Evaluation of Glucoiberin Reference Material from *Iberis amara* by Spectroscopic Fingerprinting

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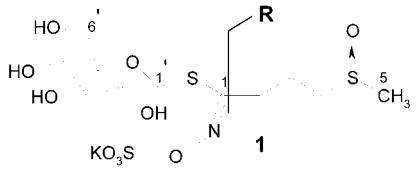
Increasing worldwide regulations require increased efforts toward validation of analytical and pharmacological reference materials. A detailed survey of glucoiberin, a prototype lead constituent of therapeutic value, using 1D/2D NMR, MS, and X-ray spectroscopy provided precise phytochemical data for structure assignment. Quantitative reference validation was achieved by the recently proposed qNMR method.

At present, a worldwide increase in the regulation of plant-derived pharmaceutical products is observed. International and national regulations for centralized and state authorization, respectively, affect the production of herbal medicinal products (HMPs), as well as related test procedures and acceptance criteria.[1] Complying with these rules, natural products reference materials are required to serve as *analytical* lead substances (markers) in the quality control (QC) of herbal medicinal products at various stages of the manufacturing process. However, there are yet no such definitions for reference materials when used as *pharmacological* lead substances for *in vitro* or *in vivo* pharmacological screening and (pre)clinical testing.

The aim of this study is to provide an exemplary survey of reference material validation for a therapeutically relevant natural product. In particular, spectroscopic fingerprinting of natural products, in addition to its chromatographic counterpart, is a method of increasing relevance in fulfilling the legal requirements, while also paying adequate attention to the often-neglected purity status of pharmacological test substances. In this context, the nuclear view offered by magnetic resonance spectroscopy (NMR) is capable of providing a specific yet rapid insight into natural products analytes and, thus, is an analytical tool supplementing the mostly molecular spectroscopic approaches.[2] This requires that qualitative and quantitative efforts work in tandem in order to unravel the wealth of fundamental information[3] contained in ^{1}H domain spectra. Accordingly, while offering an increased efficiency in quantification, the NMR approach also requires that we have a good qualitative grasp (QRDs, see below) of the ^{1}H spectroscopic details of an analyte and its potential impurities as emphasized in the recently proposed qNMR concept.[2] Being a quantitative ^{1}H NMR method, qNMR offers both options of working as a relative method (100% method, 100%-minus-impurities approach) or an absolute method. Particularly when compared with nonhyphenated chromatography, which still represents the routine method for reference standard validation, qNMR allows not only the determination of chemical purity but also the simultaneous detection, (gross) assignment, and quantification of minor impurities (e.g., triterpenoids in iridoids and flavonoids in steroids).[2] Furthermore, in contrast to chromatographic methods, it is possible to detect residual solvents and even water.

Chart 1. Names and Structures of Glucoiberin Potassium Salt (**1**) and Related Glucosinolates



Glucosinolate		R = 
chemical name	common name	R
allyl-	sinigrin	CH_2=CH–CH_2–
p-hydroxybenzyl-	sinalbin	HO–C_6H_6–
but-3-enyl	gluconapin	CH_2=$CHCH_2CH_2$–
phenylethyl	gluconasturtiin	C_6H_6–CH_2–CH_2–

Because qualitative information is such an essential component of this approach, one prerequisite of spectroscopic fingerprinting is the availability of qualitative reference dossiers (QRDs).[2] Their importance is exemplified below for the glucosinolates, comprising a group of main plant constituents of therapeutic value for which there is yet no basis for routine identification and certification of reference materials. This gap could now be filled by establishing a QRD for **1** and its derivatives, i.e., to provide a comprehensive analytical survey of spectroscopic data.

