PERACETYLATED 1-PHENYLFLAVAZOLES AS CARBOHYDRATE DERIVATIVES FOR MASS SPECTROMETRY*

PART I†. APPLICATIONS TO MONO- AND DISACCHARIDES‡**

GARY S. JOHNSON AND W. S. RULIFFSON
Department of Biochemistry, Kansas State University, Manhattan, Kansas 66502 (U. S. A.)
AND R. GRAHAM COOKS§
Department of Chemistry, Kansas State University
(Received November 23rd, 1970; accepted February 1st, 1971)

ABSTRACT

The title compounds give mass spectra that (*a*) include molecular ions of useful abundance, (*b*) allow the determination of the masses of monosaccharide constituents of disaccharides, (*c*) allow distinction between (1→4) and (1→6) interglycose bonds in disaccharides. The ion chemistry involved is interesting for the part that the 1-phenylflavazole moiety plays in elimination reactions involving hydrogen transfer.

INTRODUCTION

The use of electron-impact mass spectrometry in structural studies on carbohydrates suffers from certain well-known disadvantages, including a need for chemical derivatization, a limitation to compounds of relatively low molecular-weight (<2000), and the fact that stereochemical information is not usually obtained. Nevertheless, the method has grown rapidly in importance[2].

At the present stage in the development of mass spectrometry of carbohydrates, techniques are needed that will increase the abundances of molecular ions and ions of high mass to facilitate applications to larger and more interesting molecules. In one approach, electron impact has been used in conjunction with field ionization, sometimes with dramatic results[3]. We have opted to continue with the instrumentation more-readily available but have sought to achieve greater molecular-ion abundances by using selected derivatives. The inclusion of a group of low ionization-potential into a molecule has been suggested[4] as a general method of increasing the abundance of molecular ions. This effect operates, for example, in the spectra of 3-methyl-1-naphthyl glycosides of disaccharides[5]. In this case, moreover, the ease with which the aryloxy radical is lost channels the fragmentation of the compound

*Abstracted in part from the Ph.D. dissertation of G. S. J., Kansas State University, 1971.
†For Part II, applications to tri-, tetra-, and pentasaccharides, see following paper (p. 243).
‡A preliminary communication of this work has appeared, see Ref. 1a.
**A report on the mass spectra of 1-phenylflavazole derivatives of monosaccharides has appeared since completion of our work, see Ref. 1b.
§Address correspondence to this author at Department of Chemistry, Purdue University, Lafayette, Ind. 47907, U. S. A.

in a useful direction. The use of phenylosazones[6] and diethyl dithioacetals[7] as monosaccharide derivatives is attended by similar stabilization of the molecular ion.

In the present study 1-phenylflavazoles have been employed, in the expectation that they would show both good molecular-ion stabilizing properties and useful fragmentation-directing characteristics. Furthermore, the fluorescent and chromatographic properties of these compounds are advantageous in small scale preparations[8].

EXPERIMENTAL

D-Glucose, D-galactose, D-xylose, L-rhamnose, 4-*O*-α-D-glucopyranosyl-D-glucose (maltose), 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose), 4-*O*-β-D-galactopyranosyl-D-glucose (lactose), 6-*O*-α-D-glucopyranosyl-D-glucose (isomaltose), 6-*O*-β-D-glucopyranosyl-D-glucose (gentiobiose), and 6-*O*-α-D-galactopyranosyl-D-glucose (melibiose) were purchased from commercial sources.

The monosaccharide 1-phenylflavazoles and their acetates were synthesized according to Ohle[9]. Preparation of the 1-phenylflavazole disaccharide derivatives was based on the method of Neumuller[10], by using a sealed-tube reaction and 1–10 μmoles of disaccharide, as suggested by Nordin[8,11]. These derivatives were purified from their reaction mixtures by t.l.c. on cellulose with water-saturated butanone as eluant. Pyridine-catalyzed acetylation was used to prepare the derivatives for mass-spectrometric assay. The resulting dissaccharide 1-phenylflavazole peracetates produced spectra that were identical to those from the corresponding derivatives purified by recrystallization.

