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ACYLATED ANTHOCYANIN PIGMENTS IN *VITIS VINIFERA* GRAPES: IDENTIFICATION OF MALVIDIN-3-(6-p-COUMAROYL) GLUCOSIDE

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Abstract—A reinvestigation of the position of acylation in malvidin-3-glucoside p-coumarate from *V. vinifera* variety Tinta Pinheira grapes using GLC and NMR techniques has shown that the p-coumaric acid is attached at the six position on the glucose moiety of the pigment. The GLC analysis of 2-, 3-, 4- and 6-p-coumaroyl glucose esters as the trimethylsilyl derivatives is described. The chemical shifts of the specific aliphatic acetate protons were established using the NMR spectra of completely acetylated synthetic 3-, 4- and 6-p-coumaroyl D-glucoses and 4- and 6-p-coumaroyl-methyl-D-glucosides. Comparison of this data with the chemical shifts of the completely acetylated p-coumaroyl-D-glucose moieties of partially degraded pigment established the position of acylation.

INTRODUCTION

THE POSITIONS of acylation of various anthocyanins in several plant species have been investigated[1–4] by degradative techniques. Birkofer *et al.*[5] synthesized the different p-coumaroyl and feruloyl esters of glucose as model compounds for such studies and differentiated between them by means of paper chromatography and paper electrophoresis. Very recently Birkofer *et al.*[6] reported an extensive NMR investigation of the p-coumaroyl glucoses and a number of additional synthetic sugar esters as the trimethylsilyl derivatives and on the basis of this data established the position of acylation in neobignonoside, neopetunoside, and negretein. The previous investigation[1] in our laboratory of the position of acylation of grape anthocyanins by GLC of the methylated and methanolized pigment suggested that the glucose was acylated at the C-4 position. However the strongly acidic conditions of the methylation step left a degree of uncertainty in the results. This paper presents the results of a further investigation by NMR and GLC techniques of the position of acylation in grape anthocyanin pigments.

RESULTS AND DISCUSSION

The pigment used in this investigation, acylated band No. I isolated from the skins of *V. vinifera* variety Tinta Pinheira grapes using the techniques of Anderson *et al.*,[7] was

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[6] L. BIRKOFER, C. KAISER, B. HILLGES and F. BECKER, *Ann.* **725**, 196 (1969).
[7] D. W. ANDERSON, E. A. JULIAN, R. E. KEPNER and A. D. WEBB, *Phytochem.* **9**, 1569 (1970).

shown to consist principally (>95 per cent) of malvidin-3-glucoside acylated with p-coumaric acid. The 2-, 3-, 4- and 6-p-coumaroyl-D-glucoses, necessary model compounds for the study, were synthesized using methods similar to those in the literature.[4,5] The synthetic p-coumaroyl-D-glucoses and the p-coumaroyl-D-glucose obtained from hydrogen peroxide oxidation[8-10] and basic hydrolysis of the pigment were converted to the trimethyl silyl ethers and analyzed by GLC (see Table 1). The synthetic p-coumaroyl-D-glucoses, with the exception of the 2-derivative, apparently exist as anomeric pairs, explaining anomolies in reported melting points and yields. The silanized p-coumaroyl-D-glucose isolated from the pigment shows a pair of peaks with identical retention times to those of silanized synthetic 6-p-coumaroyl-D-glucose. Also observed was a pair of peaks with shorter retention times, apparently due to impurities in the unwashed chromatographic paper used in the

TABLE 1. GAS CHROMATOGRAPHIC DATA

Trimethylsilyl derivatives of	Relative retention times*
p-Terphenyl (unsilanized)	1·00
2-p-Coumaroyl-D-glucose	2·30
3-p-Coumaroyl-D-glucose	1·92, 2·25
4-p-Coumaroyl-D-glucose	2·00, 2·20
6-p-Coumaroyl-D-glucose	2·60, 2·74
Residue from pigment oxidation†	2·60, 2·74 and 1·88, 1·96
Residue from pigment oxidation‡	2·60, 2·74

* Relative to p-terphenyl as an internal standard; 3·7 m × 3 mm column, 0·3% OV-17, 265°, He 30 ml/min.

† Oxidation using H_2O_2 followed by basic hydrolysis. The residue was isolated using unwashed Whatman No. 1 chromatographic paper.

