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# Biflavonoids and 4,2′-epoxy-3-phenylcoumarins from the moss *Mnium hornum*☆

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**Abstract**

From gametophytes of *Mnium hornum* have been isolated a sophorotriose caffeate, three biflavonoids, among them the first biflavone methyl ether from a moss, and six derivatives of 4,2′-epoxy-3-phenylcoumarin — a group of isoflavone-related compounds — that has been found so far only in some seed plants. The structures of all compounds were elucidated and confirmed spectroscopically. © 1999 Published by Elsevier Science Ltd. All rights reserved.

*Keywords: Mnium hornum*; Mniaceae; Musci; Biflavonoids; 4,2′-epoxy-3-Phenyl-coumarin derivatives; Caffeic acid ester

## 1. Introduction

In the course of earlier chromatographic surveys it was observed that gametophytes of *Mnium hornum* (Mniaceae) exhibit on 2-D-TLC chromatograms in addition to spots attributable to biflavonoids some spots showing under the UV a very strong blue fluorescence (Anhut, 1992; Geiger, Seeger, Zinsmeister, & Frahm, 1997). Since we were unable to attribute these spots with confidence to any group of compounds known to us, we decided to study this moss species in detail.

## 2. Results and discussion

Three biflavonoids (**1**–**3**), six 4,2′-epoxy-3-phenylcoumarin derivatives (**4**–**9**) and a sugar caffeate (**10**) have been isolated from an extract of *M. hornum* by the methods described in Section 3. All, but three (**1**, **8** and **9**), of these compounds were hitherto unknown. The structures of all compounds were deduced from their mass- and NMR spectra.

Philonotisflavone (**1**) is a known compound, it was identified through a comparison of its $^{1}H$ and $^{13}C$ NMR data with those of the authentic compound (Geiger & Bokel, 1989; Geiger et al., 1993). The yield of **2** was so low that only FAB-mass, $^{1}H$ and 'inverse' C–H correlated NMR spectra could be recorded. The FAB-MS revealed a $M_r$ of 584, which is compatible with a biluteolin monomethyl ether. The NMR spectra of **2** (see Table 1) are, with the exception of the signals of a methoxyl group and considerable shifts of the 5‴ signals, nearly identical with those of **1** (see Geiger et al., 1993). The methoxyl signals prove that **2** is indeed a methyl ether; the upfield shift of C-5‴ by 4 ppm and the downfield shift of H-5‴ by 0.2 ppm relative to their position with **1** are in agreement with the shifts that would be caused by a location of the methoxyl group at C-4‴ (Markham, Chari, & Mabry, 1982; Markham & Geiger, 1986). Thus **2** is the philonotisflavone-4‴-methyl ether. This is confirmed by the NOESY spectrum, which shows all NOE interactions that can be expected for this compound. **2** is the first biflavonoid methyl ether that has been found so far in a moss.

The chromatographic spot of **3** exhibits under UV (~360 nm) the typical characteristics of a luteolin-eriodictyol dimer: it appears untreated as a dark spot, which fluoresces after spraying with diphenyl-boric acid β-aminoethylester at first yellow and after a few hours red (Geiger et al., 1997). A $[M-1]^-$ ion at 571

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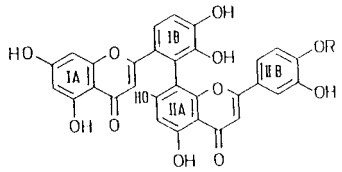
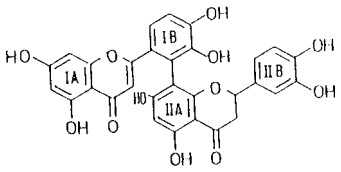
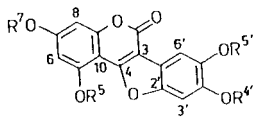


Philonotisflavone (**1**): R = H

Philonotisflavone-4'''-methyl ther (**2**): R = $CH_3$



2", 3"-Dihydrophilonotisflavone (**3**)



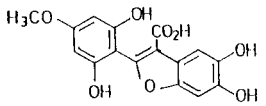
4,2'-Epoxy-5,7,4',5'-tetrahydroxy-3-phenylcoumarin (**4**): $R^5 = R^7 = R^{4'} = R^{5'} = H$

4,2'-Epoxy-4',7-dihydroxy-5,5'-dimethoxy-3-phenylcoumarin (**5**): $R^{4'} = R^7 = H$; $R^5 = R^{5'} = CH_3$