The botanical example we have chosen, *Iberis amara*, comprises a remarkable trade article, exemplified by clinically proven and patented dietary supplements. The medicinal usage of *I. amara* is twofold: The main applications make use of the motility-regulating and tonic effects on the gastrointestinal tract. Second, *Iberis* is valued for its antiphlogistic and spasmolytic properties.[4] Additional pharmacological activities are outlined elsewhere.[5−8]

For the analytical documentation of *Iberis* related preparations, phytochemical drug identification, diagnosis of adulterations, and, most significant, the standardization of extracts are usually performed using the major gluco-

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Table 1. ^{1}H and ^{13}C NMR Data of Compound **1**[a,b]

glucose	δ_C	δ_H	aglycone	δ_C	δ_H
CH 1′	82.97	4.956 (d, J = 9.9)	C 1	161.55	
CH 2′	73.55	3.342 (dd, J = 8.9, 9.9)	CH_2 2a	31.97	2.921 (m[ddd], J = 6.8, 7.5, 15.7)
CH 3′	78.65	3.492 (t/dd J = 8.9, 9.1)	CH_2 2b		2.895 (m[ddd], J = 6.8, 7.5, 15.7)
CH 4′	70.65	3.375 (dd, J = 9.1, 9.8)	CH_2 3a+b	20.87	2.191 (dddd/quint, $J \approx$ 7.3)
CH 5′	81.48	3.484 (ddd, J = 2.2, 6.1, 9.8)	CH_2 4a	52.96	3.018 (ddd, J = 6.5, 7.1, 13.4)
CH_2 6′a	62.13	3.870 (dd, J = 2.2, 12.5)	CH_2 4b		2.921 (ddd/dt, J = 7.9, 8.0, 13.4)
CH_2 6′b		3.659 (dd, J = 6.1, 12.5)	CH_3 5	37.78	2.692 (*s*)

[a] In CD_3OD with 29.3% D_2O added; chemical shifts δ in ppm; coupling pattern and constants [J in Hz] are given in parentheses. [b] All assignments were confirmed by gHSQC, gHMBC, and gCOSY maps; for numbering refer to Figure 1.

sinolate of the plant, glucoiberin **1**, as a reference standard, which comprises the prototype of an important group of these naturally occurring thioglycosides. Glucosinolates possess the general structure given in Chart 1 comprised of an amino acid derived side-chain, a sulfonated oxime moiety, and a thioglycoside residue.[9] The biological activities of glucosinolates documented so far include antinutritional and goitrogenic effects in animals (ref 10 and references therein). The main area of pharmacological effects studied in vitro is chemoprevention and the related reduction of the incidence of cancer, which has been demonstrated in animal experiments.[11]

Although **1** was known to be a plant constituent long before, the chemical constitution of **1** was only described in 1984. Since then, literature reports on glucosinolates remain sparse. Even when combined, they provide only fragmentary spectroscopic data and, therefore, are insufficient to serve as a solid reference in structure dereplication. This is particularly true with respect to the rich structural diversity of homologues, the lack of general awareness of the likely occurrence of stereoisomerism in the sugar moiety (e.g., the occurrence of allose instead of the most abundant glucose, cf. with ref 12), and the need to revise incomplete, ambiguous, or partially incorrect NMR assignments (see below). In addition, throughout the literature there is much confusion caused by applying inconsistent numbering schemes for the labeling of the aglycone skeleton and the sugar moiety of glucosinolates (cf. ref 13 vs 11). Hitherto, no X-ray crystallography data have been reported as independent evidence of glucosinolate structure, which is probably due to difficulties in the assessment of sufficiently pure material for crystallization.

In point of fact, general concerns can be raised about the quality of glycosidic reference materials, especially in case of the highly polar glucosinolates when isolated from natural sources. In particular, the (dia)stereo(iso)meric purity of a glycoside is in need of a censorious analytical mind, even if the compound is purified to crystalline material as it was intended for **1** in this study. A general uncertainty remains about the quality of such standards due to the potential presence of chemically related but chromatographically "inseparable" compounds, such as the so-called "matrix substances".[2] While the detection of enantiomers, epimers/diastereomers, and other (stereo)isomers (see also Chart 1) can hardly be achieved when using the same chromatographic methods, either in isolation or certification, such impurities might well have an impact on pharmacological effects and other parameters of *I. amara* preparations.