Mass spectra were obtained with an A. E. I. MS-9 mass spectrometer with solid-sample insertion. An ionizing voltage of 70 eV was employed, with source temperatures of 160° for the monosaccharides and 190° for the disaccharides.

RESULTS AND DISCUSSION

The mass spectra of the peracetylated 1-phenylflavazole derivatives of D-xylose, L-rhamnose, D-glucose, and D-galactose (**1–4**, respectively) exhibit molecular-ion abundances of 8–14% of the $M^+ - 102$ base peak (Table I). Major fragment-ions resulted from multiple losses of ketene and acetic acid. Rather abundant ions of mass 220, 245 and 247 were also present in each spectrum, as well as in the spectra of the corresponding nonacylated derivatives*, and can be taken as characteristic of the 1-phenylflavazole moiety. Of these three ions, the first two probably arise as illustrated, whereas *m/e* 247 is due to α-cleavage with double hydrogen-transfer to the ring.

The mass spectra of the six disaccharide derivatives studied are shown in Fig. 1. The three (1→4)-linked derivatives [maltose (**5**), cellobiose (**6**), and lactose (**7**), gave closely similar spectra, as did the three derivatives having (1→6)-glycosidic linkages [isomaltose (**8**), gentiobiose (**9**), and melibiose (**10**)]. Also included in Fig. 1 are the

*The spectra of these compounds[12] showed molecular ions of lower abundance than in compounds **1–4**. Nevertheless, relative abundances were still in the 1–10% range, which underscores the value of the 1-phenylflavazoles as compared with most other derivatives (compare Ref. 6).

TABLE I

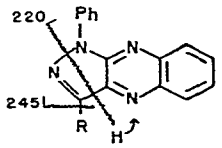
MASS SPECTRA OF THE PERACETYLATED MONOSACCHARIDE 1-PHENYLFLAVAZOLES[a]

D-Xylose derivative (**1**): 390 (9%, M^+), 331 (6), 330 (24), 305(3), 289 (23), 288 (100), 287 (3), 276 (5), 275 (27), 272 (6), 261 (4), 260 (20), 259 (10), 247 (6), 246 (7), 245 (15), 220 (14), 219 (4), 218 (4), 77 (4), 44 (3), 43 (11).

L-Rhamnose derivative (**2**): 404 (8%, M^+), 345 (7), 344 (26), 318 (4), 304 (3), 303 (23), 302 (100), 301 (32), 286 (4), 285 (8), 277 (5), 276 (33), 275 (76), 274 (4), 273 (8), 261 (3), 260 (6), 259 (6), 248 (17), 247 (37), 246 (7), 245 (18), 232 (4), 221 (3), 220 (21), 219 (4), 218 (6), 129 (3), 92 (6), 91 (3), 77 (9), 69 (3), 57 (6), 55 (7), 51 (3), 44 (6), 43 (26), 41 (4),

D-Glucose derivative (**3**)[b]: 462 (14%, M^+), 420 (3), 403 (3), 402 (10), 361 (21), 360 (100), 359 (28), 344 (3), 343 (7), 319 (9), 318 (44), 317 (37), 302 (11), 301 (50), 300 (10), 290 (6), 289 (12), 288 (6), 287 (6), 276 (23), 275 (81), 274 (6), 273 (14), 272 (4), 271 (3), 259 (6), 248 (9), 247 (24), 246 (10), 245 (21), 232 (3), 221 (4), 220 (26), 219 (4), 218 (5), 103 (3), 92 (6), 91 (3), 90 (3), 77 (15), 65 (3), 55 (3), 51 (4), 44 (4), 43 (57).

