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Quercetin and Kaempferol 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranosides from *Anthyllis hermanniae*: Structure Determination and Conformational Studies[#]

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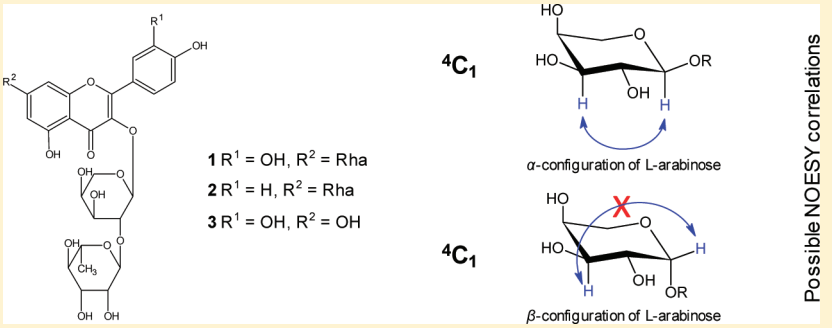
Supporting Information

ABSTRACT:



The study reports the isolation and structural identification of two new flavonol triglycosides from the methanolic extract of *Anthyllis hermanniae*, exhibiting the same glycosylation pattern: quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**1**) and kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**2**). A conformational study related to the central arabinoside moiety was carried out including the analysis of the contribution of NOE effects and acetylation to the elucidation of the 2-*O*-linked arabinoside configuration of the anomeric carbon. We also report the total synthesis of a model compound, quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), which verifies the structures of the isolated compounds.

The genus *Anthyllis* belongs to the Leguminosae family and comprises more than 170 herbaceous or shrubby species distributed in Europe, the Middle East, and North Africa. *Anthyllis hermanniae* L., which is often called yellow kidney vetch due to its yellow flowers, is a medium-sized shrub distributed in Asian countries and in Europe, especially in the northeastern Mediterranean area.[1]

Despite the large number of *Anthyllis* species, only a few have been investigated from a chemical point of view, leading mainly to the identification of flavonoids and their corresponding glycosides. Quercetin, kaempferol, isorhamnetin, and rhamnocitrin have been reported as the main aglycones in *A. vulneraria*[2] and *A. onobrychioides*, where glycosylation occurs mostly at C-3 with galactose, glucose, and arabinose.[3−5] From *A. onobrychioides*, di- and triglycosides were also identified as 3-*O*-galactoside derivatives glucosylated at C-3′ and C-4′,[6] while the phytochemical investigations of *A. sericea* led to similar flavonoid derivatives.[7] Most of these flavonoid glycosides exhibit complex glycosylation patterns at C-3, consisting of a galactopyranose linked to one or two sugar moieties, mainly glucose and rhamnose.[8] As far as *A. hermanniae* is concerned, there is only one phytochemical study reporting the presence of isoflavones and chalcones from the nonpolar fraction.[9]

The current study reports the isolation and structural identification of two new flavonol triglycosides from the methanolic extract of *A. hermanniae*. Both compounds exhibit the same glycosylation pattern and have been identified as quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**1**) and kaempferol

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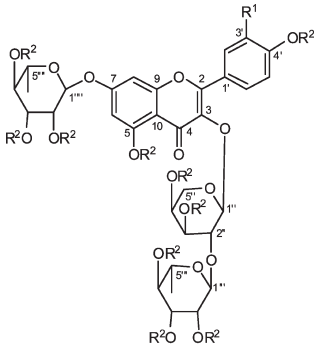
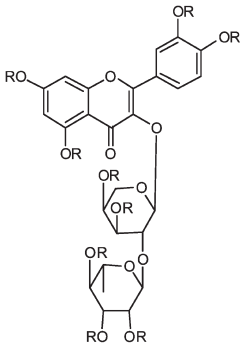
3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**2**). Additional to the phytochemical analysis, a conformational study concerning the central arabinoside moiety has been performed. The conformational equilibrium between the two possible chairlike $^4C_1$ and $^1C_4$ conformations of the arabinopyranoside unit due to steric hindrance is discussed. This study also comprises the analysis of NOE effect contributions as well as the impact of acetylation on the elucidation of the configuration of the anomeric center of 2-*O*-linked arabinopyranosides. In addition, quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), a reported natural compound, was synthesized.





1 R^1 = OH R^2 = H
1ac R^1 = OAc R^2 = Ac
2 R^1 = H R^2 = H
2ac R^1 = H R^2 = Ac

3 R = Ac
3ac R = H

RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous, yellow powder. The molecular formula was deduced from the high-resolution MS data to be $C_{32}H_{37}O_{19}$ ($[M - H]^-$ at m/z 725.1943, err. 1.13 ppm). Its UV spectrum was characteristic of a flavonol, while the addition of usual shift reagents as well as the ^{1}H NMR spectrum in methanol-d_4 indicated that **1** was a quercetin derivative. The characteristic ABX spin system of the B-ring was denoted by resonances at δ_H 6.92 (d, J = 8.4 Hz, H-5′), 7.62 (dd, J = 8.4, 2.1 Hz, H-6′), and 7.68 (d, J = 2.1 Hz, H-2′). The ^{1}H NMR spectrum also exhibited two *meta*-coupled doublets at δ_H 6.48 and 6.76 (J = 2.1 Hz) corresponding to H-6 and H-8, respectively. In addition to the quercetin moiety, three sugar residues were revealed by the corresponding anomeric protons at δ_H 5.58 (d, 5.4 Hz), 5.57 (br s), and 5.12 (d, J = 1.3 Hz). The two latter signals correspond to two α-rhamnopyranosyl moieties on the basis of their coupling constants and the presence of characteristic doublets of rhamnosyl methyl groups observed at δ_H 1.28 and 1.08, respectively. The attribution of the methyl groups to the respective rhamnopyranosides was confirmed by COSY and HMBC correlations. One of these rhamnose moieties was linked to the flavonol nucleus at C-7, as proved by the HMBC correlation between the anomeric proton at δ_H 5.57 and C-7 (δ_C 163.9). Furthermore, the chemical shifts of C-2 and C-3 (δ_C 159.7 and 135.9, respectively) indicated that another glycosylation occurred at C-3 of the quercetin nucleus. Obvious was the HMBC correlation of the most deshielded anomeric proton at δ_H 5.58 with C-3. The 1D and 2D (HSQC, COSY, and HMBC) spectra showed this sugar residue to be an arabinopyranosyl moiety.[10,11]

