Radix Aconiti Praeparata

Figure 1 A photograph of Radix Aconiti Praeparata

1. NAMES

Official Name: Radix Aconiti Praeparata

Chinese Name: 製川烏

Chinese Phonetic Name: Zhichuanwu

2. SOURCE

Radix Aconiti Praeparata is the dried and processed tuberous parent root of *Aconitum carmichaeli* Debx. (Ranunculaceae). The parent root is collected in the summer, then dried to obtain Radix Aconiti. Clean Radix Aconiti is first graded according to the size, then macerated in water until there is no more dry core, then taken out, boiled in water for 4 to 6 hours (or steamed for 6 to 8 hours) until there is no more white core in the relatively large and solid root, and the taste becomes slightly tongue-numbing. After removal from the boiling water, it is air-dried as appropriate, cut into slices, and dried to obtain Radix Aconiti Praeparata.

3. DESCRIPTION

Radix Aconiti Praeparata consists of irregular or elongated triangular slices. The outer part dark brown or yellowish-brown, the inner part with a greyish-brown cambium ring. Texture light and fragile, fracture lustrous. Odourless; the taste slightly tongue-numbing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour greyish-yellow. Stone cells nearly colourless or pale yellowish-green, subrectangular, subsquare, polygonal, or oblique at one side, 36-125 μm in diameter, 75-204 μm long, walls 5-17 μm thick, striations of the thick-walled stone cells distinct, with relatively sparse pits. Cells of metaderm brown, some with walls irregularly thickened into a bulge and intruding into the lumen. Starch grains mostly gelatinized, but ungelatinized ones occasionally visible. Bordered

pitted vessels pale yellow, 18-67 μm in diameter, the ends truncate or shortly pointed, perforated at the end or the lateral walls, some vessel elements thick and short, tortuous or connected in a crisscross pattern (Fig. 2).

4.2 Physicochemical Identification

Reagent

Potassium iodobismuthate solution R_1

Dissolve 0.85 g of bismuth subnitrate in 10 mL of glacial acetic acid and 40 mL of water, then add 20 mL of aqueous potassium iodide solution (40%, w/v).

Procedure

Weigh 2.0 g of the powdered sample and put into a test tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Transfer the solution to a test tube. Add 2 drops of potassium iodobismuthate solution R_1. An orange or orangish-brown precipitate is observed.

4.3 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solution

Benzoylmesaconine standard solution

Weigh 1.0 mg of benzoylmesaconine CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, ethyl acetate and diethylamine (6:4:0.5, v/v).

Spray reagent

Solution A

Add 2 mL of hydrogen hexachloroplatinate (IV) solution to 48 mL of water.

Solution B

Weigh 3.0 g of potassium iodide and dissolve in 50 mL of water.

Spray reagent

Mix 50 mL of solution A and 50 mL of solution B. Freshly prepare the reagent.