Glucoiberin was isolated from *Iberis amara* by means of repeated crystallization from a Soxhlet extract. For the proof of structure and determination of the quality of the isolated reference material, the ab initio interpretation of the NMR data set led to the unambiguous elucidation of the structure. Moreover, qNMR[2] was employed in order to assess the purity of the material. The purity and content

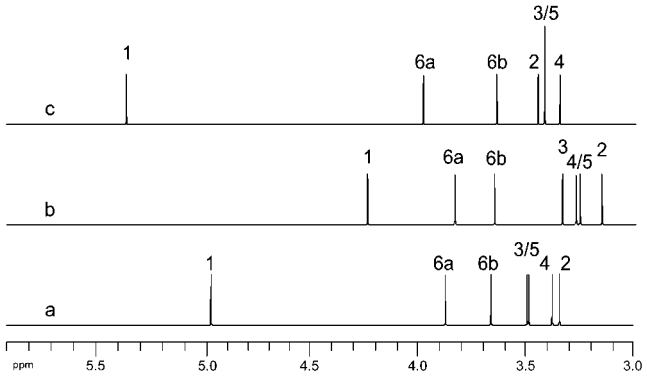

Figure 1. Distribution of the ^{1}H chemical shifts of glucose (glc) in different types of glycosides represented by singlet lines for each proton resonance: (a) the *S*-glucoside glucoiberin (**1**), (b) the *O*-glucoside allepotrioloside (**2**),[18] (c) the terminal glucose moiety of the aromatic *O*-glucoside kaempferol-3-*O*-*β*-[*β*-D-glucopyranosyl(1→6)-D-glucopyranoside]-7-*O*-α-L-rhamno pyranoside (**3**).[17] Depending on the type of their glycosidic linkage, the sugars are exposed to extremely variant chemical environments, resulting in highly different signal patterns. In **1** = a the shift of the typical anomeric doublet of proton H-1′ falls between that of **2** = b and **3** = c. This can be explained by the purely aliphatic nature of the aglycone in **2** = b, leading to only weak shift induction, as opposed to the relatively strong deshielding aromatic cone of the flavonoid B-ring located in spatial proximity to the terminal glc in **3**.[14] Furthermore, shift pattern (shift order of H-2/3/4/5) becomes a reliable indicator of glycosidic linkage and can be used to trace impurities of a different type, e.g., analogous *O*-glycosides in glucosinolates.

of a crystalline sample of **1** were investigated and two modes of calculation used for quantification: (i) the 100% method and (ii) the relative reference method with an external standard.[2] Unequivocal structural evidence was elaborated for the example of **1** leading to a valid QRD and demonstrating the feasibility of a complete fingerprint. This promotes the establishment of both glucoiberin primary standards and impurity profiles of glucosinolate analogues (homologues).

Concerning the numbering of **1**, this work follows the IUPAC recommendations with separate designation of the aglycone and the sugar. For the achievement of both, full ^{1}H signal assignment, especially in the often neglected sugar region, was a prerequisite for their interpretation and integration. As a result, the complete set of ^{1}H and ^{13}C shift values, H,H-coupling constants (J), and H,C-coupling pattern are compiled in Table 1. As demonstrated very recently for glucosides in general,[14] it was necessary to supply the 2D NMR measurements with the 1D spectral simulation of the ^{1}H data in order to allow the establishment of the true coupling constants of the sugar portion. Only by this means could the J values be determined and the presence of first-order nuclei spin systems be ascertained for quantitative analysis.

The ^{1}H NMR spectrum shows the characteristic signals of a hexose moiety and a glucosinolate backbone. While the latter is in essential accordance with the data reported by Cox et al.,[13] the authors do not provide data for the sugar.