The second rhamnosyl unit was found to be linked at the glycosidic part of the molecule and not directly to the aglycone. More specifically, the ^{1}H and ^{13}C NMR deshielded values for anomeric (δ_H 5.58, δ_C 101.6) and especially the H-2 and C-2 signals (δ_H 4.13, δ_C 77.7) indicated that the arabinosyl moiety was linked at C-2 to the second rhamnosyl unit. The HMBC correlation between the anomeric proton of the rhamnosyl (δ_H 5.12) and C-2 of the arabinosyl moiety (δ_C 77.7) unambiguously established the (1→2) linkage between the two glycosyl moieties.

However, for complete structural elucidation, the configuration of the anomeric center of the arabinopyranosyl moiety needs to be determined. In general, this is a complicated task for 2-substituted arabinosides, mainly due to the known equilibrium between the two $^4C_1$ and $^1C_4$ chair conformations of pyranosides.[12] This equilibrium with significant alteration of $^3J_{H1,H2}$ values has been observed in various 2-*O*-glycosylated pentopyranosides of bulky aglycones.[13,14] For compound **1** the $^3J_{H1,H2}$ (5.4 Hz) value favors an α-configuration of the anomeric carbon. This configuration was also described for calabricoside, a flavonol triglycoside with a similar diglycoside moiety isolated from *Putoria calabrica*.[15]

In order to obtain further structural information, the acetylation of **1** was carried out, yielding the peracetylated derivative **1ac**. The NMR data of **1ac** provided further support to the assignment of the rhamnose (1→2) arabinose linkage, since the COSY spectrum of **1ac** showed a cross-peak between the anomeric proton of arabinose (δ_H 5.58) and the upfield proton H-2 at δ_H 4.13.[16] Moreover, the presence of L-arabinopyranosyl and L-rhamnopyranosyl substituents was confirmed by acidic hydrolysis of **1** followed by TLC analysis and measurement of the optical rotation values of the sugars obtained from the water-soluble fraction and comparison with reference standards. Finally, alkaline hydrolysis of **1** was also carried out in order to selectively remove the rhamnoside at C-7, leading to quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**). Compound **3** was previously isolated from *Brassica nigra*; however its description is incomplete since only ^{13}C NMR data are given.[17] Thus, compound **1** was identified as quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside. We propose the trivial name hermannioside A for new compound **1**.

Compound **2** was obtained as an amorphous, yellow powder. Its HR-MS data indicated a molecular formula of $C_{32}H_{37}O_{18}$ on the basis of its pseudomolecular ion at m/z 709.1992 (err. 0.96 ppm). The use of UV shift reagents combined with ^{1}H NMR data indicated that **2** was a kaempferol derivative. As for compound **1**, H-6 and H-8 gave two *meta*-coupled doublets at δ_H 6.48 and 6.77 (J = 2.1 Hz). The B-ring protons appeared as two doublets of doublets at δ_H 8.10 (dd, J = 8.8, 2.0 Hz, H-2′, H-6′) and 6.94 (dd, J = 8.8, 2.0 Hz H-2′, H-6′), characteristic of an AA′BB′ spin system. ^{1}H and ^{13}C NMR data in methanol-d_4 revealed the same glycosidic signals as for **1**, i.e., three anomeric signals at δ_H 5.59 (d, J = 1.4 Hz), 5.58 (d, J = 5.1 Hz), and 5.12 (d, J = 1.1 Hz). The methyl groups of the rhamnopyranosyl moieties were observed as doublets at δ 1.28 and 1.10. Compound **2** was then assigned as a kaempferol analogue of **1**. HMBC correlations confirmed the link between the anomeric proton (δ_H 5.59) of the rhamnosyl moiety and C-7 (δ_C 163.6) of kaempferol. In the same way, there was a correlation between the anomeric proton of the arabinosyl moiety (δ_H 5.58) and C-3 (δ_C 135.9), revealing glycosylation at this position. Additionally, both C-2 and H-2 of the arabinosyl unit were deshielded (δ_C 77.1, δ_H 4.13), and the carbon correlated with the rhamnosyl anomeric proton at δ_H 5.12,

confirming the (1→2) interglycosidic linkage between the two sugar moieties. Acetylation of **2** yielded the peracetylated derivative **2ac**, while acidic hydrolysis led to kaempferol, L-arabinopyranose, and L-rhamnopyranose. Compound **2** was thus identified as kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside, a new compound for which we propose the trivial name hermannioside B.

Recently, a compound with similar NMR data to **2** was isolated from *Matthiola longipetala* and identified as kaempferol 3-*O*-(2″-α-L-rhamnopyranosyl)-*β*-L-arabinopyranoside-7-*O*-α-L-rhamnopyranoside.[18] On the basis of the low coupling constant for the anomeric proton of the arabinoside (δ 5.46, J = 4.6 Hz, DMSO-d_6), the authors claimed the *β*-configuration of the arabinosyl unit. The same authors also identified a 4′-*O*-glucopyranoside derivative for which the arabinosyl moiety had similar values (δ_H 5.48, J = 4.8 Hz, DMSO-d_6). Measuring the coupling constants of the anomeric protons of the arabinosyl units of hermannioside A (δ_H 5.48, J = 4.9 Hz) and hermannioside B (δ 5.47, J = 4.8 Hz) in DMSO-d_6, it was found that the observed values were similar to those of the compounds from *M. longipetala*. On the basis of these data it became obvious that the complete elucidation of the configuration of the anomeric center of arabinose is problematic. Indeed for the compounds isolated from *M. longipetala* an α-configuration of arabinoside with an increased population of $^1C_4$ conformers could not be excluded.

