Phytochemistry, 1972, Vol. 11, pp. 2047 to 2053. Pergamon Press. Printed in England.

A NOVEL FLAVONE-POLYSACCHARIDE COMPOUND FROM *MONOCLEA FORSTERI*

K. R. MARKHAM

Chemistry Division, D.S.I.R., Petone, New Zealand

(*Received* 24 *October* 1971)

Abstract—A flavonoid-polysaccharide compound of approximate MW 3200 (based on the presence of one flavonoid unit), in which 8-methoxy-5,7,3′,4′-tetrahydroxyflavone is chemically bound to a water soluble polysaccharide (or polysaccharides) of the hemicellulose type containing about 18 sugars, has been isolated from the liverwort *Monoclea forsteri*. The bonding is glycosidic and involves the galacturonic acid residues in the polysaccharide and the 7- and 4′-hydroxyl groups on the flavone. This is the first report from natural sources of a compound of the phenol-polysaccharide type.

INTRODUCTION

IN THE course of an investigation of the phenolic constituents of lower plants[1] it was observed that in some species of liverworts, flavonoid-like natural products behaved in a manner which suggested the existence of a bond between the flavonoid and the cell wall. Knowledge of the type of such bonding is of particular interest since it could provide, by analogy, an insight into the nature of the much investigated,[2,3] but still undefined, lignin-carbohydrate bond. Since flavonoids are closely related to lignin biosynthetically and are occasionally even found incorporated in lignin,[4,5] it appeared worthwhile to attempt an isolation of a flavonoid-polysaccharide compound from one of these liverworts.

We wish to report here the isolation and partial structure elucidation of a flavone-polysaccharide compound from *Monoclea forsteri*. This appears to be the first reported isolation of a member of the phenol-polysaccharide group of compounds, as distinct from complexes,[6,7] and simple glycosides (the most heavily glycosylated flavonoids previously known being tetra- or possibly penta-glycosylated.[8])

RESULTS AND DISCUSSION

A paper chromatographic survey of liverworts for flavonoids[9] revealed the presence of a most unusual natural product in *Monoclea forsteri*. Its appearance on the paper chromatogram as a dark UV absorbing spot, unchanged in ammonia, was typical of a 5-hydroxyflavonoid, however, its R_f values of 0·06 in TBA and 0·95 in HOAc were quite atypical of

[1] B. G. BREHM, personal communication.
[2] J. M. HARKIN, *Fortschr, Chem. Forsch.* **6**, 101 (1966).
[3] J. W. T. MEREWETHER, *Holzforschung* **11**, 65 (1957).
[4] L. J. DIETERMAN, S. H. WENDER, W. CHORNEY and J. SKOK, *Phytochem.* **8**, 2321 (1969).
[5] G. DE STEVENS and F. F. NORD, *J. Am. Chem. Soc.* **75**, 305 (1953).
[6] S. ASEN, R. N. STEWART, K. H. NORRIS and D. R. MASSIE, *Phytochem.* **9**, 619 (1970).
[7] E. BAYER, H. EGETER, A. FINK, K. NETHER and K. WEGMANN, *Angew. Chem. Intern. Ed.* **5**, 791 (1966).
[8] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 63, Academic Press, London (1967).
[9] K. R. MARKHAM, L. J. PORTER and B. G. BREHM, *Phytochem.* **8**, 2193 (1969).

normal flavonoid glycosides.[10] The extraordinarily high R_f in HOAc was suggestive of the presence of a large number of sugar residues in the molecule since in general an increase in the sugar content of a flavonoid glycoside leads to increased mobility in aqueous solvents.[10-12]

The natural product was initially isolated in 0·24% yield by paper chromatography of a dialysed aqueous plant extract. It behaved as a single compound on both paper and TLC under a wide variety of conditions, but electrophoresis revealed the presence of a small amount of polysaccharide impurity. The natural product was finally resolved into three components, MF_1, MF_2 and MF_3 by TLC on specially prepared[13] polyamide. The separation of MF_1 in any quantity however proved exceedingly difficult. Apart from eluting MF_1 from a polyamide thin-layer onto a cellulose thin-layer (on the same plate) and isolating it by water extraction, the only other successful method was repeated sephadex gel filtration of impure MF_1.

