal apiose to the glucose was confirmed by the cross-peak observed between H-1‴ (anomeric proton) of apiose and H-2″ of glucose in ROESY spectrum. In good agreement with the above evidence, acid hydrolysis of **1** gave apiose and glucose, which were identified on TLC with authentic samples. The anomeric configuration of glucose was concluded to be β from the J value (8.5 Hz) of the H-1″ signal in the ^{1}H NMR and further supported by its carbon signals (Table 1).

The IR spectrum of compound **2** showed hydroxyl group absorption as well as bands corresponding to an olefinic and a carboxylic group. The EI mass spectrum showed the $[M]^+$ peak at m/z 440 and two ionic fragments (m/z 232 $[M\text{-}207\text{-}M]^+$.) derived by retro-Diels–Alder cleavage of the β-amyrin-Δ^{12}-skeleton [11] with a carboxylic group on C-17, which was confirmed by the peak at m/z 187 $[232\text{-}COOH]^+$. ^{1}H and ^{13}C NMR suggested that **2** is a 30-nortriterpenoid with an exocyclic C 20(29)-double bond. By comparison with known compounds [12] **2** was identified as 3-hydroxy-30-norolean-12,20(29)-dien-28-oic acid, which was isolated for the first time through its methyl ester

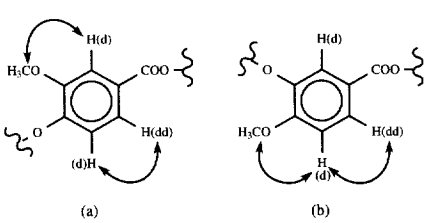

Fig. 1. Possible substitution patterns of compound **1**.

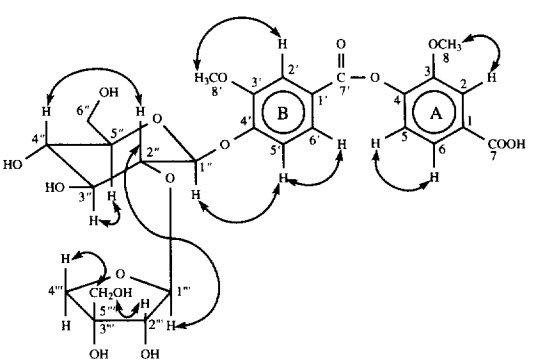

Fig. 2. ROESY correlations in compound **1**.

Table 1. ^{13}C NMR chemical shifts and HMBC data of staunoside F (1)*

C	δ [ppm]	Multiplicities (DEPT)	^1H-^{13}C Long-range connectivities (HMBC)
1	133.14	*s*	8.18 (H-2), 7.39 (H-5)
2	114.36	*d*	8.22 (H-6), 8.18 (H-2)
3	149.95	*s*	
4	148.81	*s*	8.22 (H-6), 8.18 (H-2), 7.39 (H-5)
5	114.62	*d*	
6	123.26	*d*	8.18 (H-2)
7	172.96	*s*	8.18 (H-2), 8.22 (H-6)
8	55.72	*q*	
1′	120.83	*s*	7.78 (H-2′), 7.15 (H-5′)
2′	113.32	*d*	7.81 (H-6′)
3′	148.31	*s*	7.78 (H-2′), 7.15 (H-5′)
4′	154.12	*s*	7.81 (H-6′), 7.78 (H-2′), 7.15 (H-5′)
5′	115.97	*d*	
6′	124.68	*d*	7.78 (H-2′)
7′	166.69	*s*	7.81 (H-6′), 7.78 (H-2′)
8′	55.48	*q*	
Glc-1″	99.81	*d*	4.58 (H-2″)
Glc-2″	76.61	*d*	4.43 (H-3″), 4.26 (H-4″)
Glc-3″	78.67	*d*	
Glc-4″	71.00	*d*	4.43 (H-3″)
Glc-5″	78.24	*d*	4.26 (H-4″)
Glc-6″	61.71	*t*	4.26 (H-4″)
Api-1‴	109.86	*d*	4.85 (H-2‴)
Api-2‴	78.47	*d*	4.85 (H-2‴), 5.06 (H-4‴), 4.92 (H-5‴)
Api-3‴	78.92	*s*	6.62 (H-1‴), 4.85 (H-2‴), 4.51 (H-5‴)
Api-4‴	75.05	*t*	6.62 (H-1‴), 4.85 (H-2‴), 4.51 (H-5‴)
Api-5‴	67.49	*t*	4.85 (H-2‴), 4.51 (H-5‴)