4,2'-Epoxy-4',5-dihydroxy-7,5'-dimethoxy-3-phenylcoumarin (**6**): $R^{4'} = R^5 = H$; $R^7 = R^{5'} = CH_3$

4,2'-Epoxy-4'-hydroxy-5,7,5'-trimethoxy-3-phenylcoumarin (**7**): $R^{4'} = H$; $R^5 = R^7 = R^{5'} = CH_3$

4,2'-Epoxy-5,7,4',5'-tetramethoxy-3-phenylcoumarin (**8**): $R^5 = R^7 = R^{4'} = R^{5'} = CH_3$



4,2'-Epoxy-5,4',5'-trihydroxy-7-methoxy-3-phenylcoumarinic acid (**9**)

*m*/*z* in the FAB-MS spectrum points also to a luteolin-eriodictyol dimer. The $^1H$ as well as $^{13}C$ NMR spectra of **3** reveal that this compound consists of a pair of interconverting diastereomers, the ratio of which is 1:3 at first and becomes 1:1 after storage of the DMSO-$d_6$ solution. This phenomenon has been observed also with a few other luteolin–eriodictyol dimers (Markham, Andersen, & Viotto, 1988; Geiger & Bokel, 1989); it is due to the presence of two centers of chirality (the chiral C-2 of the eriodictyol moiety and the center of atropisomerism at the interflavonyl bond round which free rotation is more or less hindered), which racemize very slowly compared with the NMR time scale. Thus the two possible diastereomers become distinguishable by NMR. Since there was only little overlapping of signals in the $^1H$ and $^{13}C$ spectra of **3**, it was possible to separate the spectra of both isomers on the basis of the signal intensities and to analyze them by HH- and HC–COSY as well as ROESY (see Table 2). Luteolin linked via C-2′ could be identified as the A-terminal unit of both isomers on the basis of the coupling patterns of the protons, the chemical shift of the correlated carbon resonances and comparison with the signals of the corresponding moiety of **1** or **2**. The coupling patterns of the $^1H$-signals, which must be assigned to the eriodictyol moiety demonstrates clearly that this part must linked via C-6 or C-8 of its A-ring. Because flavanones in contrast

Table 1
NMR data of **2**, $^{13}C$ signals of protonated carbons are derived from an 'inverse' detected C–H correlation

| Position | NOESY | $^{1}H$ NMR | $^{13}C$ NMR |
|---|---|---|---|
| 3 | H-6′ | 5.92s | 106.0 |
| 6 | | 6.03d$^{m}$ | 98.0 |
| 8 | | 5.70d$^{m}$ | 93.0 |
| 5′ | H-6′ | 6.94d$^{o}$ | 114.5 |
| 6′ | H-5′ | 7.18d$^{o}$ | 120.0 |
| 3″ | H-2‴, H-6‴ | 6.55s | 102.5 |
| 6″ | | 6.15s | 99.0 |
| 2‴ | H-3″ | 7.04d$^{m}$ | 113.0 |
| 5‴ | 4‴-$OCH_3$, H-6‴ | 6.93d$^{m}$ | 111.5 |
| 6‴ | H-3″, H-5‴ | 7.09dd$^{om}$ | 118.0 |
| 4‴-OMe | H-5‴ | 3.83s | 56.0 |

with flavones have their C-6 and C-8 as well as H-6 and H-8 signals at almost identical positions, the decision between these two possibilities must be made by comparing the chemical shifts of the B-ring proton signals of eriodictyol itself with their counterparts in the spectrum of **3**, where these signals appear 0.1–0.4 ppm upfield of their position with eriodictyol. This identifies C-8 of eriodictyol as the second bridgehead of the interflavonyl linkage (Geiger & Markham, 1992; Geiger et al., 1993). Thus **3** is 2″,3″-dihydrophilonotisflavone.

