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C-GLYCOSYLFLAVONES FROM *ORYZA SATIVA*

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Key Word Index—*Oryza sativa*, Gramineae, rice, planthopper feeding stimulants, *C*-glycosylflavones, neocarlinoside, isoscoparin 2″-glucoside, isoscoparin 2″-(6-feruloylglucoside), isoscoparin 2″-(6-coumaroylglucoside)

Abstracts—Eight flavone *C*-glycosides isolated from rice plant were found to act as probing stimulants for planthoppers They have been identified as the known compounds schaftoside, neoschaftoside, carlinoside, isoorientin 2″-glucoside and the new constituents neocarlinoside (6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylluteolin), isoscoparin 2″-glucoside (chrysoeriol 6-*C*-β-D-(2-*O*-β-D-glucopyranosyl)glucopyranoside) and its 6‴-*p*-coumaric and ferulic acid esters

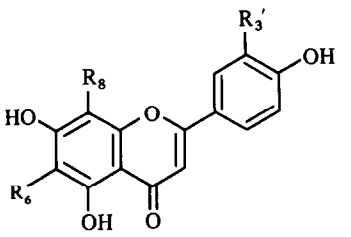
INTRODUCTION

During a study of the feeding behaviour of some planthoppers rice plant extracts were found to stimulate the probing behaviour [1] of three species (*Nilaparvata lugens*, *Sogatella furcifera*, *Laodelphax striatellus*) known as important rice pests in Asian countries Fractionation of the extracts led to the isolation of eight active *C*-glycosylflavones [2] Their structure elucidation is now reported in the present paper

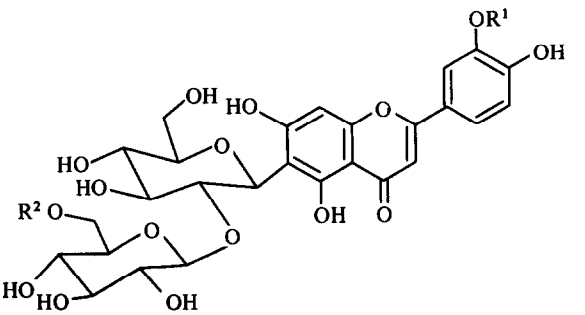
RESULTS AND DISCUSSION

When methanolic extracts of rice plant were dissolved in water and successively washed with hexane, chloroform and ethyl acetate, the probing stimulant was found to be present in the aqueous phase, which was passed through a column of cation exchange resin and extracted with *n*-butanol Three active fractions A, B and C were isolated from the *n*-butanol extract after column chromatography on polyamide (gradient H_2O–EtOH) and cellulose (30% MeOH) Further fractionation on cellulose (H_2O-satd *n*-BuOH) led to compounds **1** and **2** from fraction B, to compounds **3** and **4** from fraction C, whereas compounds **5–8** required a further separation of fraction A on polyamide (gradient H_2O–MeOH)

Both **1** and **2** showed the same UV spectrum ($\lambda_{max}^{H_2O}$ 270, 338 nm) and diagnostic shifts [3] as apigenin with free 5,7- and 4′-hydroxy groups and the chromatographic behaviour of apigenin diglycosides A 6,8-di-*C*-glycosylapigenin structure was indicated by the absence of signals for H-6 and H-8 and the presence of signals for two anomeric sugar protons in their ^{1}H NMR spectra This was confirmed by the electron impact mass spectra of the permethyl derivatives which showed the characteristic fragmentation pattern of permethyl 6-*C*-hexosyl-8-*C*-pentosylapigenins [4] for both compounds $[M]^+$ 704, $[M-15]^+$, $[M-31]^+$ (100%), $[M-47]^+$, $[M-63]^+$, $[M-103]^+$, $[M-119]^+$, $[M-131]^+$, $[M-145]^+$, $[M-163]^+$, $[M-175]^+$, $[M-189]^+$ with $[M-175]^+ > [M-131]^+$ Direct comparison with free and permethylated standard compounds on cellulose and silica gel TLC identified **1** as neoschaftoside (6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin) [5] and **2** as schaftoside (6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabino-



1 R_3' = H, R_6 = β-D-Glc*p*, R_8 = β-L-ara*p*
2 R_3' = H, R_6 = β-D-Glc*p*, R_8 = α-L-ara*p*
3 R_3' = OH, R_6 = β-D-Glc*p*, R_8 = β-L-ara*p*
4 R_3' = OH, R_6 = β-D-Glc*p*, R_8 = α-L-ara*p*



