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Chromatography of the lignans of *Thuja plicata* Donn

The ten lignans in the heartwood of western red cedar (*Thuja plicata* Donn) have been isolated and characterised in these laboratories[1–4]. With the elucidation of the structure of the last lignan[4], we now present the data on the chromatographic behaviour of all of them. These data cover paper (PC), column (CC), thin-layer (TLC), and gas–liquid chromatography (GLC) systems of these compounds or some of their derivatives.

Experimental

PC was on Whatman No. 1 paper, TLC was on Merck Silica Gel G. Solvents and detecting reagents are listed in Table I. Gas chromatography was with a 5 ft. × 1/8 in. column filled with 3 % OV-1 on Gas-Chrom Q (100–120 mesh); nitrogen carrier gas at 20 ml/min; column oven temperature programmed for 200° to 300° at 2°/min; flame ionisation detector oven at 300°; and injector block at 275°. TMS derivatives of thujaplicatins and its methyl ethers eluted from the column at 280°, plicatic acid at 290°, and plicatin at 295°. These derivatives were formed by reacting a few milligrams of the heartwood extract, etc., with bis(trimethylsilyl)acetamide (100 mg) in pyridine (1 ml). The acetates of the lignans were formed by treating them with acetic anhydride (1 ml)–pyridine (1 ml) warming to 40°, allowing the reactants to stand for 2 h, and removing the solvent under vacuum, taking care that the temperature did not exeed 40°.

Results and discussion

Isolation of the ten lignans in amounts sufficient for characterisation has been by CC on deactivated silica[2,3]. When larger yields or a preliminary separation was desired, the ethyl acetate solubles were dried and triturated several times with hot chloroform and the soluble and insoluble fractions chromatographed. This system has recently been adapted to quantitative CC[5] on a micro-scale.

This system has been supplanted[4] by CC on a polyamide (Woelm) eluting with benzene followed by a gradient elution with ethanol. This separates the above lignans in the same order as shown for the R_F values of Table I system D, and faster and cleaner than the silica gel CC.

Table I presents the chromatographic data. With the exception of the thujaplicatin methyl ethers, most of the compounds listed have the unusual monomethylated pyrogallol (3,4-dihydroxy-5-methoxyphenyl) pendant ring and would therefore be expected to complex with molybdic acid. This reaction has been incorporated[6] in a PC system in which dilute molybdic acid has been substituted for water in the usual BAW solvent (Table I, C). The system is self developing because the *o*-dihydroxy groups react to form the yellow molybdate complex. Also, the R_F values of the complexes are much smaller than in the system without molybdic acid. Two other common paper irrigation solvents (Table I, A and B) separate the lignans by classes. No system was found which separated all ten lignans by PC.

Separation by TLC on silica gel had limited success in one direction (D). By making the acetates and using a two-dimensional system (E and F), however, sepa-

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TABLE I

R_F VALUES FOR THE LIGNANS OF *Thuja plicata*

Solvent systems: (A) acetic acid–water (2:98); (B) butanol–acetic acid–water (60:15:25); (C) butanol–dilute molybdic acid–acetic acid[6]; (D) benzene–ethanol (9:1); (E) toluene–ethanol (9:1); (F) chloroform–acetone (95:5).

