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Short Communication

DNA cleavage agents from *Schisandra propinqua* var. *sinensis*

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Constituents of *Schisandra propinqua* (Wall.) Bail var. *sinensis* Oliv were investigated and 8 compounds were isolated from the stems and their structures were elucidated as (+)-catechin (1), (-)-gallocatechin (2), p-(2′-hydroxyethyl)-phenol-β-D-glucopyranoside (3), daucosterol (4), manwuweizic acid (5), cerotic acid 1-monoglyceride (6), octocosoic acid (7) and β-sitosterol (8) respectively. Based on MS and NMR techniques, (+)-Catechin, (-)-gallocatechin, p-(2′-hydroxyethyl)-phenol-β-D-glucopyranoside, manwuweizic acid, cerotic acid 1-monoglyceride and octocosoic acid were isolated from the plant for the first time. (+)-Catechin and (-)-gallocatechin mediated DNA cleavage with good efficiency at 1 μg/ml. (-)-Gallocatechin show DNA strand-scission activity for the first time.

Key words: Chemical composition, *Schisandra propinqua* var*. sinensis*, DNA cleaving activity, (-)-gallocatechin, (+)-catechin.

INTRODUCTION

DNA strand breakage process is involved in various biological stages such as inflammation, mutagenesis, carcinogenesis, or aging (Mibu et al., 2003; Chen et al., 2006). As a consequence of the clinical utility of DNA cleavage agents such as bleomycin, considerable effort has been made to identify and characterize naturally occurring molecules capable of mediating DNA strand scission, as such species may serve as lead structures for the development of novel anti-tumor drugs (Ma et al., 2004; Seo et al., 2003; Fukuhara et al., 1998). *Schisandra propinqua* (Wall.) Bail var. *sinensis* Oliv (Schisandraceae) is a plant used in folk medicine to promote blood circulation and to treat fracture, chronic gastritis, rheumatoid arthritis and irregular menstruation (Yunnan provincinal crude drugs company, 1993). Previously, 3 lignans, isoschizandrolic acid, β-sitosterol and stearic acid were isolated from the plant roots and stems (Liu et al., 1998). In the course of our search for plant derived DNA cleavage agents, we isolated 2 DNA strand-nicking principles from the plant stems of *S. propinqua var. sinensis* and the results are reported herein.

MATERIALS AND METHODS

Materials

The stems of *S. propinqua var. sinensis* was collected from E-Shan county of Yunnan province, China in November, 2001 and identified by Dr. Yong-Fa Wang, a botanist of Yunnan Institute of Traditional Chinese Medicine, where a voucher specimen (No.0102035) is deposited. Silica gel (200-300 mesh) was used for column chromatography and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co., China). Solvents were of the Indus-trial purity and distilled prior to use.

Methods

MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer and NMR spectra were measured on a Bruker DRX-500 spectrometer.

Extraction and isolation

The dried powered stems of *S. propinqua var. sinensis* (9.0 kg)

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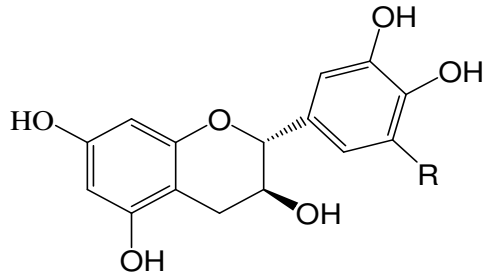
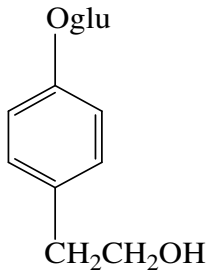
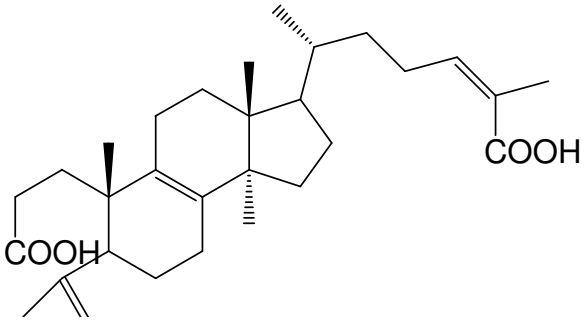


1 R= H
2 R= OH

(1) = (+)-Catechin
(2) = (-)-Gallocatechin



p-(2′-Hydroxyethyl)-phenol-β-D-glucopyranoside



Manwuweizic acid

Figure 1. Compounds from *Schisandra propinqua* var. *sinensis*.

were extracted with 95% EtOH exhaustively at room temperature. The EtOH extract was evaporated *in vacuo* to yield a dark brown residue (840 g). H_2O (2.5 l) was added to the residue and the resulting solution was extracted with chloroform, EtOAc and n-BuOH, successively. The n-BuOH extract (85 g) was applied to a silica gel column, eluting with chloroform containing increasing amounts of methanol to offer 8 fractions (A-I). Fraction B, F and G were subjected to repeated column chromatography to yield 1 g of (+)-catechin, 500 mg of (-)-gallocatechin, 9 mg of p-(2′-hydroxyethyl)-phenol-β-D-glucopyranoside and 100 mg of daucosterol (Figure 1). The EtOAc fraction (60 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc. The fractions obtained from petroleum ether- EtOAc (4:1) elution were combined and subjected to repeated column chromatography to yield 12 mg of manwuweizic acid and 200 mg of cerotic acid 1-monoglyceride. The chloroform fraction (64 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc to obtained 500 mg of octocosoic acid and 700 mg of β-sitosterol.