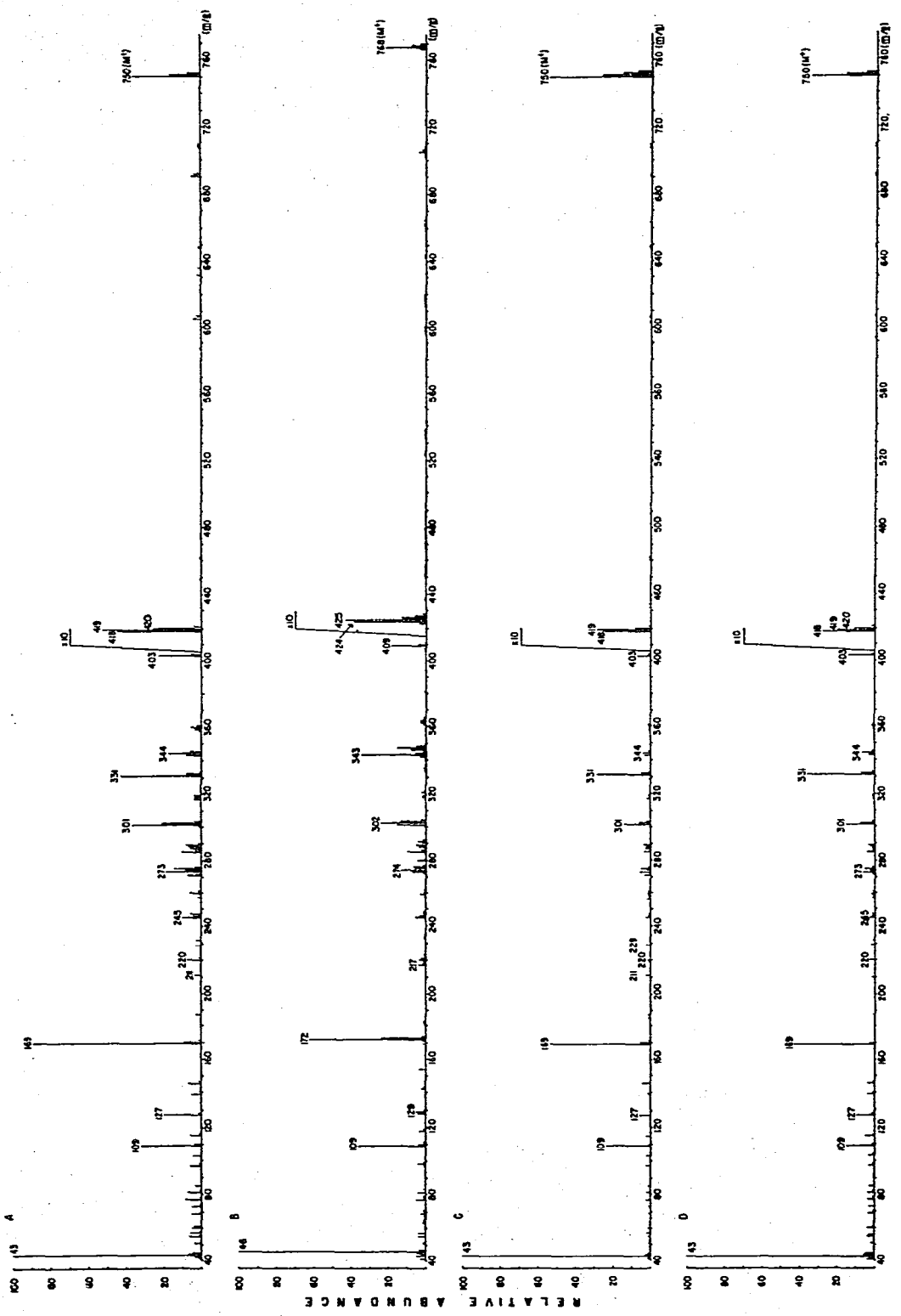
[a]All ions greater than 2% relative abundance are reported. Abundances are given in parentheses after the mass number. [b]The spectrum of D-galactose 1-phenylflavazole peracetate was very similar to that of the D-glucose derivative and is not, therefore, recorded.