* In C_5D_5N–D_2O (25:1).
Glc: β-D-Glucopyranosyl; Api: β-D-apiopyranosyl.

obtained on treatment with diazomethane from tissue cultures of *Akebia quinata* [13]. On the basis of chemical and physicochemical evidences and by comparison with authentic samples, compounds **3**–**7** were identified as oleanolic acid, hederagenin, lupeol [14, 15], betulonic acid [16, 17] and betulinic acid [14], respectively.

Oleanolic acid (**3**) has recently been proposed as an anti-arthritic and anti-inflammatory agent [18], and betulinic acid (**7**) was recommended for a preclinical development for the treatment of melanoma [19]. These results have prompted interest in the bioactivities of 30-nortriterpenoids and their derivatives. 30-Nortriterpenoids with an exocyclic double bond at C-20(29) occur in a limited number of plants. So Wang *et al.* [12] reported 30-nortriterpenoid glycosides from *Stauntonia chinensis*. It is difficult to introduce an exocyclic double bond at C-20(29) directly by chemical synthesis, because under acidic conditions, two major products (**8** and **9**) are formed easily [12]. The complexity of synthesis, the unusual structure and potential biological activities led us to investigate their biogenesis [Fig. 3(c–g)]. From the biogenetic point of view, **f**, formed from **e** by oxidation, may be proposed as the key intermediate in the biosynthesis of 30-nortriterpenoids with an exocyclic-C-20(29)-double bond. Compound **f** was not isolated nor detected in plants, perhaps due to its instability. We hope to prove this pathway of metabolism from **e** to **g** by means of incubation of labelled **e** with a plant tissue under different conditions.

EXPERIMENTAL

General. Mps: uncorr. Optical rotations: Perkin-Elmer 240 digital polarimeter. FAB MS was recorded on a Kratos MS-50 mass spectrometer in the positive ion mode with thioglycerol as matrix. ^1H (300 and 500 MHz) and ^{13}C (75 and 125 MHz) NMR spectra were taken on Varian XL-300, and Bruker AM-500 spectrometers in C_5D_5N or C_5D_5N–D_2O (25:1) or $CDCl_3$. Chemical shifts (δ) are expressed in ppm from TMS with C_5D_5N or $CDCl_3$ as the secondary standard. CC was done with Sephadex LH-20 (Pharmacia) or silica gel 60 (70–230 and 230–400 mesh, Merck). TLC was carried out on precoated silica gel $60F_{254}$ plates (Merck). Spots were detected under UV light or by spraying with 10% H_2SO_4 followed by heating.

Plant material, extraction and isolation. For collection of plant material extraction and fractionation procedures see ref [2]. Fr. XVIII (1.42 g), obtained from the CC sepn of the crude glycosidic fr. was applied to a Sephadex LH-20 column and eluted with CH_3OH–H_2O (7:1) to give 12 frs (Fr. 1–12). Fr. 6 (276 mg) was sepd by CC on silica gel and further purified by Lobar column (Lichroprep RP-18, Merck) chromatography to afford **1** (80 mg). The combined EtOAc layers were concd to dryness, affording the EtOAc extract (57 g) (yield: 1.4%) which was sepd by CC on silica gel. Elution with $CHCl_3$ containing increasing proportions of CH_3OH gave Fr. 1–5. Each fr. was

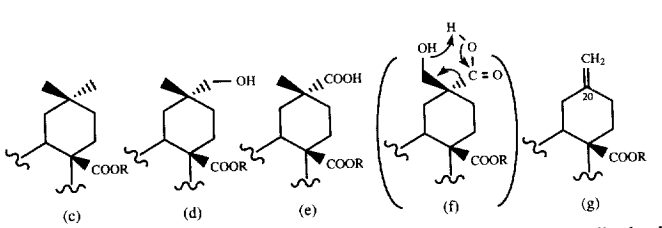

Fig. 3. Proposed biosynthesis of 30-nortriterpenoids with a C-20(29) exocyclic double bond.

sepd by CC on Sephadex LH-20 with $CHCl_3$–CH_3OH and further purified on silica gel with Hexane–EtOAc to afford **2**–**7**.