Identification of compounds

(+)-Catechin, white amorphous powder; ^{1}H NMR (CD_3COCD_3) δ 6.90 (1H, d, J= 1.8), 6.79 (1H, d, J = 8.1), 6.71 (1H, dd, J = 1.8, 8.1), 6.02 (1H, d, J = 2.3), 5.87 (1H, d, J = 2.3), 4.54 (1H, d, J = 7.9), 3.99 (1H, m), 2.92 (1H, dd, J = 5.5, 16.2), 2.50 (1H, dd, J = 8.2, 16.2); ^{13}C NMR (CD_3COCD_3) δ 157.0, 156.4, 156.2, 144.9, 131.4, 119.3, 114.9, 114.5, 99.8, 95.4, 94.6, 82.0, 67.6, 28.1; EIMS m/z (rel. int.): 290 (M^+, 90), 167 (15), 152 (77), 139 (100), 123 (78), 110 (23), 97 (1), 85 (2), 69 (14).

(-)-Gallocatechin, white amorphous powder; ^{1}H NMR (CD_3COCD_3) δ 8.15 (1H, s, OH), 7.98 (1H, s, OH), 7.82 (2H, s, OH), 7.25 (H, s, OH), 6.45 (2H, s), 6.01 (H, d, J = 2.3), 5.87 (1H, d, J = 2.3), 4.51 (1H, d, J = 7.5), 3.90 (1H, m), 2.86 (1H, dd, J = 5.4, 16.1), 2.50 (1H, dd, J = 8.2, 16.1); ^{13}C NMR (CD_3COCD_3) δ 156.8, 156.4, 156.0, 145.4, 132.4, 130.7, 106.4, 99.8, 95.3, 94.6, 81.9, 67.5, 27.6; EIMS m/z (rel. int.): 306 (M^+, 10), 168 (26) , 139 (100), 126 (24), 110 (13), 98 (16), 83 (15), 69 (23), 55 (29).

p-(2′-Hydroxyethyl)-phenol-β-D-glucopyranoside, white amorphous powder; ^{1}H NMR (CD_3OD) δ 7.17 (2H, dd, J= 6.7, 2.1 Hz), 7.04 (2H, dd, J= 6.7, 2.1 Hz), 4.87 (1H, d, J= 7.4 Hz), 4.67 (2H, t, J= 7.0 Hz), 3.88 (1H, dd, J= 12.1, 2.1 Hz), 3.68 (1H, dd, J= 12.1, 5.5 Hz), 3.41 (4H, m), 3.21 (2H, t, J= 7.0 Hz); ^{13}C NMR (CD_3OD) δ 77.5 (C-1), 33.8 (C-2), 131.7 (C-1'), 130.7 (C-2', 6'), 118.0 (C-3', 5'), 158.3 (C-4'), 102.4 (C-1''), 78.1 (C-2''), 78.0 (C-3''), 74.9 (C-4''), 71.4 (C-5''), 62.5 (C-6''); EIMS m/z 167 (70), 149 (17), 120 (100), 107 (21), 91 (29), 77 (16).

Daucosterol, white amorphous powder, mp 296-298℃; FABMS m/z 577 $[M+H]^+$; Identified by mixed melting point, co-TLC and comparison of ^{1}H and ^{13}C NMR spectrum with that of authentic sample.

Manwuweizic acid (5), white amorphous powder; ^{1}H NMR (CD_3OD) δ 5.96 (1H, t, J= 7.6 Hz, H-24), 4.92, 4.71 (each 1H, s, H-28), 1.87 (3H, d, J= 1.0 Hz, H-27), 1.78 (3H, s, H-29), 0.98 (3H, s, H-19), 0.97 (3H, s, H-30), 0.95 (3H, d, J= 6.4 Hz, H-21), 0.79 (3H, s, H-18); ^{13}C NMR (CD_3OD) δ 25.3 (C-1), 22.8 (C-2), 178.1 (C-3), 148.7 (C-4), 48.3 (C-5), 32.1 (C-6), 30.5 (C-7), 130.8 (C-8), 140.5 (C-9), 41.5 (C-10), 37.0 (C-11), 33.9 (C-12), 45.6 (C-13), 52.0 (C-14), 32.4 (C-15), 29.0 (C-16), 51.6 (C-17), 16.5 (C-18), 21.0 (C-19), 37.6 (C-20), 19.1 (C-21), 27.5 (C-22), 27.0 (C-23), 144.1 (C-24), 128.5 (C-25), 171.7 (C-26), 21.0 (C-27), 114.4 (C-28), 23.4 (C-29), 25.6 (C-30); EIMS m/z 470 (M^+, 46), 455 (14), 397 (100), 261 (7), 235 (9), 161 (15), 149 (16), 119 (20), 107 (23), 95 (37), 69 (21).