Scheme 1

mass spectra of the trideuterioacetylated analogs (**11** and **12**, respectively) of the maltose and melibiose derivatives. All compounds justified expectations by giving molecular ions (*m/e* 750) of substantial abundance (0.85–5.4% relative to the CH_3CO^+ base peak). This result is to be contrasted with the barely detectable or undetectable molecular-ions encountered by using such common disaccharide derivatives as acetic esters[5], methyl ethers[13] and trimethylsilyl ethers[14]. Fragment ions occurring in the disaccharide spectra can be divided into three groups, (*a*) those associated with glycosidic cleavage reactions, (*b*) ions characteristic of the 1-phenylflavazole group, (*c*) ions that permit assignment of linkage position. The genesis of these ions can now be discussed.

Fragmentations at the glycosidic bond result in the formation of two families of ions. One of these is composed of the familiar Type A ions* (following the nomenclature of Kochetkov and Chizhov[15]). As expected, the primary Type A ion (*m/e* 331), undergoes loss of ketene and acetic acid, forming daughter ions, *m/e* 289, 271, 247, 229, 211, 187, 169, 127, and 109. The shifts in mass seen in the per(trideuteroacetyl)-ated derivatives **11** and **12** follow expectation. The ion *m/e* 109 is not shifted, meaning that the fragmentation pathways in these compounds are more similar to those observed in *p*-nitrophenyl β-D-glucopyranoside peracetate[16] than in the *O*-acetylated and *O*-methylated peracetates of glycosides studied by Biemann and coworkers[17]. Cleavage on the other side of the glycosidic oxygen atom results in an abundant ion at *m/e* 403. Metastable fragmentations show that this ion undergoes further frag-


A
B
C
D
RELATIVE ABUNDANCE
m/z

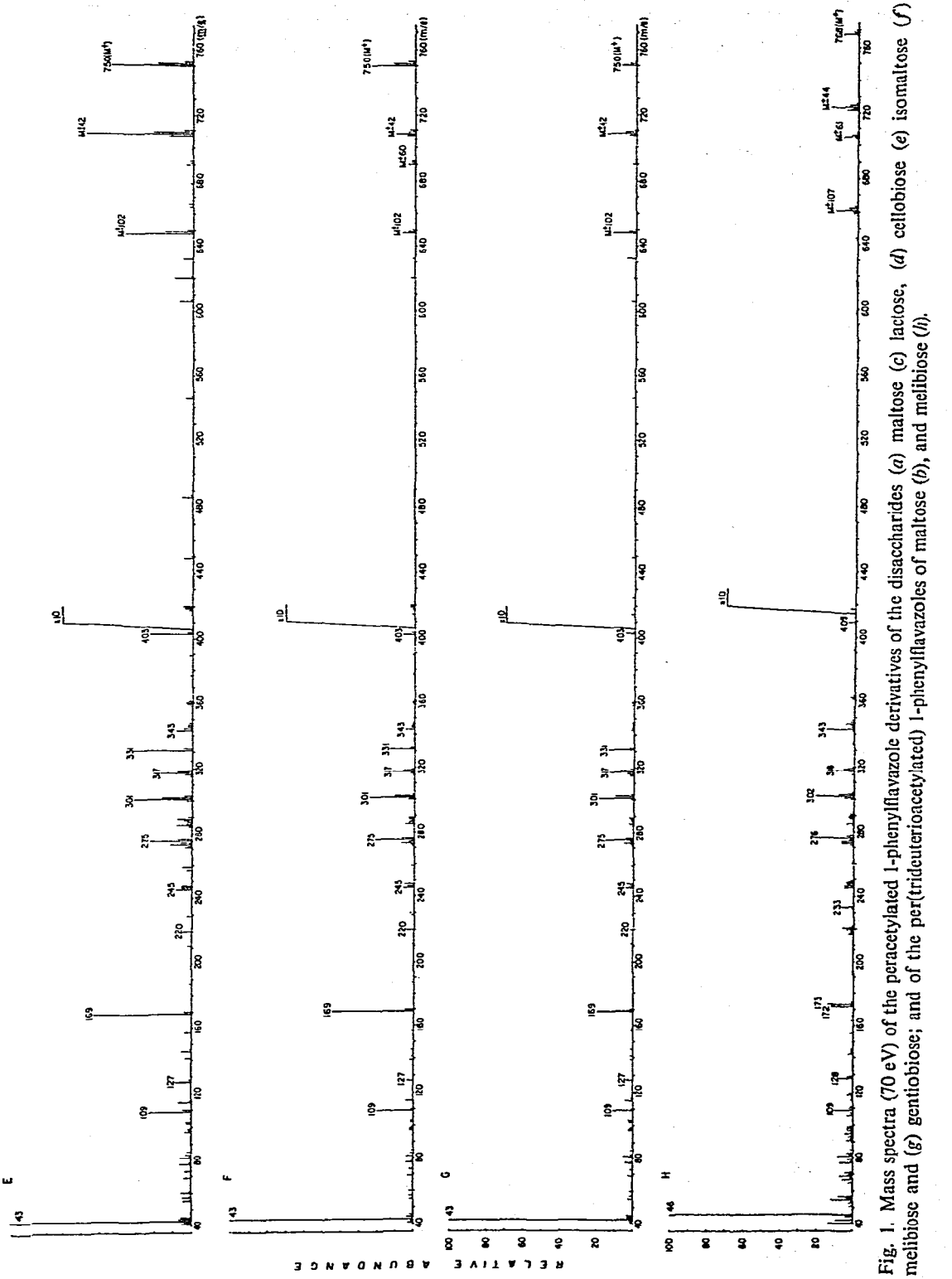
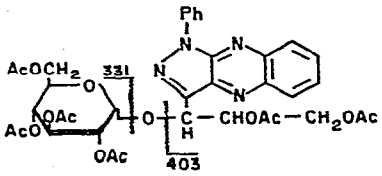


