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THE GLYCOFLAVONOID PIGMENTS OF WHEAT, *TRITICUM AESTIVUM*, LEAVES*

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Abstract—The *C*-glycosylflavone pigments found in wheat leaves are a varied mixture of compounds in the luteolin and apigenin series; iso-orientin, lutonarin, lucenin-1, lucenin-3, vicenin-2, and an unreported *C*-glycoside, wyomin, an iso-orientin derivative with rutinose at the 7-position. In addition, a 4′-*O*-glucosyl derivative of iso-swertisin was identified along with the previously reported flavone, tricin.

INTRODUCTION

C-GLYCOSYL flavones[1,2] have been isolated from several plant families including those of commercial import, the Gramineae[3,4] and Rutaceae.[5] Compounds in this series, particularly those with adjacent dihydroxy groups, have some correlation to the biochemistry of plant resistance to disease pathogens;[6,7] specifically, the resistance of certain varieties of wheat to stem rust. It is the purpose of this paper to report on the isolation and structural determination of these compounds[5,8,9] found in the leaf extracts of certain resistant and susceptible wheat varieties.

RESULTS

Separation of Pigments

The pigment mixture found in the hard, red spring wheat varieties is best illustrated by the two dimensional thin layer chromatogram (Fig. 1) using the BAW solvent system in the first direction and 5% acetic acid in water in the second direction. Separation techniques used included extraction, counter-current separation followed by Sephadex-LH-20 column chromatography and preparative TLC on cellulose layers. The absorption spectra of the isolated compounds indicated that they were either apigenin or luteolin based *C*-glycosyl flavones (Table 1) which were subsequently identified as: iso-orientin (spot 1), lutonarin

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(spot 2), lucenin-1 (spot 3), lucenin-3 (spot 4), an as yet unreported 7-*O*-rutinoside of iso-orientin, wyomin (spot 5), a new derivative of iso-swertisin,[10,11] the 4′-*O*-glucoside (spot 6), vicenin-2 (spot 8), and tricin (spot 7) which can be obtained by the method of Anderson.[12] Spots CA-1 and CA-2 have been characterized previously as the *trans* and *cis* isomers of chlorogenic acid respectively,[13] and also traces of the *C*-glycosides vitexin and orientin were observed in some counter-current *T. aestivium* extracts by TLC methods but were present in such small amounts as to be undetectable on the two dimensional chromatogram (Fig. 1).

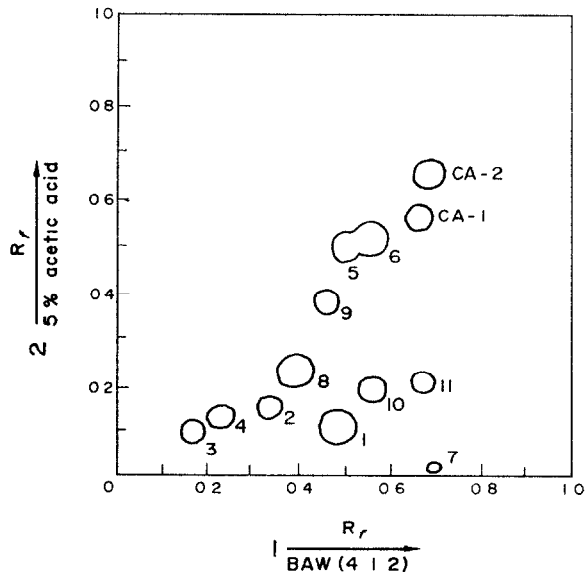

FIG. 1.