‡ Oxidation using H_2O_2 followed by acidic hydrolysis. The residue was isolated using MeOH washed Whatman No. 1 chromatographic paper.

isolation of the p-coumaroyl-D-glucose residue. Birkofer *et al.*[5,11] reported that 4-p-coumaroyl-D-glucose underwent an acyl migration to the 6-position under certain acidic and basic conditions, thus it was essential to show that the 6-p-coumaroyl-D-glucose isolated from the pigment was not formed by this type of migration. To investigate this possibility, 4-p-coumaroyl-D-glucose was subjected to hydrogen peroxide oxidation and basic hydrolysis conditions identical to those used on the pigment. GLC analysis of the silanized residue from the reaction demonstrated that, at most, 15% of the p-coumaroyl ester migrated from the C-4 to the C-6 position of glucose. Since GLC analysis of the pigment product gave only 6-p-coumaroyl-D-glucose, the evidence indicated that it was not the result of an acyl migration from C-4 to C-6 under the basic hydrolysis conditions. The oxidized pigment was also subjected to acidic hydrolysis. Since the final step in the synthesis of 4-p-coumaroyl-D-glucose involves a similar acidic hydrolysis, it was known that acyl migration from C-4 to

[8] B. V. CHANDLER and K. A. HARPER, *Australian J. Chem.* **14**, 586 (1961).
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[11] L. BIRKOFER, C. KAISER, M. DONIKE and W. KOCH, *Z. Naturforsch.* **20B**, 424 (1965).

C-6 did not occur to a significant extent under these conditions. GLC analysis again showed only silanized 6-p-coumaroyl-D-glucose present from the pigment.

Although the GLC data gave strong evidence that the pigment was acylated at C-6 on the glucose moiety, further confirmation of the position of acylation was sought using NMR techniques. Since the acylated glucose moiety of the pigment is attached to the aglycone through a β-glycosidic linkage, model compounds of acylated, acetylated glucoses, mainly with this configuration, were prepared. The model compounds were compared with the acetylated pigment and pigment derivatives using NMR methods. 6-*O*-p-Acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose,[4] 4-*O*-p-acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose,[5] methyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside, methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside, and methyl-4-*O*-p-acetoxycinnamoyl-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside were prepared and their NMR spectra recorded. The α-methyl-D-glucoside derivative was prepared by acylation of methyl-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside. The β-methyl-D-glucoside derivatives were synthesized from the respective β-acetyl derivatives by inversion of the β-acetates to the α-bromides, followed by methanolysis. To avoid ambiguity in the assigned conformations, methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside was synthesized by an alternate route. Methyl-β-D-glucopyranoside was directly acylated with one equivalent of p-acetoxy-cinnamoyl chloride to give methyl-6-*O*-p-acetoxycinnamoyl-β-D-glucopyranoside. The product after acetylation was shown to be identical to that obtained from the methanolysis of the α-bromide. The direct acylation of methyl-β-D-glucopyranoside was followed by GLC of the silylated reaction product and, upon purification, gave only one peak with a retention time slightly different from that of silylated 6-p-coumaroyl-D-glucose, which appeared as a pair of peaks because of anomerization. The fully acetylated compounds are not adaptable to GLC.

The NMR spectra of the model compounds showed excellent resolution of the acetate protons. The chemical shifts of the aliphatic acetate protons are reported in Table 2. 3-*O*-p-Acetoxycinnamoyl-1,2,4,6-tetra-*O*-acetyl-D-glucopyranoside crystallized as a mixture of α and β anomers which could not be separated. The NMR spectrum of this mixture, not shown in Table 2, showed a complex multiplet in the aliphatic acetate proton region but, significantly, there was no absorption at 2·02 ppm. With this information, it was possible to correlate the chemical shifts of the aliphatic acetate protons to the position of acetate attachment on glucose as seen in Table 2. When a p-acetoxycinnamate ester group is attached to an acetylated glucose, the corresponding acetate proton signal is absent. Molecular models were used to study the configurations of the various model compounds and anomolies between our NMR results and those reported by Heyns *et al.*[12] can be attributed to anisotropic effects. Consideration of the models indicates that the aromatic ring system of the p-acetoxycinnamoyl ester, by virtue of the ethylene linkage, is positioned such that it can have no significant anisotropic effects on an adjacent acetate ester. A p-acetoxycinnamoyl group attached at C-4 of the glucose moiety can exert anisotropic effects on acetate groups at C-6 and with a β configuration at C-1. A p-acetoxycinnamoyl group at C-6 can only effect a group attached β at C-1.