The next six compounds (**4**–**9**) are those which show on the chromatograms under UV light an intense blue fluorescence, and caused us to study *M. hornum* in more detail. The molecular formula of **4** can be derived from the low resolution MS in combination with the $^{1}H$ and $^{13}C$ NMR spectra; it is $C_{15}H_8O_7$. A blue fluorescing compound with this molecular formula — norwedelolactone — is a known constituent of the Compositae *Eclipta alba* and *Wedelia calendulacea* (Bhargava, Krishnaswamy, & Seshadri, 1970; Wagner, Geyer, Kiso, Hikino, & Rao, 1986). The $^{1}H$ and $^{13}C$

Table 2
NMR data of the two diastereomers of 2″,3″-dihydrophilonotisflavone (**3**)

| H,C-long range-coupling | NOESY with H | HH-COSY with H | $^{1}H$ NMR | | Position | | $^{13}C$ NMR | |
|---|---|---|---|---|---|---|---|---|
| | | | major | minor | H | C | major | minor |
| | | | | | | 2 | 166.7 | 166.7 |
| 2, 4, 10, 1′ | 6′ | | 6.03s | 5.88s | 3 | 3 | 106.2 | 106.3 |
| | | | | | | 4 | 181.3 | 181.1 |
| | | | | | | 5 | 161.2 | 161.2 |
| 5, 7, 8, 10 | | 8 | 6.11d$^{m}$ | 6.12d$^{m}$ | 6 | 6 | 98.6 | 98.6 |
| | | | | | | 7 | 163.8 | 163.8 |
| 6, 7, 9, 10 | | 6 | 6.03d$^{m}$ | 6.01d$^{m}$ | 8 | 8 | 93.3 | 93.5 |
| | | | | | | 9 | 157.3 | 157.3 |
| | | | | | | 10 | 103.3 | 103.3 |
| | | | | | | 1′ | 124.0 | 123.8 |
| | | | | | | 2′ | 120.2 | 120.3 |
| | | | | | | 3′ | 144.2 | 144.9 |
| | | | | | | 4′ | 148.2 | 148.2 |
| 1′, 3′, 4′ | | 6′ | 6.83d$^{o}$ | 6.81d$^{o}$ | 5′ | 5′ | 113.8 | 113.7 |
| 2, 2′, 4 | 3 | 5′ | 7.09d$^{o}$ | 7.02d$^{o}$ | 6′ | 6′ | 119.9 | 119.9 |
| | 2‴, 6‴ | $3_{eq}$″, $3_{ax}$″ | 5.27dd (2/13) | 4.78dd (2/13) | 2″ | 2″ | 78.1 | 78.4 |
| C-2″, C-1‴ [a] | 2‴, 6‴ | 2″, $3_{eq}$″ | 2.88dd (13/17) | 2.98dd (13/17) | $3_{ax}$″ | | | |
| | | | | | | 3″ | 42.3 | 42.1 |
| | 2‴ | 2″, $3_{ax}$″ | 2.67dd (2/17) | 2.54dd (2/17) | $3_{eq}$″ | | | |
| | | | | | | 4″ | 195.9 | 196.1 |
| | | | | | | 5″ | 162.6 | 162.5 |
| 5″, 7″, 8″, 10″ | | | 5.94s | 5.99s | 6″ | 6″ | 95.7 | 95.7 |
| | | | | | | 7″ | 162.6 | 162.5 |
| | | | | | | 8″ | 104.2 | 104.4 |
| | | | | | | 9″ | 160.0 | 159.4 |
| | | | | | | 10″ | 101.5 | 101.2 |
| | | | | | | 1‴ | 129.3 | 129.7 |
| 2″, 3‴, 4‴, 6‴ | 2″ | 6‴ | 6.67d$^{m}$ | 6.63d$^{m}$ | 2‴ | 2‴ | 113.9 | 113.7 |
| | | | | | | 3‴ | 144.94′ | 144.9 |
| | | | | | | 4‴ | 145.2 | 145.4 |
| 1″, 3‴, 4‴, 6‴ | | 6‴ | 6.59d$^{o}$ | 6.59d$^{o}$ | 5‴ | 5‴ | 115.1 | 114.9 |
| 2″, 2‴, 4‴ | 2″ | 2‴ | 6.51dd$^{om}$ | 6.36dd$^{om}$ | 6‴ | 6‴ | 117.1 | 117.6 |

[a]These couplings are only observed with the minor isomer!