5 R^1 = Me, R^2 = H
6 R^1 = Me, R^2 = feruloyl
7 R^1 = R^2 = H
8 R^1 = Me, R^2 = *p*-coumaroyl

Table 1 PC (Whatman 3 mm) R_f values of compounds 1–8

Compound	*n*-BuOH–*n*-PrOH–H_2O 2 1 3	H_2O-satd *n*-BuOH
1	0 27	0 28
2	0 17	0 17
3	0 20	0 13
4	0 12	0 07
5	0 30	0 32
6	0 37	0 39
7	0 23	0 25
8	0 47	0 45

pyranosylapigenin) [6], in agreement with the presence of two doublets (J = 10 Hz) at δ4 73 and 4 79 for the anomeric sugar protons in the ^{1}H NMR spectrum of the latter and the presence of one singlet at δ5 52 and one doublet (J = 10 Hz) at 4 60 in the spectrum of the former

Similarly both **3** and **4** showed the same UV spectrum ($\lambda_{max}^{H_2O}$ 255 sh, 270, 348 nm) and diagnostic shifts [3] as luteolin with free 5,7,3′- and 4′-hydroxy groups and the chromatographic behaviour of luteolin diglycosides Again a 6,8-di-*C*-glycosylluteolin structure was indicated by the absence of signals for H-6 and H-8 and the presence of signals for two anomeric sugar protons in their ^{1}H NMR spectra This was confirmed by the electron impact mass spectra of the permethyl derivatives which again showed the same characteristic fragmentation pattern of permethyl 6-*C*-hexosyl-8-*C*-pentosylluteolins $[M]^+$ 734 for both compounds Co-TLC with free and permethylated standards showed **3** and **4** to be different from lucenin-3 (6-*C*-β-D-glucopyranosyl-8-*C*-β-D-xylopyranosylluteolin) and **4** to be identical with carlinoside, a natural 6-*C*-hexosyl-8-*C*-pentosylluteolin (from the mass spectrum of its permethyl ether) isolated from *Carlina vulgaris* and assumed to be 6-*C*-glucosyl-8-*C*-arabinosylluteolin [7] The striking parallelism of their chromatographic properties in the free and in the permethylated states strongly suggested **3** and **4** to be the luteolin analogues of **1** and **2**, respectively This hypothesis was supported by the similarity of the signals observed for the anomeric sugar protons in the ^{1}H NMR spectra of **3** (one singlet at δ5 51 and a doublet J = 10 Hz, at 4 58) and **1** (see above), and of **4** (two doublets, J = 10 Hz, at δ4 72 and 4 83) and **2** (see above) In order to extend the comparison to the other carbon-bound sugar protons, **3** was perdeuteriomethylated and the ^{1}H NMR spectrum of the derivative was found to be superimposable with that of perdeuteriomethylated neoschaftoside in the field of sugar protons, between δ5 60 and 3 00. As shown in previous studies of perdeuteriomethylated *C*-arabinosylflavones [8] and neoschaftoside [5], this part of the ^{1}H NMR spectrum affords a clear distinction between the possible structures of the *C*-arabinosyl residue Final confirmation was obtained through a comparison of the ^{13}C NMR spectra of **3** and neoschaftoside which revealed near identity of the spectra in the sugar carbon region (see Table 2), and a comparison of the CD spectra of PDM **3** and PDM neoschaftoside which showed that arabinose is in the L form in both compounds [9] On the basis of the above data, **3** was considered to be 6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylluteolin and was named neocarlinoside [10] Acid treatment of both carlinoside and **3** led to the same mixture of three isomers, including the starting products and a third compound showing a lower migration in BAW and 15% HOAc on two dimensional paper chromatography (as did isoschaftoside when compared to schaftoside and neoschaftoside) This third isomer was therefore named isocarlinoside and later identified by comparison with a natural compound isolated from *Lespedeza capitata* [11] This acid isomerization result also provided indirect evidence for the structure of carlinoside (6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranosylluteolin), which has now been confirmed by the near identity of the ^{13}C NMR spectra of **4** and schaftoside in the sugar carbon region (see Table 2)

Compounds **5** and **7** showed the same UV spectrum of luteolin type ($\lambda_{max}^{H_2O}$ 255 sh, 270, 348 nm) Diagnostic shifts [3] characterized the presence of free 5,7- and 4′-hydroxyl groups in both compounds, the absence of a free 3′,4′-dihydroxy group in **5** and its presence in **7** Their chromatographic behaviour suggested a diglycoside structure No change being observed on alkaline treat-

