Higher Order and Substituent Chemical Shift Effects in the Proton NMR of Glycosides

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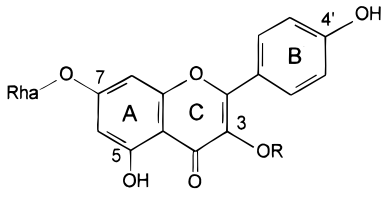
The full analysis of ^{1}H NMR spin systems and the charting of substituent chemical shifts offer great potential in the structure elucidation of glycosides. Due to the chiral nature of sugar residues, asymmetric induction causes diastereotopic shifts of aglycon resonance, while on the other hand aromatic substituent-induced shifts affect the sugar portion of the molecule. Therefore, it is possible to deduce critical structural information, such as the site of sugar linkage in bisdesmosides and oligoglycosides, through the analysis of exact chemical shifts, *J* patterns, and signal multiplicity. The potential of the method is demonstrated for three kaempferol bisdesmosides (**1**−**3**) by establishing the shift characteristics for 3-*O*-, 7-*O*-, and 6″-*O*-glycosidation. Spectral simulation and soft-pulse 1D NMR turn out to be indispensable tools in the course of refined signal and structure assignment.

Routine ^{1}H NMR spectroscopy plays a cardinal role in the structure elucidation of natural products. Especially in the case of glycosides, the proton resonances, in principle, contain a tremendous wealth of structural information that can lead to conclusions on chemical environment and relative stereochemistry as well as molecule constitution and conformation. The apparent complexity of both the complete ^{1}H NMR spectrum and the single resonances can be understood as a result of combining all this information into one spectral entity. Consequently, it becomes a challenge to extract all this information from the mostly overlapping region of routine spectra of glycosides even when obtained at high magnetic field.

While the value of exact nuclear parameters such as the chemical shift (δ) and, especially, the coupling constant (J) cannot be underestimated,[1] there is a tendency in the literature to neglect them, especially when known compounds are dereplicated based on previously published low-field NMR data. Rather these important parameters can readily be extracted with great precision using off-line data processing, optimized window functions such as Lorentzian-to-Gaussian line shape conversion, or reference deconvolution[2] techniques aided by basic 2D and soft-pulse 1D experiments.[3] Thus, the essential structural information can be unleashed from advantageously sensitive and rapid ^{1}H NMR experiments: conclusions about the stereochemistry,[4] i.e., the identity of the sugar moiety, can be drawn, conformational properties of the molecule can be recognized, and even information on sugar sequence and the site(s) of glycosidation can be obtained.

The capacity of detailed ^{1}H NMR analysis in terms of structure determination can best be demonstrated by looking at closely related compounds. Therefore, three bisdesmosidic kaempferol glycosides (**1**−**3**) containing α-L-rhamnose and β-D-glucose units, which were recently isolated from *Arabidopsis thaliana* (ecotype ws 2; Cruciferae),[5] were chosen as examples.[6] Their structures were fully established by comprehensive NMR evaluation including gradient-enhanced 2D techniques as well as [HR-] MS$^{[n]}$, UV, and chiral capillary electrophoresis for D/L sugar assignment. Fully assigned sets of proton and carbon resonances were thus obtained and are summarized in Table 1. In principle, the relative stereochemistry of the hexopyranoses in **1**−**3** can be deduced from the coupling pattern of nuclei using first-order assumptions as follows (coupling constants are given as measured line distances, see ref 5 for detailed *J* values): H-1=d (7.7 Hz), H-2=dd (7.7/9.1 Hz), H-3=t (9.1 Hz), H-4=t (9.5 Hz), H-5=ddd (2.4/5.5/9.5 Hz), H-6A=dd (2.0/11.8 Hz), H-6B=dd (5.5/11.8 Hz) for glucose (glc) and H-1=d (1.9 Hz), H-2=dd (1.9/3.5 Hz), H-3=dd (3.5/9.5 Hz), H-4=t (9.5 Hz), H-5=dq (5.9/9.5 Hz), 3H-6=d (5.9 Hz) for rhamnose (rha). However, as will be shown below, this straightforward labeling becomes imperfect in many cases due to the presence of higher order situations caused by significant substituent chemical shifts.



