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DETERMINATION OF *O*-GLYCOSIDIC BOND POSITION IN 6-*C*-GLYCOSYLGLUCOSYLFLAVONES BY MASS SPECTROMETRY

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Key Word Index—MS; *O*-glycosyl-6-*C*-glucosylflavones; 6-*C*-glycosylglucosylflavones; hexa-*O*-methyl 6-*C*-glucosylapigenins; permethylation.

Abstract—2″,3″,4″ and 6″-*O*-glycosides of 6-*C*-glucosylflavones can be differentiated by the MS of the hydrolysis products of their permethyl ethers.

In previous work [1, 2], mass spectrometry of permethyl derivatives (purified by TLC) has been shown to be useful in the structural study of *O*-glycosyl 6-*C*-glucosylflavones for indicating the nature (pentose, hexose or desoxyhexose) of the sugar linked to the *C*-glucosylflavone unit and for unambiguously differentiating the 2″-*O*-glycosyl compounds from all others from the absence of M-15 and M-31 peaks.

In the present work, we show that mass spectrometry of the hydrolysis products (again purified by TLC) of these permethyl ethers allows the complete determination of the position of the *O*″-glycosyl group.

The MS of the products derived from isovitexin *O*″-glycosides: **1** (5,7,4′,3″,4″,6″-hexa-*O*-methylisovitexin; 2″-OH free), **2** (5,7,4′,2″,4″,6″-hexa-*O*-methylisovitexin; 3″-OH free), **3** (5,7,4′,2″,3″,6″-hexa-*O*-methylisovitexin; 4″-OH free) and **4** (5,7,4′,2″,3″,4″-hexa-*O*-methylisovitexin; 6″-OH free) are listed in Table 1.

Table 1. MS data for hexa-*O*-methyl 6-*C*-glucosylapigenins **1**–**4**: relative intensities % of the main fragments (≥10%).

Compound	*m/e*	**1** (2″-OH free)	**2** (3″-OH free)	**3** (4″-OH free)	**4** (6″-OH free)
M^+	516	69	20	37	31
M-15 a_2	501	—	28	24	26
M-17	499	13	—	—	—
M-31 b_3	485	15	60	100	97
M-45	471	38	—	—	—
M-47 c_3	469	19	—	—	—
M-89 g′	427	—	—	10	22
M-103 g	413	36	17	—	—
M-105	411	19	—	—	—
M-119 f′	397	—	—	—	12
M-121	395	—	—	10	—
M-131	385	—	—	11	23
M-133 f	383	10	—	—	—
M-149 h	367	—	18	18	22
M-161 i	355	23	—	41	100
M-163	353	11	10	—	12
M-175 j	341	100	100	63	48
M-177	339	11	12	—	16
M-189	327	30	10	10	—
M-191 k	325	27	28	12	21
M-193	323	15	16	11	14
M-205 l	311	27	17	11	17

The characteristic differences observed are summarized below:

2″-OH	3″-OH	4″-OH	6″-OH
M > M-31	M-31 > M	M-31 > M	M-31 > M
M-175 ≫ M-161	M-175 ≫ M-161	M-175 > M-161	M-161 > M-175
M-103 ≫ M-89	M-103 ≫ M-89	M-89 ≫ M-103	M-89 ≫ M-103

These criteria remain valid for the corresponding hepta-*O*-methyl 6-*C*-glucosyl-luteolins derived from permethyl isoorientin *O*-glycosides as previously observed [1].

EXPERIMENTAL

Permethylation and acid hydrolysis were carried out using methods previously described [1, 3]. MS were recorded on an AEI MS 902 spectrograph to 70 eV. Temps. (sample and source in the same order) varied between 150 and 190°. Pure PM *O*-glycosyl *C*-glycosylflavones and their hydrolysis products can be obtained by TLC (Si gel) using $CHCl_3$–EtOAc–Me_2CO (5:1:4) and (5:4:1), respectively.

5,7,4′,3″,4″,6″-*Hexa*-O-*methylisovitexin* (**1**) was obtained by acid hydrolysis of the permethyl ether of synthetic 6-*C*-neohesperidosyl acacetin [4].

5,7,4′,2″,4″,6″-*Hexa*-O-*methylisovitexin* (**2**) was obtained by debenzylation of synthetic permethyl 3″-*O*-benzylisovitexin [6] and by acid hydrolysis of the permethyl ether of synthetic 6-*C*-rungiosylacacetin [5].

5,7,4′,2″,3″,6″-*Hexa*-O-*methylisovitexin* (**3**) was obtained by acid hydrolysis of the permethyl ether of synthetic 6-*C*-cellobiosylacacetin [5].

5,7,4′,2″,3″,4″-*Hexa*-O-*methylisovitexin* (**4**) was obtained by acid hydrolysis of both permethyl ether of synthetic 6-*C*-rutinosylacacetin [5] and 6-*O*-tritylisovitexin [6].

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KAEMPFERIDE 3-GLUCURONIDE FROM THE ROOTS OF *CLEOME VISCOSA*

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Key Word Index—*Cleome viscosa*; Capparaceae; kaempferide 3-glucuronide.

Cleome viscosa (Capparaceae), commonly known as 'Hurhur' in Hindi, is reputed for its medicinal properties [1, 2]. No previous chemical analysis has been carried out on the roots of this plant. In the present study we have identified a new glycoside: kaempferide 3-glucuronide in the root tissue.

EXPERIMENTAL

The air-dried powdered roots of *Cleome viscosa* were extracted exhaustively with hot EtOH, which on concn and keeping at 0° for 2 days deposited a white cpd, which is being further studied. The filtrate was diluted with H_2O and the soluble portion extracted with increasingly polar organic solvents. The MeOH soluble fraction gave a reddish coloured cpd, which on further extraction with absolute EtOH, gave the reported glycoside, mp 104–5°, crystallized from MeOH–petrol and shown to be homogeneous by PC (R_f 0.88 in *n*-BAW, 4:1:5 v/v) and TLC (R_f 0.32 in $CHCl_3$–MeOH, 7:3 v/v); yield, 300 mg (Found: C, 55.39; 4.20; —OCH_3, 6.49. Calc. for $C_{22}H_{20}O_{12}$: C, 55.46; H, 4.20; —OCH_3, 5.51%). ν_{max}^{KBr} 3375, 2975, 2870, 1700, 1680, 1410, 1370, 1225, 1170, 1020, 920 and 825 cm^{-1}. λ_{max} MeOH 265, 365; +NaOMe 285, 415; +$AlCl_3$ 276, 365; +$AlCl_3$–HCl 270, 360; +NaOAc 275, 390 and +NaOAc–H_3BO_3 265, 320, 365 nm.

150 mg of the compound on hydrolysis with H_2SO_4(20 ml; 7%) gave glucuronic acid (Co-PC) and kaempferide (kaempferol 4'-methyl ether), identified by mp, MS, demethylation, acetylation, IR, UV spectral data and NMR. As positive NaOAc and $AlCl_3$ shifts indicate that both the 5- and 7-hydroxyls are free, the glucuronic acid residue must be attached at the 3-position and emulsin hydrolysis of the glycoside confirmed that the sugar is β-linked.

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