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Diarylheptanoids from *Dioscorea villosa* (Wild Yam)†

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Abstract

A fractionation methodology aimed at the metabolomic mining of new phytoconstituents for the widely used botanical, wild yam (*Dioscorea villosa*), makes use of 1D qHNMR and 2D NMR profiles along the preparative fractionation pathway. This quantifiable and structural guidance led to the isolation of 14 diarylheptanoids (**1**–**14**), including five new compounds (**1**–**5**) with a tetrahydropyrano core skeleton. The structures, including the absolute configurations of both new and previously known diarylheptanoids, were assigned by a combination of HRESIMS, 1D and 2D NMR, ^{1}H iterative full spin analysis (HiFSA), and the Mosher's ester method. The isolation yields were consistent with yields predicted by qHNMR, which confirms the (semi-)quantifiable capabilities of NMR-based preparative metabolomic mining. The qHNMR-aided approach enabled the identification of new and potentially significant chemical entities from a small fraction of the plant extract and, thereby, facilitated the characterization of the residual complexity of the *D. villosa* secondary metabolome. LC-MS profiling of different *D. villosa* accessions further confirmed that the diarylheptanoids represent genuine secondary metabolites, which can serve as a new class of markers for botanical integrity analysis of *D. villosa*.

The genus *Dioscorea* in the family Dioscoreaceae comprises over 600 species, which are found throughout the tropical and temperate regions of the world. *Dioscorea* species are widely used as botanical dietary supplements. These plants are well known for containing steroidal saponins, which have been used as marker compounds for quality control of the botanical products.[1] The roots and rhizomes of *Dioscorea villosa* L. are known as "wild yam". This species, native to North America, is a twining tuberous vine. Since the 18th century, herbalists have been using wild yam to treat menstrual cramps and problems related to childbirth, as well as for upset stomach and coughs.[2] In 1940, the rhizomes of *D. villosa* were discovered to be an important source of diosgenin,[3] a phytoestrogen that acts on the mammary epithelium of ovariectomized mice and can be chemically converted into the mammalian hormone, progesterone.[4-6] Subsequently, diosgenin was used as starting material for the synthesis of cortisone and norethindrone.[7] The latter became known as a highly potent and orally active progestational agent and was the key ingredient in the first birth control pills in the 1960s.[7]

Today, dietary supplements containing wild yam extracts are popular among women for the alleviation of menopausal symptoms and are widely used as alternatives to hormone

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ASSOCIATED CONTENT

The IR, 1D and 2D NMR spectra of compounds **1**–**7**, the ^{1}H NMR and ^{1}H-^{1}H COSY spectra of the (*R*)- and (*S*)-MTPA esters of the compounds **1**–**6**, **8** and **9**, the ^{1}H NMR spectra of compounds **8**–**14**, and the HiFSA simulated ^{1}H NMR spectra of **1**–**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

replacement therapy, although no direct evidence exists for the estrogenic activity of wild yam extracts.[8,9] A comprehensive literature survey reveals that phytochemical information on wild yam is limited in chemical diversity: so far, only 12 steroidal saponins and two flavan-3-ol glycosides having been reported as major secondary metabolites.[1,3,10-12] From both chemotaxonomic and metabolomic perspectives, *Dioscorea* species can also be considered underexplored and likely contain other classes of secondary metabolites. The relatively narrow spectrum of known phytoconstituents limits our current understanding of the significance of wild yam as a dietary supplement, in particular with regard to the potential relief of menopausal symptoms. In addition to considering the relevance of residual complexity (RC; see ref 13 and S1, Supporting Information) in the assessment of phytoconstituents from *D. villosa*, and plant metabolomes in general, the present study also took into account the role of alternative chromatography in the discovery of new classes of secondary metabolites, as has been shown for well-studied plants such as black cohosh (*Actaea racemosa* (L.) Nutt., *syn. Cimicifuga racemosa* L.).[14] As part of the research in the UIC/NIH Botanical Center, aimed at the metabolomic mining of new and potentially interesting bioactive constituents, the present study explores preparative fractionation methodology, which utilizes the combination of both 1D and 2D NMR spectral profiles and diversified chromatography. One starting point was the ^{1}H NMR spectrum of the MeOH extract of wild yam, which is dominated by the signals of the steroidal saponins and saturated lipids in the range between δ 0.5 and 6.0. Figure 1 shows that, under higher sensitivity NMR conditions, numerous minor resonances can be detected between δ 6.0 and 8.5, which cannot be assigned to known *Dioscorea* constituents. Using qHNMR, an enrichment efficiency parameter (see Results and Discussion) was established to guide the fractionation workflow toward the isolation of the putatively new aromatic metabolites.

Primary fractionation of the crude MeOH extract used a MeOH-H_2O solvent gradient on a preparative C_{18} solid phase extraction (SPE) cartridge and effectively enriched the aromatic components into three fractions, which were further purified by VLC, MPLC, and HPLC. The resulting 14 aromatic compounds include both new tetrahydropyrano (**1**–**5**) and acyclic (**6**–**14**) diarylheptanoids, marking the first report of members of this metabolite class from *D. villosa*.[15] Structure elucidation was performed to the level of absolute configuration on the basis of 1D and 2D NMR data, ^{1}H iterative full spin analysis (HiFSA), advanced Mosher's ester analysis, and chiral HPLC. Furthermore, LC-MS profiling confirmed that the diarylheptanoids represent genuine secondary metabolites of wild yam (S2, Supporting Information).

The occurrence of diarylheptanoids in the genus *Dioscorea* was first discovered in 2004,[16] reporting three such compounds from *D. spongiosa*, a species which had been previously authenticated as *D. septemloba*.[17] Since then, two additional analogues have been reported from *D. spongiosa*[18] and one from *D. bulbifera*.[19] Diarylheptanoids are well-known and major secondary metabolites from the Zingiberaceae. They possess a 1,7-diphenylheptane skeleton and exhibit prominent pharmacological activities such as estrogenic, anticancer, antibacterial, anti-oxidative, anti-inflammation, and anti-osteoporotic properties.[15] The first diarylheptanoid to be discovered was curcumin, a yellow pigment from turmeric (*Curcuma longa* L., Zingiberaceae) first purified in 1815.[15] Curcumin has been studied extensively both in vitro and in vivo, including human clinical trials for a variety of diseases, e.g., multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colorectal cancer, psoriasis, and Alzheimer's disease.[15,20-22] Considering the immense body of existing research evidence, the present metabolomic mining of a variety of diarylheptanoids in wild yam widens the biochemical profile of *Dioscorea* plants and potentially offers new biochemical leads for the development of wild yam and other *Dioscorea* botanical products. Herein, we present the details of the isolation methodology and the structure elucidation of these new diarylheptanoids.

RESULTS AND DISCUSSION

Targeted Purification

The purification scheme that yielded the diarylheptanoids started with the EtOAc partition of the MeOH extract of *D. villosa* roots/rhizomes. A suspension of this partition in MeOH-H_2O (1:9, v/v) was loaded on a vacuum SPE cartridge containing C_{18}-RP silica gel as a packing material. Eleven primary fractions were collected, from elution with a gradient of a MeOH-H_2O solvent system from 0:10 to 10:0 in 10% intervals. The ^{1}H NMR spectra of all primary fractions were examined for the abundance of aromatic resonances. In order to establish a quantifiable measure for the enrichment of the unknown aromatic metabolites, the resonances of the steroidal methyl groups appearing between δ 0.5 and 1.3 were used as an internal reference and the integral ratio of the unexplored aromatic region (δ 6.0 to 8.5) relative to the lipophilic region (δ 0.5 to 1.3) was calculated (see Extraction and Isolation in Experimental section as well as S3, Supporting Information). This value was determined for both the extract and the primary fractions and allowed monitoring of the relative content of the aromatic target compounds, thus, serving as the enrichment efficiency parameter. The most concentrated aromatic fractions, 4-6, were combined for further purification by VLC, MPLC, and HPLC. The purification scheme eventually afforded 14 diarylheptanoids (**1**–**14**), five of which (**1**–**5)** were new compounds containing a tetrahydropyrano ring in the heptane portion of the molecule. The absolute configurations of **6** and **7** were assigned for the first time. The presence of enantiomeric pairs in four of the purified products (**2**/**3**, **4**/**5**, **6**/**7**, and **8**/**9**) was only recognized during the effort to assign their individual absolute configurations using the advanced Mosher's ester method. The generation of HiFSA data sets (see subsection General Experimental Procedures in the Experimental Section) for **1**–**7** (S4, Supporting Information) aided the unambiguous assignments of the partially complex ^{1}H NMR signals and, thus, was instrumental in the interpretation of the chiral NMR experiments. Moreover, the confirmation of δ and J values (Table 1) by HiFSA to 0.1 ppb and 0.01 Hz precision, respectively, provided valuable data for unambiguous future structure dereplication of these compounds.