The first step in the structure determination was to isolate the MF_1 aglycone via acid hydrolysis. It was found however that a significant yield of the aglycone was only produced after prolonged hydrolysis. Under normal hydrolysis conditions a number of flavonoid containing products as well as the aglycone were formed (see Fig. 1.) It was thought likely

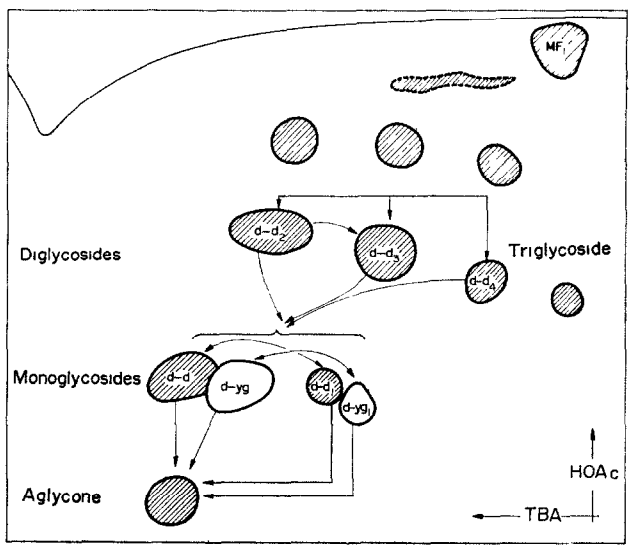
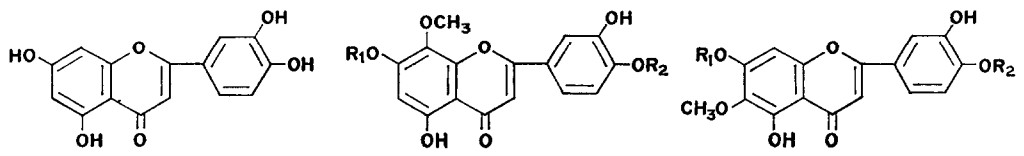


FIG. 1. A PAPER CHROMATOGRAM OF THE ACID HYDROLYSIS PRODUCTS FROM MF_1, SHOWING THE RELATIONSHIPS BETWEEN THE PRODUCTS.

that these products were a range of glycosides (mono-, di-, tri-, etc. as indicated by R_f values) formed by partial hydrolysis of the polysaccharide chain. Indeed, a shorter period of hydrolysis still, yielded additional products, the bulk of which were apparently of the polyglycoside type in that they had high R_f values in HOAc and low R_f values in TBA.

[10] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York (1970).
[11] J. B. HARBORNE, *Biochem. J.* **84**, 100 (1962).
[12] J. B. HARBORNE, see Ref. 8, p. 66 (1967).
[13] H. WYLER, H. ROSLER, M. MERCIER and A. S. DREIDING, *Helv. Chim. Acta* **50**, 545 (1967); see also Ref. 10, p. 21 (1970).

Each of the major glycosidic products was further hydrolysed to prove a relationship with the aglycone. The transformations observed are depicted in Fig. 1 (by means of arrows) and although partial hydrolysis to the aglycone occurred in all cases, isomerizations within each glycoside group also took place. The two dark UV absorbing (monoglycoside) spots which turned yellow-green in ammonia vapour (*d–yg* and *d–yg*$_1$) were found to be interconvertible as also were the two which were unaffected by ammonia (*d–d*, *d–d*$_1$). The diglycosides *d–d*$_2$ and *d–d*$_3$, in addition to producing the aglycone and all four monoglycosides, also proved to be interconvertible. Such acid catalysed interconversions are typical of those observed with 6- or 8-substituted flavones, which undergo an acid induced 6,8-isomerization known as the Wessley–Moser rearrangement,[14] and so could be accounted for by the presence of a 6- or 8-substituent in the aglycone.