Staunoside F (**1**). Amorphous powder, $[\alpha]_D - 72.2°$ (*c* 0.88, 80% EtOH). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1710 (Ph-CO-O-), 1690 (-COOH), 1600, 1650, 1610 (arom. C=C). FAB MS (positive mode): *m/z* 635 $[M+Na]^+$, 613 $[M+H]^+$. ^1H NMR [C_5D_5N–H_2O (25:1)]: δ 3.75 (3 H, *s*, CH_3O-8), 3.77 (3 H, *s*, CH_3OH-8′), 4.00 (1 H, *m*, H-5″), 4.26 (1 H, *t*, *J* = 9.0 Hz, H-4″), 4.29 (2 H, *dd*, *J* = 12.5, 4.5 Hz, H-6″), 4.43 (1 H, *d*, *J* = 9.0 Hz, H-3″), 4.51 (1 H, *d*, *J* = 9.7 Hz, H-4‴a), 4.58 (1 H, *t*, *J* = 8.5 Hz, H-2″), 4.85 (1 H, *br s*, H-2‴), 4.92 (2 H, *dd*, *J* = 11.5 Hz, H-5‴), 5.06 (1 H, *d*, *J* = 9.7 Hz, H-4‴b), 5.53 (1 H, *d*, *J* = 8.5 Hz, H-1″), 6.62 (1 H, *br s*, H-1‴), 7.15 (1 H, *d*, *J* = 8.1 Hz, H-5), 7.39 (1 H, *d*, *J* = 8.1 Hz, H-5′), 7.78 (1 H, *d*, *J* = 1.5 Hz, H-2), 7.81 (1 H, *dd*, *J* = 8.5, 1.5 Hz, H-6), 8.18 (1 H, *d*, *J* = 1.5 Hz, H-2′), 8.22 (1 H, *dd*, *J* = 8.5, 1.5 Hz, H-6′). ^{13}C NMR: Table 1.

3-Hydroxy-30-norolean-12,20(29)-dien-28-oic acid (**2**). Needles, mp 244–245°, $[\alpha]_D + 45°$ (*c* 0.7, CH_3OH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950 (C-H), 1695 (C=O, acid), 1650 (C=C). EI MS: *m/z* (rel. int.) 440 $[M]^+$. (18), 232 (100), 207 (53), 189 (80), 187 (90). ^1H NMR (C_5D_5N): δ 0.86 (*s*), 1.00 (*s*), 1.22 (each 3 H, *s*, $-CH_3$), 3.21 (1 H, *dd*, *J* = 5.5, 14.0 Hz, H-18), 3.60 (1 H, *br s*, H-3β), 4.73 (1 H, *br s*, H-29), 4.77 (1 H, *br s*, H-29), 5.49 (1 H, *br s*, H-12). ^{13}C NMR (C_5D_5N): δ 15.60 (*q*, C-25), 17.59 (*q*, C-26), 18.80 (*t*, C-6), 22.88 (*q*, C-24), 23.92 (*t*, C-11 and C-16), 26.26 (*q*, C-27), 26.52 (*t*, C-2), 28.39 (*t*, C-15), 29.51 (*q*, C-23), 30.56 (*t*, C-22), 33.36 (*t*, C-7), 33.72 (*t*, C-1), 37.67 (*s*, C-10), 38.04 (*t*, C-21), 38.53 (*s*, C-4), 40.10 (*s*, C-8), 42.10 (*t*, C-19), 42.29 (*s*, C-14), 47.16 (*s*, C-17), 48.01 (*d*, C-18), 48.06 (*d*, C-9), 49.42 (*d*, C-5), 75.23 (*d*, C-3), 107.09 (*t*, C-29), 123.02 (*d*, C-12), 144.12 (*s*, C-13), 149.11 (*s*, C-20), 179.32 (*s*, C-28).

Determination of sugar species in **1**. A soln of **1** (1 mg) in 8% HCl–dioxane (1:1) (0.8 ml) was refluxed for 3 hr. The acid soln was diluted with H_2O and then passed through a Lewatit MP 62 column. The neutralized soln was concd *in vacuo* to dryness. The residue was compared with standard sugars on silica gel-TLC [solvent: $CHCl_3$–CH_3OH–H_2O–AcOH (13:3:3:4)], which showed glucose and apiose in **1**.

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