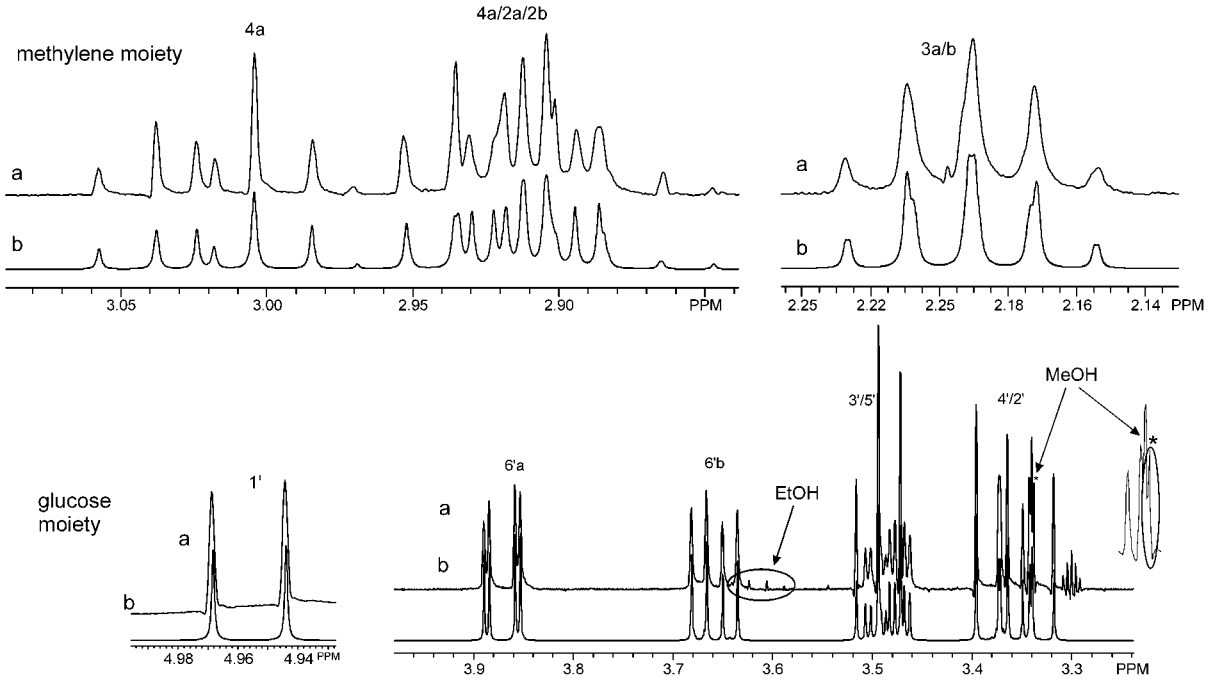

Figure 2. Fully assigned ^{1}H NMR spectrum of **1** (a) together with the completely coherent results of a ^{1}H spectral simulation (b) provide proof for the determination of refined δ and J values as given in Table 2. The arrows indicate the extraneous peaks, which are due to residual amounts of the solvents used for preparation, i.e., methanol (0.220 ± 0.007%) and ethanol (0.070 ± 0.002%).

The signal assignment given in Table 1, as it represents a partial revision of these data, provides proof for the presence of a β-glucose unit as well as a 3-methylsulfinylpropyl aglycone moiety and undoubtedly proves the structure of glucoiberin **1**. Compared with a typical aliphatic *O*-glucoside, the signal pattern of the *S*-glycosidic β-glucose unit in **1** falls into a wider shift window (3.3−5.0 vs 3.1−4.3 ppm) and exhibits a mostly reversed order of assignment. In addition **1** lacks the intramolecular aromatic *substituent*-induced shift[14] of aromatic *O*-glycosides (see Figure 1). A sharp singlet at 2.692 ppm is assigned to the completely uncoupled protons of the sulfoxide methyl function.