Compounds Isolated and Characterized

Iso-orientin (*Spot* 1). This compound was present in largest amount as the principal phenol in the mixture. The compound was isolated from tubes numbered 58–73 of a 100 tube counter-current extraction and further purified on a Sephadex LH-20 column. The UV spectra and R_f values are the same as those reported for iso-orientin in the literature (Table 1 and 2).[14] After recrystallization from hot aqueous methanol solution, the compound gave a m.p. 231–233° (lit. 235°). The acetylated derivative recrystallized from a ethanol–water mixture gave a m.p. 141–145° (lit. 140–4°). Ferric chloride oxidation according to the method of Seshadri *et al.*[15] gave a positive test for glucose. Alkaline degradation produced phloroglucinol and protocatechuic acid residues (Table 3). Spectral shifts in the UV indicated that —OH groups at the 3′,4′ and 5,7 positions were free and acid and enzymic hydrolyses produced no sugar nor alterations in the R_f values. The NMR spec-

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TABLE 1. SPECTRAL PROPERTIES OF *C*-GLYCOSYLFLAVONES FROM *Triticum aestivum* LEAVES

Compound	λ_{max} (nm) in Methanol					
	Band I				Band II	
	Alone	NaOEt	$AlCl_3$	H_3BO_3 Na-Acetate	Alone	Na-Acetate
Triticum *C*-Glycosylflavones:						
1	348	406	387	377	271 258	275
2	349	403	393	374	271 258	270
2 (Hydrolysed)	349	403	388	377	271 258	276
3	348	407	388	376	273 258	281
4	348	407	388	376	272 258	281
5	348	407	387	378	271 258	272
5 (Hydrolysed)	348	406	387	377	271 258	276
6	337	—	388	337	269	269
6 (Hydrolysed)	337	386s	384	337	269	269
8	333	399	384	344s	272	282
Authentic *C*-Glycosylflavones*:						
Iso-Orientin	349	406	388	377	272 258	276
Lutonarin	349	403	393	374	271 258	270
Lucenin-1	349	408	388	377	272 258	283
Lucenin-3	349	408	388	377	273 258	282
Swertisin	333	389	384	337	272	272
Vitexin	336	395	383	344	270	280

* Marker compounds are gifts of the USDA Forest Products Research Laboratory, Madison, Wisconsin; and of Dr. Sam Asen, USDA Agricultural Research Service, Horticulture Division, Beltsville, Md. to whom we are deeply indebted.

TABLE 2. R_f VALUES OF *C*-GLYCOSYLFLAVONES FROM LEAVES OF *Triticum aestivum*

Compound	$R_f \times 100$ in.*				
	BAW	5%-HOAc	15%-HOAc	TBA	20%-HOAc
Triticum *C*-Glycosylflavones:					
1	50	16	33	42	41
2	33	22	42	18	57
2 (Hydrolysed)	50	16	33	42	41
3	19	11	25	8	32
4	25	20	40	13	51
5	55	50	70	40	75
5 (Hydrolysed)	50	16	33	42	41
6	61	55	78	51	84
6 (Hydrolysed)	60	14	38	48	51
8	37	27	45	32	58
9	46	39	—	—	—
Authentic *C*-Glycosylflavones:					
Iso-Orientin	50	16	33	42	41
Orientin	31	5	12	23	16
Lutonarin	34	21	45	18	58
Lucenin-1	19	11	25	8	32
Lucenin-3	25	20	40	13	51
Swertisin	78	32	58	74	70
Vitexin	47	7	27	38	31
Iso-Vitexin	75	28	48	65	59
Saponarin	48	37	65	32	76

* Determined by TLC on Avicel micro-crystalline cellulose plates; solvent systems as listed in the Experimental section.

trum of the octa-acetate showed signals for acetyl methyl groups at δ1·89 (2″ acetyl) which is typical of 6-*C*-glycosylflavones.[5] Singlets at δ6·58 and δ7·41 were assigned to the H-3 and H-8 protons of the flavone molecule, respectively. A doublet at δ4·92 was assigned to the H-1″ proton ($J = 9$ Hz) which indicates trans diaxial coupling with H-2″. Thus NMR and UV spectral, alkaline degradation, ferric chloride oxidation, m.p. and chromatographic data identified compound 1 as 6-*C*-β-D-glucopyranosyl luteolin, (iso-orientin).