Table 3
NMR data of the compounds **4**–**9**

| | **4** | | **5** | | **6** | | **7** | | **8** | **9** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Position | H | C | H | C | H | C | H | C | H | H | C[a] |
| 2 | – | 157.7 | – | 157.5 | – | 157.6 | – | 157.4 | – | – | – |
| 3 | – | 100.7 | – | 101.5 | – | 101.6 | – | 102.3 | – | – | 101.5 |
| 4 | – | 155.3 | – | 158.8 | – | 159.2 | – | 158.3 | – | – | 155.0 |
| 5 | – | 159.3 | – | 156.4 | – | 155.2 | – | 156.1 | – | – | 161.0 |
| 6 | 6.33d[m] | 95.3 | 6.46d[m] | 95.8 | 6.44brs | 98.1 | 6.56d[m] | 95.5 | 6.67d[m] | 6.48s | 95.0 |
| 7 | – | 161.0 | – | 161.6 | – | 162.3 | – | 162.6 | – | – | 156.5 |
| 8 | 6.37brs | 94.7 | 6.48d[m] | 95.7 | 6.58brs | 92.9 | 6.67d[m] | 93.9 | 6.80d[m] | 6.48s | 95.0 |
| 9 | – | 155.1 | – | 155.1 | – | 155.5 | – | 155.0 | – | – | 161.0 |
| 10 | – | 99.1 | – | 96.1 | – | 96.7 | – | 97.0 | – | – | 96.0 |
| 1′ | – | 113.8 | – | 113.5 | – | 113.5 | – | 113.3 | – | – | 113.5 |
| 2′ | – | 144.1 | – | 146.4 | – | 146.4 | – | 146.6 | – | – | 144.0 |
| 3′ | 7.13 | 98.7 | 7.17s | 98.9 | 7.22s | 99.0 | 7.17s | 98.9 | 7.56s | 7.12 | 98.5 |
| 4′ | – | 148.6 | – | 149.3 | – | 149.4 | – | 149.4 | – | – | 148.5 |
| 5′ | – | 145.0 | – | 146.8 | – | 146.8 | – | 146.8 | – | – | 145.5 |
| 6′ | 7.21 | 104.5 | 7.27s | 101.6 | 7.30s | 101.7 | 7.27s | 101.6 | 7.34s | 7.22 | 104.5 |
| 5–OMe | – | – | 3.94s | 56.2 | – | – | 3.96s | 56.5 | 4.01s | – | – |
| 7–OMe | – | – | – | – | 3.80s | 55.6 | 3.85s | 55.9 | 3.87s | 3.92 | 56.0 |
| 4′–OMe | – | – | – | – | – | – | – | – | 3.86s | – | – |
| 5′–OMe | – | – | 3.885 | 56.1 | 3.87s | 56.1 | 3.86s | 56.1 | 3.88s | – | – |

[a]$^{13}$C-data taken from the H–C correlation spectra, therefore C-2 could not be observed

NMR data of **4** (see Table 3) are in accordance with the data one would expect for the structure of norwedelolactone (the structure of an isomeric coumaronochromone can be excluded, because this would be nonfluorescent and would have its carbonyl resonance farther downfield in the $^{13}$C NMR spectrum). It is, however, not possible to assign in the spectra of **4** signals with identical multiplicities and similar chemical shifts unequivocally, because this compound contains only four carbon-bound protons, which is not sufficient for meaningful 2-D NMR experiments. But with the compounds **5**–**8**, which turned out to be methyl ethers of **4** this problem is overcome, at least in part, by the additional protons of the methoxyl groups. However, the naming of these **4**-methyl ethers posed a problem, because the numbering system that was used originally with the trivial names wedelolactone, norwedelolactone, as well as coumestan (coined for the unsubstituted skeleton) had been changed later (Wong, 1975). Derivative names therefore cannot be based on these trivial names. To avoid any ambiguity and to bring the numbering in line with other flavonoids, semisystematic names based on 4,2′-epoxy-3-phenylcoumarin will be used in this paper. Thus **4** is 4,2′-epoxy-5,7,4′,5′-tetrahydroxy-3-phenylcoumarin.

The molecular formula of the main compound (**7**), $C_{18}H_{19}O_7$, was deduced from its $^1$H and $^{13}$C NMR spectra in combination with a low resolution mass spectrum. A careful analysis of its $^1$H and $^{13}$C NMR spectra (see Table 3) by means of NOESY as well as H–C one- and multiple-bond correlation spectra (the observed correlations are listed on Table 4) led to the conclusion that **7** is 4,2′-epoxy-4′-hydroxy-5,7,5′-trimethoxy-3-phenylcoumarin.