Structure Elucidation

Compound **1** was obtained as a white, amorphous powder and its molecular formula was established as $C_{19}H_{20}O_4$ on the basis of HRESIMS. Thus, the carbon skeleton required 10 indices of hydrogen deficiency. The ^{1}H NMR spectrum of **1** (Table 1) exhibited the typical *dt*-like resonances of two aromatic AA′XX′ spin system at δ_H 7.228 and 6.714 (J = 8.5 and 2.6 Hz, each 2H), as well as 7.215 and 6.754 (J = 8.4 and 2.5 Hz, each 2H), respectively, indicating the presence of two *p*-disubstituted benzene rings. Accordingly, the ^{13}C BB and DEPT NMR spectra of **1** (Table 1) exhibited four methine resonances at δ_C 128.80, 128.74, 116.09 and 116.39 (each 2C), as well as four quaternary carbon resonances at δ_C 158.27, 157.90, 135.04 and 130.02. Two proton resonances at δ_H 6.533 and 6.062 exhibiting a large coupling constant of 16.0 Hz were also observed in the ^{1}H NMR spectrum of **1**, indicating the presence of an *E* double bond. The ^{13}C DEPT experiment demonstrated three oxygenated methines at δ_C 75.20, 74.76, and 65.47, along with two methylene groups at δ_C 40.97 and 39.61. These diagnostic NMR data clearly showed that **1** is a diarylheptanoid.[15] The ^{1}H-^{1}H COSY NMR spectrum of **1** showed a continuous series of correlations from H-1 to H-7, indirectly establishing the C-C connectivity of the heptane moiety. The presence of an oxygen bridge between C-1 and C-5 and, thus, the tetrahydropyrano partial structure of the heptane moiety, were determined by the HMBC correlation from H-1 to C-5. Similarly, HMBC correlations of the H,C-pairs: H-2′/C-1, H-6′/C-1, H-1/C-1′, H-1/C-2′, H-1/C-6′, as well as H-2″/C-7, H-6″/C-7, and H-6/C-1″ linked the two benzene rings to their respective C-1 and C-7 positions of the heptane unit. Consequently, the two OH groups were assigned to C-4′ and C-4″, which is supported by their chemical shifts and the molecular

formula. ROESY correlations observed between the four pairs of H-1/H-5, H-2α/H-4α, H-3/H_2-2, and H-3/H_2-4 indicated that the tetrahydropyrano ring assumes a chair conformation. The large coupling constants between H-1 and H-2α (J = 12.0 Hz) as well as H-5 and H-4α (J = 11.7 Hz) indicated the *trans*-diaxial positions of these protons, leaving equatorial positions for H-2β and H-4β in the tetrahydropyrano ring. This was supported by the occurrence of a W-type long-range coupling constant between H-2β and H-4β (^4J = 2.1 Hz). H-3 was also demonstrated to be in an equatorial position by its quintet-like splitting pattern arising from only small coupling constants (*dddd*, J = 3.0, 2.9, 2.8 and 2.7 Hz). Finally, the advanced Mosher's ester procedure[23,24] was employed to determine the absolute configuration of C-3, bearing a secondary OH group. Treatment with (*R*)- and (*S*)-MTPA chlorides led to esterification of the secondary C-3 OH group to afford the (*R*)- and (*S*)-MTPA derivatives (**1R** and **1S**), respectively. By observing the ^{1}H NMR chemical shift difference values ($\Delta\delta_{S-R}$) of the heptane moiety, the absolute configuration of C-3 was determined to be *S* (Figure 2). Hence, the structure of **1** was elucidated as (1*S*,3*S*,5*R*,6*E*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyhept-6-ene.

Compounds **2** and **3** were purified in the form of an enantiomeric mixture by HPLC with an RP C_{18} column. As the ^{1}H NMR spectrum of this mixture showed a purity of 94.8% assuming it was a single compound, 1D and 2D NMR spectra were recorded for the elucidation of the gross structure and relative configurations. The HRESIMS gave a molecular formula of $C_{19}H_{22}O_4$. The ^{1}H and ^{13}C NMR data (Table 1) revealed that **2** and **3** were also diarylheptanoids and showed the characteristic resonances for two *p*-disubstituted benzene rings, three oxygenated methines, and four methylene groups. Using the same approach as for **1**, utilizing ^{1}H-^{1}H COSY and HMBC spectra, the gross structures of **2** and **3** were determined to be 6,7-dihydro derivatives of **1**, a finding consistent with the molecular weight and presence of two additional mass units. Upon comparison of the two ^{1}H NMR data sets of **1** and **2**/**3**, the observation of congruent splitting patterns of the signals of H-1, H-3, and H-5 indicated that the compounds have identical relative configurations, which was supported by the ROESY correlations of the pairs H-1/H-5, H-3/H_2-2, and H-3/H_2-4.

In order to determine the absolute configuration of C-3 in the original isolate (eventually determined to be an enantiomeric mixture of **2** and **3**), the (*R*)- and (*S*)-MTPA derivatives were prepared, which exhibited two very similar ^{1}H NMR spectra. The only noticeable difference was the reversed integration ratio of two sets of resonances in a ratio of 52.4:47.6, indicating that this sample is indeed an enantiomeric mixture of compounds, **2** and **3**.[25,26] In the ^{1}H NMR spectra of the two MTPA derivatives, the resonances of H-1 and H-3 could be clearly assigned based on their chemical shifts and splitting patterns (Figure 3). Owing to the MTPA acylation, the H-3 resonances of the derivatives overlapped at much lower field. The phenyl ring of the MTPA moiety exhibited the smallest shielding/deshielding effect on H-3 and did not produce diastereotopic dispersion. In contrast, the esterification widened the diastereotopic dispersion of the two H-1 resonances, yielding two separate signals with different integrations, 52.4:47.6, which was used to determine the ratio of two parent enantiomers (Figure 3). Subsequently, the chemical shift difference of the two H-1 resonances was used to assign the absolute configuration of C-3: as the $\Delta\delta_{S-R}$ values of the H-1 resonances in the major and minor diastereomers were +0.221 and –0.221 ppm, respectively, the absolute configurations of C-3 of the two parent enantiomers had to be *S* and *R*, respectively (Figure 3). Thus, the structures of **2** and **3** were deduced as (1*S*,3*S*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane and (1*R*,3*R*,5*R*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane, respectively. In further support of this finding, the enantiomeric mixture of **2** and **3** was separated by normal phase HPLC on a chiral column (Chiralcel® OJ; 10 μm, 250 × 4.6 mm) as shown in Figure 4. Using UV detection at 224 nm, the peak areas of the two enantiomers were calculated to give an

abundance ratio of 47.6:52.4, which was fully consistent with the chiral qHNMR determination (compounds **3** to **2**).