	R
1	Glc^6 → $_1$Glc
2	Glc
3	Rha

It must also be emphasized that the choice of solvent has substantial impact on the complexity of ^{1}H NMR spectra. While we have observed the most favorable signal dispersion with CD_3OD solutions of glycosides and other polar, nonglycosidic natural products,[7] most phenolic glycosides and especially flavonoids[8] are reported in DMSO-d_6 solution. On the basis of our experience it is preferable to use CD_3OD as the main solvent and to add traces of others as solubilizers only.[9] While signal dispersion and, therefore, spectral information are favorable in methanol solution, the main disadvantage of losing the hydroxyl proton resonances can still be overcome by using CD_3OH.

Substituent chemical shifts (SCS) caused by aromatic groups are mostly seen under the perspective of intermolecular interaction with the solvent and, therefore, are named aromatic solvent-induced shifts (ASIS).[10] However, phenolic glycosides additionally can show intramolecular

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Table 1. ^{1}H and ^{13}C NMR Chemical Shift Data of the Flavonoids **1**−**3**[a,b]

		δ_C			δ_H		
moiety	pos	**1**	**2**	**3**	**1**	**2**	**3**
3-*O*-glc (**1**+**2**)	1″	103.47	103.42	103.38	5.345	5.391	5.407
or	2″	75.75	75.73	71.79	3.445	3.435	4.218
3-*O*-rha (**3**)	3″	77.91	77.98	72.02	3.421	3.419	3.704
	4″	71.35	71.37	73.11	3.353	3.300	3.332
	5″	77.90	78.54	72.09	3.422	3.227	3.333
	6″(A)	69.50	62.55	17.85	3.980	3.706	0.942
	6″(B)				3.644	3.526	
6″-*O*-glc (**1**)	1‴	104.62	99.82	99.88	4.140	5.581	5.570
or	2‴	75.06	71.59	71.57	3.036	4.025	4.035
7-*O*-rha (**3**)	3‴	77.82	72.00	72.02	3.126	3.819	3.832
	4‴	71.31	73.50	73.52	3.199	3.470	3.479
	5‴	77.69	71.28	71.26	2.968	3.582	3.607
	6‴(A)	62.53	18.27	18.24	3.724	1.246	1.265
	6‴(B)				3.559		
7-*O*-rha (**1**)	1⁗	99.99			6.582		
	2⁗	71.64			4.014		
	3⁗	72.02			3.817		
	4⁗	73.54			3.468		
	5⁗	71.29			3.604		
	6⁗	18.24			1.258		
kaempferol	6	100.65	100.57	100.54	6.485	6.452	6.415
	8	95.86	95.56	95.68	6.778	6.750	6.676
	2′	132.51	132.42	131.96	8.146	8.089	7.768
	3′	116.33	116.45	116.59	6.913	6.891	6.934
	5′	116.33	116.45	116.59	6.910	6.889	6.932
	6′	132.51	132.42	131.96	8.144	8.088	7.766

[a] While the ^{1}H signal multiplicities are given in the text assuming a nuclear first-order interpretation, protons H-1−5 of glc and H-1−6 of rha have to be correctly labeled as multiplets in 3-*O*-linked hexoses. [b] 360/90 [**2**] and 600/150 MHz [**1** and **3**]; δ values in ppm; glc = β-D-glucose, rha = α-L-rhamnose.

aromatic SCS that can produce prominent changes in signal dispersion, the type of spin system, and, even more important, signal multiplicity and shape. These changes may originate from spatial interactions between two aromatic groups, such as in dicaffeoyl quinic acids,[11] or between one aromatic and one aliphatic group, e.g., a sugar residue. A typical example of an aromatic *substituent*-induced shift can be observed in the flavonoids **1**−**3**, as presented in Figure 1. Both the glc and the rha moieties exhibit remarkably different signals when comparing the 3-*O* and 7-*O* linkages. Due to the ring current effect of the B-ring which is in close proximity to the 3-*O* sugar side chain, the two glc moieties in the gentiobiose unit of **1** yield unusually well-separated sets of signals (see ref 12 for a non-phenolic gentiobioside). The prominent upfield shift of all of the terminal glc protons translates into a conformation where the terminal sugar electronically interacts with the shielding cone of the B-ring.[13] Analogous interactions with the B-ring are also observed in flavonoid 3-*O*-glucuronides and mostly affect the C/H-5/6 resonances of the glcA moiety.[14] Although no comparison data are yet available, the strength of these shift effects should vary with differences in B-ring substitution.