The NMR and MS data of **5** and **6** yield for both compounds the molecular formulae $C_{17}H_{12}O_7$, which suggests that they are both dimethyl ethers of **4**. A comparison of the NMR data of **5**–**7** which are listed on Table 3 shows that the B-ring signals of all three compounds are identical, thus one of the methoxyl groups of **5** and **6** must be in position 5′ and the difference between **5** and **6** must be whether the second methoxyl group is at position 5 or 7 of the A-ring. The NMR data of **7** demonstrate that the chemical shifts of the 5- and 7-methoxyl protons differ markedly. On this basis a comparison of the spectra of **5**–**7** in Table 3 reveals that **5** is 4,2′-epoxy-4′,7-dihydroxy-5,5′-dimethoxy-3-phenylcoumarin and **6** 4,2′-epoxy-4′5-dihydroxy-7,5′-dimethoxy-3-phenylcoumarin. On

Table 4
Important NOE and H–C long-range correlations observed in the NOESY and HMBC spectra of compound **7**

| H | NOE | H–C long-range |
|---|---|---|
| 6 | 5-OMe | C-5, C-7, C-8, C-10 |
| 8 | 7-OMe | C-6, C-7, C-9, C-10 |
| 3′ | – | C-1′, C-4′, C-5′ |
| 6′ | 5-OMe | C-3, C-2′, C-4′ |
| 5-OMe | H-6 | C-5 |
| 7-OMe | H-6, H-8 | C-7 |
| 5′-OMe | H-6′ | C-5′ |

Table 5
Important H–C long-range correlations observed in the NOESY and HMBC spectra of the compounds **5** and **6**

| 5 | | **6** | |
|---|---|---|---|
| H | H–C long-range | H | H–C long-range |
| 6 | C-5, C-7, C-8,C-10 | 6 | C-5, C-7, C-8, C-10 |
| 8 | C-6, C-7, C-9, C-10 | 8 | C-6, C-7, C-9, C-10 |
| 5-OMe | C-5 | – | – |
| – | – | 7-OMe | C-7 |
| 3′ | C-1′, C-4′, C-5′ | 3′ | C-1′, C-4′, C-5′ |
| 6′ | C-3, C-2′, C-4′ | 6′ | C-3, C-2′, C-4′, C-5′ |
| 5′-OMe | C-5′ | 5′-OMe | C-5′ |

Table 5 the H–C correlations of **5** and **6** are listed. NOESY spectra of **5** and **6** could not be recorded, because the samples were too small.

The amount of compound **8** was even smaller, only an $^{1}$H NMR and a mass spectrum could be recorded. Although the MS showed no molecular ion, but only fragments, the NMR spectrum (see Table 3) suggests that **8** is 4,2′-epoxy-5,7,4′,5′-tetramethoxy-3-phenylcoumarin.

Compound **9** yielded a FAB-mass spectrum that suggested a monomethyl ether of **4**. Its NMR data (compare Tables 3 and 6), however, are not compatible with such a structure: the equivalence of H-6 and H-8, C-5 and C-9, as well as C-6 and C-8 hints to a symmetrical A-ring resulting from a hydrolytic cleavage of the C-ring. Thus it is assumed that **9** is 4,2′-epoxy-5,4′,5′-trihydroxy-7-methoxy-3-phenylcoumarinic acid, and that the putative molecular ion was in fact a fragment ion generated by the loss of water.

The last compound (**10**) appears on the TLC chromatogram under UV also as a blue fluorescing spot, but in contrast to **4**–**9** this colour changes to turquoise after spraying with diphenylboric acid β-aminoethylester. This is reminiscent of caffeic acid and its esters. The relative molecular mass is, according to the FAB-mass spectrum, 666, which would be compatible with the caffeic ester of a trihexoside. The $^{1}$H NMR spectrum (Table 7) exhibits in the aromatic and olefinic range the signals of five protons, whose chemical shifts and coupling patterns are as expected for a caffeoyl moiety. In the aliphatic range only three signals assignable to three anomeric protons are well separated from the bulk of the other signals of the trisaccharide moiety. The $^{13}$C NMR spectrum (Table 7) exhibits 27 signals, nine of them can be readily assigned to the caffeoyl part and 17 of the sugar signals show $\delta_c$-values almost identical with those of luteolin-7-β-sophorotrioside (Brinkmeier, Geiger, & Zinsmeister, 1998), only the C-1′ signal is, expectedly, influenced by the neighbouring ester-group. Thus **10** is the 1-*O*-caffeoyl-β-sophorotriose.