Table 2 Assignments of sugar carbon signals in the ^{13}C NMR spectra of **3**, **4**, **6** and **8**

Di-*C*-glycosides					2″-*O*-Glucosyl-*C*-glucosides			
Carbon number	Schaftoside [5]	Carlinoside **4**	Neoschaftoside [5]	Neocarlinoside **3**	Carbon number	Vitexin 2″-*O*-β-D-glucopyranoside	Compound **8**	Compound **6**
G-1	73 6	73 5	72 9	73 2	G-1	71 4	71 1	71 2
G-2	70 7	70 8	70 8	71 0	G-2	81 7	81 5	81 8
G-3	78 7	78 9	79 0	79 2	G-3	78 2	78 5	78 8
G-4	69 8	70 4	69 8*	70 0*	G-4	70 1	70 1	70 3
G-5	81 2	81 7	81 6	81 8	G-5	81 1	81 5	81 2
G-6	61 5	61 2	61 6	61 9	G-6	60 9	61 4	61 4
A-1	74 7	74 4	71 4	71 5	G-1′	105 1	105 4	105 7
A-2	68 8	68 9	63 1	63 3	G-2′	74 3	74 3	74 5
A-3	75 1	75 3	72 3	72 6	G-3′	76 1	76 4	76 6
A-4	68 8	69 3	69 9*	70 2*	G-4′	69 3	68 8	69 0
A-5	70 7	70 8	67 1	67 2	G-5′	76 1	73 3	73 4
					G-6′	60 3	62 2	62 2

*Assignments bearing the same superscript may be reversed

ment, **5** and **7** were permethylated The derivatives showed the same R_f on silica gel TLC and gave the same electron impact mass spectrum The observed fragmentation pattern was characteristic of a permethyl 2″-*O*-hexosyl-6-*C*-hexosylluteolin $[M]^+$ 764, absence of $[M-15]^+$ and $[M-31]^+$ replaced by $[SO]^+$ (M − 219) and $[S]^+$ (M − 235), 100%, corresponding to the loss of the 2″-*O*-glycosyl and 2″-*O*-glycosyloxy residues, presence of $[SO_i]^+$, $[SO_j]^+$, $[SO_k]^+$, $[S-14]^+$, $[S-32]^+$ and importance (53%) of $[J]^+$ 371 (see [12] for symbols) Acid hydrolysis of **5** gave glucose (identified by GLC of the alditol acetate) and a 6-*C*-glycosylflavone, identified as isoscoparin (6-*C*-β-D-glucopyranosylchrysoeriol) by direct comparison with an authentic specimen Compound **5** is thus defined as isoscoparin 2″-glucoside, a new natural compound

Acid hydrolysis of **7** led to glucose, identified as above, and a 6-*C*-glycosylflavone identified with isoorientin (6-*C*-β-D-glucopyranosylluteolin) by direct comparison with an authentic specimen This defined **7** as isoorientin 2″-glucoside, a conclusion confirmed by direct comparison with the known compound previously isolated form *Cucumis melo* [13]

Compounds **6** and **8** resembled **5** and **7** in their chromatographic behaviour in aqueous acetic acid, but their UV spectra showed a much more intense long wavelength band **6** ($\lambda_{max}^{H_2O}$ (rel int) 271 (1 0), 295 sh, 334 (1 3) nm), **8** ($\lambda_{max}^{H_2O}$ (rel int) 271 (1 0), 295 sh, 323 (1 24) nm) Diagnostic shifts disclosed in both compounds the presence of free 5,7- and 4′-hydroxy groups and the absence of a free *ortho*-dihydroxyl group When permethylated, **6** and **8** gave derivatives which showed the same R_f and gave the same electron impact mass spectrum as those obtained from **5** and **7** All these data could be explained by the results of alkaline hydrolysis which yielded ferulic acid and compound **5** from **6** and *p*-coumaric acid and compound **5** from **8** It followed that **6** and **8** were isoscoparin 2″-glucoside ferulic and *p*-coumaric acid esters, respectively