Because the aromatic and the sugar residue electronically interact with each other, resulting effects can be observed on both sides. While the occurrence of higher order spin systems in the sugar portion is caused by the aromatic moiety, the latter is influenced by the chiral nature of the sugar residue(s), which also leads to higher order situations. A surprising example of asymmetric induction caused by chiral sugar residues is the diastereotopism of the *p*-OH aromatic B-ring protons of the flavonoid nucleus of kaempferol and analogues. While, even in very recent literature, the AA′XX′ ^{1}H signals centering around 8.0 and 6.9 ppm are erroneously reported as straight doublets with one *J* value given,[15] a detailed evaluation is necessary to establish (i) the couplings, (ii) the shift values, and (iii) the signal multiplicity (see Figure 2). The results indicate that (i) the otherwise overlooked *meta* couplings are "hidden" in sidebands of the apparent doublets (pseudo d) integrating for two protons each. Actually, the signals can be viewed as triplets with greatly shifted intensity, while *J* values cannot be calculated from the frequencies of the lines but need spectral simulation (see below). However, even the triplet character of the pseudo-doublets cannot solely be explained with active meta couplings but leads to the insight that (ii) the corresponding B-ring nuclei are *not* isochronous (AA′XX′ but not an A_2X_2 spin system). This observation is due to the asymmetric induction of the chiral sugar residue attached to C-3-OH and, again, is an expression of intramolecular interaction. Thus, the B-ring protons become diastereotopic with $\Delta\nu$ values in the range 1−2 Hz. In conclusion (iii) the apparent doublet (pseudo

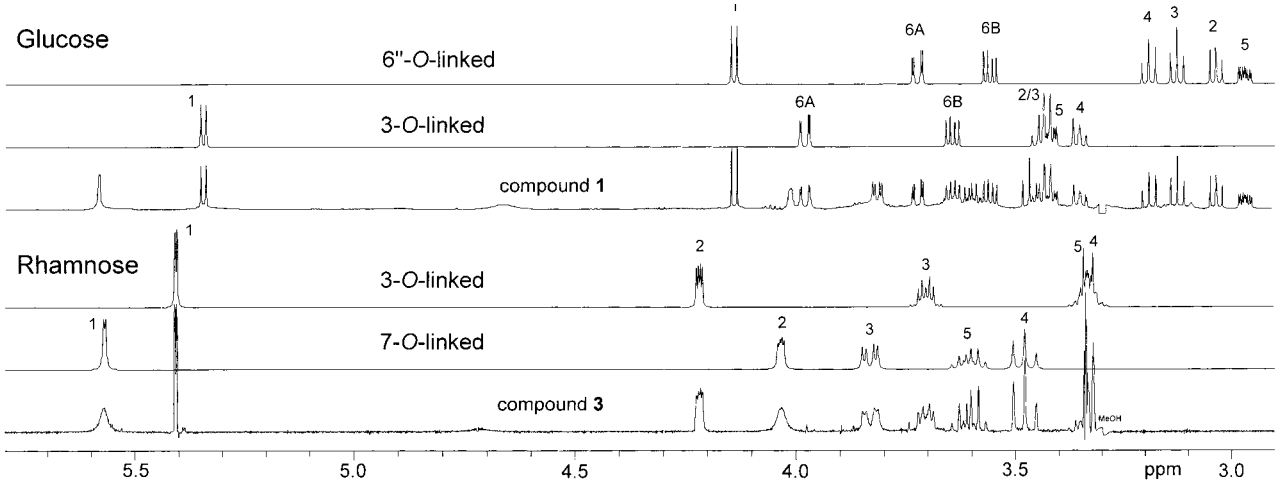

Figure 1. Proton resonances of the β-D-GLC and α-L-rha moieties in **1** and **3**. Depending on the site of linkage, the sugars are subjected to different influences of the flavonoid nucleus in terms of aromatic substituent-induced shifts arising from the ring current effect. Therefore, the resonances of glc (upper) and rha (lower) show remarkable differences concerning both chemical shift and signal multiplicity and coupling behavior. While the spectra of 7-*O*-linked rha and of 6-*O*-linked glc moieties can be interpreted as being due to nuclear first-order spin systems, this is definitively not the case for the 3-*O*-bonded sugars. Interpretation of the latter requires spectral simulation and indicates the spatial neighborhood of the 3-*O*-sugar and the B-ring. This is especially true because the glc subspectra in aliphatic glucosides differ considerably from both datasets presented here, indicating the essential influence of the aromatic group.