Furthermore, it is possible to prove the length of the side chain of the aglycone by assignment of the signals of exactly three pairs of methylene protons. This kind of approach becomes especially useful when there is no possibility to gain (direct) MS evidence for the molecular weight of the building blocks as a result of the general necessity to apply soft ionization techniques such as ESI for glycosides. In this case, routine spectra normally lack fragmentation peaks and, therefore, MS^n spectrometric analysis is needed. In **1** the methylene protons represent a higher order spin system, but it is nevertheless possible to detect the typical all ~7 Hz coupling pattern of a freely rotating aliphatic chain. Interestingly, only one of the three methylene proton pairs is homotopic (2H-3), while the others are diastereotopic, indicating the presence of asymmetric induction in the molecule. It is noteworthy that only spectral simulation could clarify the assignment of the methylene proton signals by explaining even the minor lines arising from the AMNOX$_2$ spin system of H-4$_a$4$_b$2$_a$2$_b$3$_{a+b}$ (see Figure 2). The presence of nonisochronic nuclei leading to a higher order spin system in the aliphatic side chain is an indicator of the asymmetric induction of the chiral sulfoxide moiety that mostly affects CH$_2$-4, resulting in a diastereotopic character of these methylenes. Consequently, the observed diastereotopism in **1** stands in analogy with intramolecular effects known to occur in flavonoid glycosides[14] in which significant shift effects arise from through-space effects of neighbor groups (B-ring ↔ sugar). This interpretation is supported by molecular modeling studies revealing a close proximity between the aliphatic side chain and the glucose moiety. Clear evidence for the validity of this statement came from NOE correlations: NOEs could be observed between H-1′ and H-2a/b H-3′ and H-4a as well as between H-3′ and H-2a/b.

The shifts of 11 carbon atoms recorded for **1** are in agreement with published ^{13}C NMR data,[13] with six of them showing the typical shift pattern of a β-glucose unit in *S*-glycosidic bondage. The remaining five are due to the 3-methylsulfinylpropyl aglycone consisting of one downfield C═N, three methylene, and one methyl group with a typical shift value of a sulfur-substituted carbon at 37.78 ppm. In the 2D NMR spectra the partial structure of the aliphatic side chain could be confirmed by the subsequent H,H-correlations from H$_2$-2 to H$_2$-3 and from H$_2$-3 to H$_2$-4 representing the above-mentioned aglycone spin system. HMBC couplings from H$_2$-4 to C-5 enabled us to position the methyl group, while couplings from both H$_2$-3 and H$_2$-2 to C-1 completed the establishment of the carbon skeleton of the aglycone. The (thio)glycosidic bondage is confirmed by a long-range coupling from H-1′ to the sulfur-substituted C-1 and corroborates the interpretations of the ^{1}H substituent chemical shift effects for *S*/*O*-glucosides as discussed above (see also Figure 1). Confirmation for the structure of **1** came from ESI mass spectra acquired in negative mode and showing the quasimolecular anion of the sulfonate at *m*/*z* 422. As expected, no ions were observed in the positive mode because of the strong acidic properties of free **1**. Conversely, under ESI conditions the compound shows the typical association behavior of an acid leading to the dimeric cluster ion at *m*/*z* 884. Further cluster ions centered at *m*/*z* 1346 and 1808 can be explained through the addition of one and two moles of the potassium salt of **1**, respectively, which also represented the salt species present in the X-ray sample.

Independent structural evidence for **1** came from a single-crystal X-ray diffraction analysis. The compound