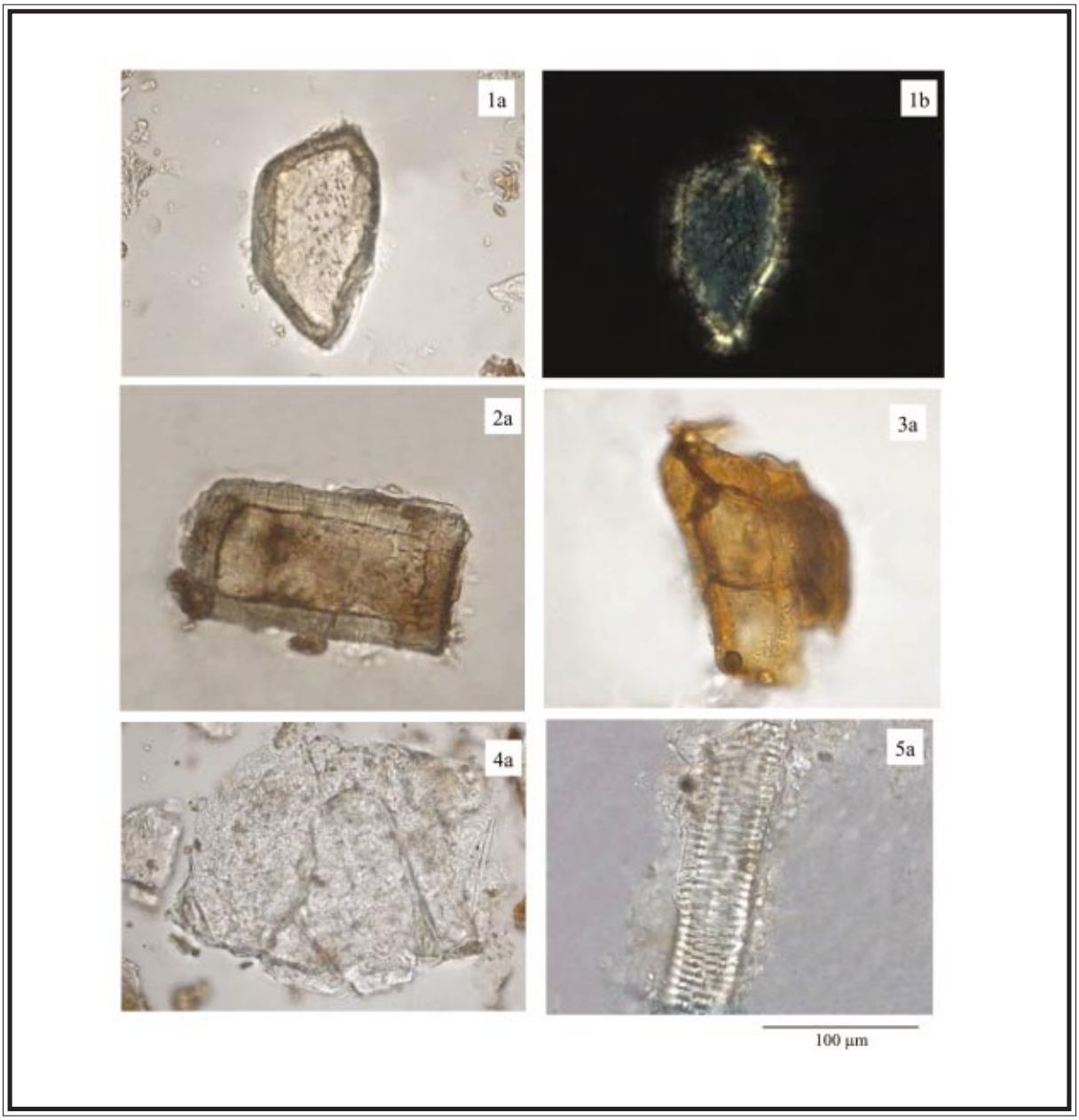

Figure 2 Microscopic features of powder of Radix Aconiti Praeparata

1. Polygonal stone cell 2. Subrectangular stone cell 3. Cells of metaderm 4. Gelatinized starch grains 5. Vessel

a. Features under the light microscope b. Features under the polarized microscope

Test solution

Weigh 3.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 2 mL of ammonium solution and 12 mL of dichloromethane. Sonicate (490 W) the mixture for 2 h. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to another tube. Evaporate the solvent to dryness with a gentle stream of nitrogen. Dissolve the residue in 1 mL of dichloromethane.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} and a freshly prepared developing solvent system as described above. Apply separately benzoylmesaconine standard solution (5 µL) and the test solution (3 µL) to the plate. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of benzoylmesaconine.

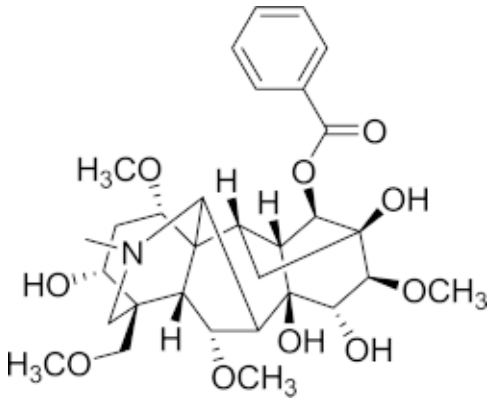

Figure 4 Chemical structure of benzoylmesaconine

4.4 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

Benzoylmesaconine standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of benzoylmesaconine CRS and dissolve in 50 mL of 0.01 M hydrochloric acid.

Test solution

Weigh 0.5 g of the powdered sample and put into a 10-mL centrifugal tube, then add 5 mL of methanol (50%). Sonicate (490 W) the mixture for 60 min. Centrifuge at about 1800 × *g* for 5 min. Filter the supernatant through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, pH: 1-12). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Ammonium bicarbonate solution* (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	95 → 40	5 → 60	linear gradient
50 – 60	40 → 0	60 → 100	linear gradient

* Ammonium bicarbonate solution

Dissolve 0.79 g of ammonium bicarbonate in 1 L of water and adjust the pH to 10 with 4 mL of ammonia solution.