(I)

(III) R_1 = H, R_2 = H
(IV) R_1 = H, R_2 = galacturonic A.
(VI) R_1 = galacturonic A., R_2 = H.
(VIII) R_1 = R_2 = galacturonic A.

(II) R_1 = R_2 = H
(V) R_1 = H, R_2 = galacturonic A.
(VII) R_1 = galacturonic A., R_2 = H.
(IX) R_1 = R_2 = galacturonic A.

Small quantities of the aglycone were isolated by paper chromatography from the hydrolysis products. The UV spectrum was identical with that of luteolin (I) and the existence of free phenolic hydroxyl groups at positions 5, 7, 3′ and 4′ was indicated by the shifts induced in the long wavelength absorption band on the addition of $AlCl_3$ and NaOMe

TABLE 1. MF_1 AND ITS HYDROLYSIS PRODUCTS

	d–d(IV)	*d–d*$_1$(V)	*d–yg*(VI)	*d–yg*$_1$ (VII)	Aglycone(II)	MF_1	*d–d*$_2$ (VIII)	*d–d*$_3$ (IX)
Spot Colour (UV + NH_3)	dk	dk	yell–gr	yell–gr	yell–gr	dk	dk	dk
R_f (TBA)	0·71	0·50	0·62	0·45	0·72	0·06	0·58	0·40
R_f (HOAc)	0·29	0·23	0·25	0·21	0·04	0·95	0·58	0·54
	d–d(IV)*		*d–gy*(VI)*		Aglycone		MF_1	
Spectral maxima (nm) in								
$MeOH/H_2O$	272, 336		253sh, 271, 341		252, 268, 346		250, 273, 332	
NaOMe	273, 295sh, 378		268, 297sh, 385		265br, 322, 406		270, 291sh, 363	
NaOAc	272, 368		268, 391		267, 399		272, 330	
$NaOAc/H_3BO_3$	271, 355		264br, 363		257, 273sh, 376		272, 330	
$AlCl_3$			276, 290sh, 347, 407		271, 294sh, 410		255sh, 282, 342, 383sh	
$AlCl_3/HCl$			256, 278, 290sh, 351, 385sh		254, 274, 292sh, 356, 384sh		255sh, 282, 342, 383sh	

* Small amounts of the 6-methoxy isomer also present.

(Table 1). The additional substituent at C-6 or C-8 in luteolin, which is required to account for the isomerizations, was shown by mass spectroscopy to be a methoxyl group. The molecular ion of *m/e* 316·0584 requires the molecular formula, $C_{16}H_{12}O_7$, and the presence of an intense M–CH_3 peak at *m/e* 301 is strongly indicative of a C-6 or C-8 methoxyl group.[15]

[14] T. R. SESHADRI, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISMAN), p. 184, Pergamon Press, Oxford (1962).
[15] J. H. BOWIE and D. W. CAMERON, *Austral. J. Chem.* **19**, 1627 (1966); J. G. NIELSON and J. MOLLER, *Acta Chem. Scand.* **24**, 724 (1970).

Direct comparison of the aglycone with authentic 6-methoxyluteolin (II) confirmed its identity as also did comparison of the respective tetramethyl ether derivatives. It is considered however that MF_1 is probably a derivative of 8-methoxyluteolin (III) and that, as is usual with this class of compounds, acid hydrolysis converts the 8-methoxyluteolin into the more stable[14,16] 6-methoxy derivative. The PMR spectrum of MF_1 for example indicates that it is an 8-methoxyluteolin derivative. The signal due to the C-6(8) proton appears at 6·75 ppm, a chemical shift which is consistent with a C-6 proton in that it is, as expected,[17] about 0·2 ppm upfield from that of a C-8 proton in a similar environment (i.e. in patulitrin[10]). The 8-methoxyluteolin structure for MF_1 is also indicated by the observation that mild acid hydrolysis consistently produces a much higher yield of the paper chromatographically more mobile[10,16a] 8-methoxyluteolin monoglycosides, than of the 6-methoxy isomers. On the basis of this evidence, MF_1 will be referred to as an 8-methoxyluteolin derivative in the remainder of this text.

