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THE IDENTIFICATION OF NATURALLY OCCURRING CINNAMIC ACID DERIVATIVES

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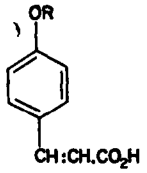
Abstract—Boron trichloride demethylates and deesterifies naturally occurring methoxy and hydroxy cinnamic acid derivatives. In the presence of methanol, the halide produces the corresponding methyl cinnamate. The procedure, in conjunction with paper chromatography and electrophoresis, may be used to classify cinnamic acids in plant tissues into one or more of three groups.

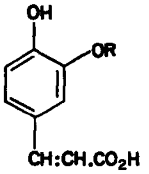
INTRODUCTION

In recent years much attention has been focussed on the derivatives of cinnamic acid which occur in higher plants. These compounds may play a role in disease resistance,[1] they are plant growth factors[2] and are probably important intermediates in the biosynthesis of flavonoids and lignin.[3] From a botanical point of view, these derivatives also have taxonomic significance.[4]

The most commonly occurring cinnamic acids are *p*-coumaric acid (I, $R=H$), caffeic acid (II, $R=H$), ferulic acid (II, $R=CH_3$) and sinapic acid (III, $R'=H$, $R=R''=CH_3$).



I



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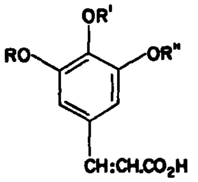

III

In addition *p*-methoxycinnamic acid (I, $R=CH_3$) and 3,4,5-trimethoxycinnamic acid (III, $R=R'=R''=CH_3$) have recently been reported,[5] and an ester of cinnamic acid itself is formed when phenylalanine is fed to potatoes.[6]

In rare instances, the cinnamic acids occur in the free state in plant tissues (e.g. Asen and Emsweller[7]) but they are more usually present in combination with sugars or quinic

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[3] A. C. Neish, *Ann. Rev. Plant Physiol.* **11**, 55 (1960).

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[5] J. J. Corner, J. B. Harborne, S. G. Humphries and W. D. Ollis, *Phytochemistry* **1**, 73 (1962).

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acid. Thus the carboxyl groups are often esterified with glucose or quinic acid and occasionally with disaccharides. In addition, the phenolic hydroxyl groups can also be glucosylated.[8]

These derivatives normally occur in low concentrations in the tissues and consequently are best identified by methods such as paper chromatography and electrophoresis and spectroscopy. Degradation of the ester derivatives can be achieved by hydrolysis with acid or alkali and by the use of enzymes such as β-glucosidase and anthocyanase. The phenolic glycosides can be hydrolysed with acid or glycosidases.[8]

In view of the facile cleavage of ethers,[9] esters[10] and glycosides[11] to the parent hydroxy compounds by boron trichloride a successful attempt has been made to convert naturally occurring cinnamic acid derivatives to the corresponding mono-, di- or tri-hydroxy cinnamic acids (isolated as methyl esters) with the aid of this reagent.

RESULTS AND DISCUSSION

Preliminary studies were designed to examine the effect of the reagent on cinnamic, *p*-coumaric, caffeic and sinapic acids. These compounds were treated with boron trichloride in methylene chloride for 2 hr at −78° followed by 20 hr at room temperature. Excess halide was removed by distillation under reduced pressure and the reaction products were then exhaustively distilled with methanol to remove borate.

Cinnamic acid itself under these conditions yielded a crystalline compound (IV) which gave the correct analysis for methyl cinnamate. The structure was confirmed by (i) hydrolysis, which produced cinnamic acid and methanol, (ii) the preparation of a dibromide derivative and (iii) comparative ultraviolet and infrared spectrophotometry.

Similarly, *p*-coumaric, caffeic and sinapic acids were converted by boron trichloride followed by methanol to methyl *p*-coumarate (V), methyl caffeate (VI) and methyl 3,4,5-trihydroxycinnamate (VII), respectively.

The paper chromatographic and electrophoretic properties of the three methyl hydroxycinnamates have been studied in some detail and compared with those of the parent cinnamic acids (Table 1). The best procedure for the resolution of mixtures of these compounds is paper chromatography using ethyl acetate–acetic acid–water solvent and molybdate-treated papers. This system clearly separates the three esters and has the added advantage that the molybdate complexes of the di- and tri-hydroxy derivatives are clearly discernible as brown spots on chromatograms. Paper electrophoresis, using borate and molybdate buffers, is also a useful procedure for separation and identification. On paper, the three esters exhibit characteristic fluorescences with ammonia under ultraviolet light and can also be located with diazotised *p*-nitroaniline.

The reaction of boron trichloride and methanol with ferulic, 3,4-dimethoxycinnamic and chlorogenic acids was also examined in small scale experiments. All these compounds gave rise to methyl caffeate (VI), which was identified in each case by paper chromatography and electrophoresis.