System suitability requirements

Perform at least five replicate injections each with 20 μL of benzoylmesaconine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of benzoylmesaconine should not be more than 3.0%; the RSD of the retention time of benzoylmesaconine peak should not be more than 2.0%; the column efficiency determined from benzoylmesaconine peak should not be less than 60000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject benzoylmesaconine Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of benzoylmesaconine peak in the chromatogram of the benzoylmesaconine Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify benzoylmesaconine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of benzoylmesaconine Std-FP. The retention times of benzoylmesaconine peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the four characteristic peaks of Radix Aconiti Praeparata extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix Aconiti Praeparata extract

Peak No.	RRT	Acceptable Range
1	0.30	±0.03
2	0.32	±0.03
3 (marker, benzoylmesaconine)	1.00	-
4	1.69	±0.03

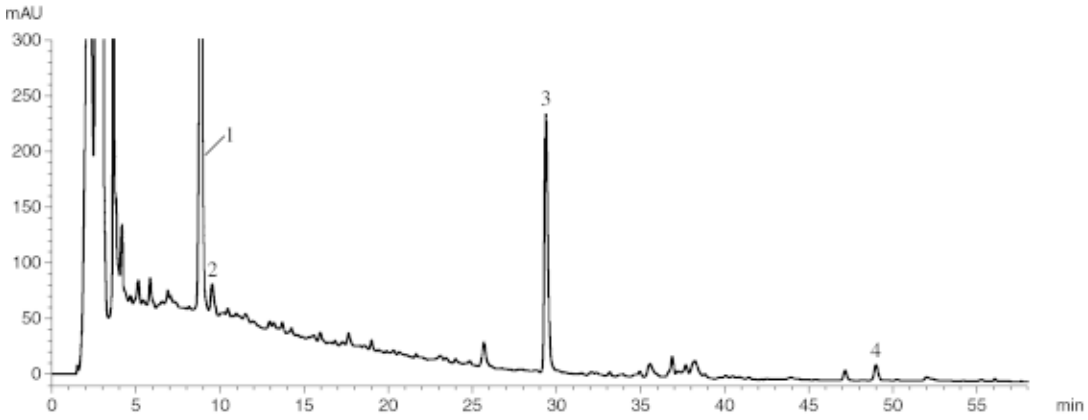

Figure 5 A reference fingerprint chromatogram of Radix Aconiti Praeparata extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 **Heavy Metals** *(Appendix V)*: meet the requirements.

5.2 **Pesticide Residues** *(Appendix VI)*: meet the requirements.

5.3 **Mycotoxins** *(Appendix VII)*: meet the requirements.

5.4 **Sulphur Dioxide Residues** *(Appendix XVII)*: meet the requirements.

5.5 **Foreign Matter** *(Appendix VIII)*: not more than 1.0%.

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*: not more than 13.0%.

5.8 Detection of Aconitine, Hypaconitine and Mesaconitine *(Appendix XIV)*

The sample contains not more than 0.022% of the total content of aconitine ($C_{34}H_{47}NO_{11}$), hypaconitine ($C_{33}H_{45}NO_{10}$) and mesaconitine ($C_{33}H_{45}NO_{11}$), calculated with reference to the dried substance.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(B).

Standard solution

Benzoylmesaconine standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of benzoylmesaconine CRS and dissolve in 5 mL of 0.01 M hydrochloric acid.

Benzoylmesaconine standard solution for assay, Std-AS

Measure accurately the volume of the benzoylmesaconine Std-Stock, dilute with 0.01 M hydrochloric acid to produce a series of solutions of 10, 20, 40, 60, 80 mg/L for benzoylmesaconine.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 10-mL centrifugal tube, then add 5 mL of methanol (50%). Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction thrice. Combine the extracts and make up to the mark with methanol (50%). Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, pH: 1-12). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Ammonium bicarbonate solution* (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	95 → 40	5 → 60	linear gradient
50 – 60	40 → 0	60 → 100	linear gradient

* Ammonium bicarbonate solution

Dissolve 0.79 g of ammonium bicarbonate in 1 L of water and adjust the pH to 10 with 4 mL of ammonia solution.

System suitability requirements

Perform at least five replicate injections each with 20 µL of benzoylmesaconine Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of benzoylmesaconine should not be more than 3.0%; the RSD of the retention time of benzoylmesaconine peak should not be more than 2.0%; the column efficiency determined from benzoylmesaconine peak should not be less than 60000 theoretical plates.

The *R* value between benzoylmesaconine peak and the closest peak in the chromatogram of the test solution should not be less than 2.5.

Calibration curve

Inject a series of benzoylmesaconine Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of benzoylmesaconine against the corresponding concentrations of benzoylmesaconine Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify benzoylmesaconine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of benzoylmesaconine Std-AS. The retention times of benzoylmesaconine peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of benzoylmesaconine in the test solution, and calculate the percentage content of benzoylmesaconine in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.035% of benzoylmesaconine ($C_{31}H_{43}NO_{10}$), calculated with reference to the dried substance.