Sulfated Metabolites of Flavonolignans and 2,3-Dehydroflavonolignans: Preparation and Properties

Kateřina Valentová ^{1,*}, Kateřina Purchartová ^{1,2}, Lenka Rydlová ^{1,2}, Lenka Roubalová ^{3,4}, David Biedermann ¹, Lucie Petrásková ¹, Alena Křenková ¹, Helena Pelantová ¹, Veronika Holečková-Moravcová ¹, Eva Tesařová ², Josef Cvačka ⁵, Jiří Vrba ^{3,4}, Jitka Ulrichová ^{3,4}, Vladimír Křen ¹

- ¹ Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 14220 Prague, Czech Republic; katerina.purchartova@gmail.com (K.P.), rydlova.l@email.cz (L.Ry.), biedermann@biomed.cas.cz (D.B.), petraskova@biomed.cas.cz (L.P.), alenka.petrickova@gmail.com (A.K.), pelantova@biomed.cas.cz (H.P.), vholeckov@gmail.com (V.H.-M.), kren@biomed.cas.cz (V.K.)
- ² Faculty of Science, Charles University, Department of Physical and Macromolecular Chemistry, Hlavova 2030/8, 12843 Prague, Czech Republic; eva.tesarova@natur.cuni.cz (E.T.)
- ³ Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, Olomouc 77515, Czech Republic; roubalova.lenka@seznam.cz (L.Ro.), vrbambv@seznam.cz (J.V.), jitkaulrichova@seznam.cz (J.U.)
- ⁴ Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, Olomouc 77515, Czech Republic
- ⁵ Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 16610 Prague, Czech Republic; josef.cvacka@uochb.cas.cz
- * Correspondence: kata.valentova@email.cz; Tel: +420-296-442-509.

Table of Contents

| Figure S1. | HPLC chromatogram of silybin A 20-O-sulfate | 3 |
|-------------|--|---|
| Figure S2. | HPLC chromatogram of silybin B 20-O-sulfate | 3 |
| Figure S3. | HPLC chromatograms of 2,3-dehydrosilybin A sulfation in time | 1 |
| Figure S4. | ESI-MS- spectrum of 2,3-dehydrosilybin 20-O-sulfate | 5 |
| Table S1. | ¹³ C and ¹ H NMR data of 2,3-dehydrosilybin-20-O-sulfate | 5 |
| Figure S5. | HPLC chromatogram of 2,3-dehydrosilybin-20-O-sulfate | 5 |
| Figure S6. | ESI-MS- spectrum of 2,3-dehydrosilybin-7,20-di-O-sulfate | 5 |
| Table S2. | ¹³ C and ¹ H NMR data of 2,3-dehydrosilybin-7,20-di- <i>O</i> -sulfate | 7 |
| Figure S7. | HPLC chromatogram of 2,3-dehydrosilybin-7,20-di-O-sulfate | 7 |
| Figure S8. | HPLC chromatograms of silychristin sulfation after 1 h of reaction | 3 |
| Figure S9. | ESI-MS- spectrum of silychristin-19-O-sulfate | 3 |
| Table S3. | ¹³ C and ¹ H NMR data of silychristin-19- <i>O</i> -sulfate | Э |
| Figure S10. | HPLC chromatogram of silychristin-19-O-sulfate | Э |
| Figure S11. | ESI-MS- spectrum of 2,3-dehydrosilychristin-19-O-sulfate |) |
| Table S4. | ¹³ C and ¹ H NMR data of 2,3-dehydrosilychristin-19- <i>O</i> -sulfate10 |) |
| Figure S12. | HPLC chromatogram of 2,3-dehydrosilychristin-19-O-sulfate | 1 |
| Figure S13. | ESI-MS- spectrum of silydianin 19-O-sulfate ([M - H] ⁻ , m/z 561.1)11 | 1 |
| Table S5. | ¹³ C and ¹ H NMR data of silydianin 19- <i>O</i> -sulfate12 | 2 |



Figure S1. HPLC chromatogram of silybin A 20-O-sulfate.



Figure S2. HPLC chromatogram of silybin B 20-O-sulfate.



