Chemotaxonomy of the Genus *Taxus*[*1]

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朱木屬의 分類學的 硏究[*1]

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Yew heartwood from different species was analysed by thin-layer chromatography for its phenolic content. A separation could be made between the species examined on the basis of the occurence of heartwood phenols.

一般的으로 識別이 어려운 朱木屬 5樹種의 心材抽出物을 薄層 chromatography로 전개후 디아조계 염료로 발색시켜 肉眼, 螢光 및 紫外線조사로 관찰한바 朱木屬의 심재추출물의 薄層크로마토그람이 수종간에 현저한 차이가 있어 朱木屬의 분류를 간단한 化學的方法으로 가능케 하였다. 그 薄層크로마토그람의 색갈에 의한 분류기초는 다음과 같다.

색갈 / 수종	黃 色	淡黃色	黃褐色	淡褐色	淡紅色	비 고
Taxus canadensis	+	−	+	+++	−	
Taxus cuspidata	+	−	++++	−	−	
Taxus baccata	+	+	++	−	−	
Taxus media	+	++	+	−	−	
Taxus brevifolia	+	−	++	−	+	

註 : +는 反應點의 個數이며, −는 無反應

INTRODUCTION

The purpose of this research was to work out a simple method for the identification of the species of yew on the basis of their heartwood phenols. Erdtman and Tsuno[1] indicate that the heartwood phenols of yew species are identical on thin-layer chromatography, and thus can not be used as a means of species identification. This is in contrast to previous work on the Genus *Pinus*[2] and the Genus *Ulmus*[3], where heartwood phenols have proven of significant taxonomic value.

METHODS AND MATERIALS

The heartwood of five species of yews(*Taxus canadensis* Marsh., *Taxus cuspidata* Sieb. & Zucc., *Taxus baccata* L., *Taxus media* Rehd., *Taxus brevifolia* Nutt.) was examined. Four different samples of each species were obtained from different areas of Ontario and other parts of Canada and examined simultaneously. A general method of extraction and subsequent chromatographic analysis is described below.

A branch of a particular yew tree with some heartwood was collected and the central heartwood portion was carefully separated from the sapwood. The hear-

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twood portion was cut into chips, dried in the oven for an hour at 100°C. and then ground into sawdust in a mill.

The heartwood sawdust(20 g) was taken in an Erlenmeyer flask and dry ether (100ml) was added to it. The flask was stoppered and left overnight at room temperature. Next day, the ether was filtered off and the solvent was removed under reduced pressure. The oily residue (15mg-20mg) was redissolved in ether (5ml) and filtered again to remove the last traces of sawdust. The filtrate was concentrated to a very small volume (1ml) and used as a chromatographic solution.

The adsorbent found to be satisfactory for our work was Silica Gel GF254 (E. Merck). Two different sizes of glass plates(5×20cm, 20×20cm) were used to make thin-layer plates. Plates were made by using a 'Quickfit' apparatus. A slurry sufficient for five large plates or twenty small plates was made of Silica Gel (35g) and water (70ml). The slurry was applied to the plates with an applicator, and the coated plates were dried at room temperature for fifteen minutes and then activated at 105°C. for an hour and a half. After cooling, the thin-layer plates were ready for spotting.

Due to the close proximity of the phenolic spots on the chromatogram, the selection of a solvent with the right polarity was essential. After experimenting with various solvents and combinations of solvents, a mixture of acetone (3) and chloroform (7) was found to give best resolution of the spots on the chromatogram. For the sake of verification of spots two-dimensional chromatography was used. In this case the same solvent system was used in both directional development. In all of the experiments dry and chromatographic quality solvents were used.

