According to recent recommendations, the rotation speed of the paddle during the test for oral suspensions should be 25 rpm, increasing up to 50 or 75 rpm for high viscosity products (Siewert et al. 2003). When the agitation rate was reduced to 50 rpm this had no major effect on the release rate from paste B, but influenced the dissolution profile of product A significantly. In addition the reproducibility of the results diminished. For example the amount of ivermectin released from paste A after 30 min was $42.0 \pm 29.$ 9% in comparison to $61.5 \pm 9.9\%$ at the higher agitation. Thus rotation speed of 75 rpm should be considered appropriate and should not be increased if the distinguishing power of the test between different preparations is to be achieved (Siewert et al. 2003).

## Experimental

### *1. Materials*

Oral pastes for horses from two different manufacturers were used containing 10 mg/g (A) or 20 mg/g (B) of ivermectin, respectively. The quantitative assay for ivermectin was performed and the content was 104.4–107.7% of the declared concentration.

### *2. Dissolution test*

The test was performed with a Ph.Eur. paddle apparatus (Pharma Test, Heinburg, Germany), at agitations of 75 rpm or 50 rpm, at $37 \pm 0.5\,^{\circ}C$. Sodium lauryl sulphate (BDH Chemicals, Poole, England) 0.5% (w/v) aqueous solution was used as a dissolution medium (900 ml). The paste (4 g of A or 2 g B) was placed into the vessel with a syringe with canule (A) or dropping the sample at the surface of the dissolution medium (B). After the time intervals (15, 30, 45, 60 min and 2 h), a sample of the dissolution medium was withdrawn (2 ml), filtered through a glass filter and analysed.

### *3. HPLC analysis*

The determination of ivermectin was performed with a modified HPLC method as described in Ph.Eur. The analysed solutions were diluted with methanol (1 : 10). The HPLC system consisted of octadecyl column ($250 \times 4$ mm; 5 μm; Lichrospher RP-18; Merck), an integrator D-12500 A, a detector UV-Vis L-4250, pump L-6200A (Merck-Hitachi, Darmstadt, Germany). A mixture of acetonitrile : methanol : water (64 : 24 : 16) was used as the mobile phase with a flow rate 1.9 ml/min. The volume of the injected sample was 20 μl and detection was performed at 254 nm.

### *4. Solubility studies*

An access of the substance was shaken in tightly closed glass flasks with a suitable solvent (Table 1) for 20 h at $37 \pm 0.1\,^{\circ}C$. The suspensions were filtered and the filtrate, after dilution in methanol, was analysed by HPLC.

### *5. Stability in acidic solution*

Solutions of ivermectin in HCl 0.1 M were stored in tightly closed glass vials at 20 °C and 37 °C. Chromatograms of the solutions were recorded at $t = 0$ and after 1, 2 and 6 h. The area of the ivermectin (retention time 10.5 min) and additional peaks were calculated.

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## Chemical properties of a mannoglucan from *Cistanche deserticola*

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A new mannoglucan from *Cistanche deserticola* from China is characterized. The compound is responsible for a mild stimulation of mitogen-induced T and B lymphocyte proliferation.