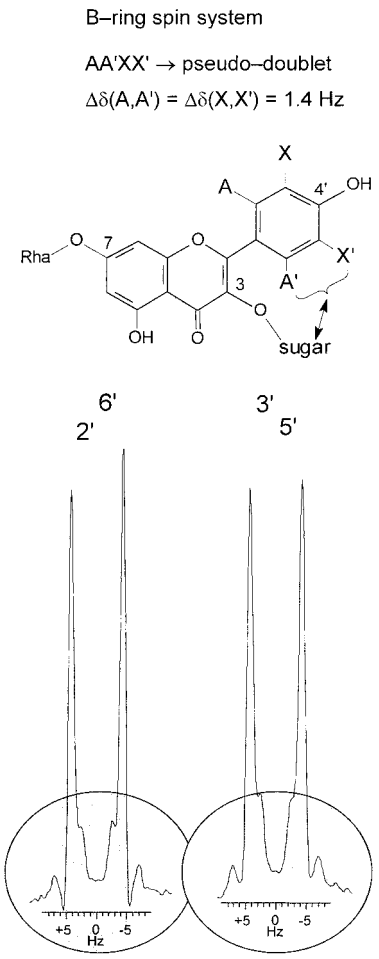

Figure 2. The four CH protons of the B-ring of kaempferol at positions 2′, 3′, 5′, and 6′ form an AA′XX′ spin system. In contrast to most of the literature, interpretation must be based on spectral simulation taking into account the nonisochronic shifts of the two pairs AA′ and XX′. In analogy to the aromatic induced shifts influencing the 3-*O*-sugar, the carbohydrate represents a chiral residue and therefore acts as an asymmetric shift inductor, causing a slight dispersion of 1.4 Hz of the otherwise isochronous pairs. Accordingly, both apparent doublet signals show significant sidebands and have to be correctly labeled as multiplets. Nevertheless, they might be best marked as pseudo-doublets with respect to their apparent shape but, keeping in mind the presence of full ortho (7.9 Hz) and meta (2.1 Hz) coupling, give rise to dt-like signals with largely shifted intensities.

d) represents a higher order multiplet and must be labeled accordingly.

As expected, asymmetric induction is not only caused by but also observed in the sugar portion of a molecule. This follows from observations made with diastereomeric pairs of cyanogenic glycosides that are derived from enantiomeric cyanohydrin aglycons, e.g., in prunasin and sumbunigrin.[16] The chiral chemical shift effects are so distinctive that two fully separated sets of sugar signals are observed in diastereomeric mixtures.

To establish the above-mentioned shift effects, spectral simulation[17] proved to be an indispensable tool for analyzing the resonances of both the aromatic and the carbohydrate moieties because of the frequent presence of higher order spin systems. While J and δ values are mostly established by measuring line distances ($\Delta\nu$) and peak centers directly in the spectra, respectively, considerable deviations can occur by neglecting higher order effects. This causes inconsistencies in the J pattern reported for a given sugar and can obscure the identification of a sugar. Moreover, minor peaks or unexpected lines that seem to be due to impurities will frequently be identified as belonging to signals of higher order spin systems ("sidebands") of the main sugar resonances (e.g., H-3 resonance in 7-*O*- vs 3-*O*-linked rha, see Figure 1). However, the known J pattern of sugars in first-order situations such as the above-mentioned sets for glc and rha serve as an excellent starting point in a simulation run.

Even when a full assignment of all sugar resonances becomes critical due to extensive signal overlap, the type of spin system on hand is still perceptible simply by looking at the very prominent, well-resolved signals. One example is the characteristic but irregular anomeric doublet of glc, and another instance is the appearance of an odd doublet for the rha Me protons, both of which are typically observed in the case of 3-O-linked sugars (see Figures 3 and 4). In most cases it is sufficient to locate only one or two more signals in order to be able to successfully simulate[18] the whole spin system and thereby determine all shift values. Consequently the number and positions of higher order sidebands, i.e., the entire shape of the signal, permit conclusions about the relative chemical shift of the coupling partners. In general, higher order effects observed for a given proton point toward a very close resonance behavior of (at least) one direct coupling partner and (!) its next neighbor. One example is the anomeric glc proton in combination with the neighboring protons H-2/3 in **2** (see Figure 3).