Fig. 1. Mass spectra (70 eV) of the peracetylated 1-phenylflavazole derivatives of the disaccharides (*a*) maltose (*c*) lactose, (*d*) cellobiose (*e*) isomaltose (*f*) melibiose and (*g*) gentiobiose; and of the per(trideuterioacetylated) 1-phenylflavazoles of maltose (*b*), and melibiose (*h*).

mentation of the type suffered by *m/e* 331, to give *m/e* 361, *m/e* 343, and *m/e* 301. The prominent primary-ions produced by cleavage on either side of the glycosidic



5

Scheme 2

oxygen atom allow determination of the masses of both the reducing and nonreducing monosaccharide constituents of the disaccharide.

The three flavazole-characteristic ions *m/e* 220, 245, and 247 appear in the spectra of all six disaccharide derivatives. The ion having *m/e* 220, presumably arises as shown for the monosaccharides derivatives; deuterium-labelling data indicating that, at least in the cases of maltose and melibiose, the hydrogen transferred has a multiple origin (Fig. 1.) The ion (*m/e* 245) resulting from α-cleavage is not expected to be shifted in the labeled compounds, and the small proportion that is shifted to *m/e* 246 points to some contribution from more complex processes. The origin of *m/e* 247 is rather complex, inasfar as the type of hydrogen atom transferred to the ring is concerned. The deuterium-labelling data for the maltose derivative (**11**) shows both HD and H_2 transfer, whereas the (1→6)-linked disaccharide (**12**) shows, in addition, appreciable D_2 transfer (Fig. 1).