Similarly, compounds **4** and **5** were also obtained as an enantiomeric mixture, recognized upon ^{1}H NMR analysis of their MTPA derivatives. The isolates gave the same molecular formula, $C_{19}H_{22}O_4$, based on HRESIMS. The ^{1}H and ^{13}C NMR spectra of **4**/**5** showed the diagnostic signals of a diarylheptanoid, in which resonances for two *p*-disubstituted benzene rings, three oxygenated methines, and four methylene groups were observed (Table 1). Analysis of the ^{1}H-^{1}H COSY and HMBC spectra led to the same gross structure as that of **2** and **3**. The coupling patterns of the tetrahydropyrano protons H-1, H-3 and H-5 indicated that all three protons assume axial positions in the chair-like conformer, which was further supported by the NOESY correlations of the pairs H-1/H-3, H-3/H-5, and H-5/H-1. Using the same method described for **2**/**3**, ^{1}H NMR analysis of the (*R*)- and (*S*)-MTPA derivatives, the absolute configurations of C-3 in **4** and **5** were determined to be *R* and *S*, respectively (Figure 2). Thus, the structures of **4** and **5** were determined as (1*S*,3*R*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5epoxy-3-hydroxyheptane and (1*R*,3*S*,5*R*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane, respectively. Shown in Figure 4, the enantiomeric pair **4**/**5** could also be separated by normal phase HPLC on a chiral column (Chiralpak® IA; 5 μm, 250 × 4.6 mm). The peak areas of the two enantiomers at UV 216 nm were used to calculate their relative abundance ratio as 83.7:16.3. The qHNMR evaluation of the (*R*)- and (*S*)-MTPA derivatives resulted in a different ratio of 76.2:23.8. This observed difference between UV and qHNMR results might partially be due to UV-active impurities, but most likely resulted from the quantification error of the qHNMR assay in this particular case. As shown only recently,[27] both the accuracy and the precision of qHNMR quantification depend on the signal to noise ratio (S/N), leading to an overestimation of content and higher errors as the S/N drops below 150. Under the chosen conditions (~300 μg sample, 600 μL, 5mm RT broadband probe, 400 MHz), the qHNMR spectra of the MTPA derivatives exhibited a S/N of 13 for the minor diastereomer (~20%) in an already mass limited sample. The conclusion that the qHNMR-based content is likely too high is consistent with observations made in the recent qHNMR validation study[27] and supports the UV-based enantiomeric ratio as being more reliable in this case.

Compounds **6** and **7** were also obtained as an enantiomeric mixture and further separated by normal phase HPLC using the same chiral column as for **2** and **3** (Figure 4). While the peak areas of HPLC at UV 213 nm showed an enantiomeric ratio of 57.2:42.8, overlap of ^{1}H NMR resonances of the pairs of (*R*)- and (*S*)-MTPA esters did not allow qHNMR quantification. The molecular formula of **6** and **7** was determined to be $C_{19}H_{20}O_4$ using HRESIMS. Interpretation of the ^{1}H and ^{13}C NMR spectra (Table 1) suggested identity with a previously reported gross structure, 5-hydroxy-1,7-bis(4-hydroxyphenyl)-1-hepten-3-one, for which no absolute configuration assignment had been made.[28] Two aliquots of the major enantiomer, **6**, were treated with (*R*)- and (*S*)-MTPA chlorides to form diastereomeric derivatives. Analysis of their ^{1}H and ^{1}H-^{1}H COSY NMR spectra indicated that **6** has the 5*R* configuration; accordingly, the minor enantiomer possesses the 5*S* configuration (Figure 2). Similarly, the structures of **6** and **7** were elucidated as (5*R*,1*E*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyhept-1-en-3-one and (5*S*,1*E*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyhept-1-en-3-one, respectively.

Seven previously reported diarylheptanoids (**8**-**14**) were characterized from *D. villosa* for the first time. Compounds **8** and **9** were obtained as enantiomeric mixtures (64.5:35.5) and determined to be (5*S*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyheptan-3-one and (5*R*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyheptan-3-one, respectively,[29,30] by analysis of their NMR and MS data as well as by a Mosher's ester analysis (Figure 2); three diarylheptanoids **10**-**12** were assigned as (4*E*,6*E*)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one,[31] (3*R**,5*S**)-1,7-

bis(4-hydroxyphenyl)-3,5-dihydroxyheptane,[32] and (3*R*,5*R*)-1,7-bis(4hydroxyphenyl)-3,5-dihydroxyheptane,[33] respectively, by comparing their NMR and optical rotation data with those in the literature; compounds **13** and **14** were obtained as a mixture (separable, but interconverting) in the ratio 87.3:12.7 as determined by qHNMR, and their structures were elucidated as (4*Z*,6*E*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyhepta-4,6-dien-3-one and (1*E*)-1,7-bis(4-hydroxyphenyl)hept-1-ene-3,5-dione, respectively,[31,34] by 1D and 2D NMR as well as MS analysis.

Stereochemical and Full Spin Analysis

While the advanced Mosher's ester method was efficient for the determination of the absolute configuration of diarylheptanoids bearing secondary OH groups (**1**–**9**), the method was unsuitable for the symmetric compounds **11** and **12**, as the proton resonances surrounding the OH group(s) in the derivatives could not be unambiguously assigned. However, the absolute configuration of enantiomers could be assigned in analogy with that of **2**/**3**, **4**/**5** and **8**/**9** by analyzing the ^{1}H NMR spectra of their mixed (*R*)- and (*S*)-MTPA esters. In general, enantiomeric ratios can be determined by integration of ^{1}H resonances, provided the isolates exhibit some degree of enantiomeric excess and the MTPA derivatives show sufficiently disperse diastereotopic proton signals.

Finally, in order to facilitate the future structural dereplication of congeneric diarylheptanoids from *Dioscorea* species, as well as analogues from other genera such as *Alpinia*, *Zingiber*, *Curcuma*, and *Alnus*, but also to support development of qHNMR standardization protocols, the precise ^{1}H NMR profiles of the newly characterized botanical markers were generated by means of HiFSA, using the PERCH software tool, for compounds **1**, **2**/**3**, **4**/**5** and **6**/**7**.[35] Molecular structures of the selected diarylheptanoids were used as starting points to analyze each discrete spin system and predict the basic ^{1}H NMR parameters (δ and J). Then, the predicted NMR parameters were optimized through iterative spin system calculations using the PERCHit iterator, until the quantum-mechanical simulations replicated the experimental ^{1}H NMR spectra (S4, Supporting Information). The final simulated HiFSA spectra exhibited excellent agreement with the observed spectra for all spectral lines and line intensities, with a total root-mean-square deviation (RMSD, "residual") of less than 0.07%. These results further validated the elucidated structures (S4, Supporting Information). The simulated HiFSA spectra represent highly precise fingerprints, which can be used to, unambiguously, identify the marker compounds and distinguish their resonances from those of impurities by comparison with the experimental spectra. This enables qHNMR-based determination of content,[35] sample purity,[36,37] and purity-activity investigation.[38] The digital HiFSA spectra of these secondary metabolomic markers can also serve as references for future metabolomic standardization of wild yam botanicals.[35]

Diarylheptanoids in *Dioscorea villosa*

While diarylheptanoids are mainly distributed in the roots, rhizomes, and bark of *Alpinia*, *Zingiber*, *Curcuma*, and *Alnus* species,[15] this is the first report of this compound class from the rhizomes/roots of *Dioscorea villosa*. Taking into account existing evidence for the presence of diarylheptanoids in the genus *Dioscorea*, it was still important to confirm that diarylheptanoids represent genuine secondary metabolites of wild yam. The LC-MS profiling performed in the present study detected the most abundant diarylheptanoid, **12**, in the crude extract of an authentic, in-house cultivated wild yam specimen (S2, Supporting Information). Additional evidence for the genuine presence of diarylheptanoids in wild yam came from characteristic correlation patterns in the aromatic region of the ^{1}H-^{1}H COSY NMR spectra of the crude extract, matching those of the isolates (S56, Supporting Information).

With respect to the preparative mining of secondary metabolites, the use of ^{1}H NMR-based information about structural fragments was shown to provide valuable guidance for the development of purification protocols. Combined with diverse chromatographic methodology, this approach facilitates the discovery of previously unknown and/or potentially interesting metabolites, even from relatively well-studied plants or other complex biological matrices. Once specific proton resonances have been identified and linked to a characteristic structural class or partial structure, a tailored and more targeted fractionation protocol can be developed, as is shown here for a series of mostly new diarylheptanoids from a widely used botanical.