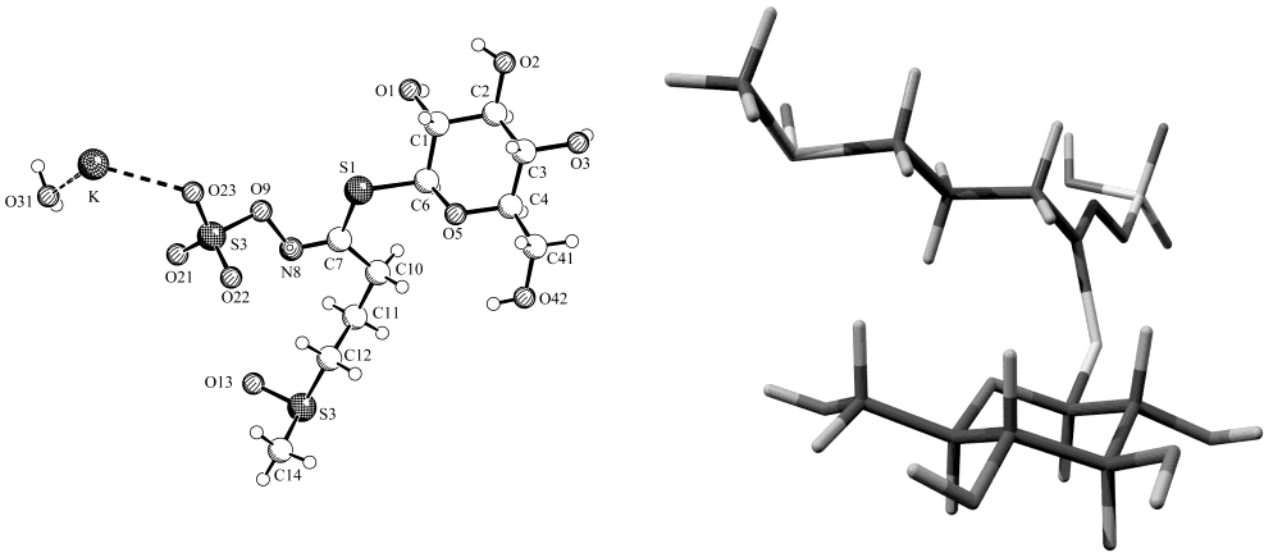

Figure 3. X-ray structure (K-salt, left) and stereoview of the probable lowest energy solution conformer (free acid, right) of **1**. The results of force-field calculations are in excellent accordance with the observed diastereotopism of the methylene protons 2/4-H_2. This can be explained by the spatial proximity to the electronically anisotropic groups including the heteroatoms that cause considerable chemical shift effects.

Table 2. Results of the qNMR Quantification[a] of the Investigated Crystalline Material of **1**

	content
relative reference method[b]	98.2 ± 3.1% glucoiberin
100% isomers methods[b]	99.3 ± 3.1% glucoiberin
indirect method	97.6(5)%
total impurity	2.3(5)%
detected impurities	1.8(3)% homologue glycoside
	0.2(3)% aromatic compounds
	0.06(5)% residual ethanol
	0.21(6)% residual methanol

[a] The relative reference method provides an absolute quantification based on an external standard, while the 100% method is related to the total integral. The indirect value reflects the result of the "100% minus known impurities" method (see discussion and ref 16). For a detailed description see ref 2. [b] Results are expressed as the mean percentage ± standard deviation.

Table 3. Selected Bond Lengths (Å) and Angles (deg) of **1**

bond	length	dihedral	angle
C6−S1	1.808(3)	C6−S1−C7	105.1(1)
S1−C7	1.753(3)	S1−C7−N8	120.3(2)
C7−N8	1.284(3)	S1−C7−C10	121.9(2)
N8−O9	1.457(3)	C10−C7−N8	117.8(3)
O9−S3	1.614(2)	C7−N8−O9	108.6(2)
S3−O21	1.435(2)	N8−O9−S3	111.8(2)
S3−O22	1.450(2)	C7−C10−C11	115.4(2)
S3−O23	1.440(3)	C10−C11−C12	113.5(2)
C7−C10	1.509(4)	C11−C12−S2	109.2(2)
C10−C11	1.528(4)	C12−S2−O13	105.5(1)
C11−C12	1.535(4)	C12−S2−C14	98.5(2)
C12−S2	1.802(3)	O13−S2−C14	105.5(1)
S2−O13	1.517(2)		
S2−C14	1.774(3)		

crystallizes with water as solvate molecule. Figure 3 shows the molecular arrangements in the solid state. Selected bond lengths and angles are compiled in Table 3, and details of the hydrogen bonding scheme are given in Table 4. The structure is built up by the anion and the six-coordinated potassium (K−O between 2.697(3) and 3.397(3) Å). Besides the oxygens of the SO_4 subunit and the hydroxyl groups of the glucose moiety, the oxygen of the solvate water is also involved into coordination. There are no unusual geometrical figures found in the structure analysis. As a result of the presence of several atoms with anomalous scattering power, the absolute structure could clearly be determined with the Flack parameter being 0.01(2). Further details of the structure analysis are given in the Experimental Section.