Features of the disaccharide spectra that allow linkage positions to be distinguished are summarized in Table 2. The contrast between the (1→4)- and (1→6)-linked compounds is most pronounced in the $M^+ - 42$, $M^+ - 102$, and *m/e* 418 ions, which are, therefore, preferred for making this assignment. Indeed, the most striking difference between the two classes of disaccharides is the fact that the high-mass region of the (1→4)-linked compounds is substantially free of fragment ions. The far greater tendency of the (1→6)-linked derivatives to eliminate ketene results in prominent

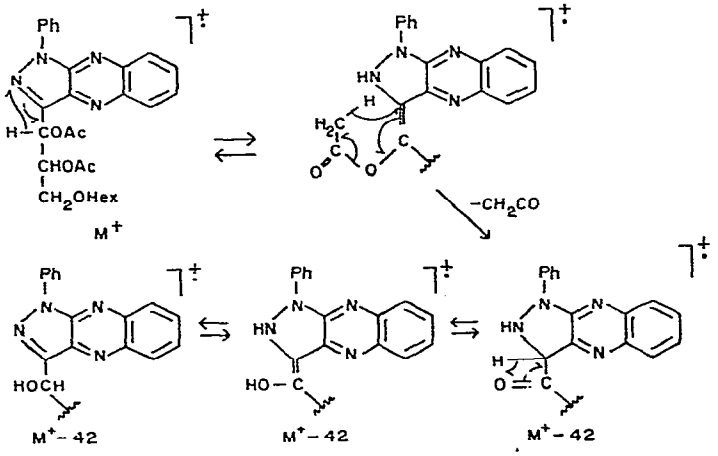
TABLE II

ASSIGNMENT OF LINKAGE POSITIONS IN PERACETYLATED DISACCHARIDE 1-PHENYLFLAVAZOLES BY MASS SPECTROMETRY[a]

Disaccharide	*Linkage*	$M^+ - 42/M^+$	$M^+ - 102/M^+$	m/e *418*	m/e *344*	m/e *318*	m/e *317*
Maltose	α-D-(1→4)	0.047	0.021	1.4	15.8	2.6	3.4
Lactose	β-D-(1→4)	0.026	0.009	1.7	5.5	0.8	0.6
Cellobiose	β-D-(1→4)	0.018	0.007	2.0	3.2	0.7	0.8
Isomaltose	α-D-(1→6)	1.34	0.84	0.2	2.3	4.7	16.5
Melibiose	α-D-(1→6)	1.89	1.18	0.0	0.7	5.1	12.5
Gentiobiose	β-D-(1→6)	0.57	0.29	0.1	0.5	3.8	12.2

[a]Ion abundances are given relative to the base peak and have been corrected for ^{13}C isotopic contributions.

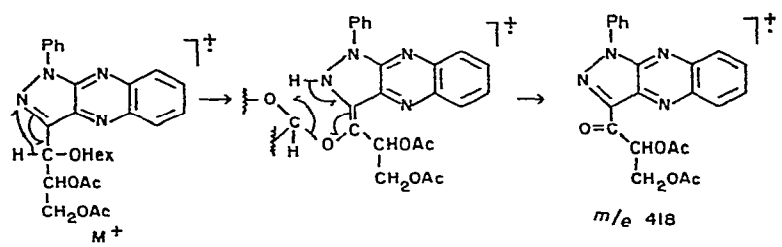
$M^+ - 42$ and $M^+ - 102$ peaks; the aromatic ring may well be involved in the hydrogen trasfer necessary for loss of ketene. In the (1→4)-linked compounds the α- carbon atom does not bear an acetyl group, and the assistance of the ring is lost. Specifically, elimination of ketene might occur in the (1→6)-linked compounds via a McLafferty rearrangement, and hence involve a 6-membered, rather than a 4-membered, transition state. The suggested process, together with the necessary tautomeric equilibria are shown in the accompanying scheme.



