Phytochemistry, 1973, Vol. 12, pp. 2269 to 2273. Pergamon Press. Printed in England.

MASS SPECTROMETRIC IDENTIFICATION OF A KAEMPFEROL TETRAGLYCOSIDE FROM *SOLANUM* SEED

ROLF D. SCHMID*
Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif-sur-Yvette, France

and

JEFFREY B. HARBORNE
Phytochemical Unit, Plant Science Laboratories, The University, Reading RG6 2AS

(*Received* 12 *March* 1973. *Accepted* 1 *May* 1973)

Key Word Index—*Solanum*; Solanaceae; mass spectrometry; kaempferol 3-sophorotrioside-7-rhamnoside; sugar identification.

Abstract—The trisaccharide present in the kaempferol 3-triglucoside-7-rhamnoside of potato seed has been identified by hydrolytic experiments and by MS measurements on the perdeuteriomethylated glycoside as sophorotriose (glucosyl-β1 → 2-glucosyl-β1 → 2-glucose).

INTRODUCTION

AN UNUSUAL kaempferol glycoside with as many as four monosaccharide moieties was earlier isolated from the seed of the potato and other tuberous *Solanum* species. It was formulated, from hydrolytical, analytical and spectral data, as the 3-triglucoside-7-rhamnoside, but the nature of the trisaccharide was not established.[1] Further work, reported in a preliminary note,[2] showed that sophorose was formed on partial acid hydrolysis. Also, a co-occurring 3-diglucoside-7-rhamnoside gave sophorose on both acid hydrolysis and H_2O_2 oxidation and was thus identified as kaempferol 3-sophoroside-7-rhamnoside.[2] H_2O_2 oxidation of the tetraglycoside gave a trisaccharide, named sophorotriose and provisionally assigned a linear β 1 → 2 linked structure; insufficient material was available to confirm this assignment by classical procedures.

That the above assignment is correct has now been confirmed by mass spectrometric measurements on perdeuteriomethylated (PDM) material, before and after hydrolysis.[3,4]

RESULTS

Sugars of the Tetraglycoside

The kaempferol 3-triglucoside-7-rhamnoside, isolated in crystalline form from potato seed,[1] gave a single disaccharide on partial acid hydrolysis, which was identified as sophorose by direct chromatographic comparison with authentic material. This shows that one of the linkages in the trisaccharide must be β 1 → 2. H_2O_2 oxidation of the tetraglycoside

* Present address: Henkel & Cie GmbH, Biochem. Abteilung, D-4 Düsseldorf, W. Germany.

[1] HARBORNE, J. B. (1962) *Biochem. J.* **84,** 100.
[2] HARBORNE, J. B. (1963) *Experientia* **19,** 7.
[3] SCHMID, R. D. (1972) *Tetrahedron* **28,** 3259.
[4] SCHMID, R. D., VARENNE, P. and PARIS, R. (1972) *Tetrahedron* **28,** 5037.

gave a sugar which from R_G values (Table 1) is clearly a trisaccharide. Furthermore, comparison of its R_G values with sophorose and with laminaribiose and laminaritriose (the related $\beta 1 \rightarrow 3$ linked oligosaccharides) support the proposition that it is linear and that it is probably glucosyl-$\beta 1 \rightarrow$ 2-glucosyl-$\beta 1 \rightarrow$ 2-glucose, or sophorotriose.

TABLE 1. R_G VALUES OF DI- AND TRI-SACCHARIDES

Sugar	R_G (×100) in *n*-BuOH–EtOH–H_2O (4:1:2·2)	R_G (×100) in *n*-BuOH–HOAc–H_2O (4:1:5)	R_G (×100) in C_6H_6–*n*-BuOH–pyridine–H_2O (4:1:2:2)
Sophorose*	65	54	58
Sophorotriose†	43	33	39
Laminaribiose‡	77	60	73
Laminaritriose‡	55	35	46

* Synthetic, from Prof. W. J. Whelan. † By H_2O_2 oxidn. of potato tetraglycoside. ‡ Koch–Light samples.

