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IDENTIFICATION OF NEOSCHAFTOSIDE AS 6-*C*-β-D-GLUCOPYRANOSYL-8-*C*-β-L-ARABINOPYRANOSYLAPIGENIN

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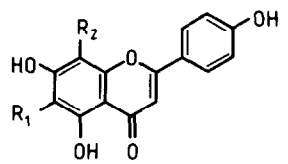
Abstract—The structure of neoschaftoside is shown for the first time to be 6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin A variety of chemical and spectroscopic techniques are involved

INTRODUCTION

Ten years ago, two di-*C*-glycosylapigenins (B and C) were isolated from *Catananche caerulea* by Proliac *et al* [1] As heating B or C with acid led to a mixture containing both compounds, the name isoschaftoside was proposed for B after identification of C as schaftoside (**1**), previously isolated from *Silene schafta* [2] Later work [3, 4] on the mass spectra of permethyl (PM) *C*-glycosylflavones showed that B and schaftoside were both 6-*C*-hexosyl-8-*C*-pentosylapigenins and B was therefore renamed neoschaftoside The name isoschaftoside was correctly given to the Wessely–Moser isomer (**2**) of schaftoside isolated from the complex mixture obtained by acid isomerization of schaftoside (which also contains neoschaftoside) This renamed isoschaftoside was identified by comparison with synthetic 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin [5], which confirmed its structure and also the 6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranosylapigenin structure earlier proposed for schaftoside [6]

Isoschaftoside was later identified as a natural product in *Flourensia cernua*, where it co-occurs with schaftoside, neoschaftoside and vicenins [7] Since then, schaftoside, isoschaftoside and neoschaftoside have been shown to be widespread in the plant kingdom [8] The structure of neoschaftoside, however, remains to be defined and in the present paper we present evidence which proves it to be 6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin (**3**)



1 R_1 = β-D-Glc$_p$, R_2 = α-L-Ara$_p$
2 R_1 = α-L-Ara$_p$, R_2 = β-D-Glc$_p$
3 R_1 = β-D-Glc$_p$, R_2 = β-L-Ara$_p$

RESULTS AND DISCUSSION

In previous work, the identity of natural neoschaftoside with the corresponding isomer of schaftoside obtained by acid treatment was based only upon comparison of absorption spectra, chromatographic properties of the free compounds and their PM ethers, and mass spectra Attempts to get larger quantities of the acid isomerization product always led to a product contaminated by schaftoside This problem was overcome, however, by TLC purification of the perdeuteromethyl (PDM) ether of the schaftoside isomer, which produced sufficient material for determination of the ^{1}H NMR spectrum This proved to be identical with that of PDM natural neoschaftoside, thus confirming their identity and the presence of D-glucosyl and L-arabinosyl‖ moieties in neoschaftoside These sugars were both shown to be in their pyranosyl forms in neoschaftoside by the Viscontini reaction [9] Thus periodate oxidation of schaftoside and neoschaftoside followed by borohydride reduction and acid hydrolysis led in both cases to a mixture of glycerol (*ex* glucose) and ethylene glycol (*ex* arabinose)

In the ^{1}H NMR spectra of PDM schaftoside and PDM isoschaftoside, the anomeric protons appeared as four doublets¶ (J = *ca* 10 Hz) in the range δ 4 8–4 5 consistent with the presence of β-D-glucopyranosyl and α-L-arabinopyranosyl residues in these compounds In the spectrum of neoschaftoside, however, only one of the anomeric protons appeared as two doublets in this range (i e δ 4 82, 4 53 in the ratio 1 2) and with the 10 Hz coupling

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‖The L-isomer of arabinose is considered to be present in the interconvertible isomers, schaftoside, isoschaftoside, neoschaftoside, on the basis that CD curves of isoschaftoside and synthetic 6-*C*-α-L-arabinopyranosylvitexin are identical (cf ref [10])

¶Due to the presence of rotational isomers In DMSO-d_6 at 90° coalescence of these signals was observed, e g the four signals in the spectrum of PDM neoschaftoside were reduced to two

characteristic of a *trans*-diaxial relationship between H-1 and H-2 in the pyranose ring The other anomeric proton gave rise to two broad singlets at δ 5 58 and 5 36 (ratio 1·2). It follows that only one sugar residue was modified in the acid-catalysed isomerization of schaftoside to neoschaftoside

The acid-modified sugar residue was considered to be the arabinosyl residue on the following basis It is well known that even prolonged acid treatment of 6- or 8-*C*-β-D-glucopyranosylflavones produces a clean Wessely–Moser rearrangement which does not affect the sugar residue In contrast, acid treatment of either 6-*C*-α-L-arabinopyranosyl- or 6-*C*-α-L-arabinofuranosyl-acacetin (synthetic) for only a short time (30–45 min) gives a mixture of the 6-*C*-α- and β-isomers of arabinopyranosyl- and arabinofuranosyl-acacetin [11] Natural 8-*C*-α-L-arabinopyranosylgenkwanin (molludistin [12]) under the same conditions gives mainly a mixture of the pyranosyl and furanosyl isomers of 8-*C*-α-L-arabinosylgenkwanin [11]

