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

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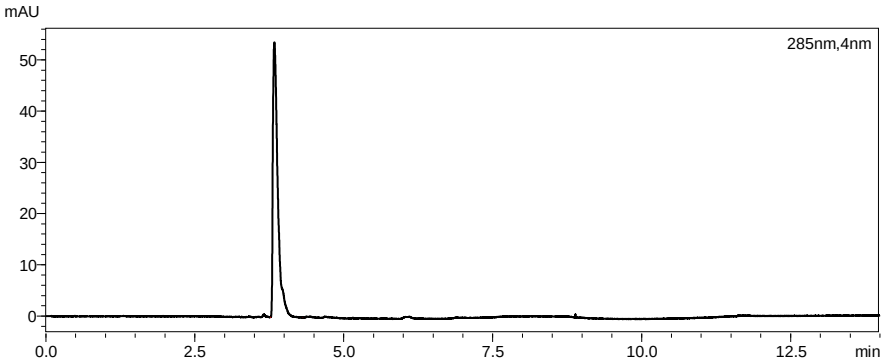



**Figure S1.** HPLC chromatogram of silybin A 20-*O*-sulfate.

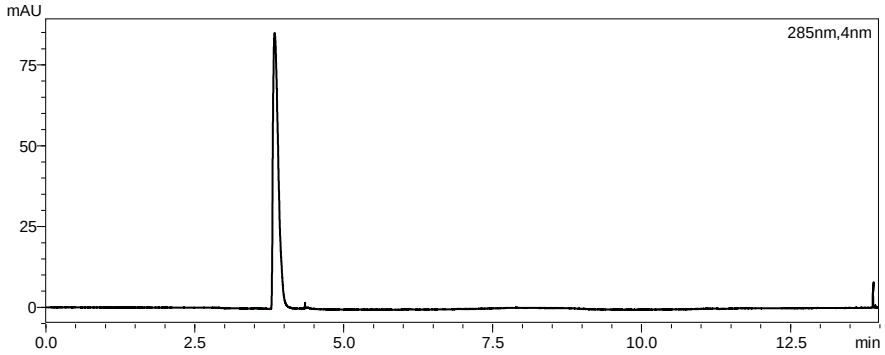



**Figure S2.** HPLC chromatogram of silybin B 20-*O*-sulfate.