	Paper[a] *chromatography*			*Thin-layer*[b] *(silica gel) chromatography*			
	A	*B*	*C*	*D*[c]	*E*[d]	*F*[d]	
Thujaplicatin (4,3′,4′-trihydroxy-3,5′-dimethoxy-lignanolid-9,9′)[f]	0.62	0.76	0.61	0.21	0.29	0.31	brown
Dihydroxy thujaplicatin (4,3′,4′,8′,9′-pentahydroxy-3,5′-dimethoxy-lignanolid-9,9′)[f]	0.62	0.76	0.61	0.17	0.10	0.05	brown
Thujaplicatin methyl ether (4,4′-dihydroxy-3,3′,5′-trimethoxy-lignanolid-9,9′)[f]	0.64	0.88	0.75	0.30	0.29	0.31	rose
Hydroxy thujaplicatin methyl ether (4,4′,8′-trihydroxy-3,3′,5-trimethoxy-lignanolid-9,9′)[f]	0.64	0.88	0.75	0.25	0.28	0.23	rose
Dihydroxy thujaplicatin methyl ether (4,8,4′,8′-tetrahydroxy-3,3′,5′-trimethoxy-lignanolid-9,9′)[f]	0.64	0.88	0.75	0.17	0.15	0.11	rose
γ-Thujaplicatene (2-(3″,4″-dihydroxy-5″-methoxybenzylidene)-3-(3′-methoxy-4′-hydroxybenzyl)-butyrolactone)	0.25[e]	0.80	0.61	0.23	0.35	0.41	green
Plicatic acid (3,4,8,4′,8′,9′-hexahydroxy-5,3′-dimethoxy-9-cyclolignanoic acid)[f]	0.85	0.45	0.22	0.00	0.29, 0.27	0.31, 0.14	red-brown
Plicatin (4,3′,4′,8′,9′-pentahydroxy-3,5′-dimethoxy-cyclolignanolid-9,9′)[f]	0.81	0.68	0.48	0.05	0.16	0.06	red-brown
Plicatinaphthalene (6-hydroxy-2-(hydroxymethyl)-7-methoxy-4-(3′,4′-dihydroxy-5′-methoxyphenyl)-3-naphthoic acid lactone)	0.14[e]	0.74	0.63	0.16	0.25	0.28	UV-yellow
Plicatinaphthol (1,6-dihydroxy-2-(hydroxymethyl)-7-methoxy-4-(3′,4′-dihydroxy-5′-methoxyphenyl)-3-naphthoic acid lactone)	0.10[e]	0.74	0.62	0.10	0.25	0.30	UV-orange

[a] Whatman No. 1 paper was used and detection was carried out with Barton's reagent.
[b] Detection was carried out with a mixed sulphuric and nitric acid spray, followed by heating and 6 *N* NaOH.
[c] On Whatman No. 1 paper.
[d] Lignans were acetylated first (see text); colour reactions are given in the margin.
[e] Fluorescent before spraying.
[f] Nomenclature of FREUDENBERG AND WEINGES[8].

ration in most cases was achieved. Where separation was difficult due to similar R_F values, *e.g.* thujaplicatin, its methyl ether, and plicatic acid, differentiation was possible by running a standard added to the mixture on a duplicate plate. Acetylation conditions were chosen so that the primary and phenolic hydroxyl groups were esterified, but the tertiary hydroxyl groups were not. The exception was plicatic acid; it has been shown that, under mild acetylation conditions, plicatic acid gave two products — the major one a mixed anhydride, tetraacetate and a minor one a pentaacetate with one tertiary hydroxyl group reacted[7].

Some effort was expended in the search for detecting reagents giving specific colour reactions. Thus, the Maüle and Pauly reagents were examined, but the former gave colours which were too fugitive and the latter gave colours which were too similar. The usual reagents were those most commonly used here, *viz.* Barton's reagent for PC and mixed acids for TLC. Spraying the lignan acetates with nitric acid gave the nitro-derivatives, and the overspray with alkali gave the sodium salts of the nitrophenols which were detectable in very small amounts, especially under UV light. Six lignans could then be identified immediately because of characteristic colour reactions.

Attempts at separating the trimethylsilyl derivatives of the lignans by GLC were unsuccessful except for plicatic acid and its lactone. No doubt a GLC method could be evolved using longer columns, etc., but the high operating temperatures necessary for the separation (295°), even with light coatings of the latest substrate, make it probable that no gas chromatograph could operate for very long.

In conclusion, the chromatographic separations were complicated by the presence of other extractives from western red cedar, such as the thujaplicins, thujic acid, etc. However, the identification of any of the western red cedar lignans can be performed using a combination of the above PC and TLC systems. The co-chromatography of specific lignans is, of course, very helpful for identification.

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