In addition to providing qualitative guidance about the partial structure of unknown metabolites, the present approach is even capable of predicting isolation yields through qHNMR-based estimation of their contents.[39] The major diarylheptanoid, **12**, was estimated to be present at a concentration of 0.045%, which compares well with the isolation yield of 0.023% (230 ppm, 205.0 mg from 900 g of extract), especially when considering unavoidable loss during the four-step isolation procedure. Similarly, minor aromatic signals, such as the typical 2H pseudo-.doublets of AA′XX′ spin system, allowed the prediction of isolation yields below 0.002% (< 20 ppm) for further diarylheptanoids, which again matches the achieved yields of 0.8-15.2 mg for the other isolates, **1**–**11**, **13**, and **14**. These preliminary results indicate that the assumptions about proton and molar ratios required for performing the qHNMR calculations are valid for practical purposes and allow a reasonably close prediction of both individual yields and required scale for the isolation procedure.

Concluding Remarks

While the biological impact and potential of the wild yam diarylheptanoids require further study, which is ongoing in our laboratory, the present report establishes a link between the ubiquitous residual complexity (RC) of crude metabolomes, such as plant extracts, and the approach of qHNMR-guided metabolomic mining. These findings not only extend the utility of qHNMR applications, but also complement previous conclusions about the relevance of RC. For example, the RC of a clinical black cohosh extract was only uncovered after using a pH-targeted approach (pH zone refinement CPC) which led to the identification of *N*-containing metabolites, including the serotonergic active principle contained in the plant.[14]

Another link relates to the dereplication and targeted analysis of individual metabolites in residually complex samples. As has been shown for the structurally diverse triterpenes of black cohosh, rapid dereplication can be achieved from standard (q)HNMR spectra of (residually) complex mixtures, provided that characteristic and coherent spectroscopic information is available.[13] By presenting the HiFSA profiles of compounds, **1**–**7**, the present study facilitates future studies of *Dioscorea* botanicals at the chemistry/biology interface by supplying comprehensive information for dereplication. As shown recently, this knowledge also enables the precise quantification of *multiple* marker compounds by a combination of HiFSA and qHNMR,[35] thus laying the groundwork for the quantifiable assessment of the RC of *Dioscorea* preparations.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 22 °C in MeOH. UV spectra were acquired on a Molecular Devices Spectra Max Plus 384 spectrophotometer. IR spectra were acquired on a Perkin-Elmer 577 IR spectrometer. For this purpose, MeOH solutions of the compounds were dripped onto the IR sample holder and evaporated to form a thin sample film. NMR spectra were obtained on a Bruker AV-400 (5 mm broadband

probe with Automatic Tuning and Matching (ATM) capability) or a DPX-400 (5 mm $^{1}H/^{13}C/^{31}P/^{19}F$ QNP probe) NMR spectrometer (Bruker, Zürich, Switzerland) using methanol-d_4 (for compound **1**, by adding 10% $CDCl_3$), pyridine-d_5, or DMSO-d_6 as the solvent. The chemical shifts of the residual solvent signals (δ_H 3.310 and δ_C 49.15 for methanol-d_4; δ_H 8.740 for pyridine-d_5; δ_H 2.500 for DMSO-d_6) were used as the chemical shift reference and also as internal calibrants for qHNMR quantification. Offline NMR data processing was performed with MestReNova software version 8.0.0-10524 for Windows OS (Mestrelab Research, Santiago de Compostela, Spain). All NMR experiments were performed using standard Bruker pulse sequences. The ^{1}H NMR data were processed with double zero-filling and Lorentz-Gauss resolution enhancement (LB −1.8 Hz and GF 0.04 = GB 1.0 Hz) prior to Fourier Transformation. Calculations for the ^{1}H NMR iterative Full Spin Analysis were performed with the PERCH software package v.2010.1 (PERCH Solutions Ltd., Kuopio, Finland). HRESIMS was carried out on a Waters Q-TOF Synapt mass spectrometer using the negative mode. LC-MS analysis for study of the genuine nature of diarylheptanoid, **12**, was performed on an AB Sciex 4000 LIT QTRAP® equipped with a Shimadzu UFLC system using a YMC-Pack ODS-AQ column (150 × 2.1 mm, 3 μm, 12 nm), with the ESI ion source operating in the positive mode.

Semi-preparative HPLC was carried out using a Waters 600 controller with a Waters 2996 photodiode array detector, using YMC-Pack ODS-AQ (250 × 10 mm, S-5, 12 nm), Chiralcel® OJ (250 × 4.6 mm, 10 μm), and Chiralpak® IA (250 × 4.6 mm, 5 μm) columns. Silica gel (230–400 mesh, Macherey-Nagel), C_{18} reversed-phase silica gel (Macherey-Nagel), Sephadex LH-20 (Sigma) and HW-40F gel (Tosoh) were used for VLC and MPLC. General fraction monitoring following preparative chromatographic separations was done by TLC analysis with pre-coated glass TLC plates (250 μm thickness, K6F Si gel 60, EM science, Germany). The compounds were visualized by spraying the dried plates with 5% H_2SO_4 in EtOH, followed by heating at 120 °C for 10 min. All solvents used for LC were of analytical and chromatographic grade purchased from Sigma-Aldrich Co., St. Louis, USA. The (*R*)- and (*S*)-MTPA chlorides were purchased from the same vendor.

Plant Material

Wild harvested rhizomes/roots of *Dioscorea villosa* L. (4.5 kg) were purchased from Mountain Rose Herbs in August 2011. Authentic rhizomes/roots of in-house cultivated *D. villosa* L. were collected in the UIC Dorothy Bradley Atkins Medicinal Plant Garden in October 2010. Both samples were authenticated by Dr. Djaja D. Soejarto of the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago. A voucher specimen of the commercial sample (accession number: BC630) has been deposited at the Field Museum of Natural History Herbarium, Chicago, IL.

Extraction and Isolation

Authentic, in-house cultivated *D. villosa* (BC 601, 5 g) was extracted with MeOH to give 980.8 mg crude extract, of which 128.5 mg was subjected to an SPE VLC using 6 g of C_{18} reversed-phase silica gel as packing material. An eleven-step gradient of MeOH-H_2O (0:10 to 10:0, v/v, 10% interval) was used as the mobile phase, and the ^{1}H NMR spectra of all 11 fractions were examined in order to systematically explore the content of aromatic compounds. The integral ratio of two ^{1}H NMR resonance regions, δ_H 6.0 to 8.5 and δ_H 0.5 to 1.3, was used as the parameter to measure the enrichment efficiency of the target compounds. The ratio was 5.3:94.7 in the MeOH extract, the ratios for the three primary fractions with the highest abundance of aromatic resonances were 45.0:55.0 (fr. 4), 34.2:65.8 (fr. 5) and 40.7:59.3 (fr. 6). Thus, fractions 4–6 were combined to give one enriched aromatic compounds fraction.

The dried and milled rhizomes/roots of *D. villosa* (BC 630, 4.5 kg) were extracted with MeOH (3 × 6000 mL) to give 900 g crude extract. This was suspended in H_2O-MeOH (9:1), then, successively partitioned at room temperature between hexanes, $CHCl_3$, EtOAc, and *n*-BuOH. Using the aforementioned enrichment strategy, the EtOAc-soluble partition (43.0 g) was subjected to a C_{18} SPE VLC, affording the enriched aromatic compounds fraction (2.4 g), which was chromatographed on a silica gel VLC eluted with a $CHCl_3$/MeOH gradient (100:1 to 5:1, v/v) to give five secondary subfractions (A to E). Sub-fraction B was further fractionated by MPLC on HW-40F gel, eluted with MeOH to afford six tertiary subfractions (B-SF-I to B-SF-VI). Subfraction B-SF-V was subjected to a silica gel VLC, eluted with an isocratic SS (hexanes/acetone 2.5:1, v/v), to afford compound **10** (15.2 mg). Subfraction B-SF-VI was subjected to a silica gel VLC eluted with the isocratic SS of hexanes/EtOAc (1/1, v/v), to yield a mixture of compounds **13** and **14** (2.1 mg). The secondary subfraction C was chromatographed over silica gel by VLC, eluted with hexanes/EtOAc (2:1, v/v), and was further purified by LPLC on Sephadex LH-20 to afford the enantiomeric mixture of **8** and **9** (4.0 mg). The secondary subfraction D was subjected to MPLC on HW-40 gel, eluted with neat MeOH, to give four tertiary subfractions, D-SF-I to D-SF-IV. Subfraction D-SF-II was further separated by silica gel VLC, eluted with hexanes/EtOAc (3:1, v/v), to give five quaternary subfractions, D-SF-II-1 to D-SF-II-5. D-SF-II-2 was purified by a semi-preparative HPLC using C_{18} reversed phase and Chiralcel® OJ chiral columns successively, to give compounds **6** (3.8 mg) and **7** (3.2 mg), respectively. Fraction D-SF-II-2 was also purified by semi-preparative HPLC using reversed phase C_{18} and Chiralpak® IA columns successively, to yield compounds **4** (2.5 mg) and **5** (0.8 mg). The secondary subfraction E was purified by silica gel VLC, using isocratic elution with hexanes/EtOAc (1:1, v/v), to give 11 tertiary subfractions, E-SF-I to E-SF-XI. E-SF-VIII was determined to be a pure compound, **12** (205.0 mg). E-SF-V was purified by semi-preparative HPLC using a C_{18} reversed phase column, to give compounds **1** (3.3 mg), **11** (3.0 mg), and the enantiomeric mixture of **2** and **3**, which was further separated into **2** (2.1 mg) and **3** (1.9 mg) by semi-preparative HPLC with a Chiralcel® OJ column. TLC fraction monitoring as described above was used throughout the separation process.