The occurrence of 8-methoxyluteolin in a liverwort is of interest in its own right, since until quite recently it was considered that flavonoids did not occur in plants more primitive than the mosses.[9] In addition, the presence of oxygenation at the C-6 or 8 position in flavonoids and of *O*-methylation have been previously considered as phylogenetically advanced characters in higher plants.[18] The only other flavones previously found in liverworts have contained the biosynthetically fundamental 5, 7, 4′ -oxygenation pattern and lack *O*-methylation.[9,19] *Monoclea* however has never been regarded as an advanced liverwort as judged by morphological features, and so the most that could be claimed on the basis of this isolation is support for Schuster's view[20] that *Monoclea* occupies an isolated position and is not closely related to other liverworts.

A molecular weight of about 3200 was established for MF_1 by UV spectroscopy using the Beer–Lambert law and the literature value[21] for the extinction coefficient of 8,3′-dimethoxy-5,7,4′-trihydroxyflavone (extinction coefficient data was not available for 8-methoxyluteolin). Hence MF_1 appears to contain approximately 18 sugars per flavonoid unit. These sugars were identified in hydrolysates of MF_1 as galactose, mannose and glucose with smaller amounts of galacturonic acid, xylose and rhamnose. The polysaccharide thus appears to be more closely related to the hemicelluloses than to any other polysaccharide group. Indeed, a similar water soluble galactoglucomannan recently isolated[22] from another bryophyte, the moss *Fontinalis antipyretica*, has been shown to be structurally similar to some softwood hemicelluloses.

The points of attachment of 8-methoxyluteolin to the polysaccharide (or polysaccharides if the sugars are not all in one chain) were shown by UV spectroscopy to be the 7- and 4′-hydroxyl groups. Reagent induced spectral shifts associated with the presence of free hydroxyl groups at these positions were absent in the spectra of MF_1. Further, the product obtained from MF_1 after complete methylation and hydrolysis gave UV spectral data consistent with the presence of free hydroxyl groups only at the 7- and 4′-positions.

This substitution pattern accounts fully for the range of mono- and diglycosides observed on hydrolysis of MF_1. Spectral data for the diglycosides d–d_2 and d–d_3 is essentially the same

[16] (a) J. B. HARBORNE, *Phytochem.* **8**, 2071 (1969). (b) N. MORITA, *Bull. Chem. Pharm., Tokyo* **8**, 66 (1960).
[17] J. T. BATTERHAM and R. J. HIGHET, *Austral. J. Chem.* **17**, 428 (1964).
[18] J. B. HARBORNE, see Ref. 8, p. 313 (1967).
[19] E. NILSSON, *Acta Chem. Scand.* **23**, 2910 (1969).
[20] R. M. SCHUSTER, *J. Hattori Botanical Lab.*, No. 26, 185 (1963).
[21] K. FUKUI and M. NAKAYAMA, *Bull. Chem. Soc., Japan* **42**, 2327 (1969).
[22] D. S. GEDDES and K. C. B. WILKIE, *Carbohyd. Res.* **18**, 335 (1971).

as for MF_1 itself (Table 1) and hence the linking sugars at the 7- and 4′-positions must still be present. The Wessely–Moser rearrangement accounts for the presence of the two isomers, the high R_f member being the 8-methoxy isomer.[16] Hydrolysis of either of these diglycosides will thus produce both 7- and 4′-monoglycosides which in turn will undergo Wessely–Moser rearrangement. These monoglycosides are the *d–yg* and *d–d* pairs, the UV spectral data (Table 1) being in accord with such assignments. The sugar in both of these monoglycoside types was shown to be galacturonic acid by enzyme hydrolysis with β-galacturonidase followed by GLC analysis of the sugar so produced. Thus *d–d*, *d–d*$_1$, *d–yg* and *d–yg*$_1$ may be assigned structures IV, V, VI and VII respectively, and the diglycosides, *d–d*$_2$ and *d–d*$_3$, structures VIII and IX.