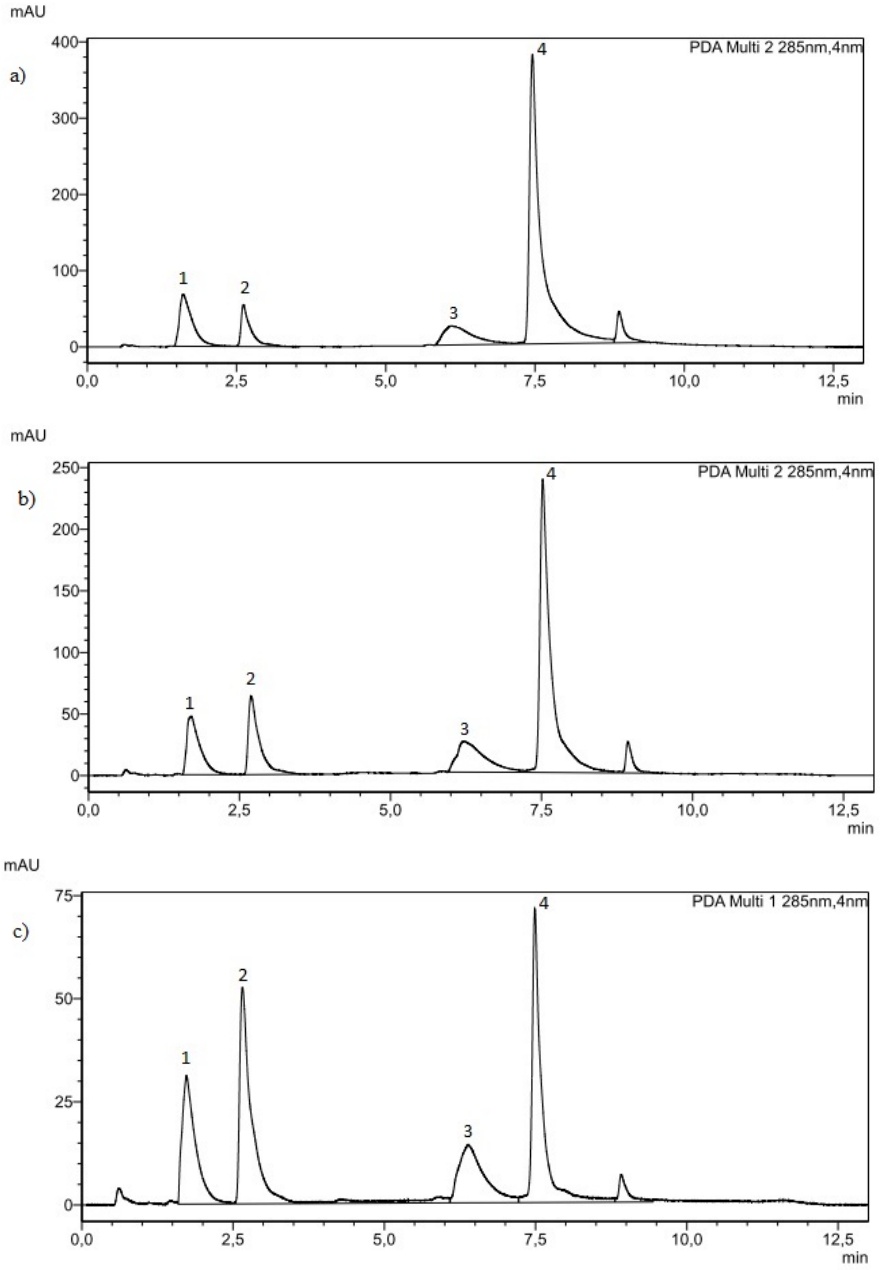



**Figure S3.** HPLC chromatograms of 2,3-dehydrosilybin A sulfation in time.
a) 1h, b) 5h, c) 12h; 1 – *p*-NPS, 2 – *p*-NP, 3 – 2,3-dehydrosilybin A sulfate, 4 – 2,3-dehydrosilybin

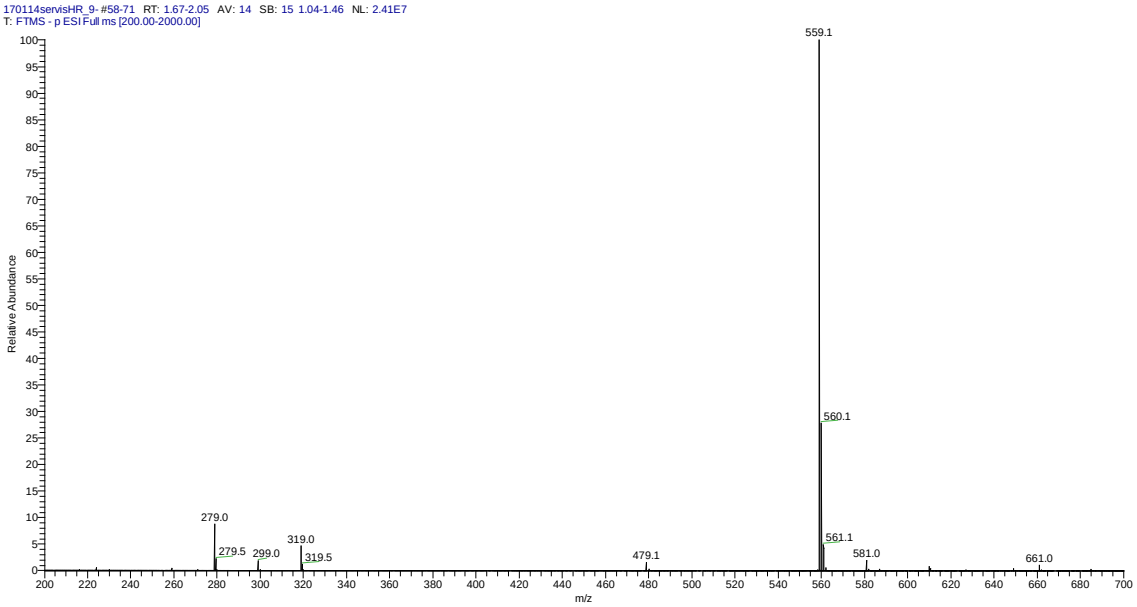
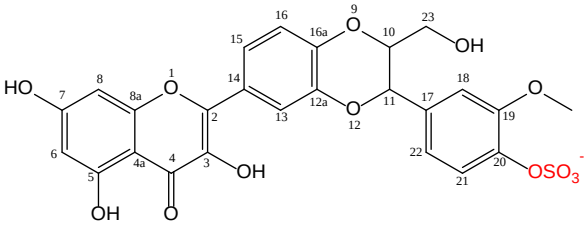