MS of the PDM Flavonol Tetraglycoside before Hydrolysis

The MS of the PDM glycoside is numerically reproduced in Table 2. Selected fragments (Table 3) are interpreted in terms of the symbols indicated in formulae Ia, b. The intensity of the molecular ion (M^+) at *m/e* 1173 is very low. Loss of PDM-glucose (4 CD_3-groups)

TABLE 2. MS PEAKS OF PDM KAEMPFEROL 3-TRIGLUCOSIDE 7-RHAMNOSIDE

1174 (1), 1173 (2), 1172 (2), 1139 (1), 964 (10), 963 (25), 962 (50), 945 (1), 748 (6), 731 (1), 718 (6), 679 (6), 657 (1), 605 (5), 578 (5), 577 (16), 576 (18), 564 (5) 548 (6), 547 (4), 544 (5), 535 (11) 534 (25), 520 (23), 519 (150), 518 (550), 517 (500), 516 (50), 515 (8), 514 (8), 502 (5), 501 (7), 488 (5), 481 (5), 444 (10), 410 (110), 409 (430), 337 (8), 322 (10), 321 (33), 320 (100), 304 (5), 291 (15), 231 (10), 207 (10), 198 (20), 197 (17), 196 (75), 179 (13), 163 (24), 161 (21), 154 (14), 149 (13), 144 (12), 138 (8), 137 (5), 135 (8), 133 (11), 128 (6), 123 (5), 122 (5), 121 (11), 119 (6), 116 (8), 114 (23), 108 (10), 107 (70), 104 (10), 102 (19), 97 (5), 96 (28), 90 (5), 88 (6), 83 (5), 81 (24), 77 (4), 76 (8), 75 (5), 74 (34), 69 (5), 62 (15), 48 (36), 47 (9), 45 (6), 44 (5), 43 (10), 41 (5), 28 (20)

Intensities relative to base peak at *m/e* 320 are in brackets. Only peaks larger 5% (0·5% > *m/e* 400) than the base peak are generally given.

from M^+ (15 CD_3-groups) should lead to a sequence peak containing 11 CD_3-groups; actually, fragment S_1 contains 12 CD_3-groups. Similarly, the S_2 peak contains 1 CD_3-group more than calculated for the loss of the two PDM-glucoses from M^+. One could rationalize this unexpected result on the assumption that PDM flavonol tetraglycoside is a mixture of the mono-, di- and tri-glucosides of PDM kaempferol rhamnoside. This is unlikely since the original material was chromatographically homogenous and since the

MS did not change after chromatographic purification of the PDM compound in 2 solvents. Therefore, methyl group migration under electron impact must have occurred. This feature is a rare phenomenon in MS.[5] A survey of MS of other PDM flavonoid glycosides might elucidate the underlying geometrical prerequisites and establish whether methyl group migration under electron impact is of diagnostic value in this field (e.g. for differentiation of β-1 $\rightarrow$ 2-linked oligosides formed by D-sugars).

Rupture of the glycosidic linkage at C-3 of the PDM kaempferol glycoside leads to the intense sequence ion S_3 and the weak trisaccharide ion OS_3. A weak disaccharide fragment OS_2 and a strong OS_2–CD_3OH are observed. The T-series (*m/e* 231, 196, 161) and T'-series (*m/e* 198, 163, 128) indicate the presence of a hexose and a deoxyhexose as terminal sugars. Mass differences between OS_3, OS_2 and T_1 confirm the presence of three hexose units in the trisaccharide residue. The presence of a flavonol diglycoside is substantiated by the mass number of the aglycone peak (A + 2H), its formation with transfer of 2H, its number of CD_3-groups and its intensity.[2]

TABLE 3. SELECTED FRAGMENTS FROM THE MS OF DERIVATIZED KAEMPFEROL 3-TRIGLUCOSIDE 7-RHAMNOSIDE

Fragment symbol*	*m/e* Values after permethylation with CD_3	CH_3I	No. of methyl groups introduced	Fragment symbol*	*m/e* Values after permethylation with CD_3I	CH_3I	No. of methyl groups introduced
M^+	1173	1128	15	OS_3	657	627	10
M^+–MeO	1139	1097	14	OS_2	444	423	7
S_1 + 2 H	962	926	12	OS_2–MeOH	409	391	6
S_2 + H	748	721	9	T_1	231	219	4
S_3	517	502	5	T_2	196	187	3
A + 2 H	320	314	2	T_1	198	189	3

* Symbols refer to formulae Ia, b. T_2 is a fragment derived from T_1 by loss of H_2O.

MS of PDM Flavonol Tetraglycoside Constituents after Acid Hydrolysis

The molecular ion (M^+) peak of the aglycone obtained by hydrolysis is at *m/e* 320 (flavone + 2x OCD_3 + 2x OH). Fragments of the a_1- and b_1-type at *m/e* 170 and *m/e* 138, respectively, show that the *A*-ring, but not the *B*-ring contained one glycosidic linkage before hydrolysis. Therefore, the sugars must have been attached through the *A*-ring and through the central pyrone ring.

Results obtained by GC–MS analysis of the PDM alditol acetate mixture derived from the PDM sugar mixture after hydrolysis are given in Table 4. GC-peak areas of peak I, III and IV were in a ratio of 1:1:2, respectively. Therefore, two 1 $\rightarrow$ 2 linked glucoses must have been present before hydrolysis.