Figure S3. HPLC chromatograms of 2,3-dehydrosilybin A sulfation in time.
a) 1h, b) 5h, c) 12h; 1 - p-NPS, 2 - p-NP, 3 - 2,3-dehydrosilybin A sulfate, 4 - 2,3-dehydrosilybin

5,0

7,5

10,0

12,5 min

2,5

0,0

Supplementary data

170114servisHR_9-#58-71 RT: 1.67-2.05 AV: 14 SB: 15 1.04-1.46 NL: 2.41E7 T: FTMS - p ESI Full ms [200.00-2000.00]



Figure S4. ESI-MS- spectrum of 2,3-dehydrosilybin 20-*O*-sulfate. $([M - H]^{-}, m/z 559.1; [M - 2H]^{2}, m/z 279.0)$

Table S1. 13 C and 1 H NMR data of 2,3-dehydrosilybin-20-O-sulfate.(600.23 MHz for 1 H. 150.95 MHz for 13 C. DMSO- d_6 . 30 °C)

| Atom δ_c m. δ_{41} n(H)m. J [Hz]2145.83S-003136.45S-004176.11S-004a103.14S-005160.74S-00698.33D6.1861d2.07164.14S-00893.64D6.4611dd7.91078.52D4.3031ddd7.9, 4.6, 2.51175.81D5.0181d7.912a143.36S-0114123.87S-0115121.42D7.7731m16a145.06S-0117131.24S-0118112.06D7.0841d8.422119.67D6.9681dd8.4, 2.02360.08T3.5941ddd12.4, 5.8, 4.63-OH9.5301br s5-OH10.8031br s19-OMe55.82Q3.7693s23-OH5.0101dd5.8, 4.8 | | (000.23 10 | 112 101 | 11, 150.55 | | C, DIVISO-06, SO CJ | |
|---|--------|-----------------|---------|-----------------|------|---------------------|----------------|
| 2 145.83 S - 0 3 136.45 S - 0 4 176.11 S - 0 4a 103.14 S - 0 5 160.74 S - 0 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 - - 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 - - 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.773 1 m - 14 123.87 S - 0 - - - - 14 123.87 S - 0 - < | Atom | $\delta_{ m c}$ | m. | $\delta_{ m H}$ | n(H) | m. | <i>J</i> [Hz] |
| 3 136.45 S - 0 4 176.11 S - 0 4a 103.14 S - 0 5 160.74 S - 0 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 - - 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 - - 10 78.52 D 4.303 1 ddd 7.9 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.780 1 m - 14 123.87 S - 0 - - - 16 116.92 D 7.127 1 m - - - 16 116.92 | 2 | 145.83 | S | - | 0 | | |
| 4 176.11 S - 0 4a 103.14 S - 0 5 160.74 S - 0 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 - - 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 - - 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.780 1 m - 14 123.87 S - 0 - - - 15 121.42 D 7.773 1 m - - - 16 116.92 D 7.084 1 d 2.0 - | 3 | 136.45 | S | - | 0 | | |
| 4a 103.14 S - 0 5 160.74 S - 0 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 . . 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 . . 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 . . 14 123.87 S - 0 . . 14 123.87 S - 0 . . . 15 121.42 D 7.773 1 m 16a 145.06 S - 0 18 12.06 D< | 4 | 176.11 | S | - | 0 | | |
| 5 160.74 S - 0 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 | 4a | 103.14 | S | - | 0 | | |
| 6 98.33 D 6.186 1 d 2.0 7 164.14 S - 0 . 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 . . 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 4.6, 2.5 11 75.81 D 5.018 1 ddd 7.9, 4.6, 2.5 13 116.22 D 7.780 1 m . 14 123.87 S - 0 . . 15 121.42 D 7.773 1 m . 16a 145.06 S - 0 17 131.24 S - 0 20 143.28 S - 0 . . . <t< th=""><th>5</th><th>160.74</th><th>S</th><th>-</th><th>0</th><th></th><th></th></t<> | 5 | 160.74 | S | - | 0 | | |