Visualisation of the spots was first accomplished by a nondestructive method, i.e., the use of U.V. light (Universal U.V. light Gilman-Camag) of 254 mu and 350 nm, whereby the compounds appeared as dark spots against a green fluorescent background(254nm) or as fluorescent spots(350nm) on a dark background. In the next step the plates were sprayed first with a diazotised solution of benzidine followed by spraying with a solution of sodium hydroxide (IN). The diazo solution was made in the cold by mixing equal volumes of benzidine solution (0.18g benzidine in 50 ml of 0.5N hydrochloric acid) and sodium nitrite solution (1g of sodium nitrite in 100 ml of water). The diazo solution was then allowed to lose its colour after which it was diluted with an equal volume of water. The various phenolic compounds showed up as differently-coloured spots(Fig. 1).

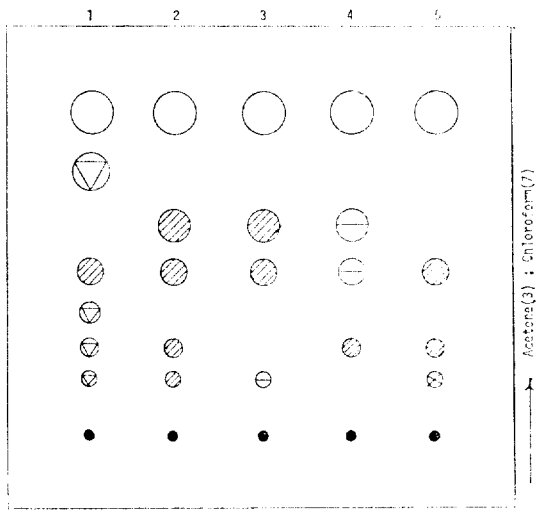

Fig. 1. Master chromatogram of the *Taxus* species showing the relative positions of the phenolic spots after spraying with diazotised benzidine solutions.

Species of *Taxus*

1. *Taxus canadensis* Marsh.
2. *Taxus cuspidata* Sieb. & Zucc.
3. *Taxus baccata* L.
4. *Taxus media* Rehd.*
5. *Taxus brevifolia* Nutt.

*Hybrid of *Taxus baccata* and *Taxus cuspidata*

Legend of Colours

1. Yellow
2. Pale yellow
3. Yellowish brown
4. Light Brown
5. Pink
6. Origin

In each case the plates were developed to a definite height(ca. 14cm) from the origin. An ordinary cylindrical chromatographic jar saturated with the developing solvent was used as a developing chamber for

the smaller plates. In the case of large plates a special Desaga chamber was used. To avoid lateral diffusion and distortion of the spots, the activated plates were pre-equilibrated by keeping them for a few hours in a saturated atmosphere of the solvent used for developing. The solvent front was allowed to overrun for a few minutes (ca. 5 min.) after it had reached the desired height from the origin.

Spotting was done using a fine capillary tube in order to make the original application in as small an area as possible. If the developed chromatogram was too faint for detection, or was streaking or tailing, the concentration of the spotting solution was changed accordingly. To get all of the spots depicted in the master chromatogram a certain degree of overloading of the plate with the spotting solution was necessary.

The coloured spots in the chromatogram were reproduced on paper for comparison with other chromatograms. The plates were also photographed for ready reference.

DISCUSSION AND CONCLUSION

The master chromatogram (Fig. 1) shows a very similar qualitative content of hearwood phenols of the different yew species. however, each species has its own characterististic chromatogram. In all cases the highest yellow spots are identical as shown by their behaviour on mixed thin-layer chromatography. The mixed thin-layer chromatography involves superposition of spotting solution thought to be identical. *Taxus canadensis* has the highest number of phenolic spots. For the sake of comparison each species of yew was chromatographed separately, followed by their geometrical projection on one plane, to show the relative positions of different spots.

The investigation of the phenolic constituents of the heartwood of the yew series has revealed a normal chemotaxonomic pattern within the Genus *Taxus* as the results may be extended to show that the number and type of phenolic compounds can be directly correlated with the morphological classification of yews.

The conditions for the thin-layer chromatography are given for the best resolution of spots but Rf values have been omitted because they are non-reproduceable on unstandardized plates.

Considering the results of Erdtman and Tsuno[1] the method has revealed hitherto undescribed phenols in the heartwood of yews which will be studied further.

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