Independent from spectral simulation, the higher order multiplets can be extracted by means of the 1D selective pulse TOCSY technique named SelTOCSY in the following. On the basis of the selective excitation[20] of resolved resonances, e.g., an anomeric proton, the spin system can be evaluated in a stepwise fashion through the adjustment of the mixing time. Thus, even very complex and strongly overlapped signals such as H-2/3 in the 3-*O*-glc **2** or H-5/4 in the 3-*O*-rha **3** are accessible and support both the results of spin simulation studies (see Figures 3 and 4, respectively) and the identification of the sugar.

Within one group of natural products, as shown here for flavonoids, the exact chemical shifts and the type of the spin systems are predetermined by the overall substituent chemical shift effects occurring in a glycoside. Therefore, there is a real need for comprehensive NMR data being reported not only for novel but also for well-known compounds if not yet available. In particular, standard NMR datasets should then include δ values with adequate digital resolution (better than 0.2−0.5 Hz/point or 0.001 ppm) and provide J values that are derived from fully assigned sets of signals and verified by spin simulation. Finally, it must be mentioned that the definitive assignments of all observed sugar resonances are an essential part of the comprehensive interpretation of gHSCQ and gHMBC maps, which today are routine tools in the structure elucidation of glycosides. This is especially true for the critical distinction of epimeric sugars whenever both the proton and the carbon nuclei resonate in a narrow spectral window.[21]

Experimental Section

General Experimental Procedures. UV, IR, and optical rotation as well API- and HR-MALDI-TOF spectra were measured as described previously.[5] The NMR spectra, in addition to the previously reported[5] data, were recorded on a Varian Unity 600 (5 mm multinuclear probe, gradient unit) operating at 600 and 150 MHz for ^{1}H and ^{13}C, respectively. Norell 508-UP 5 mm tubes were filled with nondegassed 800 μL CD_3OD solutions of the compounds **1**−**3** containing 5% of DMSO-d_6. The manufacturer's software was used for APT, gradient-enhanced COSY, as well as for the inverse-detected