| 7 164.14 S - 0 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 - - 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.780 1 m - 14 123.87 S - 0 - - - 15 121.42 D 7.773 1 m - - - 16a 145.06 S - 0 - <t< th=""><th>6</th><th>98.33</th><th>D</th><th>6.186</th><th>1</th><th>d</th><th>2.0</th></t<> | 6 | 98.33 | D | 6.186 | 1 | d | 2.0 |
| 8 93.64 D 6.461 1 d 2.0 8a 156.29 S - 0 0 0 7.9, 4.6, 2.5 1 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 1 11 75.81 D 5.018 1 d 7.9 1 12a 143.36 S - 0 0 7.9 1 m 14 123.87 S - 0 0 7.127 1 m 16 116.92 D 7.127 1 m 16 116.92 D 7.084 1 d 2.0 13 112.06 D 7.084 1 d 2.0 13 13.24 S - 0 1 14 12.05 D 7.084 1 d 2.0 1 13.24 S - 0 1 14 2.0 1 14 2.0 1 14 2.0 1 14 12.0 1 14 14 2.0 | 7 | 164.14 | S | - | 0 | | |
| 8a 156.29 S - 0 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.780 1 m - 14 123.87 S - 0 - - 15 121.42 D 7.773 1 m - 16 116.92 D 7.127 1 m - - 16a 145.06 S - 0 - - - 17 131.24 S - 0 - - - - 18 112.06 D 7.084 1 d 8.4 - 0 20 143.28 S - 0 - - - - - - - - - - - - - - | 8 | 93.64 | D | 6.461 | 1 | d | 2.0 |
| 10 78.52 D 4.303 1 ddd 7.9, 4.6, 2.5 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 - - 13 116.22 D 7.780 1 m - 14 123.87 S - 0 - - 15 121.42 D 7.773 1 m - 16 116.92 D 7.127 1 m - - 16a 145.06 S - 0 - - - 17 131.24 S - 0 - - - - 18 112.06 D 7.084 1 d 2.0 - 20 143.28 S - 0 - | 8a | 156.29 | S | - | 0 | | |
| 11 75.81 D 5.018 1 d 7.9 12a 143.36 S - 0 0 0 13 116.22 D 7.780 1 m 0 14 123.87 S - 0 0 0 15 121.42 D 7.773 1 m 0 16 116.92 D 7.127 1 m 0 16a 145.06 S - 0 0 0 0 17 131.24 S - 0 0 0 0 0 18 112.06 D 7.084 1 d 2.0 0 19 150.44 S - 0 0 0 0 0 20 143.28 S - 0 | 10 | 78.52 | D | 4.303 | 1 | ddd | 7.9, 4.6, 2.5 |
| 12a 143.36 S - 0 13 116.22 D 7.780 1 m 14 123.87 S - 0 0 15 121.42 D 7.773 1 m 16 116.92 D 7.127 1 m 16a 145.06 S - 0 0 17 131.24 S - 0 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 0 2.0 19 150.44 S - 0 0 2.0 20 143.28 S - 0 0 2.0 21 120.59 D 7.513 1 dd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.0H - - 9.530 1 br s 5-OH - - 10.803 1 <td< th=""><th>11</th><th>75.81</th><th>D</th><th>5.018</th><th>1</th><th>d</th><th>7.9</th></td<> | 11 | 75.81 | D | 5.018 | 1 | d | 7.9 |
| 13 116.22 D 7.780 1 m 14 123.87 S - 0 0 15 121.42 D 7.773 1 m 16 116.92 D 7.127 1 m 16a 145.06 S - 0 0 17 131.24 S - 0 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 0 2.0 20 143.28 S - 0 0 2.0 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 5.8, 4.6 3-OH - - 9.530 1 br s 5 5-OH - 10.803 1 br s 19-OMe 55.82 Q <th>12a</th> <th>143.36</th> <th>S</th> <th>-</th> <th>0</th> <th></th> <th></th> | 12a | 143.36 | S | - | 0 | | |
| 14 123.87 S - 0 15 121.42 D 7.773 1 m 16 116.92 D 7.127 1 m 16a 145.06 S - 0 - 17 131.24 S - 0 - 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 - - 20 143.28 S - 0 - - 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 5.8, 4.6 - - 3.391 1 ddd 12.4, 5.8, 4.6 3-OH - - 9.530 1 br s - - 5.0H - - 10.803 1 br s - 5-OH - - 10.803 1 br s -< | 13 | 116.22 | D | 7.780 | 1 | m | |
| 15 121.42 D 7.773 1 m 16 116.92 D 7.127 1 m 16a 145.06 S - 0 17 131.24 S - 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 0 20 143.28 S - 0 0 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 ddd 12.4, 5.8, 4.6 3-0H - - 9.530 1 bddd 12.4, 5.8, 4.6 3-OH - - 12.406 1 s 5.01 br s 5-OH - - 10.803 1 br s 5.8, 4.8 19-OMe 55.82 Q 3.769 3 s 5.010 1 dd 5.8, 4.8 | 14 | 123.87 | S | - | 0 | | |