TABLE 3. ALKALINE DEGRADATION PRODUCTS OF *Triticum aestivum* *C*-GLYCOSYLFLAVONES

Compound	$R_f \times 100$ in* CAW	BzAW	BAW	Color DSA Reagent†
Triticum *C*-glycosylflavones:				
1	50, 22	10, 0	84, 72	Buff, Orange
2	50, 22	10, 0	84, 72	Buff, Orange
3	50, 22 (tr)	10, 0 (tr)	84, 72 (tr)	Buff, Orange
4	50, 22 (tr)	10, 0 (tr)	84, 72 (tr)	Buff, Orange
5	50, 22	10, 0	84, 72	Buff, Orange
6	83, 22 (tr)	37, 0 (tr)	91, 72 (tr)	Yellow, Orange
Authentic compounds:				
Phloroglucinol	22	0	72	Orange
p-OH acetophenone	96	63	88	Yellow
p-OH benzoic acid	83	37	91	Yellow
Protocatechuic acid	50	10	84	Buff
Vanillic acid	95	88	95	Yellow-Brown
Syringic acid	99	83	93	Orange
Anisic acid	98	99	98	UV (F1) Purple
p-OH coumaric acid	88	50	92	Yellow
Caffeic acid	60	12	82	Buff

* Determined by TLC on Avicel Micro-crystalline cellulose plates; solvent systems as listed in the Experimental section.

† All Chromatograms visualized in color with DSA reagent spray.

Lutonarin (*Spot* 2). A very small amount of this compound was isolated from a TLC chromatogram strip developed in the BAW system of a mixture obtained from counter-current tubes 31–48 which was further purified on a Sephadex LH-20 column. The UV spectral data (Table 1) indicated a luteolin based compound with the 7-position substituted. Acid and enzymic hydrolysis (anthocyanase-B) yielded glucose and iso-orientin (Tables 2 and 4). Co-chromatography with authentic lutonarin also showed that this compound was 7-*O*-β-D-glucopyranosyl-iso-orientin, (lutonarin).[16,17]

Lucenin-1 (*Spot* 3). This compound was isolated in very small amounts in the same manner as lutonarin. The UV spectral data (Table 1) indicated a luteolin derivative with the 5,7 and 3′4′—OH positions unoccupied. Migration on TLC, however, showed a diglycosyl derivative and co-chromatographic data with authentic lucenin-1 matched R_f values for the marker compound (Table 2). Furthermore, digesting the compound in 2 N HCl produced no sugar but gave two spots matching those for lucenin-1 and lucenin-3 (Wessely-Moser shift). Therefore, it was concluded that this compound be identified as lucenin-1.[18]

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[18] M. K. SEIKEL, J. H. S. CHOW and L. FELDMAN, *Phytochem.* **5**, 439 (1966).

TABLE 4. SUGARS FROM HYDROLYSED *Triticum aestivum* C-GLYCOSYLFLAVONES

	$R \times 100$*		Color by Partridge Reagent
	S_I*	S_{II}†	
Triticum *C*-Glycosylflavones:			
1 ($FeCl_3$ oxidation)	15	36	Brown
2	15	36	Brown
5	15, 60	36, 61, 23 (Anthocyanase-B)	Brown‡
6	15	36 (β-Glucosidase)	Brown§
Authentic Compounds:			
Glucose	15	36	Brown
Galactose	12	30	Brown
Arabinose	28	46	Red
Xylose	33	49	Red
Rhamnose	60	61	Brown
Cellobiose	—	14	Brown
Rutinose (from Hesperidin)	—	23	Brown
Neo-Hesperidose (from Naringin)	—	25	Brown

* S_I Determined by TLC on silica gel plates buffered with 0·3 M NaH_2PO_4 in *n*-butanol–acetone–water (4:5:1 v/v).

† S_{II} Determined by TLC on silica gel plates buffered with 0·1 M KH_2PO_4 in $CHCl_3$–acetic acid–water (30:35:5 v/v) and developed twice upwards.