Table 6
Important NOE and H–C long-range correlations observed in the NOESY and HMBC spectra of Compound **9**

| H | NOE | C–H long-range |
|---|---|---|
| 6/8 | 7-OMe | C-4, C-5/9, C-7, C-8/6, C-10 |
| 7-OME | H-6/8 | – |
| 3′ | – | C-1′, C-2′, C-4′, C-5′ |
| 6′ | – | C-3, C-2′, C-4′, C-5′ |

Table 7
$^{1}$H and $^{13}$C NMR data of 1-*O*-caffeoyl-β-sophotriose (**10**)

| Position | $^{1}$H | $^{13}$C |
|---|---|---|
| 1 | – | 125.8 |
| 2 | 7.06 d (2.0) | 114.9 |
| 3 | – | 145.5 |
| 4 | – | 148.6 |
| 5 | 6.76 d (8.1) | 115.8 |
| 6 | 7.02 dd (2.0/8.1) | 121.6 |
| γ | 7.54 d (15.6) | 146.2 |
| β | 6.31 d (15.6) | 113.6 |
| α | – | 164.9 |
| 1′ | 5 d (8.0) | 92.4 |
| 2′ | 3.00–3.75 m | 82.5 |
| 3′ | 3.00–3.75 m | 75.5 |
| 4′ | 3.00–3.75 m | 68.6 |
| 5′ | 3.00–3.75 m | 76.0 |
| 6′ | 3.00–3.75 m | 60.2 |
| 1″ | 4.52 d (7.9) | 102.4 |
| 2″ | 3.00–3.75 m | 83.0 |
| 3″ | 3.00–3.75 m | 76.0 |
| 4″ | 3.00–3.75 m | 69.1 |
| 5″ | 3.00–3.75 m | 77.3 |
| 6″ | 3.00–3.75 m | 60.4 |
| 1‴ | 4.50 d (7.8) | 104.1 |
| 2‴ | 3.00–3.75 m | 74.6 |
| 3‴ | 3.00–3.75 m | 76.3 |
| 4‴ | 3.00–3.75 m | 69.7 |
| 5‴ | 3.00–3.75 m | 77.7 |
| 6‴ | 3.00–3.75 m | 60.9 |

The most remarkable result of the present work is the identification of the isoflavone-related 4,2′-epoxy-3-phenylcoumarins (is coumestans) as constituents of *M. hornum*. Since 2-D chromatograms of three other *Mnium* species — *M. ambignum*, *M. spinosum* and *M. stellare* — are reminiscent of that of *M. hornum*, 4,2′-epoxy-3-phenylcoumarins might turn out to be useful chemical characters of the genus *Mnium*. With regard to chemotaxonomic studies, however, it must be kept in mind that the concentration of individual constituents may undergo drastic seasonal changes (Brinkmeier, Hahn, Seeger, Geiger, & Zinsmeister, submitted). Any conclusion in this field must be based therefore on a variety of samples that have been collected at different times of the year.

## 3. Experimental

### 3.1. Plant material

500 g gametophytes of *Mnium hornum* Hedw. were gathered in late summer 1993 in the Stadtwald near the 'Universität des Saarlandes'. A voucher is deposited at SAAR (No. 3062).

### 3.2. Extraction and isolation

Extraction and removal of chlorophyll and lipids by solid-phase extraction was performed as described l.c. (Rampendahl, Seeger, Geiger, & Zinsmeister, 1996). MPLC on Lichroprep RP 18 (40–63 μm, Merck, Darmstadt) with a MeOH–$H_20$ gradient ranging from 10–70% MeOH gave eight fractions containing **10**, **3** + **4**, **1**, **2** + **9**, **5** + **6**, **6**, **7** and **8**, respectively. **3** and **4** as well as **2** and **9** could be separated by CC on Sephadex LH-20 with $Me_2CO$–MeOH–$H_2O$ (2:1:1). An attempt to separate **5** and **6** with the same system led to a crystalline mixture of **5** and **6** that was very sparingly soluble in most solvents. Therefore this mixture could not be separated chromatographically. The yields were 26 mg **1**, 1.5 mg **2**, 8 mg **3**, 8 mg **4**, 10 mg **5** + **6**, 5 mg **6**, 18 mg **7**, 1 mg **8**, 2 mg **9** and 100 mg **10**.

FAB-MS (neg. mode): 4–7 keV Xe and glycerol as matrix. NMR: DMSO-$d_6$, ambient temperature, 400 MHz ($^1$H) and 100 MHz ($^{13}$C) for 1-D spectra, and 500 MHz for proton detected 2-D spectra.

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