| 16 116.92 D 7.127 1 m 16a 145.06 S - 0 17 131.24 S - 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 - - 20 143.28 S - 0 - - 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 5.8, 4.6 - - 3-OH - - 9.530 1 bdd 12.4, 5.8, 4.6 3-OH - - 12.406 1 s - 5-OH - - 10.803 1 br s - 19-OMe 55.82 Q 3.769 3 s - 23-OH - - 5.010 1 dd 5.8, 4.8 | 15 | 121.42 | D | 7.773 | 1 | m | |
| 16a 145.06 S - 0 17 131.24 S - 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 0 2.0 20 143.28 S - 0 0 0 21 120.59 D 7.513 1 dd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 5.8, 4.6 3.391 1 ddd 12.4, 5.8, 4.6 3-OH - - 9.530 1 br s 5 5-OH - - 12.406 1 s 5 7-OH - - 10.803 1 br s 5 19-OMe 55.82 Q 3.769 3 s 5 23-OH - - 5.010 1 dd 5.8, 4.8 | 16 | 116.92 | D | 7.127 | 1 | m | |
| 17 131.24 S - 0 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 0 0 20 143.28 S - 0 0 0 21 120.59 D 7.513 1 dd 8.4 22 119.67 D 6.968 1 ddd 12.4, 4.8, 2.0 23 60.08 T 3.594 1 ddd 12.4, 5.8, 4.6 3-OH - - 9.530 1 br s 5 5-OH - - 12.406 1 s 7-OH - - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 16a | 145.06 | S | - | 0 | | |
| 18 112.06 D 7.084 1 d 2.0 19 150.44 S - 0 - - 0 - - - 0 - - - 0 - - - 0 - | 17 | 131.24 | S | - | 0 | | |
| 19 150.44 S - 0 20 143.28 S - 0 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 ddd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.0H - - 9.530 1 br s 5-OH - - 12.406 1 s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 18 | 112.06 | D | 7.084 | 1 | d | 2.0 |
| 20 143.28 S - 0 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 dd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3-0H - - 9.530 1 br s 5-OH - 12.406 1 s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 19 | 150.44 | S | - | 0 | | |
| 21 120.59 D 7.513 1 d 8.4 22 119.67 D 6.968 1 dd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.0H - - 9.530 1 ddd 12.4, 5.8, 4.6 3-OH - - 12.406 1 s 5-OH - 10.803 1 br s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 20 | 143.28 | S | - | 0 | | |
| 22 119.67 D 6.968 1 dd 8.4, 2.0 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.0H - - 9.530 1 br s 5-OH - 12.406 1 s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 21 | 120.59 | D | 7.513 | 1 | d | 8.4 |
| 23 60.08 T 3.594 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 4.8, 2.5 3.391 1 ddd 12.4, 5.8, 4.6 3-OH - 9.530 1 br s 5-OH - 12.406 1 s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 22 | 119.67 | D | 6.968 | 1 | dd | 8.4, 2.0 |
| 3.391 1 ddd 12.4, 5.8, 4.6 3-OH - 9.530 1 br s 5-OH - 12.406 1 s 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 23 | 60.08 | т | 3.594 | 1 | ddd | 12.4, 4.8, 2.5 |
| 3-OH - - 9.530 1 br s 5-OH - - 12.406 1 s 7-OH - - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | | | | 3.391 | 1 | ddd | 12.4, 5.8, 4.6 |
| 5-OH - 12.406 1 s 7-OH - - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 3-OH | - | - | 9.530 | 1 | br s | |
| 7-OH - 10.803 1 br s 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 5-OH | - | - | 12.406 | 1 | S | |
| 19-OMe 55.82 Q 3.769 3 s 23-OH - - 5.010 1 dd 5.8, 4.8 | 7-OH | - | - | 10.803 | 1 | br s | |
| 23-OH 5.010 1 dd 5.8, 4.8 | 19-OMe | 55.82 | Q | 3.769 | 3 | S | |
| | 23-OH | - | - | 5.010 | 1 | dd | 5.8, 4.8 |