**Figure S4.** ESI-MS− spectrum of 2,3-dehydrosilybin 20-*O*-sulfate.
([M – H]$^-$, *m/z* 559.1; [M – 2H]$^2$, *m/z* 279.0)

**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 | $\delta_C$ | m. | $\delta_H$ | n(H) | m. | *J* [Hz] |
|---|---|---|---|---|---|---|
| **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 | 7.780 | 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 |
| **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 | 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-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 |

m. multiplicity

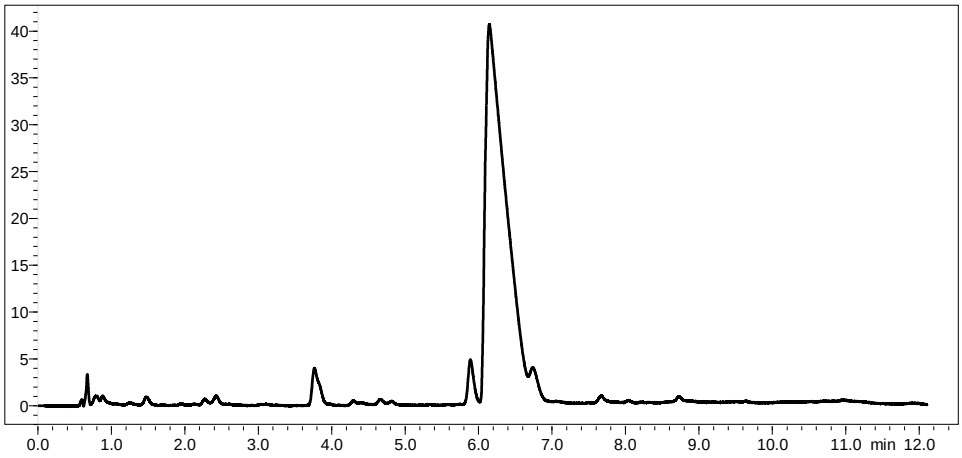



**Figure S5.** HPLC chromatogram of 2,3-dehydrosilybin-20-*O*-sulfate.

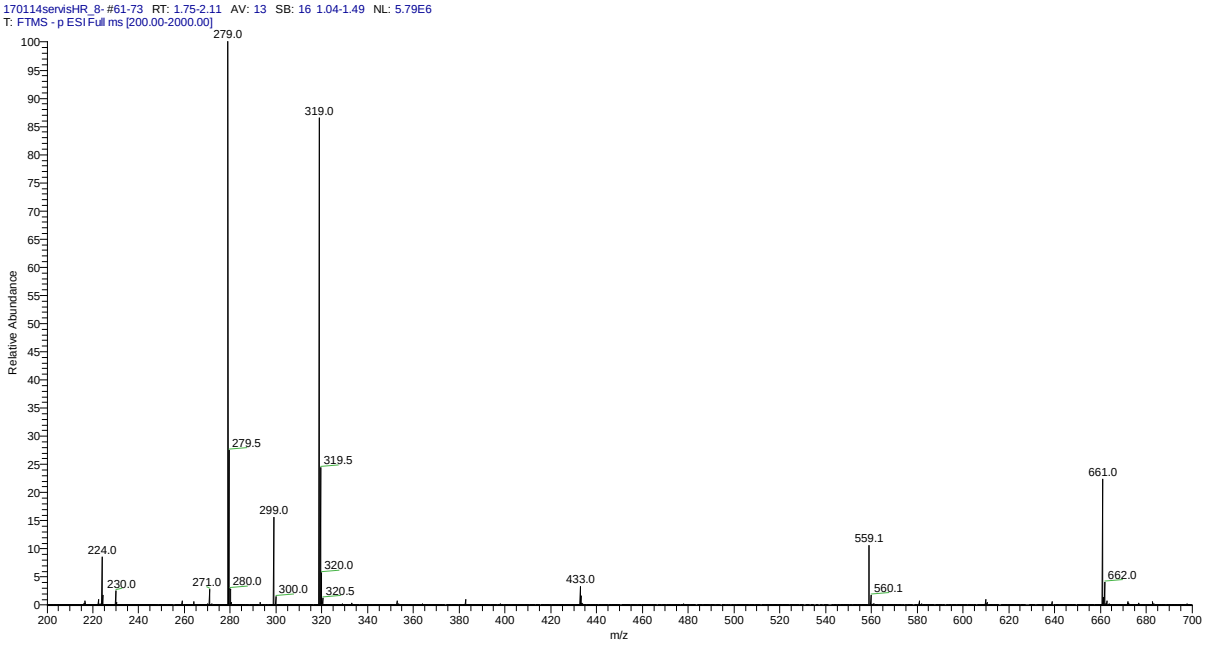
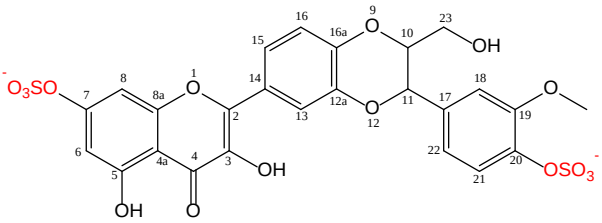



**Figure S6.** ESI-MS− spectrum of 2,3-dehydrosilybin-7,20-di-*O*-sulfate.
([M – 2H + Na] *m/z* 661.0; $[M - H - SO_3]^-$, *m/z* 559.1; $[M - 2H]^{2-}$, *m/z* 319.0; $[M - 2H - SO_3]^{2-}$, *m/z* 279.0)

**Table S2.** $^{13}C$ and $^{1}H$ NMR data of 2,3-dehydrosilybin-7,20-di-*O*-sulfate. (600.23 MHz for $^{1}H$, 150.95 MHz for $^{13}C$, DMSO-$d_6$, 30 °C)




| Atom | $\delta_C$ | m. | $\delta_H$ | n(H) | m. | *J* [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 | 1 | 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 | T | 3.591 | 1 | ddd | 12.4, 5.0, 2.5 |
| | | | 3.385 | 1 | ddd | 12.4, 5.9, 4.6 |
| **3-OH** | - | - | 9.644[a] | 1 | br s | |
| **5-OH** | - | - | 12.325 | 1 | s | |
| **7-OH** | - | - | n.d.[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