The structural elucidation of flavonoid glycosides with two, three, or even more sugar moieties is not easy to achieve due to the complexity of NMR spectra in the sugar region. The difficulty in complete assignment concerns both the position and configuration of the interglycosidic linkage.[19] Previous studies have reported that 2-*O*-glycosyl-L-arabinopyranosides exhibit unusual glycosylation shifts.[20,21] Significant alteration of $^3J_{H1,H2}$ values has been observed in various 2-*O*-glycosylated arabinopyranosides of bulky aglycones. These atypical values could be explained by a specific conformational equilibrium between the two possible chairlike conformations $^4C_1$ and $^1C_4$. Indeed, when a bulky substituent is linked to C-2 of an arabinopyranosyl unit, the equilibrium between $^4C_1$ and $^1C_4$ conformers is altered in favor of the latter, since the 1- and 2-substituents adopt the stereochemically and thermodynamically more stable *anti* orientation.[12] Durette and Horton analyzed this phenomenon using low-temperature NMR to freeze this equilibrium so that the interconversion is slow enough and the anomeric signals due to both $^1C_4$ and $^4C_1$ conformers could be distinguished.[22] They proposed that the coupling constant $J_{H1,H2}$ observed at room temperature results from the weighted average between the coupling value J_e and J_a, when the proton is exclusively equatorial (theoretical J = 1 Hz) or axial (theoretical J = 8 Hz), respectively, and could be calculated according to the following equation: $J_{obs} = N_eJ_e + N_aJ_a$, where N_e and N_a represent the mole fractions of both conformers.

The $^3J_{H1,H2}$ value of the anomeric proton of methyl-α-L-arabinoside decreased from 6.9 to 4.9 Hz[12] after a 2-*O*-glucosyl linkage was added. Interestingly, when the steric hindrance is particularly strong, due to a bulky 1-*O*-aglycone, the observed $^3J_{H1,H2}$ value could reach low values similar to those observed with a *β*-configuration (around 2−3 Hz).[12,23,24] Therefore, it appears that in the case of 2-*O*-linked arabinopyranosides an intermediate or low value of the coupling constant does not permit the accurate assignment of the configuration of the anomeric proton.

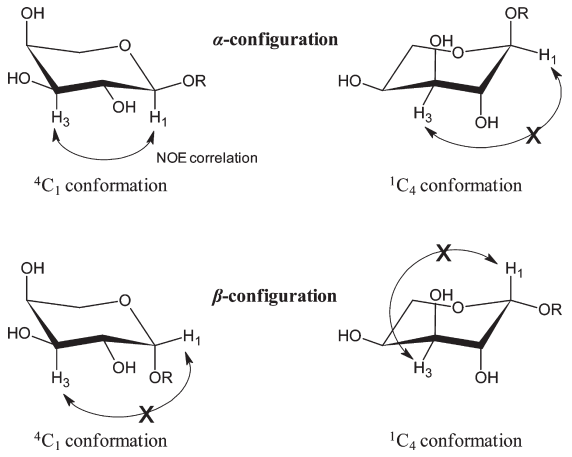


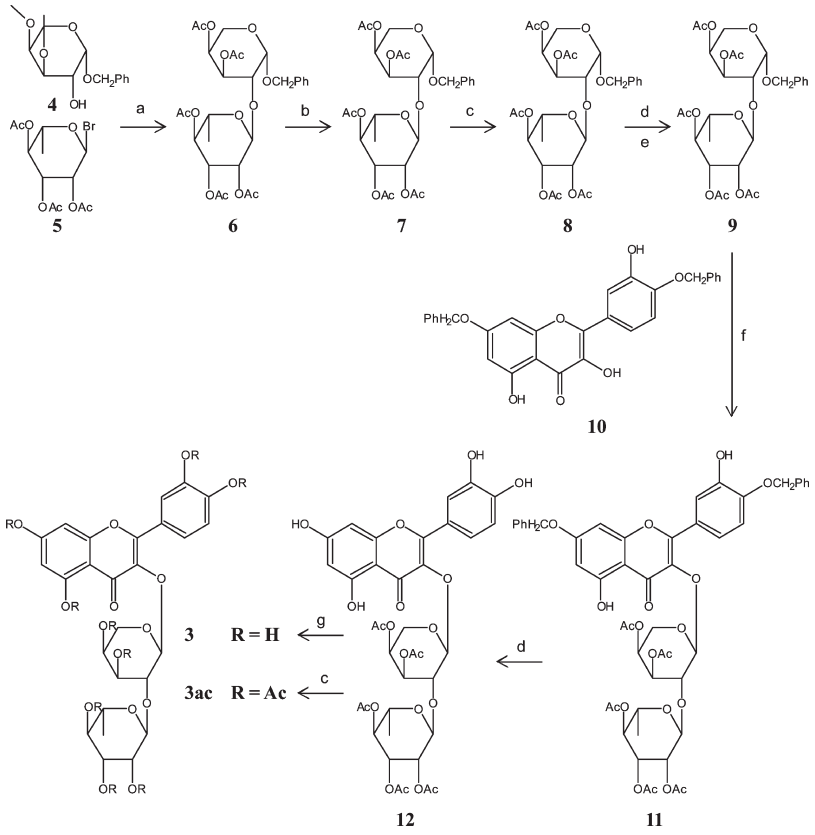
Figure 1. Possible NOESY correlations between H-1 and H-3 of the arabinopyranosyl moiety.