The pigment was exhaustively acetylated and the NMR spectrum of the acetylated pigment was recorded. If the p-coumaroyl group is on the C-6 position of the glucose moiety of the pigment, the configurations of the acylated glucose moiety of the acetylated pigment and

[12] K. Heyns, W. P. Trautwein and F. G. Espinosa, *Angew. Chem. Intern. Ed.* **6**, 966 (1967).

TABLE 2. NMR-ASSIGNMENT OF THE POSITION OF THE ACETATE GROUPS

	Chemical shifts of the acetate protons, ppm				
Compound	C-1	C-2	C-3	C-4	C-6
A	2·09	2·04	2·02	1·99	
B	2·10	2·05	2·02		1·96
C		2·05	2·02	2·00	1·97
D		2·04	2·02	1·98	
E		2·04	2·02	1·99	
F		2·04	2·02		1·93
G		2·04	2·02	1·99	

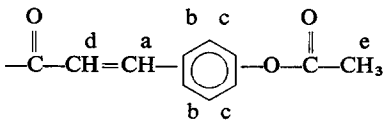
A 6-*O*-p-Acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose.
B 4-*O*-p-Acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose.
C Methyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside.
D Methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside.
E Methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside.
F Methyl-4-*O*-p-acetoxycinnamoyl-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside.
G Acetylated pigment.

methyl-6-*O*-p-acetoxycinnamoyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (E) should be identical and the chemical shifts of their aliphatic acetate protons should be similar. The results in Table 2 show the absence of a C-6 acetate proton peak in acetylated pigment (G) and excellent agreement of the chemical shifts of the acetate proton peaks present with those of the model compound (E), further confirming the position of acylation at C-6.

In the direct acetylation of the pigment there was some question as to the fate of the benzopyrylium ring system. To eliminate this problem, the pigment was oxidized with hydrogen peroxide and exhaustively acetylated, leaving the glucose moiety with a configuration identical to that of 6-*O*-p-acetoxy-cinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (A). The NMR spectrum of oxidized and acetylated pigment (H) was recorded and compared with the NMR spectra of 6-*O*-p-acetoxycinnamoyl-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (A) and 4-*O*-p-acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (B). Table 3 lists the chemical shifts of the p-acetoxycinnamate protons and of three sugar ring protons for the three compounds. Although no attempt was made to identify the specific sugar protons, the signal at approximately 5·7 ppm is probably due to the sugar proton at C-1.[13] The chemical shifts of the sugar protons of model compound A and the pigment derivative (H) are in excellent agreement and differ from those of model compound B which has a p-acetoxycinnamate linkage at C-4 instead of C-6. The p-acetoxycinnamate protons are in excellent agreement for all three compounds with the exception of proton (d). The olefinic proton (d) adjacent to the carbonyl group in p-acetoxycinnamate esters is subject to a significant change in chemical shift due to inductive effects which would be dependent on the position of attachment on the glucose moiety. The excellent agreement of the chemical shifts in the NMR spectra of model compound A and the oxidized, acetylated pigment (H) again confirms that the position of acylation by p-coumaric acid is at the C-6 position of the glucose moiety of the pigment.

[13] L. D. HALL, *Advan. Carbohydrate Chem.* **19**, 51 (1964).

TABLE 3. COMPARISON OF SELECTED CHEMICAL SHIFTS OF SYNTHETIC GLUCOSE DERIVATIVES WITH OXIDIZED AND ACETYLATED PIGMENT



	Chemical shifts, ppm							
	p-Acetoxycinnamate protons					Sugar ring Protons		
	a	b	c	d	e			
A	7·63	7·52	7·07	6·35	2·28	5·71	5·15	4·25
B	7·65	7·52	7·10	6·28	2·28	5·77	5·27	4·20
H	7·66	7·53	7·08	6·36	2·28	5·69	5·16	4·26

A 6-*O*-p-Acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose.
B 4-*O*-p-Acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose.
H Oxidized and Acetylated Pigment.