Cerotic acid 1-monoglyceride, white amorphous powder; ^{1}H NMR ($CDCl_3$) δ 4.16 (2H, m, H-1), 3.91 (1H, m, H-2), 3.68 (1H, dd, J= 11.1, 5.0 Hz, H-3), 3.58(1H, dd, J= 11.1, 4.5 Hz, H-3), 2.32 (2H, t, J= 7.5 Hz, H-2'), 1.61(2H, m, H-3'), 0.87 (3H, t, J= 6.7 Hz, H-26'); EIMS m/z 470 (M^+, 2), 452 (M^+-H_2O, 1), 442 (2), 424 (5), 411 (15), 396 (16), 368 (25), 351 (33), 340 (16), 323 (21), 294 (6), 134 (56), 112 (45), 98 (71), 57 (100).

Octocosoic acid, white amorphous powder; ^{1}H NMR ($CDCl_3$) δ 2.39 (2H, *t*, J= 7.5 Hz, H-2), 1.67 (2H, *m*, H-3), 1.30 (48H, *br s*, H-4~ H-27), 0.93(3H, t, J= 7.0Hz, H-28); EIMS *m/z* 424 (28, M^+), 396 (100), 368 (94), 354 (14), 297 (4), 241 (3), 185 (6), 129 (30), 73 (39), 57 (48).

β-Sitosterol (8), white needles, mp 136 - 137℃; EIMS m/z 414 (M^+); Identified by mixed melting point, co-TLC and comparison of ^{1}H and ^{13}C NMR spectrum spectrum with that of authentic sample.

DNA cleavage assay

The DNA strand-scission assay was performed based on a modified

Hecht procedure (Deng et al., 2000; Huang et al., 1996). Compounds were dissolved in DMSO-MeOH (1:1); 1 µl of each sample was added to a 13 µl reaction mixture (total volume) containing 600 ng of PBR 322 DNA and 20 µM $CuCl_2$ in 10 mM tris-HCl, pH 8.0. The reac-tions were incubated at 37°C for 1 h, terminated by addition of 2 µl of 0.125% bromophenol blue in 30% gly-cerol and applied to a 1% agarose gel containing 0.7 µg/ ml ethidium bromide. The gel was run in 89 mM Tris containing 8.9 mM boric acid and 2.0 mM Na_2-EDTA at 110 V for 1 h, then visualized by UV irradiation. Agarose gels were quantified for percent DNA cleavage utilizing 1D Image Analysis software, Windows Extra Package, Version 3.5.

RESULTS AND DISCUSSION

The stems of *S. propinqua* var. *sinensis* was extracted with 95% EtOH and then fractionated into chloroform, ethyl acetate and n-butanol soluble fractions successively. The n-butanol fraction was subjected to repeated column chromatography on silica gel to yield (+)-catechin, (-)-gallocatechin, p-(2′-hydroxyethyl)-phenol-β-D- glucopyranoside and daucosterol identified by spectroscopic analysis (NMR and MS), literature comparison and comparison with authentic samples (Nonaka et al., 1977, 1981; Zhang et al., 1995; Tanahashi et al., 1997). The ethyl acetate fraction was subjected to repeated column chromatography on silica gel to yield manwuweizic acid and cerotic acid 1-monoglyceride and the chloroform fraction obtained octocosoic acid and β-sitosterol by similar chromatography (Liu et al., 1988; Chen et al., 2001).

(+)-Catechin, (-)-gallocatechin and p-(2′-hydroxyethyl)-phenol-β-D-glucopyranoside and manwuweizic acid, cerotic acid 1-monoglyceride and octocosoic acid were isolated from the plant for the first time.

In view of the complete DNA cleaving activity of n-butanol fraction at 100 µg/ml in the presence of 20 µM Cu^{2+} in initial bioassay test, (+)-catechin, (-)-gallocatechin and p-(2′-hydroxyethyl)-phenol-β-D-glucopyranoside were tested for their ability to relax PBR 322 plasmid DNA, a supercoiled, covalently closed, circular DNA in the presence of Cu^{2+}, using a cell free DNA cleavage assay (Deng et al., 2000). (+)-Catechin, showed 52% relaxation of supercoiled DNA (Form I) to nicked DNA (Form II) at 1 µg/ml in the presence of 20 µM Cu^{2+}, while (-)-gallocatechin was found to relax 39% of form I DNA to form II DNA at 1 µg/ml in the presence of 20 µM Cu^{2+}. p-(2′-Hydroxyethyl)-phenol-β-D-glucopyranoside showed no activity. (-)-Gallocatechin was reported to show DNA strand-scission activity for the first time.

The results of this study provided some understanding on the chemical constituents of *S. propinqua* var. *sinensis* and its use in folk medicine to treat fracture, chronic gastritis and rheumatoid arthritis.

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