Hex = peracetylated hexopyranosyl group

Scheme 3

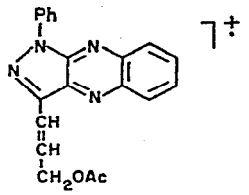
Formation of the linkage-characteristic ions *m/e* 418 and *m/e* 344 also appears to be favored when hydrogen transfer to the heteroaromatic system is possible. Thus *m/e* 418 arises by hydrogen transfer to the pyranoside sugar, which is eliminated as a neutral molecule. The mechanism shown accounts for the shift of six mass units in the labelled compound (**11**) and for the restriction to the (1→4)-linked disaccharides. The ion of *m/e* 344, also characteristic of the (1→4)-glycosidically linked



Scheme 4

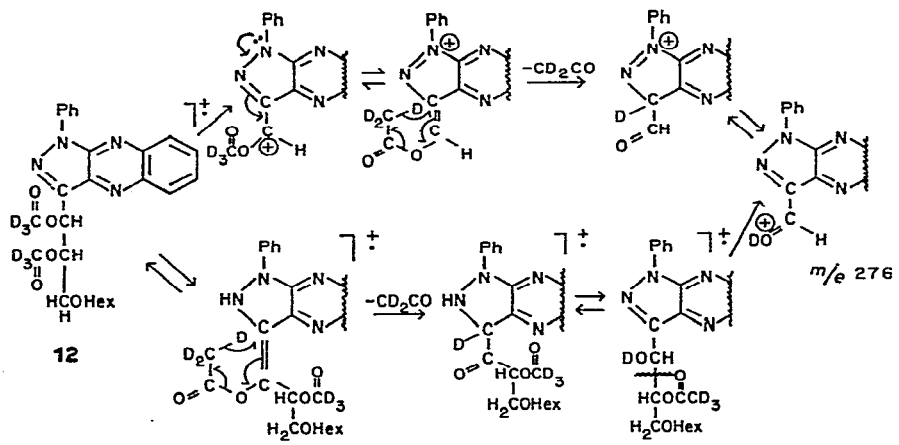
compounds, shifts to *m/e* 347 in the d_{18}-derivative (**11**) indicating retention of the flavazole moiety and just one acetyl group in the ion. This suggests an ion structure

(as illustrated) that could arise by elimination of neutral molecules of ketene and monosaccharide. In addition to participation by the ring in loss of the monosaccharide, the impossibility of forming a similarly conjugated ion in the (1→6)-linked compounds may favor the process in the (1→4)-bonded compounds.



Scheme 5

Ions that remain to be discussed are those at *m/e* 318 and 317 and the associated ions, *m/e* 276 and 275. The most obvious explanation for these ions in the (1→6)-linked compounds is that they are due to β-cleavage, with and without hydrogen rearrangement, followed by loss of ketene. Although labelling data for compound **12** provides clear evidence that *m/e* 275, which is shifted cleanly to *m/e* 276, does arise by loss of ketene following or preceding β-cleavage (as illustrated), it also shows that *m/e* 317 does not represent the product of simple β-cleavage or *m/e* 318 that of

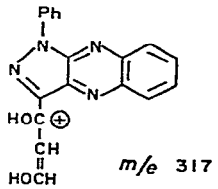


Hex = per(trideuteroacetyl)hexopyranosyl

Scheme 6

β-cleavage with hydrogen rearrangement to the ring. At first sight, these observations on the (usually favored) processes of β-cleavage are unexpected; indeed, it may be that they do occur with facility to give *m/e* 318 and 317, which then must undergo rapid fragmentation to give *m/e* 276 and 275. It is also noteworthy that β-cleavage, with or without hydrogen rearrangement, does not give rise directly to a product-ion in at least two of the monosaccharide derivatives (**1** and **2**). In the other cases, elimination of two molecules of ketene and one of acetic acid satisfactorily accounts for the

observed ions (*m/e* 317 and 318). All of the monosaccharide derivatives do show abundant ions at *m/e* 275, the origin of which presumably does involve β-cleavage, as with the (1→6)-linked disaccharides. The ion observed at *m/e* 317 in the (1→6)-linked disaccharides may have the structure shown, and probably arises by processes related to those suggested for the monosaccharide derivatives. These include elimination of ketene from the molecular ion, an unfavorable process in (1→4)-linked disaccharides (see earlier arguments), which explains the lower abundance of this ion in



m/e 317

Scheme 7

the (1→4)-linked class. The lower abundances of *m/e* 275 in the (1→4)-linked compounds can be explained simply, since β-cleavage here leads to *m/e* 605 and not *m/e* 317.