The results presented by Albach *et al.*,[1] indicating the position of acylation at C-4, can undoubtedly be ascribed to incomplete methylation. The boron trifluoride-etherate catalyst, necessary for the methylation of the hydroxyl groups with diazomethane, probably also catalyzed the cleavage of the acyl linkage. Complete methylation then gave the observed fully methylated glucose, detected as the principle product by gas chromatography. If methylation was incomplete, the C-4 position, being the most hindered, would remain unmethylated, leading to the observation of methyl-2,3,6-tri-*O*-methyl-D-glucoside and suggesting that the acyl group was removed from the C-4 position by the hydrolysis following the exhaustive methylation. Even less complete methylation would lead to partially methylated glucoses which could not be observed under the gas chromatographic conditions employed in the study.

EXPERIMENTAL

Plant Material

Fruit from *Vitis vinifera* var. Tinta Pinheira specimen vines in the experimental vineyards, University of California, Davis, was harvested, placed in polyethylene bags, and kept frozen until used.

Isolation of Acylated Anthocyanin Pigments

The extraction and chromatographic separation of the pigments were done in a manner similar to that described for investigation of the acylated anthocyanin pigments in *V. cinerea* grapes[7] with the following changes. After the pentanol–acetic acid extract was evaporated and the pigment residue dissolved in MeOH, the MeOH solution was extracted with mixed hexanes (10 × 150 ml) to remove waxlike plant materials. The crude pigment was first chromatographed on Whatman 3 MM paper with 20% HOAc in descending fashion before using the CAW solvent system. Acylated pigment band No. I was further purified by rechromatography on Whatman 3 MM paper with 20% HOAc in descending fashion.

Gas Chromatographic Investigation of the Position of Acylation

Samples (1–2 mg) of the synthetic coumaroyl glucoses were placed in a Microflex bottle (Kontes Glass Co., Vineland, New Jersey) which was sealed with a cap containing a teflon-faced rubber septum. TRI-SIL 'Z' (Pierce Chemical Co., Rockford, Illinois) (150 μl) was injected into the bottle and the mixture heated at 60° for 5 min. The solution (4 μl) was injected immediately onto a 3·7 m × 3 mm OV-17 column, 0·3% on 60/80 mesh Chromosorb G AW/DMCS, in an Aerograph HY-FI Model 600 D gas chromatograph with flame ionization detection; column oven temp. 265°; injector temp. 325°; nitrogen carrier gas at 30 ml/min.

The band I pigment (10 mg) was dissolved in MeOH, oxidized with H_2O_2 by the method of Chandler and Harper,[8] and hydrolysed with NH_4OH. The residue obtained from evaporation *in vacuo* was chromatogramed on Whatman No. 1 paper using BAW (4:1:2, v/v) in descending fashion. The UV absorbing band with an R_f value similar to that of the synthetic coumaroyl glucoses was isolated, silanized, and analysed by GLC as described above. A portion of the residue from H_2O_2 oxidation of the pigment was also subjected to an acidic hydrolysis by refluxing for 1 hr in 50% aqueous MeOH (4 ml) containing conc. HCl (1 drop). The solution was neutralized with Amberlite IR-4B and the coumaroyl glucose was isolated on MeOH washed Whatman No. 1 paper, silanized, and analysed by GLC.

Acetylation of Band I Pigment

Band I pigment (20 mg) was dissolved in pyridine (2 ml) and acetic anhydride (0·2 ml), stirred overnight at room temp., the solution diluted with MeOH, and evaporated *in vacuo*. The residue was dissolvedin $CHCl_3$ and the solution washed with H_2O, dried and evaporated to dryness *in vacuo*. Yield: 28 mg (90%).

A second sample of pigment (50 mg) was dissolved in MeOH (5 ml) and 30% H_2O_2 (5 ml). After 5 hr at room temp., the solution was treated overnight with Pd catalyst and evaporated *in vacuo*. The residue was dissolved in pyridine (2 ml) and Ac_2O (0·5 ml) at 0° and stored overnight at −10°. The reaction mixture was worked up as described above, yielding a tan oil. Yield: 39 mg (55%).

NMR Spectral Measurements

A Varian A-60A Spectrometer (Varian Associates, Palo Alto, California) was used to record the spectra of the samples dissolved in $CDCl_3$ with tetramethylsilane as an internal standard. Spectra were recorded at 50 Hz and 500 Hz sweep width.

Syntheses of Reference Compounds

The model compounds and key intermediates in the syntheses were characterized by measurement of i.r. and NMR spectra.