Anthocyanase-B was obtained as a gift from Rohm & Haas Co., Philadelphia, Pa. to whom we are indebted. The enzyme was purified by $(NH_4)_2SO_4$ ppt. and subsequent gel chromatography on DEAE-Cellulose (Sigma Chemical Co., St. Louis, Mo.).

β-Glucosidase (Sigma Chemical Co., St. Louis, Mo.).

Lucenin-3 (*Spot* 4). The same isolation procedure and analytical data (Table 1 and 2) for the determination of lucenin-1 indicated that compound 4 be identified as lucenin-3.

Wyomin (*Spot* 5). Samples of this as yet unreported compound were obtained from the counter-current extraction in tubes 6–21 of a 100 tube separation which were further purified on a Sephadex LH-20 column followed by streaking onto TLC cellulose plates and developing in 5% acetic acid. Attempts to recrystallize the compound were unsuccessful but an acetate derivative gave a m.p. of 144–145°. The UV spectra indicated luteolin derivative with the 7-OH position occupied as indicated by the sodium acetate shift (Table 1); otherwise, these data are similar to those of iso-orientin. Enzymic (anthocyanase-B) and acid hydrolysis showed a disaccharide moiety present which was found to be rutinose by TLC; the aglycone was determined as iso-orientin by the same techniques. NMR spectrum of the acetate derivative in deuterated chloroform showed the following signals: δ1·83 (2″ acetyl), δ0·98 (6″ —CH_3 of rhamnose), δ4·4 (1″-rhamnose), δ4·9–5·2 (1″-glucose) and other signals which conformed to the iso-orientin acetate NMR spectrum and which also integrated for 13 acetyls. Thus, NMR, UV, spectral and chromatographic data (Tables 1, 2, and 4) identified *wyomin* as the 7-*O*-rutinosyl derivative of iso-orientin, or 7′-*O*-rutinosyl-6-*C*-β-D-glucopyranosyl luteolin.

4′-O-*Glucosyl iso-swertisin* (*Spot* 6). A very small amount of this compound was isolated from a mixture containing *wyomin* (above) by column chromatography using a long Sephadex LH-20 column (25 × 600 mm). The desired compound was eluted from the column before *wyomin* and was further purified on TLC cellulose in 5% acetic acid. UV spectral data indicated an apigenin derivative with the 7-OH position occupied both before and after hydrolysis reactions (sodium acetate shift). The sodium borate shift was negligible

but sodium ethoxide treatment after hydrolysis indicated a free 4'-OH (Table 1). Enzymic (β-glucosidase) and acid hydrolysis produced the aglycone iso-swertisin (iso-flavocommelitin) and glucose by TLC (Tables 2 and 4). Alkaline degradation studies (Table 3) with both fused potassium hydroxide and 15% barium hydroxide yielded *p*-hydroxybenzoic acid but very little phloroglucinol. Spectral data in the IR (KBr disc) yielded peaks ν_{max} 3420 (—OH), 2920 and 2846 (—OCH_3), 1625 (CO), 1380 (—CH_3), 1246 (—OCH_3), 1182 (—OCH_3) and 832 cm^{-1} (—OH) very similar to swertisin.[19] Thus chromatographic and spectral data characterized this compound as the 4'-*O*-glucosyl derivative of iso-commelitin, or 4'-*O*-β-D-glucopyranosyl-8-*C*-β-D-glucopyranosylgenkwanin.

Vicenin-2 (*Spot* 8). This compound was isolated in very small yield by counter-current extraction (Tubes 6–21), further separated on the Sephadex LH-20 column with wyomin (above) and then obtained in pure form by preparative TLC on micro-crystalline layers developed with 5% acetic acid. UV spectral data indicated (Table 1) a vitexin derivative with the 5,7 and 4' —OH positions unsubstituted; however TLC data (Table 2) indicated the presence of two glycosyl moieties which must therefore be present at the *C*-6 and/or *C*-8 positions. Prolonged acid hydrolysis (2 hr) yielded no sugar nor was the R_f of the glycoside changed in any way, thus excluding the possibility of a *C*-glycosyl-*O*-glycoside or the vicenin-1,3 or 4 isomers.[18] These data, then, indicated that (Spot 8) be characterized as the 6,8-*C*-diglycosylapigenin, vicenin-2.