(1*S*,3*S*,5*R*,6*E*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyhept-6-ene (1)— white, amorphous powder; $[\alpha]_D^{22}$ +14.6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 263 (4.49) nm; IR (MeOH) ν_{max} 3342, 1611, 1515, 1238, 1020, 828 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 311.1289 $[M - H]^-$ (calcd for $C_{19}H_{19}O_4$, 311.1283).

(1*S*,3*S*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (2)—white, amorphous powder; $[\alpha]_D^{22}$ +190.2 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.90), 224 (4.65) nm; IR (MeOH) ν_{max} 3310, 2922, 1614, 1515, 1236, 1172, 1070, 1031, 827 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 313.1446 $[M - H]^-$ (calcd for $C_{19}H_{21}O_4$, 313.1440).

(1*R*,3*R*,5*R*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (3)—white, amorphous powder; $[\alpha]_D^{22}$ –166.7 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.90), 224 (4.65) nm; IR (MeOH) ν_{max} 3336, 2922, 1614, 1515, 1232, 1172, 1069, 1030, 827 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 313.1446 $[M - H]^-$ (calcd for $C_{19}H_{21}O_4$, 313.1440).

(1*S*,3*R*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (4)—white, amorphous powder; $[\alpha]_D^{22}$ +32.9 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.54), 216 (4.39) nm; IR (MeOH) ν_{max} 3336, 2944, 1614, 1515, 1449, 1367, 1236, 1066, 831 cm^{-1}; ^1H

and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 313.1448 [M – H]$^-$ (calcd for $C_{19}H_{21}O_4$, 313.1440).

(1*R*,3*S*,5*R*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (5)—white, amorphous powder; $[\alpha]_D^{22}$ –30.6 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.54), 216 (4.39) nm; IR (MeOH) ν_{max} 3336, 2944, 1614, 1515, 1449, 1367, 1236, 1066, 831 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 313.1448 [M – H]$^-$ (calcd for $C_{19}H_{21}O_4$, 313.1440).

(5*R*,1*E*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyhept-1-en-3-one (6)—yellow, amorphous powder; $[\alpha]_D^{22}$ +21.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 327 (4.37), 213 (4.38) nm; IR (MeOH) ν_{max} 3309, 2943, 1600, 1582, 1514, 1242, 1170, 1021, 819 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 311.1289 [M – H]$^-$ (calcd for $C_{19}H_{19}O_4$, 311.1285).

(5*R*,1*E*)-1,7-bis(4-hydroxyphenyl)-5-hydroxyhept-1-en-3-one (7)—yellow, amorphous powder; $[\alpha]_D^{22}$ –18.1 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 327 (4.37), 213 (4.38) nm; IR (MeOH) ν_{max} 3310, 2940, 1598, 1581, 1514, 1204, 1169, 821 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS negative mode: *m*/*z* 311.1289 [M – H]$^-$ (calcd for $C_{19}H_{19}O_4$, 311.1285).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1

Two aliquots of compound **1** (0.3 mg each in 50 μL) were transferred into two NMR tubes and dried under vacuum overnight at room temperature. Then, 6 μL of (*R*)- or (*S*)-MTPA chloride and 600 μL of pyridine-d_5 were successively added. The NMR reaction tubes were immediately sealed, shaken vigorously to ensure even mixing, and stored in a desiccator overnight until the reaction was complete. ^{1}H NMR spectra were used to monitor the reaction. The ^{1}H NMR spectra of the final (*R*)- and (*S*)-MTPA adducts were recorded directly after each reaction, and the chemical shifts were assigned based on ^{1}H-^{1}H COSY NMR experiments. Ambiguous signals were excluded from the calculation of $\Delta\delta_{S\text{-}R}$ values [23,24].

^{1}H NMR data of the (*R*)-MTPA ester of **1** (400 MHz, pyridine-d_5) (S38, Supporting Information): δ 6.814 (1H, d, *J* = 15.9 Hz, H-7), 6.449 (1H, dd, *J* = 15.9, 5.5 Hz, H-6), 5.757 (1H, br quintet, *J* = 2.6 Hz, H-3), 4.715 (1H, dd, *J* = 11.8, 1.1 Hz, H-1), 4.621 (1H, m, H-5), 2.228 (1H, m, H-2a), 2.194 (1H, m, H-4a), 1.953 (1H, m, H-4b), 1.922 (1H, m, H-2b).

^{1}H NMR data of the (*S*)-MTPA ester of **1** (400 MHz, pyridine-d_5) (S40, Supporting Information): δ 6.764 (1H, d, *J* = 16.3 Hz, H-7), 6.430 (1H, dd, *J* = 16.3, 5.5 Hz, H-6), 5.765 (1H, br quintet, *J* = 2.8 Hz, H-3), 4.952 (1H, dd, *J* = 12.0, 2.2 Hz, H-1), 4.469 (1H, m, H-5), 2.264 (1H, m, H-2a), 2.169 (1H, m, H-4a), 1.993 (1H, m, H-2b), 1.903 (1H, m, H-4b).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 2 and 3

The (*R*)- and (*S*)-MTPA esters of the mixture of **2** and **3** obtained from the isolation scheme were produced by following the general Mosher reaction procedure described for **1**.

^{1}H NMR data of the (*R*)-MTPA ester of **2** (400 MHz, pyridine-d_5) (S42, Supporting Information): δ 5.683 (1H, m, H-3), 4.603 (1H, dd, *J* = 11.6, 1.6 Hz, H-1).

^{1}H NMR data of the (*S*)-MTPA ester of **2** (400 MHz, pyridine-d_5) (S43, Supporting Information): δ 5.683 (1H, m, H-3), 4.824 (1H, dd, *J* = 11.6, 1.6 Hz, H-1).

^{1}H NMR data of the(*R*)-MTPA ester of **3** (400 MHz, pyridine-d_5) (S42, Supporting Information): δ 5.683 (1H, m, H-3), 4.824 (1H, dd, J = 11.6, 1.6 Hz, H-1).

^{1}H NMR data of the (*S*)-MTPA ester of **3** (400 MHz, pyridine-d_5) (S43, Supporting Information): δ 5.683 (1H, m, H-3), 4.603 (1H, dd, J = 11.6, 1.6 Hz, H-1).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 4 and 5

The (*R*)- and (*S*)-MTPA esters of mixture of **4** and **5** were produced using the general Mosher reaction procedure.

^{1}H NMR data of the (*R*)-MTPA ester of **4** (400 MHz, pyridine-d_5) (S44, Supporting Information): δ 5.506 (1H, m, H-3), 4.557 (1H, dd, J = 11.2, 1.4 Hz, H-1), 3.532 (1H, m, H-5), 2.775 (2H, m, H-7), 2.441 (1H, m, H-2a), 2.092 (1H, m, H-4a), 1.727 (1H, m, H-2b), 1.453 (1H, m, H-4b), 1.913 (1H, m, H-6a), 1.772 (1H, m, H-6b).