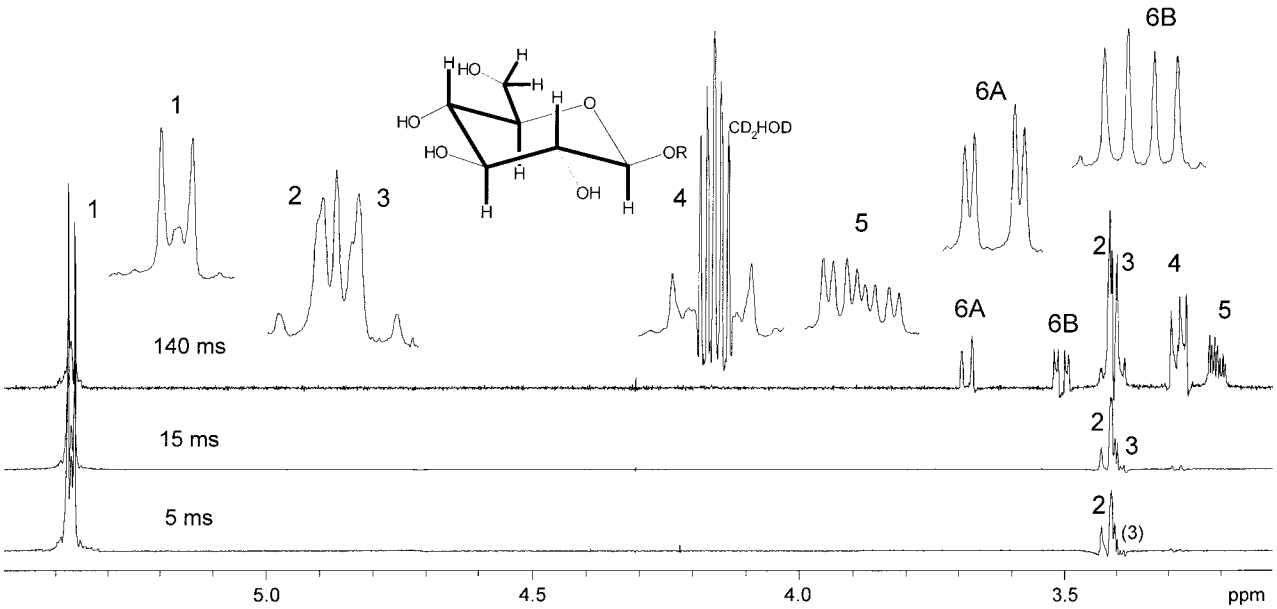

Figure 3. 1D selective TOCSY experiments for the 3-*O*-glc moiety of **2** upon excitation of H-1. Acquisitions were performed using increasing mixing times (τ_{mix}) of 5, 15, and 140 ms, with the latter giving rise to a complete subspectrum of the sugar. With the help of short τ_{mix} definitive proof is gained for the close proximity and the higher order shape of the signals of H-2 and H-3. This resonance behavior causes prominent higher order effects for the signals of the neighbors, especially the anomeric proton, which, in this case, is not observed as the typical doublet. A complete set of glc ^{1}H resonances is depicted on the top and allows establishment of the full relative stereochemistry, with an all-trans diaxial coupling pathway allowing easy magnetization transfer within the carbohydrate moiety. The 1D TOCSY also allows determination of the H-4 signal, which otherwise is exactly hidden beneath the solvent peak (600 MHz, in CD_3OD + 5% DMSO-d_6).

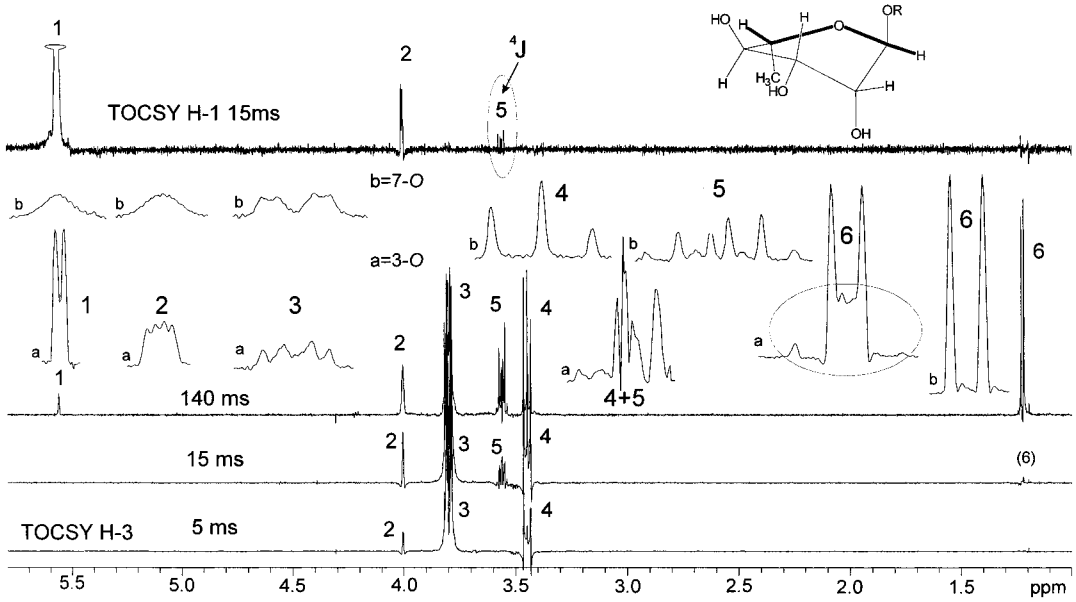

Figure 4. Evaluation of the rha proton spin systems in **3** using 1D selective TOCSY experiments. Because of the small couplings $J_{1,2}$ and $J_{2,3}$, sufficient magnetization transfer for the whole pyranose ring system is achieved best upon excitation of H-3. Unlike the all-trans coupling pathway in glc, signal intensity of the 140 ms full rha TOCSY is greatly influenced by the different magnetization transfer of the scalar couplings. Interestingly, the 1D TOCSY spectra lead to the identification of an unusual long-range coupling between the anomeric proton and H-5 (see arrow), which can be understood in terms of a *W*-type ^4J, indicating a twisted, nonchair conformation of the pyranose skeleton. While this coupling cannot be recognized by signal splitting in the regular 1D spectra, which is probably due to multiple additional signal splitting caused by slight higher order effects, its value can be estimated to be well below 1 Hz. Like in the glc moieties attached to C-3-OH, rha sugars linked to this position also tend to form higher order proton spin systems as clearly indicated by the set "a" of resonances (see H-4+5 and H-6). On the other hand, the 7-O-rha moiety marked "b" shows dynamic signal broadening of H-1−3. (600 MHz, in CD_3OD + 5% DMSO-d_6).