The method has been applied to plant material containing cinnamic acid derivatives. Thus, powdered coffee beans, which contains chlorogenic acid,[12] gave rise to (VI) and compounds (V) and (VI) were produced by treating a crude extract of *Antirrhinum majus*

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TABLE 1. CHROMATOGRAPHIC AND ELECTROPHORETIC PROPERTIES AND COLOUR REACTIONS OF CINNAMIC ACIDS AND METHYL CINNAMATES

Compound	R_f values in:			
	BEW		EAW	
	Plain paper	Borate-treated paper	Plain paper	Molybdate-treated paper
p-Coumaric acid	0·89	0·36	0·96	0·96
Caffeic acid	0·71	0·03	0·89	0·29
Sinapic acid	0·86	0·28	0·90	0·89
Methyl *p*-Coumarate	0·94	0·96	0·97	0·96
Methyl caffeate	0·86	0·61	0·92	0·68
Methyl 3,4,5-trihydroxycinnamate*	0·80	0·48	0·87	0·34

Compound	M_{SA} values† in:			Appearance in U.V. light and NH_3	Colour with diazotised *p*-nitroaniline +NaOH
	Borate	Molybdate	Acetate		
p-Coumaric acid	0·98	0·44	0·35	Violet	Mauve
Caffeic acid	1·04	0·72	0·20	Light blue	Yellow-brown
Sinapic acid	0·64	0·28	0·21	Green	Red
Methyl *p*-Coumarate	0·38	0	0	Violet	Mauve
Methyl caffeate	0·45	0·78	0	Light blue	Yellow-brown
Methyl 3,4,5-trihydroxycinnamate*	0·61	0·82	0	Blue	Brown

* This compound tends to streak on paper chromatograms and electrophoretograms.
† Mobility relative to that of salicylic acid.

flowers with boron trichloride and methanol. These flowers contain glucose esters of *p*-coumaric, caffeic and ferulic acids.[8] The action of boron trichloride on *O*-glucosides of cinnamic acids has not been investigated but there is no doubt that these would also be cleaved by the reagent (cf. Bonner, Bourne and McNally[11]).

The procedure described is an additional aid to the identification of cinnamic acids in plants. The method classifies those compounds present in the tissues into one or more of three hydroxycinnamic acid "families" and it is extremely simple to carry out.

EXPERIMENTAL

Paper Chromatography

The solvents used were: BEW (butan-1-ol, ethanol, water 40 : 11 : 19, v/v), EAW (ethyl acetate, acetic acid, water 9 : 2 : 2, v/v). Whatman No. 3 paper was used; impregnation with molybdate and borate was achieved by dipping papers into 0·1M sodium molybdate and 0·1M sodium borate solutions, respectively, blotting and air-drying.

Paper Electrophoresis

This was carried out, as described by Pridham,[13] with the following electrolyte solutions: 0·2M sodium borate (pH 10·0), $8{\cdot}1\times10^{-3}$M ammonium molybdate (pH 5·2) and 0·2M sodium acetate (pH 5·2).

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Location of Compounds

Chromatograms and electrophoretograms were viewed under ultraviolet light ("Chromatolite", Hanovia Ltd., Slough, Bucks) after exposure to ammonia vapour. They were also sprayed with diazotised *p*-nitroaniline followed by N NaOH solution.[14]

Absorption Spectra

Ultraviolet spectra were measured with a Unicam SP.500 spectrophotometer using 1 cm silica cells. Infrared measurements were made with a Perkin–Elmer (model No. 137) spectrophotometer.

Standard Conditions for Reaction with Boron Trichloride

The dried cinnamic acid was dissolved or suspended in methylene chloride and placed in a flask fitted with a silica gel trap. The flask was cooled to −78° and boron trichloride (12 ml) at the same temperature added. After 2 hr at −78° the reaction mixture was allowed to stand for approximately 20 hr at room temperature. Excess boron trichloride was then removed under reduced pressure and the borate ions removed by repeated distillation with dry methanol. The mixture was then concentrated to dryness under reduced pressure.

Reaction with cinnamic acid

Cinnamic acid (4·3 g) in methylene chloride (80 ml) under the above conditions yielded a residue which was distilled in a Jackson unit at 0·1 mm pressure. A pale yellow oil came over at 68–72° and on cooling, crystals (IV, 2·9 g) m.p. 35°, were produced. The compound (IV) gave a positive ester test with ferric chloride and hydroxylamine and had ultraviolet and infrared spectra identical to those of methyl cinnamate (lit. m.p. 34·7°[15]), and a molecular weight determination (166; determined cryoscopically in benzene) confirmed this. (Found: C, 74·5; H, 6·3; OMe, 19·1. Calc. for $C_{10}H_{10}O_2$: C, 74·1; H, 6·2; OMe, 19·1%.)