That the L-arabinopyranosyl residue is the one modified on acid isomerization of schaftoside was also evident from a comparison of the ^{1}H NMR spectra of PDM neoschaftoside with that of PDM 6-*C*-β-L-arabinopyranosylacacetin (synthetic) In the latter, the anomeric proton appeared as a broad singlet (i e lacking *trans*-diaxial coupling) at δ 5 26 instead of the doublets observed in the spectra of PDM 6-*C*-α-L-arabinofuranosylacacetin (δ 5 47, J = 7.6 Hz), PDM 8-*C*-α-L-arabinofuranosyl-acacetin (δ 5 68, J = 7 6 Hz) and PDM 6-*C*-β-L-arabinofuranosylacacetin (δ 5 80, J = 7 2 Hz) Broad singlets at δ 5 58 and 5 36 in the spectrum of PDM neoschaftoside represented the anomeric proton of the acid-affected sugar residue, whereas the other anomeric proton was represented by doublets (J = 10 Hz) at δ 4.82 and 4 53, similar to that of the glucose H-1 in PDM 6-*C*-β-D-glucopyranosylapigenin (PDM isovitexin, δ 4 80, J = 10 Hz)

On this basis, neoschaftoside is defined as 6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin, a structure which was also supported by the chemical shifts of the other sugar protons Thus, whereas the spectrum of PDM schaftoside (in the δ 4 4–3 2 region) was equivalent to a superimposition of the spectra of PDM 6-*C*-β-D-glucopyranosylapigenin (PDM isovitexin) and PDM 8-*C*-α-L-arabinopyranosylgenkwanin (PDM molludistin), the spectrum of PDM neoschaftoside in this same region was equivalent to a superimposition of the spectra of PDM isovitexin and PDM 6-*C*-β-L-arabinopyranosylacacetin (ignoring the 4′-methoxyl signal in the latter)

Subsequent to much of the above work, one of us (R M) had discovered a new source of neoschaftoside, the liverwort *Radula complanata*, which made available for the first time substantial quantities of neoschaftoside This enabled ^{13}C NMR studies to be carried out which provide further confirmation of the conclusions based on the evidence discussed above

The ^{13}C NMR spectra of schaftoside and neoschaftoside were found to be essentially identical apart from the sugar carbon resonances These are presented in Table 1 and assignments listed for the schaftoside spectrum are as reported by Markham *et al* [13] It is clear that the β-D-glucopyranosyl residue of schaftoside is also present in neoschaftoside and that it is the arabinosyl signals that are different None of the signals representing the arabinosyl residue occurred at lower field than δ 72 3 and this excludes the possibility that arabinose is present in the furanose form [14] Of the two remaining possible structural variants of arabinose, α-L-arabinopyranose is present in schaftoside thus leaving only β-L-arabinopyranose for neoschaftoside Although no published data are available, the pattern of arabinose signals in the ^{13}C NMR spectrum of neoschaftoside was approximately that expected for a *C*-linked β-L-arabinopyranosyl residue Thus, with the exception of the C-4 signal, all others occurred at higher field than the equivalent carbon signals in the spectrum of *C*-linked α-L-arabinopyranose (e g in schaftoside, Table 1) This is the type of difference observed in *O*-linked glycosides of these two arabinose isomers [14] On the basis of the above data, neoschaftoside is assigned the structure 6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin (**3**)

Table 1 Assignments of sugar carbon signals in the ^{13}C NMR spectra of schaftoside and neoschaftoside

Ara	Schaftoside	Glc	Neoschaftoside*	Ara
	81 2	G-5	81 6	
	78 7	G-3	79 0	
A-3	75 1			
A-1	74 7			
	73 6	G-1	72 9	
			72 3	A-3
			71 4	A-1
	70 7	G-2	70 8	
A-5	70 7			
			69 9	A-4†
	69 8	G-4†	69 8	
A-2	68 8			
A-4	68 8			
			67 1	A-5
			63 1	A-2
	~ 61 5	G-6	61 6	

*Neoschaftoside assignments A-5, assignment by 'GASPE' [15], A-2, the only possible assignment for this high field signal, A-1, tentatively assigned by selective decoupling of the arabinose H-1, A-3, A-4, tentatively assigned on the basis of additive chemical shift correction (calculated from differences between β- and α-*O*-L-arabinosides [14]) applied to α-*C*-L-arabinoside