m. multiplicity



Figure S5. HPLC chromatogram of 2,3-dehydrosilybin-20-O-sulfate.



Figure S6. ESI-MS- spectrum of 2,3-dehydrosilybin-7,20-di-*O*-sulfate. ([M - 2H + Na] *m*/*z* 661.0; [M - H - SO₃]⁻, *m*/*z* 559.1; [M - 2H]²⁻, *m*/*z* 319.0; [M - 2H - SO₃]²⁻, *m*/*z* 279.0)

| Table S2. | ¹³ C and ¹ H NMR data of 2,3-dehydrosilybin-7,20-di- <i>O</i> -sulfate. |
|-----------|--|
| | (600.23 MHz for ¹ H, 150.95 MHz for ¹³ C, DMSO- <i>d</i> ₆ , 30 °C) |

| Atom | $\delta_{ m c}$ | m. | $\delta_{ m H}$ | n(H) | m. | <i>J</i> [Hz] |
|--------|-----------------|----|-----------------|---------|------|----------------|
| 2 | 146.60 | S | - | 0 | | |
| 3 | 136.78 | S | - | 0 | | |
| 4 | 176.42 | S | - | 0 | | |
| 4a | 105.13 | S | - | 0 | | |
| 5 | 159.80 | S | - | 0 | | |
| 6 | 101.45 | D | 6.607 | 1 | d | 2.0 |
| 7 | 159.49 | S | - | 0 | | |
| 8 | 97.70 | D | 6.958 | 1 | d | 2.0 |
| 8a | 155.27 | S | - | 0 | | |
| 10 | 78.56 | D | 4.298 | 1 | ddd | 7.9, 4.6, 2.5 |
| 11 | 75.79 | D | 5.026 | 1 | d | 7.9 |
| 12a | 143.30 | S | - | 0 | | |
| 13 | 116.68 | D | 7.869 | 1 | m | |
| 14 | 123.75 | S | - | 0 | | |
| 15 | 121.25 | D | 7.757 | 1 | m | |
| 16 | 116.99 | D | 7.136 | 61 m | | |
| 16a | 145.19 | S | - | 0 | | |
| 17 | 131.22 | S | - | 0 | | |
| 18 | 111.98 | D | 7.087 | ' 1 d | | 2.0 |
| 19 | 150.43 | S | - | 0 | | |
| 20 | 143.24 | S | - | 0 | | |
| 21 | 120.59 | D | 7.510 | 1 | d | 8.4 |
| 22 | 119.64 | D | 6.969 | 1 | dd | 8.4, 2.0 |
| 23 | 60.07 | Т | 3.591 1 dd | | ddd | 12.4, 5.0, 2.5 |
| | | | 3.385 | 385 1 0 | | 12.4, 5.9, 4.6 |
| 3-OH | - | - | 9.644ª | 1 | br s | |
| 5-OH | - | - | 12.325 | 1 | S | |
| 7-OH | - | - | n.d.ª | .a _ | | |
| 19-OMe | 55.80 | Q | 3.768 | 3 | S | |
| 23-OH | - | - | 5.015 | 1 | dd | 5.9, 5.0 |



^a - tentative assignment, m. - multiplicity



Figure S7. HPLC chromatogram of 2,3-dehydrosilybin-7,20-di-*O*-sulfate.



