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An Acidic Polysaccharide from *Tribulus terrestris*

Hai Sheng CHEN[1]*, Wing Nang LEUNG[2] , Yi Xin XU[1]

[1]Collage of Pharmacy, Second Military Medical University, Shanghai 200433
[2]Department of Biology, Hong Kong Baptist University, Hong Kong

Abstract: An aqueous acidic polysaccharide, named rhamnogalacturonan (designated as TTP-D_2) was isolated from *Tribulus terrestris* L by means of DEAE-cellulose chromatography and gel filtration. The molecular mass of TTP-D_2 was estimated to be 26 KDa by gel filtration. TTP-D2 is composed of galacturonic acid, rhamnose, arabinose, galactose, fucose, mannose, xylose and glucose in a ratio of 71.4 : 13.5 : 5.6 : 4.9 : 3.1 : 1.9 : 1.9 : 1.0. The main chain structure of TTP-D_2 was elucidated as an acidic hetero-polysaccharide with the connection of α-(1-4) galacturonic acid with α-(1-3) rhamnose by GC analysis of partially hydrolyzed products and the determination of ^{1}H, ^{13}C-NMR spectra.

Keywords: *Tribulus terrestris L.*, rhamnogalacturonan, structural characterization.

Tribulus terrestris L. is a traditional Chinese medicine. It has been used to treat dizziness, headache, hypertensive and nettle rash. The results of recent research indicated that the crude steroidal saponins from *T. terrestris* had significant bioactivities in the treatment of cardiovascular and cerebrovascular disease, longevity and improving the sexual function. We have reported a new derivative of cinnamicamide, two new steroidal sapogenins and two new saponins [1-3] isolated from *T. Terrestris.* Now we report the isolation and purification of an aqueous acidic polysaccharide (TTP-D_2) from *T. Terrestris.*

Experimental

The NMR spectra with Varian INOVA 500; JNM-GX 270 FT. Optical rotation was measured with JASCO DIP-1000. GC was carried out on HP 6890. IR was obtained with Nicolet FTIR-550.

Tribulus terrestris L. was collected from Anyang district in Henan province, and identified by Professor Zheng Han-Chen, Dept. of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai.

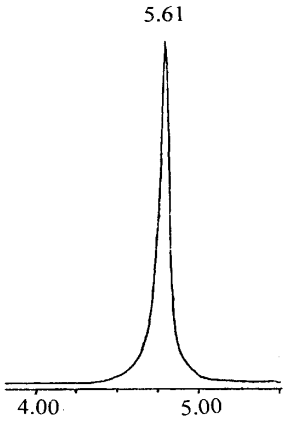
The stems and leaves (1000g) of *T. terrestris* were cut into fine pieces and extracted with 70% ethanol for three times to remove lipid substance. The residue was extracted with water at 100°C. The extract was concentrated under vacuum and 95% ethanol was added to obtain the crude polysaccharides (70g). The crude polysaccharide

(40g) was dissolved in 800 mL of hot water. Then 2400 mL of 95% ethanol was added to obtain the yellowish crude polysaccharide 23 g (TTP). TTP (11 g) was dissolved in water (100 mL) and the solution was applied to a column (4 × 30 cm) of DEAE-cellulose DE22 (OAc^-), eluting with H_2O; 0.1 mol/L ; 0.2 mol/L and 0.5 mol/L NaOAc to obtain fractions TTP-A, B, C and D, respectively. Fraction D was dissolved in water and chromatographied on a column of Sephadex G-75 (2.5 × 70 cm) and eluted with H_2O, collected fractionally (1 mL/min., 10 mL/tube), detected by colorimetry (Ph-OH/ H_2SO_4). The fractions 11-13 were combined and lyophilized to obtain TTP-D1 and fractions 26-29 to obtain TTP-D_2.

Purification of TT-D_2

TTP-D_2 was purified by HPLC with TSK G2000SW (7.5×300 mm, Pharmacia, LKB) column, eluting with H_2O (flow rate: 1 mL/min; UV λ=190 nm). The purity of TTP-D_2 was shown in **Figure 1**.

Figure 1 HPLC of TT-D_2



The molecular weight of TTP-D_2 was estimated by comparison with the authentic dextrans T_{10}, T_{40}, T_{70}, T_{90} (Pharmacia) on HPLC. The molecular weight was calculated based on the logarithm of Y=8.533-0.665X (r = -0.9874).

Analysis of monosaccharide composition of TTP-D2

TTP-D2 (5 mg) was hydrolyzed completely with 1 mol/L H_2SO_4 (2 mL) in a sealed tube at 100°C for 4 h. The hydrolyzed monosaccharides were converted into the corresponding alditol acetates and analyzed by GC for neutral sugars [4]. The authentic monosaccharides (Rha. Fuc. Ara. Xyl. Man. Glu. Gal.) were dealed with the same as

above. The conditions of GC were: capillary column (carbowax 1.53 m×30 m, film thickness 1.33 μm); the flow rate of He 3.1 mL per min.; H_2 35 mL per min.; air 300 mL per min.; the temperature of the column 210°C (keep 0.5 min.) →220°C → 230°C; the injector entrance temperature 250°C; the detector temperature 270°C, and the detector FID. All the above monosaccharides could be detected by GC, except galacturonic acid, which was estimated according to reference 5.

TTP-D_2 (10 mg) was partially hydrolyzed with 0.25 mol/L H_2SO_4 (4 mL) in a sealed tube at 100°C for 1h. Then, five volumes of absolute ethanol was added into the hydrolyzed solution to get the precipitate which was determinated by HPLC with TSK G2000SW (7.5×300 mm, Pharmacia, LKB) column, eluting with H_2O (flow rate : 1 mL/min; UV λ=190 nm). After that, the precipitate was hydrolyzed completely with 1 mol/L H_2SO_4 (2 mL) in a sealed tube at 100°C for 4h and analyzed by GC according to reference 4.