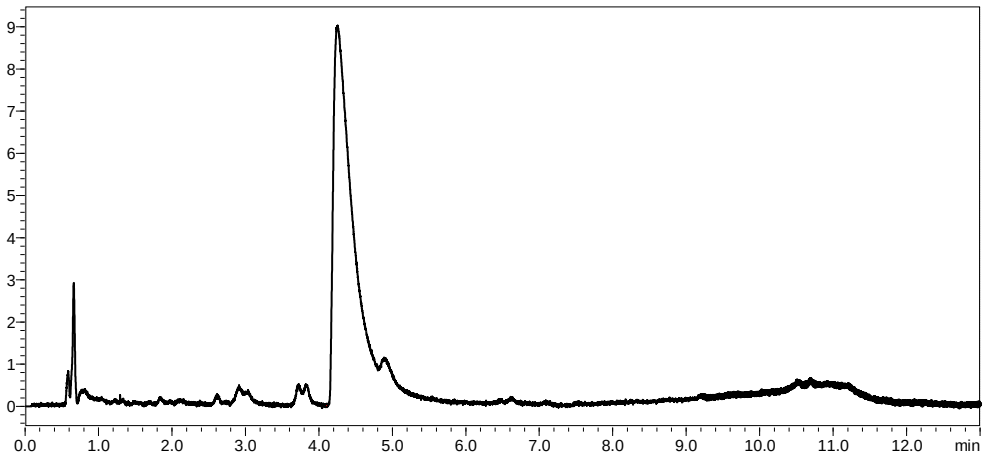



**Figure S7.** HPLC chromatogram of 2,3-dehydrosilybin-7,20-di-*O*-sulfate.

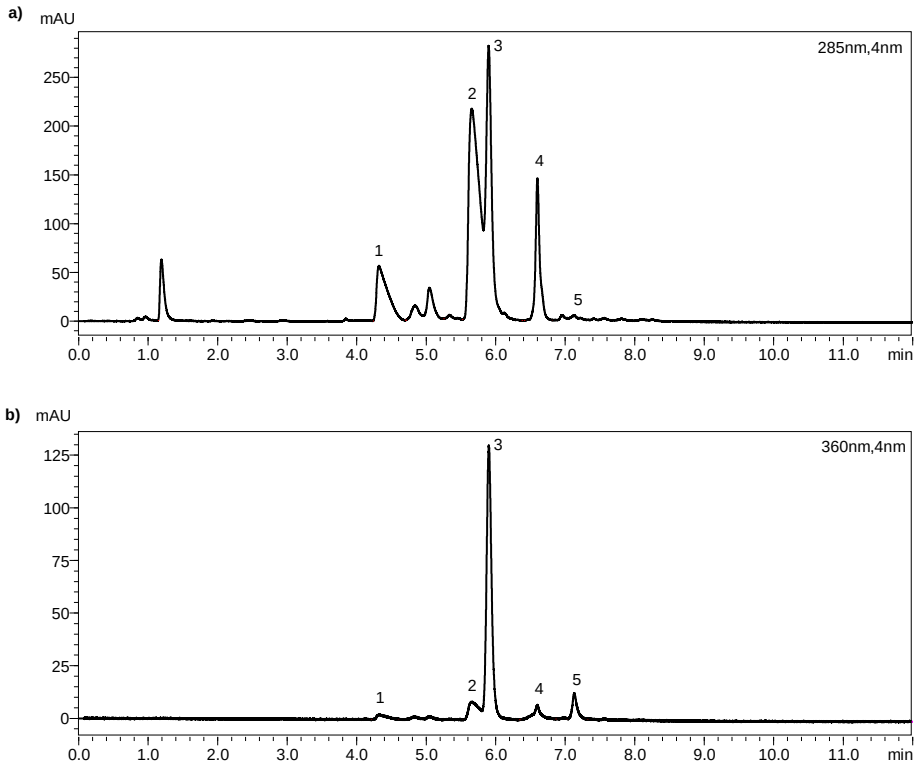



**Figure S8.** HPLC chromatograms of silychristin sulfation after 1 h of reaction under Ar atmosphere. Recorded at a) 285 nm, b) 360 nm; 1 – *p*-NPS, 2 – silychristin-19-*O*-sulfate, 3 – *p*-NP, 4 – silychristin, 5 – 2,3-dehydrosilychristin-19-*O*-sulfate