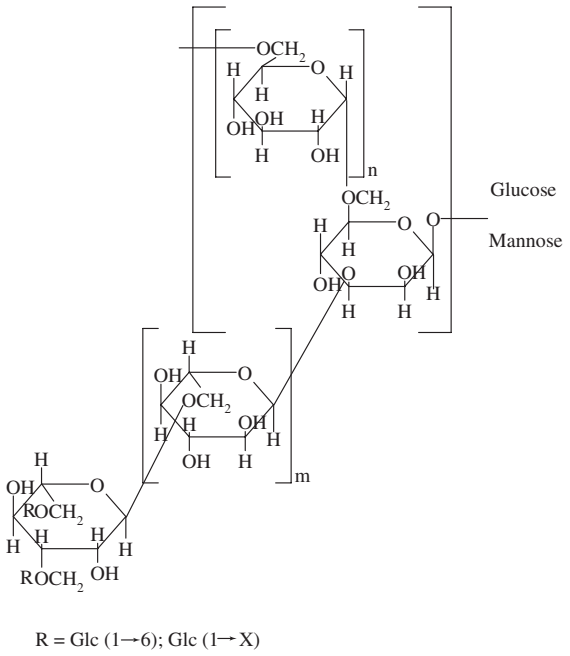
*Cistanche deserticola* Y. C. Ma is a holoparasite growing on the roots of the dicotyledonous plant *Haloxylon ammodendron* Bunge which is widely distributed in the northwest of China. As it is an important tonic in oriental medicine, many active components and pharmacological activities have been claimed (Karasawa et al. 1986; Yoshizawa et al. 1990). These properties might be associated with some of the polysaccharide components of *Cistanche* which have not yet been investigated. Mitogenic pectic polysaccharides and neutral polysaccharides have been isolated and characterized from *Cistanche deserticola* from Mongolia (Radna et al. 1996; Anna et al. 1997; Bozena et al. 1999). Here we report the chemical properties of a new mannoglucan from *Cistanche deserticola* from China, which is responsible for mild stimulation of mitogen-induced T and B lymphocyte proliferation.

After the stems of *Cistanche deserticola* Y. C. Ma were extracted with 95% ethanol, cold distilled water was used to extract the residue. The cold water extract was precipitated with EtOH, protein was removed and dialyzed, obtaining the crude polysaccharide CCDP. The crude polysaccharide was fractionated on a DEAE column and a Sephadex G-150 column to afford a protein-free polysaccharide (CDP-6), which comprised glucose (89.2%) and mannose (10.4%), and of which the average $M_r$ was estimated to be $6.8 \times 10^4$. The polysaccharide CDP-6 showed $[\alpha]_D^{20} + 42^{\circ}$ (c 1, $H_2O$). The absolute configurations of the sugars were determined by GC of TMSi (−)-2-butylglycosides. CDP-6 was methylated (Needs et al. 1993), hydrolyzed, converted to the alditol acetates, and analyzed by

**Table: Methylation analysis of CDP-6 mannoglucan**

| Methylated alditol acetate derivatives | Molar ratio[a] CDP-6 | CDP-6-1 | CDP-6-12 |
|---|---|---|---|
| 2,3,4-tri-OMe Glc | 22.8 | 20.1 | 4.1 |
| 2,3,4,6-di-OMe Glc | 0.3 | 3.7 | 1.0 |
| 2,4-di-OMe Man | 2.3 | 1.2 | 0.8 |
| 2,3,4-di-OMe Man | | 0.9 | |

[a]Calculated from peak areas and molecular weight of derivatives




Proposed structure of CDP-6 mannoglucan

GC and GC-MS (Table). This result together with molar ratio data from methylation analysis suggested that CDP-6 has a predominantly 1,6-linked Glc*p* and a backbone 1,6-linked to a smaller extent branched at O-3 of mannose. The methylation analysis of two polymers (CDP-6-1, CDP-6–2) from partial hydrolysis confirmed that CDP-6 has a backbone consisting of 1,6-linked glucosyl residues and 1,6-linked mannosyl residues, which are substituted at O-3 of mannose by branches consisting of terminal, 1,6-linked glucosyl residues and 1,3,6-linked mannosyl residues. According to molar ratios and the literature (Bozena et al. 1999; Bacon et al. 1996), the $^{13}$C NMR spectrum of CDP-6 showed signals due to anomeric carbons of the α-D-Glc*p* (C-1 at 98.4 ppm), β-D-Man*p* (C-1 at 104.3–104.9 ppm) and α-D-Man*p* (C-1 at 100.0 ppm). The $^{1}$H NMR spectrum of CDP-6-2 in $D_2O$ showed proton signals corresponding to the α-D-Glc*p* (H-1 at 4.94–4.95 ppm) and α-D-Man*p* (H-1 at 4.96–5.00 ppm). The $^{13}$C NMR spectrum of CDP-6-2 in $D_2O$ showed proton signals corresponding to the α-D-Glc*p* (C-1 at 98.6 ppm) and α-D-Man*p* (C-1 at 98.4ppm).