gradient selected heteronuclear correlations gHMBC (8.5 Hz) and gHSQC (145 Hz). Chemical shifts (δ in ppm) were referenced to the solvent as internal standard (3.300 and 49.00 ppm, respectively), and the coupling constants (J) are given in Hz. The digital resolution was better than 0.4 Hz equivalent to 0.00067 ppm (16K real datapoints, 10 ppm spectral width) in the ^{1}H, and 1.2 Hz equivalent to 0.008 ppm (32K real data points, 250 ppm spectral width) in the ^{13}C domain. The 1D selective TOCSY and NOE experiments were performed using eburp1/25 selective pulses. The data were processed offline with the Nuts program package.

Plant Material, Extraction, and Isolation. The compounds were isolated from the combined 80% MeOH, MeOH, and MeOH−NH_3 extracts prepared from 240 g of pulverized dried *A. thaliana* L. ecotype Wassilewskija (ws 2, seeds from NASC, University of Nottingham, Nottingham, U.K.) cultivated in the greenhouse. Voucher specimens are deposited at the Department of Pharmaceutical Biology, University of

Würzburg. Purification steps (see ref 5 for details) involved partitioning with EtOAc and column chromatography on polyamide (Macherey & Nagel SC-6), followed by repeated passage over Sephadex LH-20 using EtOH−H_2O mixtures, and yielded 9 mg of pure **1**, 25 mg of **2**, and 56 mg of **3**.

Chiral Sugar Analysis. The D/L-configuration of the sugars was determined by chiral capillary zone electrophoresis on a Beckman P/ACE 5010 instrument using an uncoated fused silica capillary (570 mm, 50 μm i.d., 30 kV, 27 °C) and a DAD UV detector (λ 200 nm) operated with System Gold software. For further details see ref 5.

Kaempferol 3-*O*-*β*-[*β*-D-glucopyranosyl(1→6)-D-glucopyranoside]-7-*O*-α-L-rhamnopyranoside (1), Kaempferol 3-*O*-*β*-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (2), and Kaempferol 3-*O*-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (3). Analytical data have been reported previously.[5]