The ^{13}C NMR spectra of **6** and **8** confirmed the presence both of the acyl functions and of the 2″-*O*-β-linked glucopyranosyl residues In addition, analysis of the sugar carbon signals with reference to those of vitexin 2″-*O*-β-D-glucopyranoside (Table 2) revealed that the only significant differences occurred in the C-5 and C-6 signals of the terminal glucose (G-5′ and G-6′ respectively) In the spectra of both **6** and **8**, G-5′ had shifted upfield by 2 7–2 8 ppm and G-6′ had shifted downfield by 1 9 ppm Such shifts typically indicate acylation at the C-6 (G-6′) position [14] Since there is no indication of acylation at any other sites in either **6** or **8**, it is concluded that both are mono-acylated Accordingly, **6** is assigned the structure isoscoparin 2″-*O*-β-(6-*O*-feruloylglucopyranoside) and **8**, isoscoparin 2″-*O*-β-(6-*O*-*p*-coumaroylglucopyranoside)

EXPERIMENTAL

Plant Oryza sativa cv Nihonbare

Isolation of compounds See ref [2]

6-*C*-β-D-*glucopyranosyl*-8-*C*-β-L-*arabinopyranosylapigenin* (*neoschaftoside*, **1**) ^1H NMR (90 MHz, DMSO-d_6) δ 13 41 (1H, *br s*, OH-5), 8 01 (2H, *d*, *J* = 9 Hz, H-2′, 6′), 6 90 (2H, *d*, *J* = 9 Hz, H-3′, 5′), 6 79 (1H, *s*, H-3), 5 52 (1H, *s*, H-1 Ara), 4 60 (1H, *d*, *J* = 10 Hz, H-1 Glc)

6-*C*-β-D-*glucopyranosyl*-8-*C*-β-L-*arabinopyranosylluteolin* (*neocarlinoside*, **3**) ^1H NMR (90 MHz, DMSO-d_6) δ 13 44 (1H, *br s*, OH-5), 7 51 (1H, *dd*, *J* = 8 and 2 Hz, H-6′), 7 43 (1H, *d*, *J* = 2 Hz, H-2′), 6 87 (1H, *d*, *J* = 8 Hz, H-5′), 6 64 (1H, *s*, H-3), 5 51 (1H, *s*, H-1 Ara), 4 58 (1H, *d*, *J* = 10 Hz, H-1 Glc) ^{13}C NMR (20 MHz, DMSO-d_6) δ 182 2 (C-4), 163 5 (C-2), 163 2 (C-7), 160 1 (C-5), 153 0 (C-9), 150 1 (C-4′), 146 0 (C-3′), 121 6 (C-1′), 119 2 (C-6′), 116 1 (C-5′), 113 7 (C-2′), 109 3 (C-6), 103 1 (C-10), 102 5 (C-3, 8), for sugar carbon resonances see Table 2

Permethyl derivative of **3** EIMS 70 eV, *m/z* (rel int) 734 $[M]^+$ (18), 719 $[M-15]^+$ (30), 703 $[M-31]^+$ (100), 687 $[M-47]^+$ (7), 673 $[M-61]^+$ (7), 631 $[M-103]^+$ (13), 615 $[M-119]^+$ (3), 603 $[M-131]^+$ (11), 589 $[M-145]^+$ (2), 573 $[M-161]^+$ (16), 571 $[M-163]^+$ (28), 559 $[M-175]^+$ (32), 545 $[M-189]^+$ (8)

Perdeuteriomethyl derivative of **3** ^1H NMR (250 MHz, $CDCl_3$) δ 7 76 (1H, *dd*, *J* = 8 and 2 Hz, H-6′), 7 48 (1H, *d*, *J* = 2 Hz, H-2′), 6 94 (1H, *d*, *J* = 8 Hz, H-5′), 6 62 (1H, *s*, H-3), 5 58 and 5 37 (1H, 2*s*, H-1 Ara), 4 82 and 4 54 (1H, 2*d*, *J* = 10 Hz, H-1 Glc), 4 20 (1H, *m*, H-Glc), 4 08 (1H, *m*, H-Ara), 3 83 (3H, *m*, 3H-Ara), 3 58 (3H, *m*, 2H-Glc, 1H-Ara), 3 40 (1H, *m*, H-Glc), 3 25 (2H, *m*, 2H-Glc) CD (MeOH) $[\theta]_{355}$ + 5700, $[\theta]_{308}$ − 16 100, $[\theta]_{265}$ + 14 200, PDM neochaftoside $[\theta]_{345}$ + 19 000, $[\theta]_{310}$ − 11 700, $[\theta]_{262}$ + 26 700