^{1}H NMR data of the (*S*)-MTPA ester of **4** (400 MHz, pyridine-d_5) (S46, Supporting Information): δ 5.509 (1H, m, H-3), 4.545 (1H, dd, J = 11.2, 1.6 Hz, H-1), 3.550 (1H, m, H-5), 2.789 (2H, m, H-7), 2.382 (1H, m, H-2a), 2.173 (1H, m, H-4a), 1.960 (1H, m, H-6a), 1.811 (1H, m, H-6b), 1.636 (1H, m, H-2b), 1.567 (1H, m, H-4b).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 6

The (*R*)- and (*S*)-MTPA esters of **6** were produced by following the general Mosher reaction procedure.

^{1}H NMR data of the (*R*)-MTPA ester of **6** (400 MHz, pyridine-d_5) (S48, Supporting Information): δ 7.872 (1H, d, J = 16.7 Hz, H-1), 7.040 (1H, d, J = 16.7 Hz, H-2), 6.083 (1H, m, H-5), 3.205 (1H, dd, J = 17.2, 4.1 Hz, H-4b), 2.642 (2H, m, H-7), 2.097 (2H, m, H-6).

^{1}H NMR data of the (*S*)-MTPA ester of **6** (400 MHz, pyridine-d_5) (S50, Supporting Information): δ 7.785 (1H, d, J = 16.5 Hz, H-1), 6.949 (1H, d, J = 16.5 Hz, H-2), 6.103 (1H, m, H-5), 3.144 (1H, dd, J = 17.1, 4.4 Hz, H-4b), 2.816 (2H, m, H-7), 2.186 (2H, m, H-6).

Preparation of the (R)- and (S)-MTPA Ester Derivatives of 8 and 9

The (*R*)- and (*S*)-MTPA esters of the mixture of **8** and **9** obtained from the isolation scheme were produced by following the same general Mosher reaction procedure. The ^{1}H NMR spectra of the (*R*)- and (*S*)-MTPA esters of **8** and **9** (400 MHz, pyridine-d_5), see Figures S52 and S54 in Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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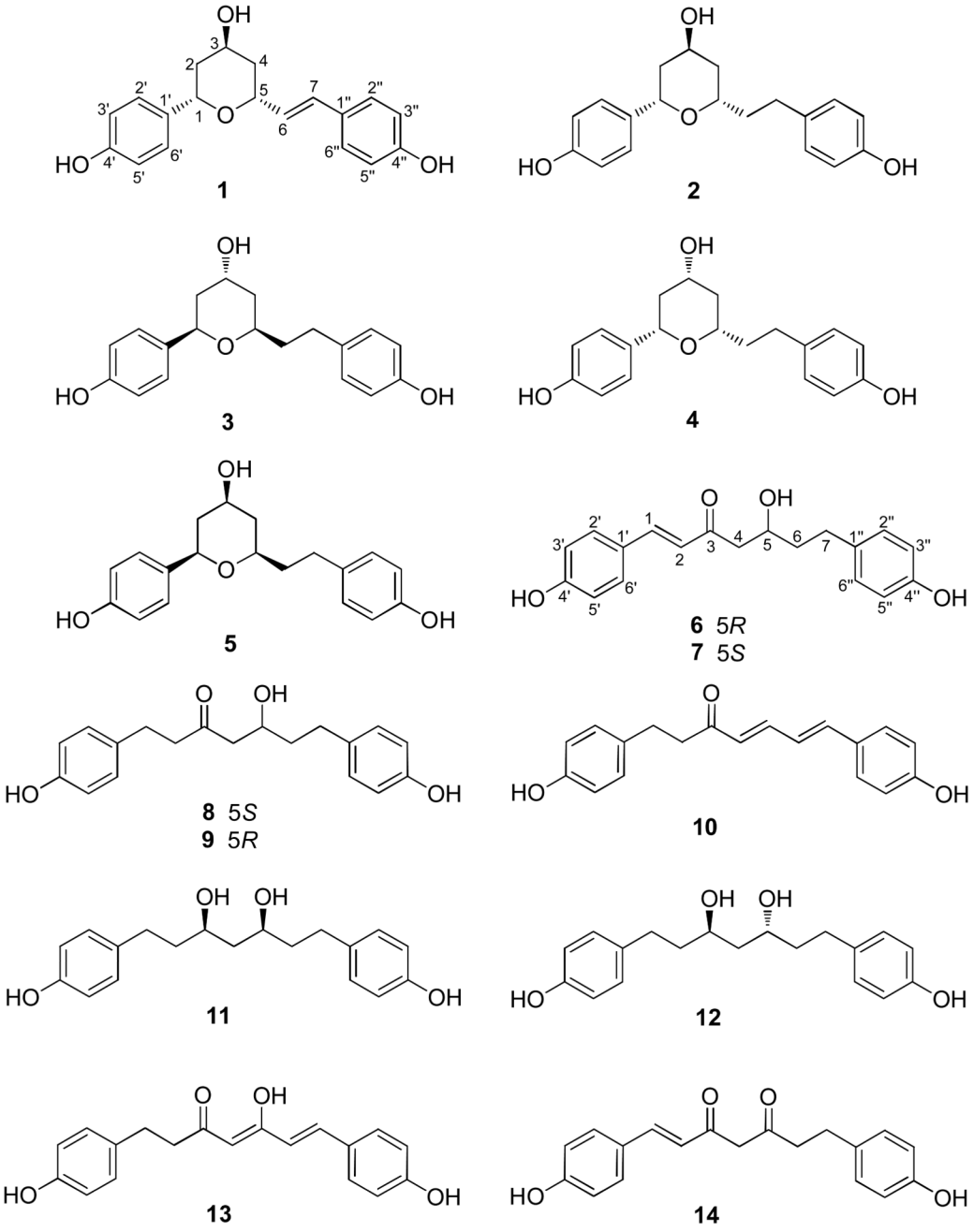

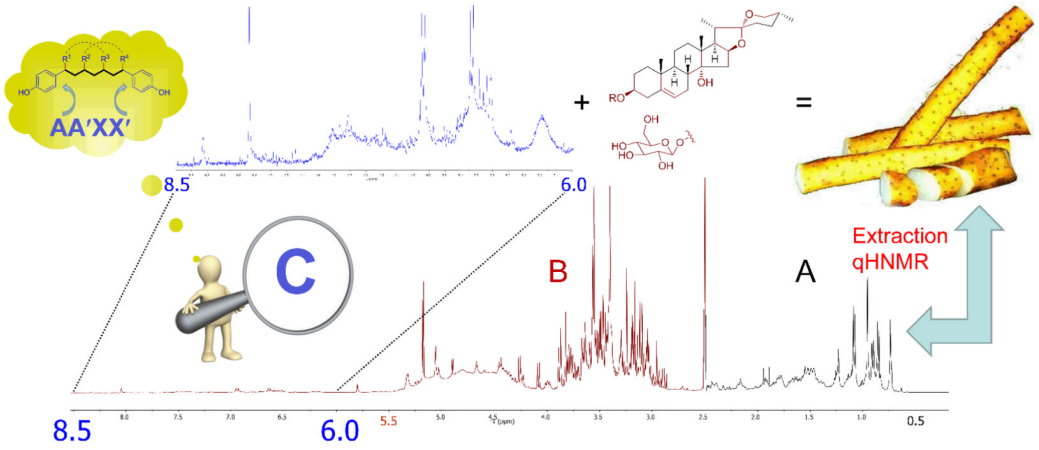

Figure 1.

Representation of the concept of ^{1}H NMR-guided fractionation for the metabolomic mining of *Dioscorea* phytoconstituents. A 30 mg aliquot of crude MeOH extract of wild yam was carefully dried in vacuo, dissolved in 600 μL of DMSO-d_6, and a high S/N ^{1}H NMR spectrum collected (400 MHz, 5 mm broadband probe with ATM, NS = 5 K). Under these conditions, signals in the aromatic region between δ_H 6.0 and 8.5 (2.47%) were readily detected and some of them could even be recognized as diagnostic AA′XX′ resonances of the subsequently isolated diarylheptanoids.