2-*p*-*Coumaroyl*-D-*glucose*. Using Birkofer's method,[5] 4,6-*O*-butylidene-α-D-glucopyranose[14] (2·4 g, 0·01 moles) in pyridine was acylated with a solution of p-acetoxycinnamoyl chloride (2·2 g, 0·01 moles) in $CHCl_3$. Acid hydrolysis and subsequent workup gave 2-p-coumaroyl-D-glucose, recrystallized from EtOH. Yield: 0·3 g (22%) m.p. 193–6° dec.

3-*p*-*Coumaroyl*-D-*glucose*.[5] 1,2,5,6-Di-*O*-*iso*propylidene-α-D-glucofuranose[15] (7·0 g, 0·017 moles) in pyridine (85 ml) was treated at 0° for 3 days with p-acetoxycinnamoyl chloride (6·1 g, 0·017 moles) in $CHCl_3$. Partitioning the reaction solution between H_2O and $CHCl_3$ gave a semisolid residue (10·0 g, 82%) upon evaporation of the $CHCl_3$ layer. The residue was refluxed in 50% aqueous dioxane (100 ml) and conc. HCl (1·0 ml) for 3 hr, the solution neutralized with Amberlite IR-4B resin, extracted with Et_2O, and the aqueous phase evaporated *in vacuo*. The residue was triturated with ethyl acetate (2 × 50 ml) until solid, collected and washed with $CHCl_3$. Recrystallization from boiling H_2O gave 3-p-coumaroyl-D-glucose. Yield: 4·0 g (55%). M.p. 184·5–6·5° dec.

4-*p*-*Coumaroyl*-D-*glucose*.[5] 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[16] (12·8 g, 0·037 moles) in pyridine (128 ml) was treated for 2 days at −10° with p-acetoxycinnamoyl chloride (8·3 g, 0·037 moles) in $CHCl_3$ (90 ml) which, after workup, gave 4-*O*-p-acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose which was crystallized from EtOH. Yield: 12·2 g (62%) m.p.: 128·0–9·5°. 4-*O*-Acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (1·0 g, 1·9 moles) was refluxed with 50% aqueous dioxane (25 ml) and conc. HCl (0·23 ml) for 3 hr, the solution neutralized with Amberlite IR-4B resin, extracted with ethyl acetate and the aqueous phase concentrated to 5 ml *in vacuo*. Chilling overnight gave white crystals of 4-p-coumaroyl-D-glucose which were recrystallized from H_2O. Yield: 0·28 g (48%) m.p.: 220–3° dec. Paper chromatography: homogeneous, $R_f = 0{\cdot}65$, Whatman No. 1 developed with BAW.

6-*p*-*Coumaroyl*-D-*glucose*.[5] 1,2,3,4-Tetra-*O*-acetyl-β-D-gluropyranose[17,18] (10·0 g, 0·029 moles) in pyridine (100 ml) was treated dropwise at 0° with p-acetoxycinnamoyl chloride (7·0 g, 0·029 moles) in $CHCl_3$ (100 ml) which, after workup, gave 6-p-acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose which was crystallized from EtOH. Yield: 8·9 g (55%). M.p.: 156–9°. An analytical sample from EtOH showed m.p.: 160–2°, resolidifying then remelting at 172·5–3·5°. 6-p-Acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (5·0 g, 9·3 mmoles) was dissolved in MeOH (250 ml) and treated for 3 days at room temperature with 1% NaOMe in MeOH (7·5 ml). The solution was neutralized with Amberlite IRC-50 resin, evaporated *in vacuo*, the residue triturated with ethyl acetate (2 × 25 ml), then recrystallized from boiling H_2O.

[14] R. L. MELLIES, C. L. MEHLTRETTER and C. E. RIST, *J. Am. Chem. Soc.* **73**, 294 (1951).
[15] O. T. SCHMIDT, *Methods in Carbohydrate Chemistry*, Vol. II, pp. 318–25, Academic Press, New York (1963).
[16] B. HELFERICH and W. KLEIN, *Ann.* **455**, 173 (1927).
[17] B. HELFERICH amd W. KLEIN, *Ann.* **450**, 219 (1926).
[18] D. REYNOLDS and W. L. EVANS, *J. Am. Chem. Soc.* **60**, 2559 (1938).