DISCUSSION

Most of the flavonoid glycoside pigments found in grasses occur as *C*-glycosyl flavones, a characteristic feature of the family.[1,20] This proved to be the case in the hard, spring wheat varieties examined, as *C*-glycosides both in the luteolin and apigenin series were found, some of which had mono and disaccharide moieties attached. Purification and separation of wyomin (Spot 5) and the iso-flavocommelitin glycoside (Spot 6) was difficult because these two compounds had very similar R_f chromatographic values in the solvent system employed and could not be recrystallized because of the large amount of sugar present. Reasonable separation could be obtained, however, by using a longer Sephadex LH-20 column (12·5 × 600 mm).

Some correlation of this work to the results reported by Harborne and Hall[3] on grasses, especially those results which pertain to the *T. dicoccum* cv. 'Khapli' extracts, is relevant to this discussion. There is no doubt that the two Triticum taxa both contain iso-orientin, Harborne and Hall's compound T-2·1, in the cv. 'Khapli' extracts and present as the principal phenol in the *T. aestivum* cultivars (Spot 1). It would likewise appear that wyomin (Spot 5) and Harborne's compound T-5 are the same substance; and his T-2·2 and T-2·3 compounds bear a close resemblance to the lucenin-3 (Spot 4) and lucenin-1 (Spot 3) compounds respectively, detected in this work. Also, (Spot 9), unidentified in this paper seems to have the same chromatographic data (Table 2) as saponarin which was identified as compound T-6 in the cv. 'Khapli' extracts of Harborne and Hall. Although vicenins 1 and 3 have been detected in the cv. 'Khapli' extracts, the vicenin compound found in the *T. aestivum* cultivars was characterized as vicenin-2 previously identified in the wood of Vitex lucens by Seikel *et al.*[18] Surprisingly, traces of iso-flavone glycosides were detected

[19] L. H. BRIGGS, L. D. COLEBROOK, H. M. FALES and W. C. WIDMAN, *Analyt. Chem.* **29**, 904 (1957).
[20] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp.241–247, Academic Press, New York (1967).

in our extracts (Spots 10 and 11) which is significant enough to report because these compounds, although not characterized in this work due to very limited amounts, have potential physiological effects because of their estrogen-like structure. Thus, most compounds found in the *T. aestivum* cultivars correspond closely with those found in the cv. 'Khapli' extracts reported by Harborne and Hall.[3]

The iso-flavocommelitin glycoside (Spot 6) proved to be an interesting compound as it is the first reported occurrence of a 7-methyl ether flavone in the Gramineae although it appears in *Iris tingitana* hybrids (Prof. Blaauw) as swertisin,[21] and in the spiderwort, *Commelina communis*, as the 4′-*O*-β-D-glucopyranosyl derivative of swertisin, flavocommelinin.[10] When iso-flavocommelitin glycoside (Spot 6) was hydrolysed and R_f values of its aglycone compared with the iso-flavocommelitin obtained by Takeda *et al.*[10] after acid digestion of their swertisin glycoside, it was found that the R_f values of the two compounds closely matched each other despite the fact that the Japanese workers used Toyo-50 paper. This also proved to be the case when swertisin and iso-swertisin (Spot 6-H) were compared in the same chromatographic solvent systems (*in actuo*) as the same two spots were obtained after acid digestion of both compounds. These data also correlated well with the spectral data on both swertisin and iso-swertisin from hydrolysed (Spot 6) in the UV. The IR spectra of both compounds gave methoxy peaks at ν_{max} 2920, 2846, 1246, and 1182 cm^{-1} and other absorption peaks were similar in both compounds. Unfortunately, meager amounts of material precluded NMR analysis. Spot 6, then, was concluded to be the 4′-*O*-glucosyl derivative of iso-swertisin, an 8-*C*-glucoside of genkwanin.