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(3) Pauli, G. F.; Nauman, M.; Fischer, N. H. *Spectrosc. Lett.* **1995**, *28*, 903−913.
(4) It shall be noted that in routine application NMR represents a nonchiral method. Therefore, no distinction can be made between the D- and L-series of sugars. However, the utilization of chiral lanthanide shift reagents offers the possibility of discrimination between, but not the direct assignment of, enantiomers. The distinction of D/L-sugars on the level of glycosides, in theory, is possible due to diastereotopism caused by asymmetric induction but, in practice, requires yet unavailable reference data of compounds with known absolute stereochemistry of the sugar, e.g., by preparing synthetic D/L-glycosides.
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(6) In the prominent case of flavonoids, besides ^{1}H NMR there are numerous other spectroscopic techniques such as UV, ^{13}C NMR, and MS available for structure elucidation that have been extensively reviewed[8] (see also: (a) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier: Amsterdam, 1989; Vol. 39. (b) Mabry, T.; Markham, K.; Thomas, M. *The Systematic Identification of Flavonoids*; Springer: Berlin, New York, 1970). However, these methods do not allow the establishment of sugar sequences and locations in bisdesmosides and oligoglycosides. Consequently, detailed ^{1}H NMR spectral analysis not only simplifies routine analysis of well-known groups of glycosides but also offers valuable information that is hard to obtain in the case of less prominent or unknown natural products.
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(8) For reviews see: (a) Harbone, J. *The Flavonoids−Advances in Research since 1980*; Chapman and Hall: New York, 1988. (b) Harbone, J. *The Flavonoids−Advances in Research since 1986*; Chapman and Hall: London, 1994.
(9) Whenever solvent mixtures are used, the inter-laboratory reproducibility of the exact proton chemical shifts may still be poorer than the digital resolution even when their composition has precisely been reported. However, such data are still very meaningful in terms of the chemical shift *differences*, which are measures for the resolution of the individual signals, their relative position in the spectrum, and, therefore, possible higher order effects. Practical experience shows that many polar compounds such as flavonoids that are hard to dissolve in CD_3OD can be easily solubilized with a few drops of DMSO-d_6 (5% or less), which, in turn, does not cause severe shift differences.
(10) For the theory of ASIS refer to (a) Laszlo, P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1967**, *3*, 231−402 For a practical guide see: (b) Braun, S.; Kalinowski, H.-O.; Berger, S. *150 and more basic NMR experiments*; 2nd ed.; Wiley-VCH: Weinheim, 1998.
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(13) Because of the mostly coplanar arrangement of the chromene A/C ring system and the sugar or sugar chains attached to 5/7-OH positions, the SCS in those sugars are much less pronounced compared to 3-*O*-linked sugars due to the distance from the shielding aromatic cone. However, A-ring glycosidation can be recognized from resonance shifts of protons H-6/8 using empirical rules.[8]
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(15) The interpretation as a straight doublet is obviously wrong because each proton shares at least two prominent couplings, i.e., one ^3J(ortho) and one ^4J(meta), beside possible long-range ^5J(para) connectivities. But also in those few instances where doublets are reported as resulting from AA′XX′ situations (e.g., in Fossen, T.; Larsen, A.; Kiremirec, B. T.; Andersen, O. M. *Phytochemistry* **1999**, *51*, 1133−1137) this labeling is misleading because only one coupling constant is reported for the magnetically inequivalent pairs of protons.
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(18) Perkins, S.; Johnson, L.; Phillips, D. *Carbohydr. Res.* **1977**, *59*, 19−34.
(19) The term virtual coupling has been coined in the early days of NMR spectroscopy (e.g., Musher, J. I.; Corey, E. J. *Tetrahedron* **1962**, *18*, 791−809) and has been used to describe the occurrence of "irregular" signals due to higher order spin coupling. However, there are two situations that need to be distinguished: (a) higher order shift effects that give rise to mostly very complex "extra lines" or "sidebands" and (b) higher order spin coupling that leads to simple signal splitting, e.g., into small doublets. To avoid possible misinterpretation, e.g., conclusions about the presence of long-range couplings in the molecule, it is suggested that the term *virtual coupling* today should preferably be used in case (b), while the phenomena observed for the glycosides discussed here fall within category (a). One example of *virtual coupling* belonging to category (b) is the fine doublet splitting observed for the H-2/6eq signals in quinic acid derivatives, which must be *virtual* due to the definitive lack of a corresponding long-range coupling partner in the molecule (see ref 11 for details).
(20) For a survey on soft pulse 1D experiments see: Kessler, H.; Oschkinat, H.; Griesinger, C. *J. Magn. Reson.* **1986**, *70*, 106−133, while Gaussian pulses are addressed in: Bauer, C.; Freeman, R.; Frenkiel, T.; Keller, J.; Shaka, A. *J. Magn. Reson.* **1984**, *58*, 442−457. To achieve the acquisition of pure in-phase signals, an improved technique applying field-gradient selection has been proposed by: Fäcke, T.; Berger, S. *GIT Lab. Fachzeitschr.* **1998**, *42*, 206−208.
(21) This is of greater importance since the (nonchiral) chromatographic distinction of, for example, the 8 epimeric aldohexoses or 6-desoxy-aldohexoses, which is often used in combination with hydrolysis, can be cumbersome. At the same time, epimeric sugars give rise to very similar sets of ^{13}C resonances (e.g., mannose and galactose), while their ^{1}H spectra suffer from severe overlap. Therefore, unambiguous sugar identification is in need of full signal assignment, which, at least for model compounds, should be provided based on gHSQC/gHMBC maps. Transferred to the example of the flavonoid glycosides **1**−**3**, distinction of the following critical resonances must be achieved: C/H-2/3/4/5 of rha (also compare with rha given in ref 12) and C/H-3/5 of glc.

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