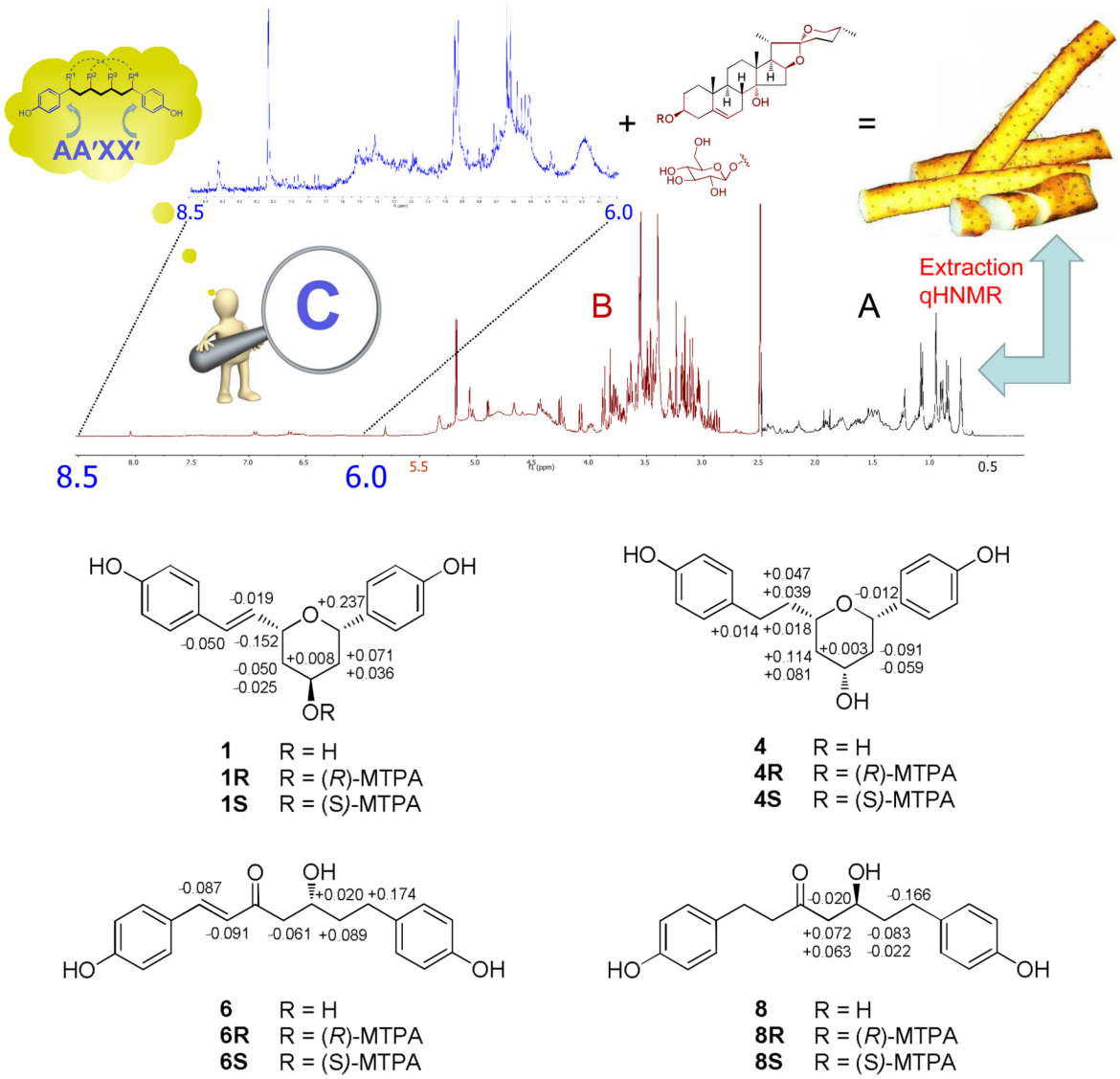

Figure 2.

The $\Delta\delta_{S\text{-}R}$ values of MTPA esters of **1**, **4**, **6** and **8** used for the determination of absolute condiguration.

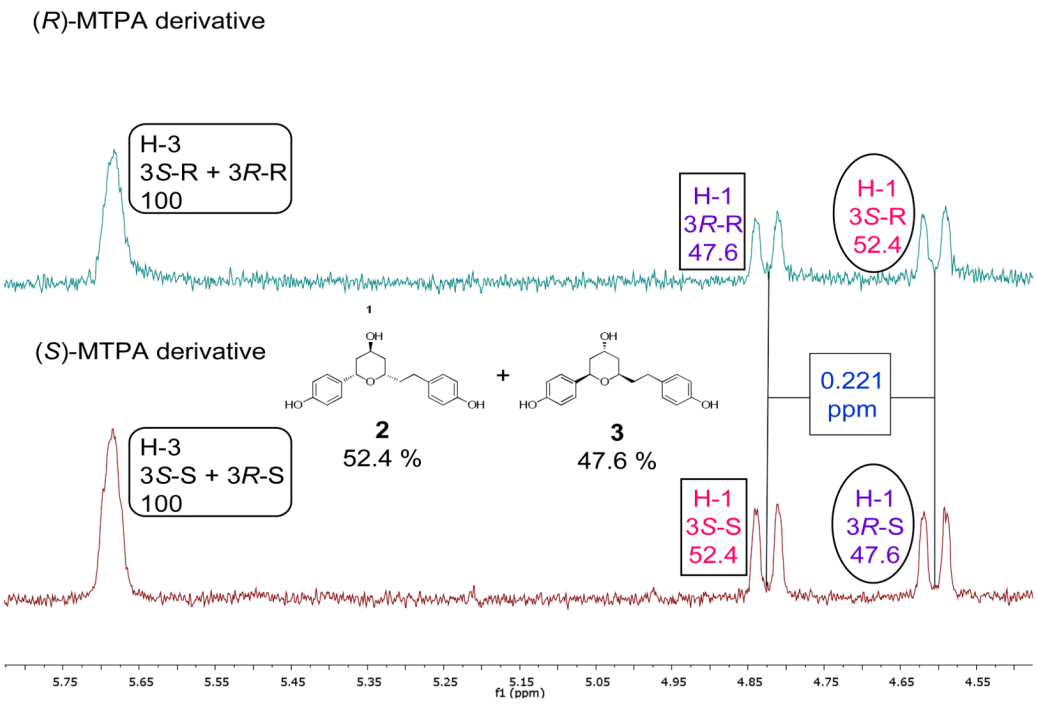

Figure 3.

Comparison of the ^{1}H NMR spectra of the MTPA esters of the enantiomeric mixture of **2** and **3** (δ_H 4.5 to 5.8). The resonances of H-1 and H-3 could be readily assigned based on their chemical shifts and splitting patterns. However, the four resonances of H-1 revealed a small but significant difference in their integrals (47.6:52.4, and vice versa), providing evidence for the fact that the isolate was an enantiomeric mixture of **2** and **3** rather than a pure single enantiomer.