Although a great deal of work has been expended on the identity of the *C*-glycosyl flavones in wheat, it is still not known what specific role these phenolic compounds perform in the plant in relation to plant disease resistance. In this study, the number of different phenols and their total amounts showed no significant difference in the rust susceptible (cv. 'Marquis') and rust resistant (cv. 'Selkirk') varieties tested so undoubtedly other factors are involved.[13] Further studies are needed to determine how physiologic mechanisms of disease resistance are affected by phenolic compounds or their oxidation products.

EXPERIMENTAL

Plant Material

Two *Triticum aestivum* hard, red, spring wheat varieties were used in the investigation; 'Selkirk', a rust resistant variety and 'Marquis', a rust susceptible variety. The plants were field grown and harvested at the boot stage, dried in moving air in a dehydrator at 150° for 6–8 hr and subsequently ground in a Wiley mill and stored in air tight containers.

Chromatographic Procedures

Two dimensional TLC chromatograms were developed on Avicel microcrystalline cellulose, using: BAW, *n*-BuOH–HOAc–H_2O (4:1:2 v/v) in the first direction and 5% HOAc in the second direction.

Analytical TLC was performed on Avicel microcrystalline cellulose layers (250 μ), in the following solvent systems: 5% HOAc; 15% HOAc; 20% HOAc; BAW; TBA, *t*-BuOH–HOAc–H_2O (3:1:1 v/v); CAW, $CHCl_3$–HOAc–H_2O (50:45:5 v/v); BzAW, benzene–HOAc–H_2O (6:7:3 v/v) upper phase.

C-Glycosyl flavones were visualized in UV (366 nm) light and in the UV light in the presence of ammonia fumes. Phenolic compounds were sprayed with DSA, diazotized sulfanilic acid.[22] Sugars were sprayed with Partridge's Reagent and subsequently heated at 105° for 15 min.[23]

Column chromatography was carried out on Sephadex LH-20 columns (25 × 400 mm) and developed

[21] S. Asen, R. N. Stewart, K. H. Norris and D. R. Massie, *Phytochem.* **9**, 619 (1970).
[22] R. N. Ames and H. K. Mitchell, *J. Am. Chem. Soc.* **74**, 252 (1952).
[23] S. M. Partridge, *Nature, Lond.* **164**, 443 (1949).

in an equivolume mixture of MeOH and $CHCl_3$. Usually, 30 × 5 ml fractions were collected in each separation.

Spectral Analyses

UV spectra were determined in MeOH using a Beckman DB-G UV spectrophotometer. Diagnostic shifts were recorded 1 min after the addition of the following reagents to 3 ml solutions of the *C*-glycosyl flavones: (1) 5 drops of 5% $AlCl_3$ in MeOH; (2) 3 drops of 0·3% NaOEt in MeOH; (3) excess of coarsely powdered NaOAc; (4) solution 3 plus 5 drops of saturated H_3BO_3 in MeOH.[24]

IR spectra were measured in a Perkin–Elmer Model 621 spectrophotometer using KBr pellets.

NMR spectra were determined on a Varian A-60 spectrometer using either $CDCl_3$ or DMSO (d_6) as solvents and tetramethylsilane used as internal standard.

Extraction and Separation

The powdered leaves were extracted twice by stirring in H_2O (80°) for 2 hr and then allowed to cool and stand overnight at room temp.; 10 and then 5 ml per gram being used. The water extracts were combined and concentrated to 0·1 the extract volume and extracted with *n*-BuOH until colorless. The BuOH extract was concentrated under reduced pressure at 40° to dryness, dissolved in H_2O and freeze-dried. The freeze-dried mixture was redissolved in 25 ml of a mixture of 15% *n*-BuOH in EtOAc and applied to a 100 tube counter-current distribution machine and developed using the solvent system: 15% *n*-BuOH in EtOAc–H_2O (1:1 v/v).