Three minor additional peaks observed in GC contained no alditol acetates, according to MS evidence.

[5] HAMMING, M. C. and FOSTER, N. G. (1972) *Interpretation of Mass Spectra of Organic Compounds*, pp. 327–8, Academic Press, New York.

[6] SCHMID, R. D., MUES, R., MCREYNOLDS, J. H., VANDER VELDE, G., NAKATANI, N., RODRÍGUEZ, E. and MABRY, T. J. (1973) *Phytochemistry* **12**, in press.

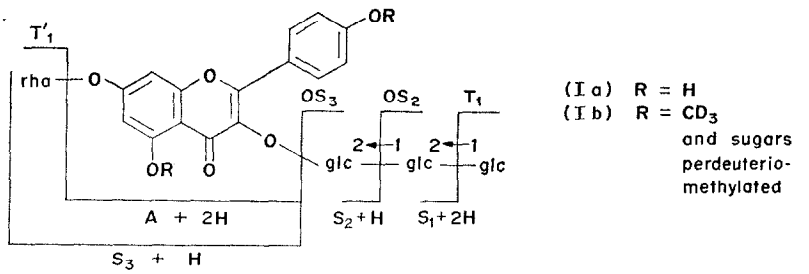
TABLE 4. GC–MS DATA OF PDM ALDITOL ACETATES DERIVED FROM FLAVONOL TETROSIDE AFTER ACID HYDROLYSIS

No. of GC-peak	RR_t*	Prominent fragments observed *m/e*	Structure	Blocked positions†
I	0·50	168, 134, 121, 108	1,5-di-*O*-acetyl-2,3,4-tri-*O*-deuteriomethyl-rhamnitol	1,5
III	1·00	214, 168, 167, 154, 132, 131, 108, 107	1,5-Di-*O*-acetyl-2,3,4-tri-*O*-deuteriomethylglucitol	1,5
IV	2·00	193, 167, 133, 132	1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-deuteriomethylglucitol	1,2,5

Reduction step with $NaBD_4$.

* Retention time relative to 1,5-di-*O*-acetyl-2,3,4-tri-*O*-deuteriomethyl-glucitol. GC on 3% OV-225 at 160°.

† Positions blocked in the glycoside and liberated by hydrolysis of its PDM derivative.



DISCUSSION

The information from MS and GC–MS analysis before and after hydrolysis indicates that the original tetraglycoside contains rhamnose and a linear trisaccharide in which glucose is joined by 1 → 2 linkages. These data do not at present show which sugar is attached to which hydroxyl, or what is the nature of the sterochemistry at C-1 of the sugars. However, the hydrolytic data indicate the definite presence of one $\beta 1 \rightarrow 2$ linkage and the failure to detect kojibiose (glucosyl-$\alpha 1 \rightarrow$ 2-glucose) on partial acid hydrolysis suggests that the second linkage is also β- not α-. Thus, the combined data confirm that the flavonol tetraglycoside of potato seed is kaempferol 3-sophorotrioside-7-rhamnoside (Ia).

Sophorotriose (glucosyl-$\beta 1 \rightarrow$2-glucosyl-$\beta 1 \rightarrow$ 2-glucose) appears to be new as a naturally occurring trisaccharide, although it has been obtained as a degradation product of a $\beta 1 \rightarrow 2$ glucan present in *Agrobacterium* spp.[7] It is probably not restricted to the kaempferol glycoside of *Solanum* seed, since preliminary data[2,8] indicate that it is also present in the acylated kaempferol and quercetin 3-triglucosides of *Pisum sativum* leaf.[9] Work to confirm this latter identification is in progress.

[7] GORIN, P. A. T., SPENCER, J. F. T. and WESTLAKE, D. W. S. (1961) *Can. J. Chem.* **39**, 1067.
[8] HARBORNE, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, Academic Press, London.
[9] FURUYA, M., GALSTON, A. W. and STOWE, B. B. (1962) *Nature* **193**, 456.

EXPERIMENTAL

Kaempferol 3-triglucoside-7-rhamnoside from potato seed[1] was available as colourless needles, m.p. 196–198°. Partial acid hydrolysis, H_2O_2 oxidation and chromatographic distinction between oligosaccharides were carried out by previously described procedures (see Ref. 8).

Acknowledgements—R.D.S. thanks Professor E. Lederer and Dr. B. C. Das, for the hospitality of their Institute, P. Varenne, for techical assistance in recording MS on an A.E.I. MS 9 and for GC–MS analysis on a LKB 9000S, Dr. C. Cone, for recording several MS of purified PDM flavonol tetroside on a CEC 110 B and Deutsche Forschungsgemeinschaft for a scholarship.