The actual linkage of the uronic acid to the flavone in each case is considered to be that of a normal glycoside, that is, through the C-1 aldehydic position of galacturonic acid. This was indicated by the successful hydrolyses with β-galacturonidase, and incidentally, accounts for the difficulty experienced in hydrolyzing MF_1 through to the aglycone, since flavone uronides are known to be extraordinarily resistant to acid hydrolysis.[23] That the carboxyl group is not involved in the linkage was confirmed by isolating the sugar from the *d–yg* monoglycoside after it had been treated with diazomethane. The production of the methyl ester of galacturonic acid by this means precluded the possibility that the carboxyl group is involved in the phenol-polysaccharide bonding.

A particularly interesting feature of the structure of MF_1 is that the uronic acids which link the polysaccharide to the flavone appear to be the only uronic acid residues in the polysaccharide. A quantitative estimation of the amount of galacturonic acid per flavonoid unit, using the carbazole colorimetric method, indicated that there are only two uronic acid residues in the polysaccharide. This is supported by the fact that no aldobiuronic acids were formed on hydrolysis, which means that there are no further uronic acids present which are glycosidically linked to the neutral sugars of the chain.

It is concluded that MF_1 is a flavone-polysaccharide compound of approximate MW *n*(3200), where *n* is the number of flavone units, in which a polysaccharide (or polysaccharides) of the hemicellulose type is linked glycosidically via its only galacturonic acid residues, to 8-methoxyluteolin at the 7- and 4′-positions. As such, it appears to be the first example of a discrete phenol-polysaccharide compound to be isolated from natural sources.

EXPERIMENTAL

UV spectra were determined in AR MeOH or H_2O using diagnostic reagents made up as directed in Ref. 10. Paper chromatograms were run on Whatman 3MM paper (46 × 57 cm) using *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA), and 15% HOAc (HOAc). Electrophoreses were carried out on glass fibre paper in 0·1 M borax using a Shandon Electrophoresis Apparatus (After Kohn) model U77 fitted with a Vokam (300 V) d.c. power supply. An MS9 mass spectrometer was used for the determination of mass spectra. GLC of sugars was carried out on a Hewlitt–Packard 5750 gas chromatograph using an SE 52 column and a column temperature of 200°. PMR spectra were measured on a Varian DA60I spectrometer fitted with a Varian C1024 time averaging computer.

Extraction procedure and isolation of MF$_1$. Air-dried *Monoclea forsteri* gametophyte tissue (Voucher specimen No. H399, Dominion Museum, Wellington. N.Z.) (33 g) was mixed with water (1·4 l.) and pulverized in a Waring-Blendor. After 3 days standing, the slurry was filtered through glass wool and the extract was then applied to cellulose powder (MN 100) which had been packed and prewashed in a Buchner funnel. Under vacuum, a clear yellow–orange solution was obtained which on evaporation yielded gum-like material (6·0 g). This, in solution, was dialysed against frequently changed distilled water, with stirring, for 3 days (H. B. Selby & Co. Ltd. cellulose casing dialysis tubing was used). The solution remaining in the tubing yielded 0·8 g of solid containing 80% of the required compound (as determined by UV spectroscopy), and the

[23] J. B. Harborne, *Phytochem.* **4**, 107 (1965).