The counter-current separations were combined into appropriate fractions as monitored by TLC in 5% HOAc. The organic phase was removed by rotary evaporation and the aqueous phase freeze-dried. The freeze-dried fractions were further separated on a Sephadex LH-20 column and then further separated if necessary on TLC layers using 5% HOAc. The separated compounds were then scraped from the cellulose layers and eluted with 80% aqueous MeOH, freeze-dried and stored for analytical use.

Investigation of C-Glycosyl flavones

Acid hydrolysis. 1–2 mg of compound was brought to reflux in 5 ml of an equivolume mixture of MeOH and 2 N HCl for 1 hr. The MeOH was evaporated and the acid solution extracted with H_2O saturated *n*-BuOH, condensed and spotted onto cellulose plates and subsequently developed in 5% HOAc with appropriate markers. The acidic sugar solutions were neutralized with 10% N-methyldioctylamine in $CHCl_3$ to pH 6; separated and condensed to small volume and spotted onto buffered silica plates and developed in S_I or S_{II} solvents[25] along with appropriate markers (Tables 2 and 4).

Enzymic hydrolysis. 1–2 mg of compound was incubated overnight in 0·1 M acetate buffer, pH 5, with 1 mg of enzyme (β-glucosidase or anthocyanase B, Rohm and Haas) at 30°. The mixture was then extracted with *n*-BuOH, condensed to small volume and chromatographed as in acid hydrolysis above with appropriate aglycone markers. The residual aqueous mixture was condensed to small volume and cold MeOH added to precipitate the enzyme which was then removed by centrifugation. The supernate was condensed to small volume and spotted onto buffered silica gel plates along with appropriate markers and developed with S_{II} solution[26] (Table 4).

Alkaline degradation. 1–2 mg of compound was fused with 200 mg of KOH for 10 min at 205–210° using a silicone oil bath. The cooled melt was neutralized with 2 N HCl, extracted with Et_2O, condensed to small volume and spotted onto cellulose TLC along with appropriate markers and developed in CAW, BzAW and BAW solvent systems (Table 3).[27]

Mild alkaline degradation. 1–2 mg of compound was brought to reflux for 1 hr in 15% $Ba(OH)_2$ under N_2.[28] The products were isolated and chromatographed as above (Table 3).

Acetylation. 50 mg of compound were allowed to stand overnight in a mixture of 1·5 ml pyridine and 2·5 ml Ac_2O at room temp. The mixture was then concentrated to a thick syrup and H_2O was added dropwise to give a white precipitate. The acetylated compound was recrystallized from EtOH–H_2O mixtures.

Ferric chloride oxidation [15] 25 mg of compound was treated with 200 mg $FeCl_3$ in 0·8 ml water which was heated in an autoclave at 115° for 15 min and then at 125° for 6 hr. The mixture was diluted to 10 ml with H_2O and 10% NaOH added dropwise to pH 8 to precipitate the excess Fe salts. The filtrate was treated with 2 N HCl to pH 6 and chromatographed successively on Amberlite IR-120 (H-form) (Rohm and Haas) and

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[25] V. S. OVODOV *et al.*, *J. Chromatography* **26**, 111 (1967).
[26] R. M. HOROWITZ and B. GENTILI, *J. Org. Chem.* **26**, 2899 (1961).
[27] R. HANSEL and H. RIMPLER, *Archiv der Pharmazie* **296**, 598 (1963).
[28] D. Y. C. LYNN and B. S. LUH, *J. Food Sci.* **29**, 735 (1964).

Duolite A-4 (OH-form) (Chemical Process Co., Redwood City, Calif.) columns (10 × 100 mm) to remove interfering ions. The eluate was concentrated to small volume and chromatographed onto buffered silica gel thin layers in S_1 solvent system along with appropriate sugar markers (Table 4).

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