The identification of glucoiberin is straightforward when making use of the plentiful structural information achievable by mass spectrometry and mainly NMR. However, because there is a great potential of impurities with homologues, it is not safe to rely on mass spectrometry alone when judging their absence, but rather to deconvolute the methylene proton NMR resonances. The elucidation of the sugar moiety needs special attention and, like other complex spin systems, requires detailed analysis, including ^{1}H spectral simulation. Like any reference compound isolated from a natural source, **1** carries a considerable potential of being contaminated with biogenetically related compounds other than the homologues, e.g. glucocheirolin (3-(methylsulfonyl)propylglucosinolate). As demonstrated here for the investigated sample, (repeated) crystallization does not necessarily avoid this problem, and it must be taken into account that such impurities may be hard, or even impossible, to sense when using chromatographic validation techniques. Two methods of the qNMR concept were applied for quantification:[2] the relative reference method representing a "semi"-absolute quantification, and the 100% integral method, which is comparable to the quantification by relative peak areas in chromatography. Both methods permitted the determination of the compound content (98.2%) and the exact amount as well as the probable nature of impurities (see Table 2). By this means it could be shown that even in the crystals traces of uncharacteristic contamination, i.e., those belonging to a different class of compounds, were accompanied by a small but significant amount of a characteristic glycosidic impurity. On the basis of two extraneous peaks in the glycosidic (3.71 ppm) and the H_2-3 methylene range (2.25 ppm), the amount was calculated to be 1.8(3)% assuming that it is a homologue with a molecular weight of 447 g mol^{-1}. Furthermore, 0.23% of an unidentified aromatic impurity with a supposed molecular weight of 300 g mol^{-1} could be detected. Accordingly, the 98.2% purity, although determined with a relatively poor relative standard deviation of 3.1%,[15] is valid because the 1.8% difference from 100% purity matches very well with the sum of the aforementioned minor impurities. The minor components/impurities

Table 4. Hydrogen Bonding Scheme of **1**

bond	Å	bond	Å	distance	Å	H bond	angle [deg]
O1−H1a	0.83	H1a···O22[a]	1.95	O1···O221	2.774	O1−H1a···O22[a]	173.9
O2−H2a	0.83	H2a···O1	2.42	O2···O1	2.845	O2−H2a···O1	112.7
		H2a···O13[b]	2.20	O2···O132	2.916	O2−H2a···O13[b]	141.5
O3−H3a	0.83	H3a···O42[c]	1.94	O3···O423	2.772	O3−H3a···O42[c]	178.3
O42−H42	0.83	H42···O13[d]	1.88	O42···O134	2.667	O42−H42···O13[d]	157.5
O31−H31a	0.86	H31a···O22[e]	2.00	O31−O225	2.854	O31−H31a···O22[e]	178.0
O31−H31b	0.70	H31b···O31	2.15	O31−O31	2.802	O31−H31b···O31	155.4

[a] [$x-0.5, -y+0.5, -z$]. [b] [$x+0.5, -y+0.5, -z$]. [c] [$-x+1, y+0.5, -z-0.5$]. [d] [$x+0.5, -y-0.5, -z$]. [e] [$-x+1, y+0.5, -z+0.5$].

could be determined with the same relative precision and are flawed by relative errors below <3%, resulting in better absolute precision (<0.03%), as determined by internal validation[2] using ^{13}C satellites (see Table 2). Accordingly, the present study supports the proposal of an indirect definition of purity (indirect purity, see Table 2) as being 100% minus all perceptible impurities in the sample,[16] i.e., 97.7% in the present sample of **1**. Finally, to complete the file of reference compound validation, it was possible to detect the residual solvents contained in the investigated sample by qNMR. Thus, the content of ethanol (0.065%) and methanol (0.216%) was determined from their characteristic ^1H NMR signals.