Yield: 1·6 g (55%) m.p.: 183–5°. dec. at 200°. Paper chromatography: homogeneous, $R_f = 0{\cdot}65$, Whatman No. 1 developed with BAW.

Methyl-6-O-*p-acetoxycinnamoyl*-2,3,4-*tri*-O-*acetyl*-α-D-*glucopyranoside*. Methyl-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside[19] (1·0 g, 3·1 m-moles) in pyridine (10 ml) was treated at 0° by dropwise addition with a solution of p-acetoxycinnamoyl chloride (0·75 g, 3·3 moles) in $CHCl_3$ (10 ml). After 2 days at −10°, the solution was poured into ice H_2O (100 ml), stirred and separated. After further $CHCl_3$ extractions (2 × 20 ml), the combined $CHCl_3$ extracts were washed with H_2O (2 × 50 ml) and evaporated *in vacuo* to give a quantitative yield of an oil. The oil was chromatographed on ChromAR 1000 Sheet (Mallinckrodt Chemical Works, Saint Louis, Mo.) in ascending fashion with $CHCl_3$, giving a 66% yield of chromatographically pure oil. (Found: C, 56·50; H, 5·59. $C_{24}H_{28}O_{12}$ required: C, 56·69; H, 5·55%.)

Methyl-6-O-*p-acetoxycinnamoyl*-2,3,4-*tri*-O-*acetyl*-β-D-*glucopyranoside*. 6-*O*-p-Acetoxycinnamoyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose[4] (4·0 g, 7·5 m-moles) was stirred at 0° for 45 min with a solution of $CHCl_3$ (8 ml), Ac_2O (0·8 ml) and 30% HBr in acetic acid (24 ml). The reaction mixture was poured into ice H_2O and immediately extracted with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ extracts were washed with ice H_2O (4 × 300 ml), dried, and evaporated *in vacuo*. The oily residue was stirred overnight at room temp. with MeOH (50 ml) and Ag_2CO_3 (1·0 g). After workup, the residue was recrystallized twice from EtOH. Yield: 2·15 g (56%). M.p.: 156·5–159°. (Found: C, 56·59, H, 5·43. $C_{24}H_{28}O_{12}$ required: C, 56·69, H, 5·55%.)

Methyl-4-O-*p-acetoxycinnamoyl*-2,3,6-*tri*-O-*acetyl*-β-D-*glucopyranoside*. 4-*O*-p-Acetoxycinnamoyl-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[5] (2·0 g, 3·7 m-moles) was treated in a manner identical to the 6-p-acetoxycinnamoyl derivative. The product crystallized as a gelatinous solid from EtOH and dried to a white powder. Yield: 1·5 g (77%). M.p. 91–92·5°. (Found: C, 56·85, H, 5·38, $C_{24}H_{28}O_{12}$ required: C, 56·69; H, 5·55%.)

Methyl-6-O-*p-acetoxycinnamoyl*-β-D-*glucopyranoside*. Methyl-β-D-glucopyranoside (1·0 g, 5·2 m-mole) in pyridine (25 ml) was treated in the usual manner with a solution of p-acetoxycinnamoyl chloride (1·15 g, 5·2 m-mole) and $CHCl_3$ (30 ml) at 0°. After workup, the residue was recrystallized twice from EtOH. Yield: 0·4 g (20%). M.p. 158·5–160°. (Found: C, 57·71; H, 5·67. $C_{18}H_{22}O_9$ required: C, 56·55; H, 5·80%.)

3-O-*p-Acetoxycinnamoyl*-1,2,4,6-*tetra O-acetyl*-D-*glucopyranose*. 3-p-Coumaroyl-D-glucose (0·5 g, 1·5 m-mole) in pyridine (10 ml) was stirred overnight at room temp. with acetic anydride (5 ml). The excess acetic anhydride was decomposed with MeOH, the solution evaporated *in vacuo*, and the residue partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was washed, dried, and evaporated *in vacuo* .The residue was recrystallized repeatedly from EtOH. Yield: 0·34 g (44%). M.p.: 102·5–111°. (Found: C, 56·12; H, 5·20. $C_{25}H_{28}O_{13}$ required: C, 55·96; H, 5·25%.)

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[19] B. Helferich, H. Brederich and A. Schneidmuller, *Ann.* **458**, 111 (1927).