Figure S8. HPLC chromatograms of silychristin sulfation after 1 h of reaction under Ar atmosphere. Recorded at a) 285 nm, b) 360 nm; 1 - p-NPS, 2 - silychristin-19-O-sulfate, 3 - p-NP, 4 - silychristin, 5 - 2,3-dehydrosilychristin-19-O-sulfate



Figure S9. ESI-MS- spectrum of silychristin-19-*O*-sulfate. $([M - H]^{-}m/z 561.1; [M - H - SO_3]^{-}, m/z 481.1)$

| Atom | δς | m. | δн | n(H) | m. | J [Hz] |
|--------|--------|----|---------------------|---------------------------|----------------|---------------|
| 2 | 83.29 | D | 4.981 | 1 | d | 11.3 |
| 3 | 71.74 | D | 4.498 | 1 | dd | 11.3, 6.2 |
| 4 | 197.56 | S | - | 0 | | |
| 4a | 100.32 | S | - | 0 | | |
| 5 | 163.36 | S | - | 0 | | |
| 6 | 96.17 | D | 5.878 1 d | | 2.0 | |
| 7 | 167.2ª | S | - | 0 | | |
| 8 | 95.17 | D | 5.844 | 1 | d | 2.0 |
| 8a | 162.56 | S | - | 0 | | |
| 10 | 86.85 | D | 5.509 | 1 | d | 7.0 |
| 11 | 53.60 | D | 3.489 | 1 | ddd | 7.0, 6.8, 5.5 |
| 11a | 128.96 | S | - | 0 | | |
| 12 | 115.36 | D | 6.871 | 1 | d | 1.7 |
| 13 | 130.19 | S | - | 0 | | |
| 14 | 115.78 | D | 6.831 | 1 | d | 1.7 |
| 15 | 140.79 | S | - | 0 | | |
| 15a | 147.11 | S | - | 0 | | |
| 16 | 136.66 | S | - | - 0 | | |
| 17 | 110.68 | D | 6.997 | 1 | d | 2.0 |
| 18 | 150.55 | S | - | 0 | | |
| 19 | 142.53 | S | - | 0 | | |
| 20 | 120.93 | D | 7.438 | 1 | d | 8.3 |
| 21 | 117.74 | D | 6.882 | 1 | dd | 8.3, 2.0 |
| 22 | 62.99 | Т | 3.659 1 ddd | | 10.9, 6.8, 5.7 | |
| | | | 3.747 1 ddd 1 | | 10.9, 5.5, 5.2 | |
| 3-OH | - | - | 5.707 1 d | | 6.2 | |
| 5-OH | - | - | 11.916 | 11.916 1 s | | |
| 7-OH | - | - | 10.747 ^b | 0.747 ^b 1 br s | | |
| 15-OH | - | - | 10.315 ^b | 1 | S | |
| 18-OMe | 55.83 | Q | 3.737 | 3 | S | |
| 22-OH | - | - | 5.010 | 1 | dd | 5.7, 5.2 |

| Table S3. | ¹³ C and ¹ H NMR data of silychristin-19- <i>O</i> -sulfate. |
|-----------|--|
| | (600.23 MHz for ¹ H, 150.95 MHz for ¹³ C, DMSO- <i>d</i> ₆ , 30 °C) |



^a - HSQC readout; ^b - might be interchanged, m - multiplicity



Figure S10. HPLC chromatogram of silychristin-19-O-sulfate.



([M – H], *m*/z 559.1; [M – 2H]², *m*/z 279.0.)

| Table S4. | ¹³ C and ¹ H NMR data of 2,3-dehydrosilychristin-19-O-sulfate. |
|-----------|--|
| | (600.23 MHz for ¹ H, 150.95 MHz for ¹³ C, DMSO- d_6 , 30 °C) |