Furthermore, it is important to note that almost all the natural 3-*O*-arabinosylated flavonoids are reported with an α-L-arabinosyl moiety. There are a few publications referring to flavonoids *O*-linked to *β*-L-arabinosyl moieties, and on the basis of the above discussion many of them might be reappraised. Indeed, some authors have confused L-arabinose with D-arabinose, and so they assigned a *β*-configuration when they observe high $^3J_{H1,H2}$ values (around 7 Hz). In some other cases the *β*-configuration is assigned on the basis of quite low to intermediate J values (<∼5 Hz). Most of the time, authors evade this problem and do not mention the configuration of the arabinosyl unit, or even the ^{1}H NMR data for the sugar moiety.[26] The most in-depth study is the one of Abdel-Shafeek et al.,[14] reporting the isolation and characterization of a quercetin derivative linked at C-3 to a 2-*O*-substituted arabinosyl moiety. They observed a $J_{H1,H2}$ value of 1.8 Hz, which could be characteristic of a *β*-configuration; however, an α-configuration of arabinose with a strong predominance of $^1C_4$ conformers population (>90%) could not be excluded.

Surprisingly, no one has attempted to clarify these issues for flavonoid glycosides using NOE effects. NOESY could be a useful spectrometric experiment to solve the problem of anomeric proton configuration of 2-*O*-linked arabinose. Indeed, for α-arabinopyranosides, a strong NOE correlation should be observed only between the anomeric proton (H-1) and H-3 in the $^4C_1$ conformer. In contrast, this correlation is weak or impossible in the $^1C_4$ conformer of α-arabinose as well as in the case of *β*-arabinose whatever its conformation (Figure 1). In the case of compounds **1** and **2**, the NOESY spectrum exhibited correlation between the anomeric protons of the arabinosyl moiety (δ_H 5.58 and 5.48) and protons 3 (δ_H 3.91 and 3.67), respectively, an observation in agreement with an α-configuration for the anomeric center of arabinoside, in both compounds.

In order to unequivocally confirm the structures of hermanniosides A and B compared to the compounds isolated from *Matthiola longipetala*,[18] but mainly to investigate further the configuration of the arabinosyl moiety, the synthesis of a model compound was performed. Quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), previously isolated from *Brassica nigra*[17] and also obtained by alkaline hydrolysis of **1**, was selected to be synthesized in a regio- and stereoselective manner.

Figure 2. Scheme for the synthesis of **3** and **3ac**.



(a) $Hg(CN)_2$, benzene−nitromethane; 68%; (b) acetone, H_2SO_4, reflux; 53%; (c) pyridine, Ac_2O; 92%; (d) H_2−Pd−C; 100%; (e) HBr/OHAc, HOAc−$CHCl_3$; 0 °C, 43%; (f) $PhCH_2N^+(Et)_3Br^-$, KOH−$CHCl_3$ anh., 30%; (g) MeONa−MeOH; 80%.

The starting material chosen for the synthesis of **3** was benzyl 3,4-*O*-isopropylidene-β-L-arabinopyranoside (**4**), readily available from L-arabinose in two steps by a classical procedure.[27,28] Condensation with 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl bromide (**5**)[29] in the presence of mercuric cyanide in benzene-nitromethane gave benzyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-L-arabinopyranoside (**6**) (Figure 2).[30] Removal of the isopropylidene protecting group of **6** by treatment with acetone and H_2SO_4 afforded **7**, which after acetylation gave compound **8**. Debenzylation of **8** through hydrogenolysis, followed by treatment with HBr in HOAc, furnished 2,3,4-tri-*O*-acetyl-(α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-β-L-arabinopyranosyl bromide (**9**),[13,31,32] which was immediately used for the glycosidation of 7,4′-di-*O*-benzylquercetin (**10**),[33] which under stereospecific phase-transfer-catalyzed conditions led exclusively to the α-arabinoside (**11**).[34] The resulting 7,4′-di-*O*-benzylquercetin 3-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-L-arabinopyranoside] (**11**) was hydrogenolyzed to remove the benzyl group to afford the corresponding glycoside **12**. A portion of **12** via Zemplen deacetylation yielded the targeted quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), identical to the natural compound previously isolated from *Brassica* nigra,[17] while another portion was acetylated to yield peracetylated quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3ac**) (Figure 2). The ^1H NMR study of **3** and its peracetylated derivative **3ac** revealed the anomeric proton signal of the arabinosyl moiety in **3** at δ_H 5.43 with a coupling constant of 5.0 Hz (DMSO-d_6) and at δ_H5.59 with a coupling constant of 7.0 Hz ($CDCl_3$) in **3ac**.

According to these data, the proposed structures for hermanniosides A and B were confirmed since the $J_{H1,H2}$ for the arabinose unit observed in DMSO-d_6 of **3** (5.0 Hz) was similar to that of **1** (J = 4.9 Hz) and **2** (J = 4.8 Hz). These observations confirm the initial hypothesis of enhancement in the population of the $^1C_4$ conformers due to steric hindrance. Regarding the acetylated derivative **3ac**, the coupling constant of the anomeric proton was significantly increased (J = 7.0 Hz, $CDCl_3$), a phenomenon that is also observed in the acetylated products of hermanniosides **1ac** (J = 6.6 Hz, $CDCl_3$) and **2ac** (J = 6.8 Hz, $CDCl_3$), suggesting that *O*-acetylation stabilizes the $^4C_1$ conformation.[35]

Overall, it is evident that the observed coupling constants of 4−5 Hz for the anomeric proton of an arabinopyranosyl moiety linked to a rhamnopyranosyl unit at C-2 correspond to the α-configuration of the anomeric carbon of arabinoside and is the result of the alternation of the equilibrium between the $^4C_1$ and $^1C_4$ conformations of arabinose. Consequently, on the basis of these data the proposed β-configuration for the anomeric carbon of arabinose in the flavonol glycosides isolated from *Matthiola longipetala*[18] should be revised to α. Finally, taking into consideration the observed coupling constants as well as the equation of Durette and Horton we conclude that the population of the $^4C_1$ and $^1C_4$ conformers is respectively 67% and 33% for hermannioside A (**1**) and 60% and 40% for hermannioside B (**2**). Likewise, for the corresponding acetylated derivatives **1ac** and