combined outside solutions yielded 5 g containing the other 20% of MF_1. The dialysed solution was then paper chromatographed in TBA, repeatedly, on the same chromatogram, until the required compound (visible as a dark band in UV light, 360 nm) had moved well clear of the origin. Elution of the UV absorbing band with water yielded 0·065 g of a light brown crusty solid after evaporation and freeze-drying. A further 0·015 g was recovered from material which had passed through the dialysis tubing. The product so isolated was very soluble in water, insoluble in methanol, and chromatographed as a single spot in a variety of solvents on paper, and on silica gel, cellulose and polyamide TLC. Electrophoresis at 100 V/20 mA also failed to resolve the sample into more than one spot; however when the electrophoretogram was treated with a *p*-anisidine spray reagent an additional pale yellow–orange band appeared. The original material was finally resolved into 3 separate spots, R_f 0·27, 0·21, 0·16 (MF_1, MF_2, and MF_3, in the visually estimated ratio of 10·0:1·0:0·7) by TLC on polyamide prepared according to Wyler *et al.*,[13] in MeOH–HOAc–H_2O, 14:3:3 (run twice). To isolate these compounds TLC plates were used on which one half was polyamide and the other cellulose. MF_1, MF_2 and MF_3 were separated on the polyamide and then run through to the cellulose, from which they were extracted with water. Alternatively the mixture, in water, was passed down a column of Sephadex G-50 and the MF_1 rich fractions were rechromatographed on the same column. This process was repeated 5 times before TLC indicated that pure MF_1 had been obtained. MF_1 (2 mg) isolated by this means was found to have the physical properties listed in Table 1 and a PMR spectrum (d_6-DMSO, 55°) with signals at 7·50, 7·28 (m, H-2′6′), 6·90 (d, J = 10 Hz, H-5′) and 6·75 (s, H-3 and H-6; position unchanged at 20°) ppm, in the aromatic proton region. The presence of an intense signal due to the sugar protons obliterated the methoxyl signal. For the MW determination, MF_1, at a concentration of 0·1368 g/l. in water, had an optical density at 332 nm of 0·85.

Methylation and hydrolysis of MF_1. MF_1 (0·5 mg) in MeOH–H_2O was methylated repeatedly at 0° with freshly distilled ethereal CH_2N_2 until methylation was complete. The product was then hydrolysed in MeOH–3 N HCl (1:2) for 3·5 hr at 100°. The methylated aglycone was separated by 2D paper chromatography and then isolated by MeOH extraction of the blue UV fluorescent spot, R_f 0·78 (TBA), 0·16 (HOAc). It had λ_{max} (MeOH) 264, 334 nm; (NaOMe) 254, 330, 395, nm; (NaOAc) 268, 318 sh, 378 nm; (NaOAc–H_3BO_3) 263, 333, nm.

Acid hydrolysis of pure MF_1. (a) Pure MF_1 from a sephadex column separation was dissolved in 5 ml of MeOH–2 N HCl (1:1) and heated at 100° for 1 hr. The products were analysed by 2D paper chromatography in TBA and HOAc (see Fig. 1). (b) Hydrolysis of MF_1 was carried out as in (a) and samples were removed after 0, 5, 10, 17 and 30 min of hydrolysis. Paper chromatographic analysis revealed that as the period of hydrolysis increased, the R_f values (in HOAc) of the major flavonoid products decreased in a series of steps from 0·95 to 0·15.

MF_1 Aglycone II. The aglycone (2 mg) was isolated by paper chromatography (HOAc) from the products of hydrolysis of crude MF_1 (20 mg) in MeOH–3 N HCl (1:1) for 5 hr. It was isolated as a yellow semi-solid and had the physical properties listed in Table 1, IR absorption as reported by Brieskorn and Michel,[24] and *m/e* 316·0584 (100%) M^+, 301 (72%) M–Me, 298 (44%) M–H_2O, 273 (44%), M–CH_3CO, 167 (16%), 139 (20%), 135 (36%), ($C_{16}H_{12}O_7$ requires M^+ 316·0583). Its identity with authentic 6-methoxyluteolin was established by paper chromatography, TLC (SiO_2 and polyamide), IR, UV and MS spectroscopy. The tetramethyl ether was prepared by treating the aglycone in MeOH with ethereal CH_2N_2, and was purified by paper chromatography in TBA. It had R_f 0·83 (TBA), *m/e* (M^+) 372·1208 ($C_{20}H_{20}O_7$ requires M^+ 372·1209), λ_{max} (MeOH) 238sh, 263, 327 nm and was indistinguishable from authentic 5,6,7,3′,4′-pentamethoxyflavone by paper chromatography, TLC (SiO_2 and polyamide) and UV spectroscopy.