†Assignments could be reversed

EXPERIMENTAL

Isolation of neoschaftoside from Radula complanata Air-dried *R complanata* gametophytes with sporophytes (49 g) were ground and extracted with deionized H_2O at room temp The extracted flavonoids were separated and purified by CC (a) on microcrystalline cellulose (Merck) with 3% and 15% HOAc, (b) Sephadex LH-20 with 20% aq MeOH and MeOH, and finally repeated PC on Whatman 3 MM paper with 3% and 15% HOAc, and BAW Schaftoside (38 mg) was crystallized from MeOH–H_2O and neoschaftoside (35 mg) was precipitated with EtOAc–EtOH following final purification on LH-20 (20% aq MeOH) Neoschaftoside $[\alpha]_D^{20}$ −66 5° (c = 0 2%, H_2O), [schaftoside $[\alpha]_D^{22}$ + 110° (c = 0 5%, H_2O) [6]

PDM neoschaftoside from schaftoside acid isomerization Schaftoside (200 mg), from *Silene schafta*, in 8 ml MeOH–4 M

HCl (1 1) was refluxed for 9 hr and the resulting products were separated by 2D-PC in BAW (4 1 5) and 2% HOAc on Whatman 3 MM paper The spot corresponding to neoschaftoside was cut out, eluted and rechromatographed on Whatman No 1 paper in BAW (4 1 5) The band corresponding to neoschaftoside was eluted and perdeuteromethylated (method, ref [16]) Prep TLC (silica gel) of the resulting mixture in $CHCl_3$–EtOAc–Me_2CO (5 4 1) and elution of the band corresponding to PDM neoschaftoside gave a product identical (^1H NMR) with the latter

PDM neoschaftoside ^1H NMR (250 MHz, $CDCl_3$) δ 8 06 (2H, *d*, J = 9 Hz, H-2', 6'), 6 99 (2H, *d*, J = 9 Hz, H-3', 5'), 6 63 (1H, *s*, H-3), 5 58 and 5 36 (1H, *s*, H-1, Ara), 4 82 and 4 53 (1H, *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)

PDM schaftoside ^1H NMR (250 MHz, $CDCl_3$) δ 8 06 (2H, *d*, J = 9 Hz, H-2', 6'), 7 01 (2H, *d*, J = 9 Hz, H-3', 5'), 6 62 (1H, *s*, H-3), 4 79, 4 74, 4 66, 4 58 (*d*'s, J = 10 Hz, H-1, Glc and Ara), 4 30 (2H, *m*, 2H-Ara) 4 10 (1H, *m*, H-Glc), 3 79 (1H, *s*, H-Ara), 3 58 (3H, *m*, 2H-Glc, 1H-Ara), 3 42 (2H, *m*, 1H-Glc, 1H-Ara), 3 24 (2H, *m*, 2H-Glc)

PDM isovitexin ^1H NMR (250 MHz, $CDCl_3$) δ 7 91 (2H, *d*, J = 9 Hz, H-2', 6'), 7 00 (2H, *d*, J = 9 Hz, H-3', 5'), 6 82 (1H, *s*, H-8), 6 67 (1H, *s*, H-3), 4 80 (1H, *d*, J = 10 Hz, H-1, Glc), 4 08 (1H, *m*, H-Glc), 3 60 (2H, *m*, 2H-Glc), 3 42 (1H, *m*, H-Glc), 3 24 (2H, *m*, 2H-Glc)

PDM molludistin ^1H NMR (250 MHz, $CDCl_3$) see ref [12]

PDM 6-C-β-L-*arabinopyranosylacacetin* ^1H NMR (250 MHz, $CDCl_3$) δ 7 84 (2H, *d*, J = 9 Hz, H-2', 6'), 7 02 (2H, *d*, J = 9 Hz, H-3', 5'), 6 81 (1H, *s*, H-8), 6 60 (1H, *s*, H-3), 5 26 (1H, *s*, H-1, Ara), 4 04 (1H, *dd*, J = 8 5 Hz, J' = 3 5 Hz, H-Ara), 3 89 (3H, *s*, OMe-4'), 3 82 (1H, *br s*, H-Ara), 3 74 (2H, *m*, 2H-Ara), 3 64 (1H, *m*, H-Ara)

Periodate oxidation according to ref [9] was carried out on 0 4–0 7 μmol of molludistin, cytisoside, adenosine, neoschaftoside and schaftoside After $NaBH_4$ reduction and acid hydrolysis, the resulting polyols were separated on silica gel TLC in EtOAc–pyridine–H_2O (7 2 1) and detected by spraying with $NaIO_4$ and benzidine Cytisoside and adenosine gave one spot R_f 0 48 (glycerol), molludistin one spot R_f 0 68 (ethylene glycol), neoschaftoside and schaftoside two spots R_f 0 48 and 0 68

^{13}C NMR spectra were measured on a Varian FT-80A spectrometer using DMSO-d_6 solns of neoschaftoside (22 mg) and schaftoside (19 mg) at 30° Data for neoschaftoside (ppm from TMS) δ 182 1 (C-4), 163 0/162 8 (C-2/7), 161 2 (C-4'), 159 8 (C-5), 152 5 (C-9), 128 5 (C-2', 6'), 121 0 (C-1'), 116 0 (C-3', 5'), 109 1 (C-6), 102 9/102 5/102 2 (C-3/8/10), for sugar carbon resonances see Table 1

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