Table 1. ^{1}H and ^{13}C NMR Assignment for 1 and 2 (600 MHz, in methanol-d_4) and 1ac and 2ac (600 MHz, in $CDCl_3$)

	hermannioside A (1)		1ac	hermannioside B (2)		2ac
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2		159.7			159.7	
3		135.9			135.9	
4		180.1			180.1	
5		164.4			163.4	
6	6.48 (d, 2.1)	100.9	6.74 (d, 2.5)	6.48 (d, 2.1)	100.6	6.74 (d, 2.5)
7		163.9			163.6	
8	6.76 (d, 2.1)	95.9	7.04 (d, 2.5)	6.77 (d, 2.1)	95.6	7.07 (d, 2.5)
9		158.4			158.0	
10		107.9			107.9	
1′		123.4			123.1	
2′	7.68 (d, 2.1)	117.7	7.94 (d, 2.1)	8.10 (dd, 8.8, 2.0)	132.1	8.10 (dd, 8.8, 2.0)
3′		146.6		6.94 (dd, 8.8, 2.0)	116.5	7.18
4′		150.5			150.1	
5′	6.92 (d, 8.4)	116.8	7.28 (d, 8.5)	6.94 (dd, 8.8, 2.0)	116.5	7.18
6′	7.62 (dd, 8.4, 2.1)	123.8	6.74 (dd, 8.5, 2.1)	8.10 (dd, 8.8, 2.0)	132.1	8.10
3-*O*-L-Ara						
1	5.58 (d, 5.4)	101.6	5.60 (d, 6.6)	5.58 (d, 5.1)	100.8	5.60 (d, 6.8)
2	4.13 (dd, 6.6, 5.4)	77.7	4.01 (dd, 9.9, 6.6)	4.13 (dd, 6.9, 5.2)	77.1	4.02 (dd, 9.2, 6.8)
3	3.84 (m)	72.9	5.04 (dd, 9.9, 3.3)	3.83 (dd, 9.5, 3.5)	70.2	5.05 (dd, 9.2, 3.4)
4	3.82 (m)	69.0	5.15 (m)	3.81 (m)	68.8	5.15 (m)
5a	3.81 (m)	66.0	3.70 (dd, 12.8 2.4)	3.82 (m)	65.4	3.69 (dd, 12.6, 2.7)
5b	3.42 (dd, 13.5, 4.7)		3.47 (dd, 12.8 1.7)	3.40 (m)		3.46 (dd, 12.8 1.7)
3-*O*-L-Rha						
1	5.12 (d, 1.3)	102.7	4.96 (br s)	5.12 (d, 1.1)	102.1	4.96 (br s)
2	3.94 (dd, 3.2, 1.3)	73.4.	5.16 (m)	3.91 (dd, 3.2, 1.6)	72.1	5.16 (m)
3	3.73 (dd, 9.5, 3.2)	72.7	5.42 (dd, 10.0, 3.3)	3.73 (dd, 9.6, 3.3)	72.3	5.42 (dd, 10.2, 3.4)
4	3.37 (t, 9.5)	74.1	5.00 (t, 10.0)	3.38 (t, 9.6)	73.9	5.00 (t, 10.2)
5	3.82 (m)	70.6	4.30 (dd, 10.0, 6.2)	3.82 (m)	69.2	4.30 (m)
6	1.05 (d, 6.2)	18.0	0.96 (d, 6.2)	1.10 (d, 6.2)	17.7	0.94 (d, 6.3)
7-*O*-L-Rha						
1	5.57 (br s)	100.4	5.54 (br s)	5.59 (d, 1.4)	99.6	5.53 (br s)
2	4.04 (dd, 3.2, 1.7)	73.0	5.40 (dd, 3.3, 1.6)	4.04 (dd, 3.3, 1.7)	71.7	5.39 (dd, 3.4, 1.7)
3	3.86 (m)	71.9	5.35 (dd, 9.9, 3.3)	3.85 (dd, 9.5, 3.3)	71.4	5.35 (dd, 10.2, 3.4)
4	3.50 (t, 9.5)	74.5	5.12 (t, 9.9)	3.50 (t, 9.5)	73.6	5.11 (m)
5	3.61 (m)	71.7	3.88 (dd, 9.9, 6.2)	3.61 (dd, 9.4, 6.2)	71.3	3.87 (m)
6	1.26 (d, 6.2)	18.5	1.27 (d, 6.2)	1.28 (d, 6.2)	17.9	1.27 (d, 6.2)

2ac, these populations are altered by stabilization of the $^{4}C_1$ conformation and are calculated to be 80% and 20% for the first and 82% and 18% for the latter.

In conclusion, caution should be exercised in determining the identity of pentopyranosides and especially in the elucidation of their configurations. Nevertheless, the NOESY correlations and $J_{H1,H2}$ coupling constants of the acetylated derivatives of the compounds under investigation could be useful tools for their complete structure elucidation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a Perkin-Elmer 141 polarimeter. UV spectra were obtained using spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer. The 2D experiments (COSY, HSQC, HMBC, and NOESY) were performed using standard Bruker microprograms. The residual ^{1}H and ^{13}C signals of methanol-d_4 (δ_H 3.31, δ_C 49.5, respectively), DMSO-d_6 (δ_H 2.50, δ_C 39.4, respectively), and chloroform-d (δ_H 7.26, δ_C, respectively) were used as internal standards. FABMS (thioglycerol) was performed on a VG Micromass 70-70 spectrometer, LR-ESIMS spectra were obtained in negative mode using a Thermo-Finnigan MSQ mass spectrometer, and HR-ESIMS spectra were obtained on a hybrid Thermo Scientific LTQ-Orbitrap Discovery mass spectrometer (negative mode). Analytical TLC was performed on Merck precoated silica gel 60 F_{254} plates. Spots were visualized using UV light and vanillin−H_2SO_4 reagent. Column flash chromatography was carried out using Si gel, Merck, 0.04−0.06 mm. A Thermo Finnigan HPLC system (Thermo Finnigan, San Jose, CA) connected to a Spectral System UV2000 PDA detector was employed for the isolation procedure. The reagents used for the synthesis of the model compound were reagent grade and were used without further purification, while solvents were distilled from the appropriate drying

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agents before use when needed. All reactions involving air- or moisture-sensitive reagents or intermediates were performed under a nitrogen or argon atmosphere.