Compound (IV) (0·1 g) was heated on a boiling water bath for 2 hr with 0·025N NaOH (30 ml). The hydrolysate was then distilled and the methanol, which came over in the distillate, was characterized by the preparation of the 3,5-dinitrobenzoyl derivative (m.p. 108°; lit. m.p. 108°[16]). The residue remaining after distillation was dissolved in water and acidified with 2N H_2SO_4. Cinnamic acid (m.p. and mixed m.p. 133°) crystallized out.

Treatment of (IV) (0·15 g) with bromine in carbon tetrachloride followed by concentration of the reaction mixture yielded crystalline methyl α,β-dibromodihydrocinnamate (0·08 g) which on recrystallization from light petroleum (b.p. 60–80°) had m.p. 117° (lit. m.p. 117°[17]). (Found: C, 37·8; H, 3·5; Br, 48·8. Calc. for $C_{10}H_{10}O_2Br_2$: C, 37·3; H, 3·1; Br, 49·6%).

Reaction with p*-Coumaric Acid*

p-Coumaric acid (4·9 g) in methylene chloride (80 ml), when treated with boron trichloride and methanol, yielded a compound (V), which was removed from the reaction mixture by distillation at 75–80° (0·1 mm). The resulting oil, on cooling, yielded crystals (3·65 g, m.p. 137°). (Found: C, 67·3; H, 5·6. Calc. for $C_{10}H_{10}O_3$; C, 67·4; H, 5·7%).

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[14] T. Swain, *Biochem. J.* **53**, 200 (1953).

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[17] A. Michael, *Ber.* **34**, 3640 (1901).

Hydrolysis of (V) with 0·025N NaOH followed by acidification yielded *p*-coumaric acid (identified by paper chromatography (BEW solvent) and paper electrophoresis (acetate buffer). The ultraviolet and infrared spectra of (V) were consistent with it being methyl *p*-coumarate (lit. m.p. 137°[18]).

Reaction with caffeic acid

Chromatographic resolution (cellulose column, BEW solvent) of the mixture obtained by reacting caffeic acid (1·02 g) in methylene chloride (20 ml) with boron trichloride and methanol gave a fluorescent compound (VI, 0·62 g) which, after recrystallization from aqueous ethanol, had m.p. 152° (lit. m.p. for methyl caffeate is 152°–3[19]). (Found: C, 61·7; H, 5·0. Calc. for $C_{10}H_{10}O_4$; C, 61·9; H, 5·2%). Alkaline hydrolysis of (VI) yielded caffeic acid, which was identified by paper chromatography (BEW solvent) and paper electrophoresis (acetate buffer). The infrared and ultraviolet spectra confirmed the identification of (VI) as methyl caffeate.

Reaction with Sinapic Acid

Sinapic acid (2·13 g) in methylene chloride (40 ml) was treated with boron trichloride and methanol. The mixture was fractionated on a cellulose column (BEW solvent) and a fluorescent compound (VII, 1·01 g) obtained which was further purified on thick paper chromatograms. Hydrolysis of (VII) with N H_2SO_4 (2 hr, 100°) produced a phenolic compound which formed a brown molybdate complex and which tended to streak on paper chromatograms and electrophoretograms but nevertheless had the characteristics expected of a trihydroxycinnamic acid.

Treatment of (VII) with acetic anhydride and anhydrous zinc chloride yielded an acetate which, after repeated recrystallizations from glacial acetic acid and finally from a chloroform–light petroleum (b.p. 60–80°) mixture, had m.p. 170° (lit. m.p. for methyl 3,4,5-triacetoxycinnamate is 168°[20]). (Found: C, 57·4; H, 4·8. Calc. for $C_{16}H_{16}O_8$: C, 57·1; H, 4·8%).

Reactions with Ferulic, 3,4-*Dimethoxycinnamic and Chlorogenic Acids*

Approximately 0·05 g of each of the above compounds in methylene chloride (*ca.* 20 ml) was allowed to react with boron trichloride and methanol. The reaction mixtures were then placed directly on paper chromatograms (BEW and EAW solvents) and electrophoretograms (borate, molybdate and acetate buffers). In all three cases, the major product was methyl caffeate (VI).

Reaction with Coffee Bean Powder

The green beans were frozen in liquid nitrogen and then ground to a fine powder. This was dried at 60° under reduced pressure and then treated with the halide and methanol. The concentrated reaction products were taken up in chloroform and shown to contain methyl caffeate by chromatographic analysis.

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[19] F. Mauthner, *J. prakt. Chem.* **142**, 33 (1935).

[20] T. Boehm and K. W. Rosenmund, *Ann. Chem. Liebigs.* **437**, 125 (1924).

Reaction with Antirrhinum majus *Cinnamic Acid Derivatives*

An aqueous methanolic (80%) extract of flowers from this plant was concentrated to dryness at 60° under reduced pressure. Treatment and examination, as with the coffee bean powder, showed that methyl *p*-coumarate (V) and methyl caffeate (VI) had been formed.

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