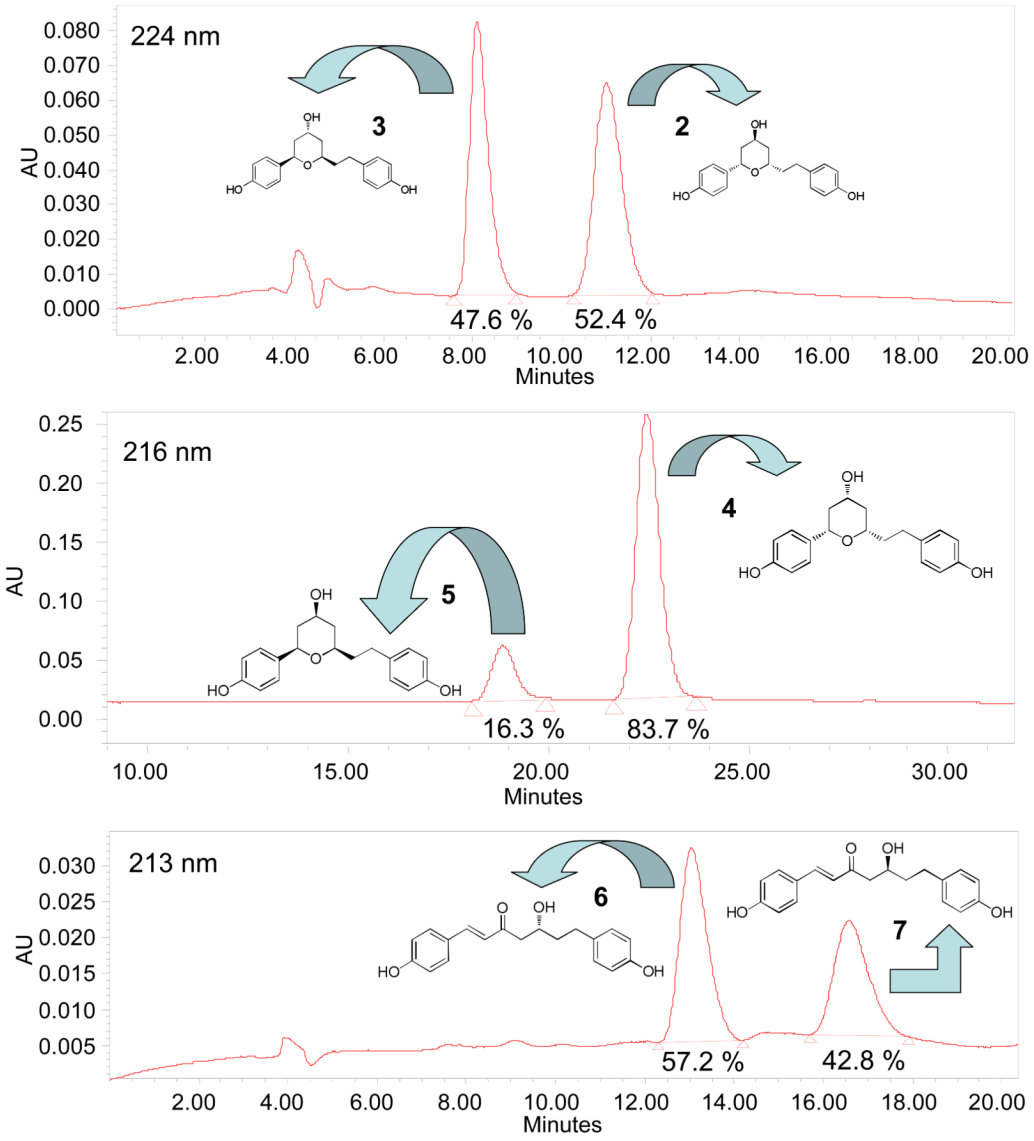

Figure 4. Chiral separation of three pairs of enantiomers (**2**/**3**, **4**/**5** and **6**/**7**) by HPLC (**2**/**3** and **6**/**7**: Chiralcel® OJ, 10 μm, 250 × 4.6 mm; **4**/**5**: Chiralpak® IA, 5 μm, 250 × 4.6 mm)

Table 1

^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**–**7**[a,b]

Pos.	1[c]		2/3		4/5		6/7	
	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult (*J* in Hz)	δ_C, mult.
1	4.8376, dd (11.96, 2.06)	75.20, CH	4.7013, dd (11.87, 2.12)	75.03, CH	4.2506, dd (11.42, 1.98)	78.87, CH	7.5634, d (16.12)	145.69, CH
2α	1.8153, ddd (13.62, 11.96, 2.88)	40.97, CH_2	1.7637, ddd (13.88, 11.87, 2.74)	41.03, CH_2	1.4305, ddd (12.42, 11.42, 11.01)	43.73, CH_2	6.6649, d (16.12)	124.64, CH
2β	1.8396, dddd (13.62, 3.00, 2.06, 2.05)		1.8334, dddd (13.88, 3.32, 2.12, 2.09)		2.0715, dddd (12.42, 4.51, 1.98, 1.82)			
3	4.2864, dddd (quintet) (3.00, 2.88, 2.82, 2.67)	65.47, CH	4.2303, dddd (quintet) (3.32, 2.89, 2.74, 2.40)	65.74, CH	3.8294, dddd (11.01, 11.00, 4.60, 4.51)	69.19, CH		202.08, C
4α	1.7286, ddd (13.96, 11.70, 2.82)	39.61, CH_2	1.5415, ddd (13.82, 11.59, 2.89)	39.37, CH_2	1.2226, ddd (12.30, 11.22, 11.00)	41.99, CH_2	2.8516, dd (15.41, 8.26)	48.89, CH_2
4β	1.8323, dddd (13.96, 2.67, 2.28, 2.05)		1.7070, dddd (13.82, 2.55, 2.40, 2.09)		1.9539, dddd (12.30, 4.60, 1.97, 1.82)		2.7743, dd (15.41, 4.35)	
5	4.5942 dddd (11.70, 6.43, 2.28, 1.09)	74.76, CH	3.9099, dddd (11.59, 9.37, 5.25, 2.55)	72.81, CH	3.4428, dddd (11.22, 7.88, 5.27, 1.97)	76.49, CH	4.0991, dddd (8.26, 7.84, 4.47, 4.35)	68.98, CH
6	6.0619 dd (15.96, 6.43)	128.45, CH	1.7815, dddd (13.75, 8.97, 8.52, 5.25)	39.66, CH_2	1.8507, dddd (13.59, 9.17, 7.36, 5.27)	39.41, CH_2	1.7671, dddd (13.56, 9.53, 6.90, 4.47)	40.71, CH_2
			1.6553, dddd (13.75, 9.37, 7.73, 4.73)		1.7270, dddd (13.59, 9.18, 7.88, 5.02)		1.7531, dddd (13.56, 9.11, 7.84, 6.30)	
7	6.5327, dd (br d) (15.96, 1.09)	131.57, CH	2.6582, ddd (13.95, 8.97, 4.73)	31.94, CH_2	2.6630, ddd (13.80, 9.17, 5.02)	31.99, CH_2	2.7028, ddd (13.87, 9.11, 6.90)	32.14, CH_2
			2.6012, ddd (13.95, 8.52, 7.73)		2.6102, ddd (13.80, 9.18, 7.36)		2.5912, ddd (13.87, 9.53, 6.30)	
1′		135.04, C		135.36, C		134.77, C		127.39, C
2′,6′	7.2145, dd (dt-like) (8.37, 2.47)	128.74, CH	7.2005, dd (dt-like) (8.41, 2.54)	128.71, CH	7.1994, dd (dt-like) (8.43, 2.38)	128.69, CH	7.4891, dd (dt-like) (8.52, 2.52)	131.74, CH
3′,5′	6.7539, dd (dt-like) (8.37, 2.70)	116.09, CH	6.7592, dd (dt-like) (8.41, 2.54)	116.10, CH	6.7623, dd (dt-like) (8.43, 2.71)	116.11, CH	6.8160, dd (dt-like) (8.52, 2.51)	117.07, CH
4′		157.90, C		157.94, C		158.07, C		161.76, C
1″		130.02, C		134.56, C		134.37, C		134.30, C
2″,6″	7.2283, dd (dt-like) (8.52, 2.63)	128.80, CH	6.9984, dd (dt-like) (8.27, 2.73)	130.54, CH	6.9941, dd (dt-like) (8.29, 2.32)	130.51, CH	7.0243, dd (dt-like) (8.29, 2.74)	130.47, CH
3″,5″	6.7141, dd (dt-like) (8.52, 2.74)	116.39, CH	6.6766, dd (dt-like) (8.27,	116.20, CH	6.6803, dd (dt-like) (8.29, 2.76)	116.23, CH	6.6918, dd (dt-like) (8.29,	116.27, CH

Pos.	1[c]		2/3		4/5		6/7	
	δ_H, mult. (*J* in Hz)	**δ_C, mult.**	**δ_H, mult. (*J* in Hz)**	**δ_C, mult**	**δ_H, mult. (*J* in Hz)**	**δ_C, mult.**	**δ_H, mult (*J* in Hz)**	**δ_C, mult.**
			2.41)				2.46)	
4″		158.27, C		156.45, C		156.50, C		156.53, C

[a] Measured in methanol-d_4 at 400 and 100 MHz, respectively.

[b] Four and two decimal places were determined by HiFSA analysis for δ_H and *J*, respectively.

[c] In order to improve the solubility, 10% $CDCl_3$ was added.