Experimental Section

General Experimental Procedures. UV, IR, and optical rotation spectra were measured as described elsewhere.[17] The ESIMS spectrum was measured on a Finnigan LC-Q spectrometer in the negative ion direct inlet mode. For NMR spectroscopy a 30 mg sample of **1** was dissolved in CD_3OD containing 29.3% of D_2O, both with isotopic purity of 99.8% D, to give a final volume of 1 mL, corresponding to a filling height of 50 mm in 5 mm tubes. The spectra were recorded at 303 K on Bruker ARX 300 and DMX400 spectrometers. 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 ^1H and 1.2 Hz, equivalent to 0.008 ppm (32K real datapoints, 250 ppm spectral width), in the ^{13}C domain. For signal assignment 2D correlations were performed as standard gradient-selected COSY, HSQC (145 Hz), and HMBC (8.5 Hz) experiments. Off-line data analysis was performed with the NUTS NMR software package, Acorn NMR Inc. Molecular modeling calculations were performed using the Hyperchem software package, version 5.0, from Hypercube Inc. (Gainesville, FL). Molecular mechanics procedures included steepest descent and conjugated gradient optimizations; for conformational searches systematic calculations of rotamers along the single bonds of C-1 were performed.

Plant Material, Extraction, and Isolation. Glucoiberin was isolated from dried seeds of *Iberis amara* L (Voucher No. Steigerwald 981644). Powdered plant material (385 g) was defatted with petroleum ether (pb 40−60 °C) and extracted with methanol for 12 h in a Soxhlet apparatus. The solution was concentrated by evaporation (1 L), and upon storage at 8 °C a precipitate was formed. Repeated crystallization from EtOH gave 1.05 g of colorless crystals. They were evaporated to dryness *in vacuo* and after that dried under a nitrogen stream.

Crystal Structure of Glucoiberin (1). X-ray crystal structure analysis of **1**: formula $C_{11}H_{20}NO_{10}S_3^-K^+ \cdot H_2O$, M_{rel} = 479.58, colorless crystal 0.35 × 0.25 × 0.10 mm, a = 9.080(1) Å, b = 9.391(1) Å, c = 22.993(2) Å, V = 1960.6(3) Å^3, ρ_{calc} = 1.625 g cm^{-3}, μ = 58.82 cm^{-1}, empirical absorption correction via ψ scan data ($0.233 \le T \le 0.591$), Z = 4, orthorhombic, space group $P2_12_12_1$ (No. 19), λ = 1.54178 Å, T = 223 K, $\omega/2\theta$ scans, 2294 reflections collected ($+h, +k, +l$), [(sin θ)/λ] = 0.62 Å^{-1}, 2294 independent and 2246 observed reflections [$I \ge 2\sigma(I)$], 256 refined parameters, R = 0.034, wR_2 = 0.094, max. residual electron density 0.33 (−0.39) e Å^{-3}, Flack parameter 0.01(2), hydrogens at the solvate molecule from difference Fourier calculations, others calculated and all refined as riding atoms. Data sets were collected with an Enraf Nonius CAD4 diffractometer. Programs used: data reduction MolEN, structure solution SHELXS-97, structure refinement SHELXL-97, graphics SCHAKAL-92. The crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CSD-157216. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. code +44(1223) 336-033, e-mail: deposit@ccdc.cam.ac.uk).

Glucoiberin (1): colorless needles (EtOH); mp 141−143 °C (lit. 142−144 °C); TLC R_f 0.23; $[\alpha]_D^{20}$ −55.3° (c 4.9, H_2O); IR (KBr) ν_{max} 3700, 3450, 2984, 2980, 1650, 1552, 1260, 1210, 1048, 976, 795 cm^{-1}; UV (H_2O) λ_{max} 235 nm; ^1H (400 MHz, CD_3OD + 29.3% D_2O) and ^{13}C NMR (100 MHz, CD_3OD + 29.3% D_2O), see Table 1; negative ion ESIMS (MeOH) m/z 422 [$M_{acid} - H$]$^-$ (100), 884 [$M_{acid} + M_{acid}K - H$]$^-$ (9), 1346 [$M_{acid} + 2M_{acid}K - H$]$^-$ (4), 1808 [$M_{acid} + 3M_{acid}K - H$]$^-$ (2).

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Supporting Information Available: Tables 1−5 of crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) The following guidelines demonstrate the rising importance of validated analytical techniques for impurity testing of natural products reference materials: European Agency for the Evaluation of Medicinal Products, EMEA, www.emea.eu.int; Committee for Proprietary Medicinal Products, CPMP, www.eudra.org; International Conference for Harmonization, ICH, www.emea.eu.int.
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