CONCLUSION

As emphasized in the discussion, the acetylated 1-phenylflavazole derivatives meet the requirements of providing molecular ions of adequate abundance for structural studies on disaccharides. Their spectra also allow, very simply, the determination of the masses of the constituent monosaccharides and the position of the glycosidic linkage.

Several limitations of this method need emphasis. First, the conditions of derivatization could cause a serious problem of hydrolysis with disaccharides having more-labile glycosidic linkages. Second, the epimers **3** and **4** gave virtually identical spectra. In addition, the differences observed within the groups of compounds **5**–**7** and **8**–**10** could not be correlated with stereochemical features. However, by examining a wider range of compounds such correlations might be found. A third, and serious, limitation is the restriction on the types of disaccharides that can form 1-phenylflavazole derivatives. For this reason, other aromatic derivatives that incorporate fewer carbohydrate carbon atoms in the aromatic nucleus might be preferable.

ACKNOWLEDGMENT

The authors acknowledge financial support provided by NASA University Sustaining Grant NSG-692, Kansas Agriculture Experiment Station Organized Research Fund 797, and the Research Corporation.

REFERENCES

1 (a) G. S. JOHNSON, W. S. RULIFFSON, AND R. GRAHAM COOKS, *Chem. Commun.*, (1970) 857; (b) L. DOLEJŠ, Z. VEISOVÁ, AND J. FARKAS, *Org. Mass Spectrom.*, 3 (1970) 1535.
2 For a general discussion, see R. G. COOKS AND G. S. JOHNSON, in *Specialist Periodical Report, Mass Spectrometry of Organic and Organometallic Compounds*, (1971), in press.
3 P. BROWN, F. R. BRUSCHWEILER, G. R. PETTIT, AND T. REICHSTEIN, *J. Amer. Chem. Soc.*, 92 (1970) 4470.
4 A. N. H. YEO, AND D. H. WILLIAMS, *J. Chem. Soc. (C)*, (1968) 2666.
5 J. KARLINER, *Tetrahedron Lett.*, (1968) 3545.
6 T. ITO, *Agr. Biol. Chem.*, 33 (1968) 1217.
7 D. C. DEJONGH, *J. Org. Chem.*, 30 (1965) 1563.
8 P. NORDIN AND D. FRENCH, *J. Amer. Chem. Soc.*, 80 (1958) 1445.
9 H. OHLE AND R. LIEBIG, *Ber.*, 75 (1942) 1536.
10 G. NEUMULLER, *Arkiv Kemi Mineral. Geol.*, 21*A* (1946) No. 45.
11 P. NORDIN, *Methods Carbohyd. Chem.*, 2 (1963) 136.
12 G. S. JOHNSON, W. S. RULIFFSON, AND R. G. COOKS, unpublished results.
13 (a) O. S. CHIZHOV, L. A. POLYAKOVA, AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 158 (1964) 979; (b) J. KÄRKKÄINEN, *Carbohyd. Res.*, 14 (1970) 27.
14 (a) N. K. KOCHETKOV, O. S. CHIZHOV, AND N. V. MOLODTSOV, *Tetrahedron*, 24 (1968) 5587, (b) J. KÄRKKÄINEN, *Carbohyd. Res.*, 11 (1969) 247.
15 N. K. KOCHETKOV AND O. S. CHIZHOV, *Advan. Carbohyd. Chem.*, 21 (1966) 39.
16 T. C. SMALE AND E. S. WAIGHT, *Chem. Commun.*, (1966) 680.
17 (a) K. BIEMANN, D. C. DEJONGH AND H. K. SCHNOES, *J. Amer. Chem. Soc.*, 85 (1963) 1763; (b) D. C. DEJONGH AND K. BIEMANN, *J. Amer. Chem. Soc.*, 85 (1963) 2289.