Plant Material. Whole plants of *Anthyllis hermanniae* L. were collected in May 2007 in the vicinity of Athens, Greece, on Ymittos hill (250 m alt.), and identified by Dr. Eleftherios Kalpoutzakis. A voucher specimen (No. NEK 001) was deposited at the Herbarium of the Division of Pharmacognosy, University of Athens.

Extraction and Isolation. Air-dried powdered plant (1.1 kg) was extracted at room temperature successively with CH_2Cl_2 (3 × 2 L) and MeOH (3 × 2 L) for 2 days each time. The MeOH extract was concentrated to give a residue (58 g), which was applied to a silica gel column and eluted with a CH_2Cl_2/MeOH gradient to yield 18 fractions. Fractions 15−17 were combined and concentrated (7.8 g). An aliquot (1 g) was dissolved in 50% aqueous MeCN (20 mg/mL), passed through nylon acrodisc filters (0.45 μm, Waters), and subjected to semipreparative HPLC-PDA using a reversed-phase C_{18}, Supelcosil SPLC-18 column (250 × 10 mm, 5 μm, Supelco, Sigma-Aldrich). The gradient conditions were as followed: eluent A: H_2O, B: MeCN; gradient: 4% to 12% B in 30 min, then to 20% in 15 min, followed by a 30 min gradient to 50% B, to finish with 70% B within a total analysis time of 80 min; flow rate: 3 mL/min. Two peaks were collected to afford **1** (34 mg, t_R 45.0 min) and **2** (32 mg, t_R 47.4 min).

Acetylation and Hydrolysis of 1 and 2. *Acetylation.* Compounds **1** (5 mg) and **2** (5 mg) were treated with Ac_2O (0.5 mL) and pyridine (0.5 mL), at room temperature, overnight and gave the peracetylated derivative **1ac** (91%) and the peracetylated derivative **2ac**, respectively (^{1}H NMR, Table 1).

Acidic Hydrolysis. Compounds **1** (5 mg) and **2** (5 mg) were dissolved in 2 N HCl (2.0 mL) and heated at 100 °C for 3 h. After evaporation of the solvent under vacuum, the residue was dissolved in H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The residue of the aqueous phase was redissolved after evaporation in 50% MeOH (5 mg/mL). This fraction and standards of the sugars L-arabinopyranose and L-rhamnopyranose (Sigma-Aldrich) were applied on normal-phase TLC, and $CHCl_3$−MeOH−H_2O (128:80:16) was used as the mobile phase. Using preparative TLC with the same solvent system the sugars were obtained, and their specific rotation values were compared with those of the standards. Therefore, the identity of both of L-arabinopyranosyl and L-rhamnopyranosyl moieties was confirmed for **1**. In a similar manner, the presence of the same sugars was also verified for **2**.

Alkaline Hydrolysis. Compound **1** (10 mg) was dissolved in 0.5% KOH (10 mL) and heated for 30 min under an N_2 atmosphere. After neutralization with 2 M HCl and filtration, the mixture was evaporated to dryness and **3** was obtained (2 mg) by preparative TLC using MeOH−EtOAc (80:20) as solvent.

Synthesis of 3 and 3ac. A solution of 7,4′-di-*O*-benzylquercetin 3-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-L-arabinopyranoside (**11**) (15.0 mg, 0.015 mmol) in MeOH (5 mL) containing 10% Pd−C (0.04 g) was submitted to hydrogenolysis (H_2, 1 atm), at 20 °C, for 4 h. The catalyst was removed by filtration over Celite, and the solvent was evaporated under reduced pressure to give crude quercetin 3-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-L-arabinopyranoside (**12**). A portion of **12** (5 mg) was dissolved in 1 N NaOMe in MeOH (3 mL) and was stirred for 3 h at 20 °C. After neutralization by addition of Amberlite IRC 50 H^+ ion-exchange resin and filtration, the solvent was removed by evaporation to afford **3** as an amorphous solid (3.0 mg). The other part of **12** was dissolved in pyridine (1 mL), Ac_2O (1 mL) was added under stirring, and the stirring was continued for 72 h at room temperature. Evaporation of the solvent under reduced pressure afforded **3ac** as a foam (5.0 mg).

*Quercetin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-O-α-L-rhamnopyranoside, hermannioside A (**1**):* amorphous, yellow solid; $[\alpha]^{20}_D$ −101.7 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 256.2 (4.8), 270.2 sh (4.3), 353.6 (4.3) nm; + NaOMe, 265.2, 400.5; + NaOAc, 258.0, 358.4; + NaOAc/H_3BO_3, 260.0, 374.0; + $AlCl_3$, 274.5, 439.5; + $AlCl_3$/HCl, 271.5, 402.0; ^{1}H and ^{13}C NMR Table 1; HR-ESIMS *m/z* 725.1943 $[M - H]^-$ (calcd for $C_{32}H_{38}O_{19}$, 725.1935).

*Peracetyl quercetin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-O-α-L-rhamnopyranoside (**1ac**):* amorphous, brown solid; $[\alpha]^{20}_D$ −23.8 (*c* 0.1, MeOH); ^{1}H NMR Table 1; HR-ESIMS *m/z* 1187.3069 $[M - H]^-$ (calcd for $C_{54}H_{60}O_{30}$, 1187.3085).