Analyses of sugars from MF_1. Pure MF_1 (1 mg) was hydrolysed in 2 N HCl for 3 hr at 100°. The solution was evaporated to dryness and the residue dissolved in 1 ml H_2O to provide a stock solution. Paper chromatographic analyses were each carried out on 0·02 mg of hydrolysate using EtOAc–pyridine–H_2O (12:5:4), *i*PrOH–Pyr–H_2O (3:1:1), *i*PrOH–H_2O (4:1) and the spray reagents, *p*-anisidine HCl, aniline phthalate and Tollens reagent. Sugars positively identified by comparison with authentic samples were rhamnose, xylose, glucose, galactose and mannose. A uronic acid was also present. GLC analysis was carried out using 0·1 mg of hydrolysate. The sugars were run as their trimethylsilyl ethers (see Ref. 25 for details) and were identified (semi-quantitatively) by direct comparison as: galactose (6), mannose (4), glucose (3), xylose (2), galacturonic acid (2) and rhamnose (1). No aldobiuronic acid peaks were observed. The level of uronic acid in MF_1 was checked using the carbazole method.[26] MF_1 (0 07 mg) gave 0·009 mg of galacturonic acid. H_2O. Assuming a MW of 3200 for MF_1, the presence of two galacturonic acid residues should give rise to 0·0093 mg of galacturonic acid. H_2O.

Hydrolysis products of MF_1 (d–d, d–d_1, d–d_2, d–d_3, d–yg, d–yg_1). Compounds *d–d*, *d–d_1*, *d–d_2*, *d–d_3* *d–yg* and *d–yg_1* were isolated by 2D paper chromatography from the products of hydrolysis of crude MF_1 in MeOH–2 N HCl (1:1) for 1·5 hr. The chromatographic and spectral properties noted for each of these compounds are listed in Table 1, and the interrelationships indicated in Fig. 1 were established by hydrolysing

[24] C. H. BRIESKORN and H. MICHEL, *Tetrahedron Letters* 3447 (1968).
[25] P. E. REID, B. DONALDSON, D. W. SECRET and B. BRADFORD, *J. Chromatog.* **47**, 199 (1970).
[26] E. A. MCCOMB and R. M. MCCREADY, *Analyt. Chem.* **24**, 1630 (1952).

each compound independently in MeOH–2 N HCl (1 : 1) for about 1 hr and then analysing the products by 2D paper chromatography. Enzyme hydrolyses were carried out on *d–d*, *d–yg*, and $d–d_2$ using β-glucuronidase (Koch-Light ex Marine mollusc) and β-galacturonidase (Pectinase, Koch-Light ex *A. niger*) in distilled water at 20°, for a period of 3 days. Hydrolysis to the aglycone occurred in all cases. GLC analyses of the trimethylsilylated products were carried out, and when peaks due to the enzymes themselves were discounted only one peak remained. This was shown to be due to galacturonic acid (and not glucuronic acid) by cochromatography with trimethyl silylated authentic material. The glucuronidase hydrolysates were found to be the most suitable for the GLC analyses. Methylation of *d–yg* in MeOH with ethereal CH_2N_2, followed by hydrolysis in MeOH–3 N HCl (1 : 2) at 100° for 2 hr, produced a sugar which on GLC had the same retention time as methyl galacturonate.

Acknowledgements—The author wishes to thank Professor R. Hodges, Massey University, Palmerston North for mass spectra measurements; Professor C. H. Brieskorn, University of Wurzburg and Professor K. Fukui, Hiroshima University, for samples of 6-methoxyluteolin; and Drs. L. J. Swift and J. H. Tatum, U.S.D.A., Winterhaven, Florida for a sample of sinensetin (5,6,7,3′,4′-pentamethoxyflavone).

Key Word Index—Monoclea forsteri; Hepaticae; 8-methoxyluteolin derivative; flavone-polysaccharide compound.