Results

TTP-D_2 gave a single peak on HPLC which indicated that TTP-D_2 was a homogeneous component which was consisted of D-galacturonic acid, L-rhamnose, L-arabinose, D-galactose, D-fucose, D-mannose, D-xylose, D-glucose in a ratio of 71.4: 13.5: 5.6: 4.9: 3.1: 1.9: 1.9: 1.0 by GC analysis in **Table 1** and photocolorimetry determination of uronic acid. The molecular mass of TTP-D_2 was estimated to be 2.6 x 10^4 Dalton by comparison of the analytical gel filtration chromatography on HPLC with authentic dextrans. $[\alpha]_D^{24.2}$ of TTP-D_2 was -439.78 (c=0.0667, H_2O).

Table 1 GC analysis of hydrolyzates of TTP-D_2 (min.)

	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
standard sugars	2.673	3.030	3.308	3.509	5.506	5.907	6.597
TTP-D2	2.661	3.208	3.292	3.486	5.497	5.872	6.564

TTP-D_2 was hydrolyzed partially (0.25 mol/L H_2SO_4, 100°C for 1 h), then poured into five volumes absolute ethanol. The precipitate was centrifuged and detected by HPLC . One peak was shown on HPLC and its molecular mass was 10 KD. The ^{1}H-NMR spectrum of the precipitate showed two anomeric proton signals: δ 5.14 (d, J=1.5Hz, anomeric proton of galacturonic acid) and δ 5.24 (d, J=1.6Hz, anomeric proton of rhamnose) and the ratio of peak area was about 3 :1, which indicated that the main chain of TTP-D_2 was composed of galacturonic acid and rhamnose.

The ^{1}H-NMR of TTP-D2 showed a methoxy signal at δ 3.794 (s), two methyl signals at δ 1.244 (d, J=7.2Hz) which were attributed to the methyl of rhamnose and fucose and five anomeric proton signals at δ 5.140 (d, J=1.5Hz), δ 5.244 (d, J=1.6Hz), δ 4.884 (d, J=1.3Hz), δ 4.744 (d, J=1.5Hz), δ 4.948 (d, J=1.1Hz) represented the α-configurations of the five sugars.

The ^{13}C-NMR of TTP-D_2 showed the signals of OCH_3 and several main

saccharides in **Table 2**. In **Table 2** δ 101.69 of C_1 of galacturonic acid indicated a α-linked bound which was different from the value of β-linked bound (δ 105 ppm). The chemical shift of C_4 of D-galacturonic acid showed about 6.0 ppm upfield shift which indicated that the C_4-OH was connected with another sugar. Meanwhile, δ 102.74 of C_1 of rhamnose belonged to α-linked bound and the chemical shift of C_3 of L-rhamnose (δ 81.45) indicated that the C_3-OH was connected with another sugar.

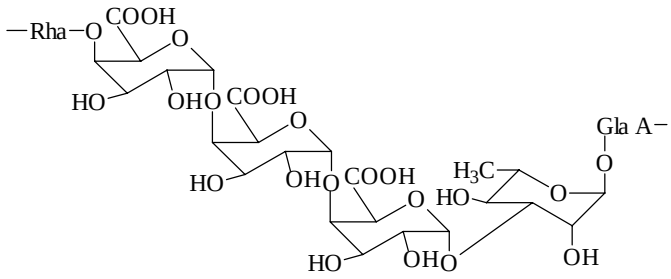
Table 2 ^{13}C-NMR data of TTP-D_2 (D_2O, ppm)

sugars	C_1	C_2	C_3	C_4	C_5	C_6
Gal A	101.69	70.71	71.45	80.74	74.05	177.66
Rha	102.74	70.71	81.45	73.31	68.66	19.34

The derivatives of aditols acetates of monosaccharides from TTP-D_2 after completely hydrolyzing were analyzed by GC-MS. The result showed that the fragments: *m/z* 315 (M^+), 314 (M-1) belonging to the alditol acetate derivatives of penta-carbon sugar; *m/z* 329 (M^+), 328 (M-1) belonging to 6-deoxy sugar; *m/z* 387 (M^+), 386 (M-1) belonging to hex-carbon sugar, which indicated that the hydroxyls of the saccharides were not methylated. The signal of OCH_3 (3.79 ppm) in ^{1}H-NMR spectrum could be from the $COOCH_3$ indicating that partial of COOH was methylated.

Based on the above results, it was suggested that the minimal repeating unit of the main chain of TTP-D2 was composed of L-rhamnose α (1-4) D-galacturonic acid α (1-4) D- galacturonic acid α (1-4) D- galacturonic acid α (1-3) L- rhamnose α (1-4) D-galacturonic acid as shown in **Scheme 1**.

Scheme 1 Repeating unit of main chain of TTP-D_2



References

1. Y. J. Ren, H. S. Chen, G. J. Yang, *Acta Pharm Sini.* , **1994,** *29* (3),204.
2. Y. X. Xu, H. S. Chen, H. Q. Liang, *Phytochemistry*, **1998**, *49* (1), 199.
3. Y. X. Xu, H. S. Chen, H. Q. Liang, *Planta Med.* , **2000,** *66* (6), 545.
4. T. L. Li, C. X. Wu, Y. X. Zhang, *Chin J Anal Chem.* , **1982,** *10* (5), 272.
5. M.Y. Tian., Z. Q. Zhang, *Prog Biochem Biophys.* , **1987,** *6*, 67.

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