*Kaempferol 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-O-α-L-rhamnopyranoside, hermannioside B (**2**):* amorphous, yellow powder; $[\alpha]^{20}_D$ −86.9 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 225.0 (4.5), 265.0 sh (4.3), 348.5 (4.2) nm; + NaOMe, 274.0, 387.0; + NaOAc, 269.5 sh, 348.5; + NaOAc/H_3BO_3, 270.0 sh, 359.5 sh; + $AlCl_3$, 233.0 sh, 273.5, 348.5; + $AlCl_3$/HCl, 230.0 sh, 273.0; ^{1}H and ^{13}C NMR Table 1; HR-ESIMS *m/z* 709.1992 $[M - H]^-$ (calcd for $C_{32}H_{37}O_{18}$, 709.1985).

*Peracetyl kaempferol 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-O-α-L-rhamnopyranoside (**2ac**):* amorphous, brown solid; $[\alpha]^{20}_D$ −20.3 (*c* 0.1, MeOH); ^{1}H NMR Table 1; HR-ESIMS *m/z* 1129.3045 $[M - H]^-$ (calcd for $C_{52}H_{57}O_{28}$, 1129.3031).

*Quercetin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**):* $[\alpha]^{20}_D$ −58.8 (*c* 0.9, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) 7.55 (1H, dd, *J* = 8.2, 2.2 Hz, H-6′Q), 7.56 (1H, d, *J* = 2.2 Hz, H-2′Q), 6.78 (1H, d, *J* = 8.2 Hz, H-5′Q), 6.28 (1H, d, *J* = 2.0 Hz, H-8Q), 6.05 (1H, d, *J* = 2.0 Hz, H-6Q), 5.43 (1H, d, *J* = 5.0 Hz, H-1), 4.95 (1H, br s, H-1′), 4.10 (1H, dd, *J* = 8.0, 5.3 Hz, H-2), 3.70 (1H, m, H-2′), 3.68 (1H, m, H-5′), 3.67 (1H, m, H-3), 3.66 (1H, m, H-4), 3.64 (1H, m, H-5a), 3.42 (1H, dd, *J* = 9.2, 3.1 Hz, H-3′), 3.42 (1H, dd, *J* = 13.5, 4.0 Hz, H-5β), 3.21 (1H, t, *J* = 9.2, Hz, H-4′), 0.95 (3H, d, *J* = 6.0 Hz, CH_3-6′); ^{13}C NMR (50 MHz, DMSO-d_6) 176.6 (C-4Q), 168.5 (C-7Q), 160.8 (C-5Q), 156.4 (C-2Q), 155.2 (C-9Q), 149.8 (C-4′Q), 145.7 (C-3′Q), 132.9 (C-3Q), 121.1 (C-6′Q), 120.2 (C-1′Q), 116.0 (C-5′Q), 115.6 (C-2′Q), 102.1 (C-10Q), 99.9 (C-1), 99.6 (C-1′), 99.4 (C-6Q), 93.9 (C-8Q), 74.5(C-2), 71.9 (C-4′), 71.0 (C-2′), 70.6 (C-3′), 69.9 (C-3), 68.4 (C-5′), 66.6 (C-4), 64.1 (C-5), 17.4 (C-6); FABMS (*m/z*) 581 $[M + H]^+$; *anal.* calcd for $C_{26}H_{29}O_{15}$.

*Peracetyl quercetin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3ac**):* $[\alpha]^{20}_D$ −48.6 (*c* 0.3, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) 8.05 (1H, dd, *J* = 8.5, 2.2 Hz, H-6′Q), 7.95 (1H, d, *J* = 2.2 Hz, H-2′Q), 7.30 (1H, d, *J* = 8.5 Hz, H-5′Q), 7.22 (1H, d, *J* = 2.0 Hz, H-8Q), 6.80 (1H, d, *J* = 2.0 Hz, H-6Q), 5.59 (1H, d, *J* = 7.0 Hz, H-1), 5.38 (1H, dd, *J* = 10.1, 4.0 Hz, H-3′), 5.15 (1H, dt, *J* = 3.5, 2 Hz, H-4), 5.12 (1H, dd, *J* = 4.0, 2.0 Hz, H-2′), 5.06 (1H, dd, *J* = 10.0, 3.5 Hz, H-3), 5.05 (1H, t, *J* = 10.0 Hz, H-4′), 5.00 (1H, d, *J* = 2.1 Hz, H-1′), 4.31 (1H, dq, *J* = 10.0, 6.0 Hz, H-5′), 4.03 (1H, dd, *J* = 10.0, 7.0 Hz, H-2), 3.73 (1H, dd, *J* = 13.0, 3.5 Hz, H-5eq), 3.49 (1H, dd, *J* = 13.0, 2.0 Hz, H-5ax), 1.94−2.55 (27H, 9 × s, 9 × −OCOCH_3), 0.97 (3H, d, *J* = 6.0 Hz, CH_3-6′); ^{13}C NMR (50 MHz, $CDCl_3$) 177.3 (C-4Q), 164.2 (C-7Q), 161.2 (C-5Q), 156.1 (C-2Q), 154.2 (C-9Q), 148.5 (C-4′Q), 144.9 (C-3′Q), 133.6 (C-3Q), 122.1 (C-6′Q), 120.8 (C-1′Q), 115.2 (C-2′Q), 115.5 (C-5′Q), 103.8 (C-10Q), 100.0 (C-1), 99.9 (C-1′), 98.6 (C-6Q), 93.9 (C-6Q), 76.5 (C-2), 71.9 (C-4′), 70.5 (C-3), 70.5 (C-2′), 70.5 (C-3′), 68.5 (C-5′), 66.1 (C-4), 63.5 (C-5), 20.6−22.7 (9 × CH_3CO−), 17.4 (C-6′); FABMS (*m/z*) 959 $[M + H]^+$; *anal.* calcd for $C_{44}H_{47}O_{24}$.

■ ASSOCIATED CONTENT

Supporting Information. Detailed description of the synthesis of compounds **3** and **3ac**, ^{1}H NMR and ^{13}C NMR data for the intermediate synthetic products, as well as NMR, MS, and HRMS spectra for the isolated compounds **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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DEDICATION

#This paper is dedicated to the memory of our late colleague Professor François Tillequin.