6-*C*-β-D-*glucopyranosyl*-8-*C*-α-L-*arabinopyranosylluteolin* (*carlinoside*, **4**) ^{13}C NMR (20 MHz, DMSO-d_6) δ 182 3 (C-4), 164 0 (C-2), 162 0 (C-7), 159 8 (C-5), 154 2 (C-9), 150 0 (C-4′), 145 8 (C-3′), 121 6 (C-1′), 119 9 (C-6′), 116 2 (C-5′), 113 8 (C-2′), 108 8 (C-6), 104 2 (C-8), 103 3 (C-10), 102 5 (C-3), for sugar carbon resonances see Table 2

Isoscoparin 2″-glucoside (**5**) TLC (cellulose) R_f 0 79 (15% HOAc) UV λ_{max}^{MeOH} nm 239 sh, 252 sh, 270, 344, + $AlCl_3$ 231 sh, 261 sh, 278, 296 sh, 360, 385, + $AlCl_3$ + HCl 259 sh, 278, 293 sh, 353, 382, + NaOAc 234 sh, 277, 319, 365, + NaOAc + H_3BO_3 238 sh, 272, 346, + NaOH 259 sh, 269 sh, 278, 340 sh, 406 *Permethyl derivative* EIMS 70 eV, *m/z* > 300 (rel int) 764 $[M]^+$ (2), 589 $[SO_i]^+$ (3), 575 $[SO_j]^+$ (4), 559 $[SO_k]^+$ (3), 545 $[SO]^+$ (31), 529 $[S]^+$ (100), 515 $[S-14]^+$ (6), 497 $[S-32]^+$ (8), 427 $[f]^+$ (5), 399 $[n]^+$ (2), 397 $[h]^+$ (3), 385 $[i]^+$ (8), 371 $[j]^+$ (53), 355 $[k]^+$ (18), 341 $[l]^+$ (11)

Isoscoparin-2″-glucoside-6‴-ferulic ester 6-*C*-β-D-(2-*O*-β-D-(6-*O*-feruloyl)glucopyranosyl)glucopyranosylchrysoeriol (**6**) TLC (cellulose) R_f 0 75 (15% HOAc) UV λ_{max}^{MeOH} nm 273, 299 sh, 331, + $AlCl_3$ 281, 297, 334, 386, + $AlCl_3$ + HCl 282, 296, 335, 382, + NaOAc 279, 325, + NaOAc + H_3BO_3 273, 330, + NaOH 279, 327, 392 ^{13}C NMR (20 MHz, DMSO-d_6) δ 181 9 (C-4), 166 4 (Fer-CO_2R), 163 5 (C-2, 7), *ca* 161 (C-5), 156 5 (C-9), 150 7 (C-3′), 149 3 (Fer-4), 147 9 (Fer-3, C-4′), 144 6 (Fer-β), 125 6 (Fer-6), 123 0 (Fer-1), 121 8 (C-1′), 120 4 (C-6′), 115 6 (C-5′, Fer-5,α), 114 0 (Fer-2), 110 3 (C-2′), 108 1 (C-6), 103 2 (C-3, 10), 93 6 (C-8), 55 7 (OMe), for sugar carbon resonances see Table 2

Isoscoparin-2″-glucoside-6‴-p-coumaric ester 6-*C*-β-D-(2-*O*-β-D-(6-*O*-*p*-coumaroyl)glucopyranosyl)glucopyranosylchrysoeriol (**8**) TLC (cellulose) R_f 0 74 (15% HOAc) UV λ_{max}^{MeOH} nm 274, 299 sh, 320; + $AlCl_3$ 282, 301, 322, 384, + $AlCl_3$ + HCl 281, 300, 322, 382, + NaOAc 280, 311, + NaOAc + H_3BO_3 275, 319, + NaOH 269 sh, 278, 332 sh, 386 ^{13}C NMR (20 MHz, DMSO-d_6) δ 181 8 (C-4), 166 2 (Cou-CO_2R), 163 5/163 3 (C-2/7), 160 9 (C-5), 159 6 (Cou-4), 156 3 (C-9), 150 5 (C-3′), 147 8 (C-4′), 144 4 (Cou-β), 129 9 (Cou-2, 6), 124 9 (Cou-1), 121 6 (C-1′), 120 2 (C-6′), 115 5 (C-5′, Cou-3,5), 113 8 (Cou-α), 110 1 (C-2′), 107 9 (C-6), 102 9 (C-3, 10), 93 3 (C-8), 55 7 (OMe), for sugar resonances see Table 2

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