| Atom | δ_{c} | m. | $\delta_{\!H}$ | n(H) | m. | <i>J</i> [Hz] |
|--------|--------------|----|----------------|------|----------|---------------|
| 2 | 146.84 | S | | 0 | | |
| 3 | 135.84 | S | | 0 | | |
| 4 | 175.91 | S | | 0 | | |
| 4a | 103.08 | S | | 0 | | |
| 5 | 160.77 | S | | 0 | | |
| 6 | 98.26 | D | 6.193 | 1 | d | 2 |
| 7 | 163.98 | S | | 0 | | |
| 8 | 93.44 | D | 6.420 | 1 | d | 2 |
| 8a | 156.22 | S | | 0 | | |
| 10 | 87.30 | D | 5.602 | 1 | d | 6.6 |
| 11 | 53.27 | D | 3.578 | 1 | m | |
| 11a | 129.74 | S | | 0 | | |
| 12 | 115.58 | D | 7.610 | 1 | dd | 1.8, 1.0 |
| 13 | 123.94 | S | | 0 | | |
| 14 | 116.00 | D | 7.647 | 1 | dd | 1.8, 0.4 |
| 15 | 140.98 | S | | 0 | | |
| 15a | 148.76 | S | | 0 | | |
| 16 | 136.43 | S | | 0 | | |
| 17 | 110.72 | D | 7.007 | 1 | d | 2.1 |
| 18 | 150.61 | S | | 0 | | |
| 19 | 142.64 | S | | 0 | | |
| 20 | 121.01 | D | 7.457 | 1 | d | 8.3 |
| 21 | 117.78 | D | 6.894 | 1 | ddd | 8.3, 2.1, 0.4 |
| 22 | 63.01 | Т | 3.774 | 1 | m | |
| | | | 3.722 | 1 | m | |
| 3-OH | - | - | 9.347 | 1 | S | |
| 5-OH | - | - | 12.457 | 1 | S | |
| 7-OH | - | - | 10.757 | 1 | S | |
| 15-OH | | | 0 500 | 1 | <i>c</i> | |
| 0 | - | - | 9.399 | T | 5 | |
| 18-OMe | 55.87 | Q | 3.745 | 3 | S | |
| 22-OH | - | - | 5.081 | 1 | dd | ΣJ = 10.8 |





Figure S12. HPLC chromatogram of 2,3-dehydrosilychristin-19-*O*-sulfate.



Figure S13. ESI-MS- spectrum of silydianin 19-*O*-sulfate. $([M - H]^{-}, m/z 561.1)$

15

15a

16

17

18

19

20

21

22

3-0H

5-OH

7-OH

15a-OH

18-OMe

| Table S5. | ¹³ C and ¹ H (600.23 M | NMR da Hz for ¹ | ata of silyc H, 150.95 | lianin 19 MHz foi | ∂- <i>O</i> -sul r ¹³ C, D | fate MSO-d ₆ , 30 °C) | |
|-----------|---|-------------------------------|---------------------------|----------------------|--|-------------------------------------|----|
| Atom | δ_{c} | m. | $\delta_{	extsf{H}}$ | n(H) | m. | <i>J</i> [Hz] | |
| 2 | 81.66 | D | 4.835 | 1 | dd | 10.9, 0.7 | HO |
| 3 | 70.82 | D | 4.446 | 1 | d | 10.9 | |
| 4 | 196.19 | S | - | 0 | | | 6 |
| 4a | 99.95 | S | - | 0 | | | |
| 5 | 163.38 | S | - | 0 | | | |
| 6 | 96.53 | D | 5.86ª | 1 | m | | |
| 7 | 167.74 | S | - | 0 | | | |
| 8 | 95.34 | D | 5.85ª | 1 | m | | |
| 8a | 162.07 | S | - | 0 | | | |
| 10 | 72.79 | Т | 4.143 | 1 | dd | 7.9, 3.3 | |
| | | | 3.799 | 1 | d | 7.9 | |
| 11 | 44.18 | D | 2.769 | 1 | m | ΣJ = 9.2 | |
| 11a | 48.58 | D | 3.481 | 1 | dd | 4.2, 2.2 | |
| 12 | 139.79 | S | - | 0 | | | |
| 13 | 124.39 | D | 6.101 | 1 | dd | 7.1, 2.0 | |
| 14 | 53.15 | D | 3.231 | 1 | dd | 6.8, 2.9 | |

0

0

0

1

0

0

1

1

1

1

1

3

d

d

dd

m

br s

br s

S

2.1

8.4

8.4, 2.1

ΣJ = 4.6

-

-

-

6.809

-

-

7.340

6.673

3.356

5.788

11.77

n.d.

n.d.

3.723

S

S

S

D

S

S

D

D

D

-

-

-

-

Q

201.76

96.81

137.32

112.94

150.11

141.39

120.58

119.71

46.24

-

-

-

-

55.60



^a ... HSQC readout

p. 12/12

റടറ