REFERENCES

(1) Brullo, S.; Giusso del Galdo, G. *J. Bot. Nomen.* **2006**, *16*, 304–314.
(2) Gonnet, J.-F.; Jay, M. *Phytochemistry* **1972**, *11*, 2313–2316.
(3) Gonnet, J.-F. *Phytochemistry* **1975**, *14*, 823–828.
(4) Gonnet, J.-F. *Phytochemistry* **1978**, *17*, 1319–1323.
(5) Marco, J A.; Barbera, O.; Sanz, J. F.; Sanchez-Parareda, J. *Phytochemistry* **1985**, *24*, 2471–2472.
(6) Barbera, O.; Sanz, J. F.; Sánchez-Parareda, J.; Marco, J. A. *Phytochemistry* **1986**, *25*, 2361–2365.
(7) Adell, J.; Barbera, O.; Marco, J. A. *Phytochemistry* **1988**, *27*, 2967–2970.
(8) Marco, J. A.; Adell, J.; Barbera, O.; Strack, D.; Wray, V. *Phytochemistry* **1989**, *28*, 1513–1516.
(9) Pistelli, L.; Spera, K.; Flamini, G.; Mele, S.; Morelli, I. *Phytochemistry* **1996**, *42*, 1455–1458.
(10) Beier, C. R.; Mundy, P. B. *J. Carbohydr. Chem.* **1984**, *3*, 253–266.
(11) Pawan, K. A. *Phytochemistry* **1992**, *31*, 3307–3330.
(12) Mizutani, K.; Hayashi, A.; Rasai, K.; Tanaka, O.; Yoshida, N.; Nakajima, T. *Carbohyd. Res.* **1984**, *126*, 177–189.
(13) Razanamahefa, B.; Demetzos, C.; Skaltsounis, A. L.; Andriantsiferana, M.; Tillequin, F. *Heterocycles* **1994**, *38*, 357–361.
(14) Abdel-Shafeek, A. K.; El-Messiry, M. M.; Shahat, A. A.; Apers, S.; Pieters, L.; Seif-El Nasr, M. M. *J. Nat. Prod.* **2000**, *63*, 845–847.
(15) Çalis, I.; Heilmann, J.; Tasdemir, D.; Linden, A.; Ireland, M. C.; Sticher, O. *J. Nat. Prod.* **2001**, *64*, 961–964.
(16) Stoi, D.; Gorunovi, M.; Skaltsounis, A. L.; Tillequin, F.; Koch, M. *Helv. Chim. Acta* **1988**, *71*, 348–353.
(17) Geiger, H.; Maier, H.; Markham, K. R. *Z. Naturforsch.* **1983**, *38c*, 490–491.
(18) Marzouk, M. M.; Kawashty, S. A.; Ibrahium, L. F.; Saleh, N.; A, M.; Al-Nowaihi, A-S. M. *Nat. Prod. Commun.* **2008**, *3*, 1325–1328.
(19) Veit, M.; Pauli, G. F. *J. Nat. Prod.* **1999**, *62*, 1301–1303.
(20) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389–1397.
(21) Liptàk, A.; Szurmai, P.; Nànàsi, P.; Neszmélyi, A. *Tetrahedron* **1982**, *38*, 3489–3497.
(22) Durette, P. L.; Horton, D.; Bhacca, N. S. *Carbohydr. Res.* **1969**, *10*, 565–577.
(23) Mizutani, K.; Ohtani, K.; Kasai, R.; Tanaka, O.; Matsura, H. *Chem. Pharm. Bull.* **1985**, *33*, 2266–2272.
(24) Mukhopadhyay, B.; Field, R. A. *Carbohydr. Res.* **2004**, *339*, 1285–1291.
(25) Kim, M.-R.; Lee, J.-Y.; Lee, H.-H.; Aryal, D.-Q.; Kim, Y.-G.; Kim, S.-K.; Woo, E.-R.; Kang, K.-W. *Food Chem. Toxicol.* **2006**, *44*, 1299–1307.
(26) Hosoi, S.; Shimizu, E.; Ohno, K.; Yokosawa, R.; Kuninaga, S.; Coskun, M.; Sakushima, A. *Phytochem. Anal.* **2006**, *17*, 20–24.
(27) Sivakumaran, T.; Jones, K. N. *Can. J. Chem.* **1967**, *45*, 2493–2500.
(28) Ballou, C. E. *J. Am. Chem. Soc.* **1957**, *79*, 165–166.
(29) Fleet, G. W. J.; Gough, M. J. *Tetrahedron Lett.* **1982**, *23*, 4509–4512.
(30) (a) Liptàk, A.; Nànàsi, P.; Neszmélyi, A.; Riessmaurer, I.; Wagner, H. *Carbohydr. Res.* **1981**, *93*, 43–52. (b) Liptàk, A.; Nànàsi, P.; Neszmélyi, A.; Riessmaurer, I.; Wagner, H. *Carbohydr. Res.* **1981**, *93*, 43–52.
(31) Boughandjioua, R. C.; Skaltsounis, A. L.; Seguin, E.; Tillequin, F.; Koch, M. *Can. J. Chem.* **1992**, *70*, 1956–1965.
(32) Schroeder, L. R.; Counts, K. M.; Haigs, F. C. *Carbohydr. Res.* **1974**, *37*, 368–372.
(33) Jurd, L. *J. Org. Chem.* **1962**, *27*, 1294–1297.
(34) Demetzos, C.; Skaltsounis, A. L.; Tillequin, F.; Koch, M. *Carbohydr. Res.* **1990**, *207*, 131–135.
(35) Kitagawa, H. I. I.; Matsushita, K.; Shirakawa, K.; Tori, K.; Tozyo, T.; Yoshikawa, M.; Yoshimura, Y. *Tetrahedron Lett.* **1981**, *22*, 1529–1532.