Mannoglucans are reported to have a 1,4-linked glucopyrannan and 1,4-linked mannopyrannan backbone (Radjabi-Nassab et al. 1984; Tanabe et al. 2000). CDP-6 has some different structural features by its α-1,6-linked Glc*p* and β-1,6-linked Man*p* backbone, and its substituted by mannoglucan branches (Fig.).

In order to investigate the use of *Cistanche deserticola* in folk medicine, the effect of CDP-6 on the T and B cell system was evaluated. It was found to mildly stimulate mitogen-induced T and B lymphocyte proliferation.

## Experimental

### *1. General procedures*

Optical rotation was measured with a W22-1S automatic polarimeter. IR spectra were determined on a Perkin-Elmer 599B spectrometer. GC analysis was performed on an Agilent HP6890N instrument, equipped with a FID detector. GC-MS was performed on an Finnigan Trace GC-MS instrument. Homogeneity and $M_r$ of CDP-6 were estimated from the calibration curve of the elution volume of standard dextrans, which was performed on a Agilent 1100 series apparatus with a Shodex KS-805 column.$^{1}$H NMR and $^{13}$C NMR spectra were recorded with an INOVA-500 instrument. Protein content was analyzed by the method of Bradford (Andre et al. 1975). Monosaccharides were analyzed as their alditol acetate after they had been hydrolysed (2 M TFA, 2 h, 110 °C), reduced and acetated. The hydrolysates were analyzed using TLC on a silica gel plate containing 5% sodium dihydrogen phosphate in BuOH-EtOAc-isopropyl alcohol-HOAC–$H_2O$-pyridine (3.5 : 10 : 6 : 3.5 : 3 : 3) and sugars were detected by spraying with orcinol-$H_2SO_4$. The resulting alditol acetates were analyzed by GLC using a HP-5 capillary column (30 m 0.32 mm). CDP-6 was methylated by the Ciucanu method, followed by hydrolysis, and was analyzed as partially methylated alditol acetates by GC-MS. After partial hydrolysis, the products were dialyzed and separated, obtaining two polymers, CDP-6-1 and CDP-6–2.

### *2. Plant material*

The plant was collected from Inner Mongolia Province in China in August 2001 and was identified by Hu-Biao Chen, Department of Natural Medicine, School of Pharmaceutical Sciences, Peking University Health Science Center. A voucher specimen [E-1-(9)] of this plant was deposited at the School of Pharmaceutical Sciences, Peking University.

### *3. Extraction, isolation and purification of polysaccharide CDP-6*

The stems of *Cistanche deserticola* Y. C. Ma (1500 g) were extracted with 95% ethanol (6 L) under reflux. The dried ethanol-insoluble residue was macerated three times with cold distilled water per 12 h. The cold water extract was filtered and the filtrate centrifuged. After precipitation with four volumes of EtOH (stirring and standing for 24 h at 4 °C), the precipitate product (35.5 g) was obtained. The protein in the precipitate was removed by Savage's method. After dissolution in distilled water, the residue (25.5 g) was dialyzed and lyophilized (yield: 10.6 g). The crude polysaccharide (8 g) was dissolved in distilled water (100 mL, 8%w/v) and separated using a DEAE column (70 × 5 cm, gradient of 0–2 M NaCl, per 1.5 L). A water eluted fraction (600 mg) was obtained. A sample (250 mg) was purified by column gel-permeation chromatography on a Sephadex G-150 column (4 × 80 cm, 1600 mL, 5 mL per fraction). Fractions of CDP-6 (1500–1600 mL) were pooled, dialyzed and freeze-dried. After desalted by Sephadex G-25 (60 × 1 cm). CDP-6 (110 mg) was obtained and determined as a single peak by HPSGC.

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