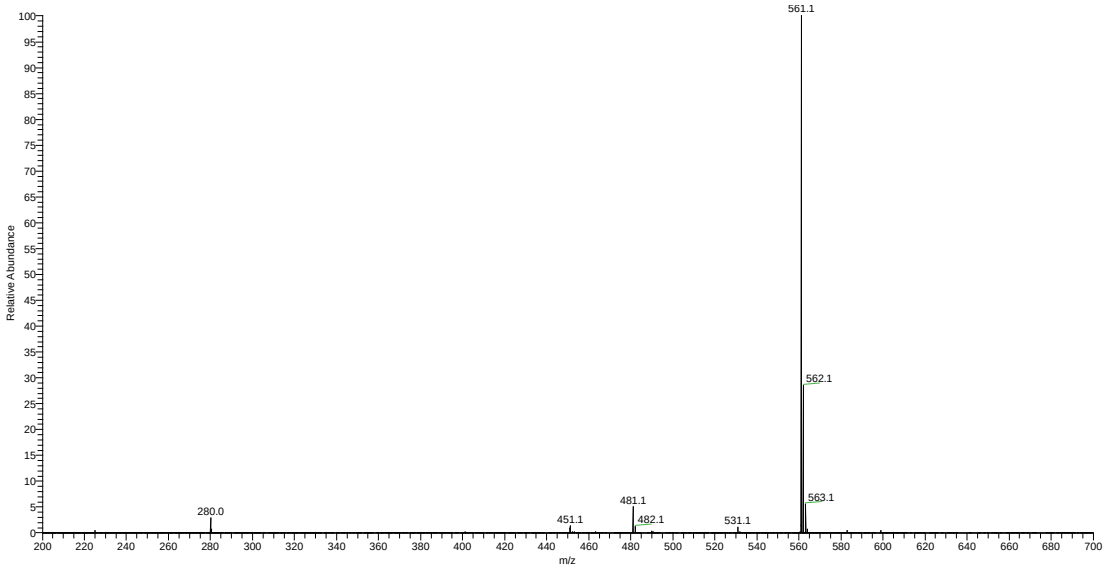
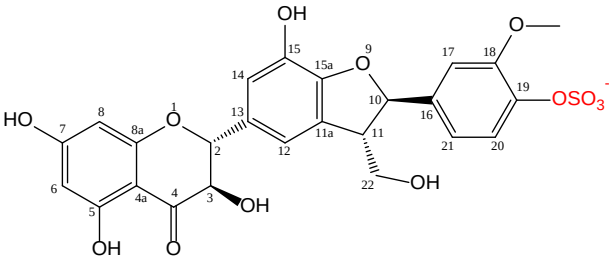



**Figure S9.** ESI-MS− spectrum of silychristin-19-*O*-sulfate. ($[M - H]^-$ *m/z* 561.1; $[M - H - SO_3]^-$, *m/z* 481.1)

**Table S3.** $^{13}C$ and $^{1}H$ NMR data of silychristin-19-*O*-sulfate.
(600.23 MHz for $^{1}H$, 150.95 MHz for $^{13}C$, DMSO-$d_6$, 30 °C)




| Atom | $\delta_C$ | m. | $\delta_H$ | 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** | 167.2[a] | 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 | T | 3.659 | 1 | ddd | 10.9, 6.8, 5.7 |
| | | | 3.747 | 1 | ddd | 10.9, 5.5, 5.2 |
| **3-OH** | - | - | 5.707 | 1 | d | 6.2 |
| **5-OH** | - | - | 11.916 | 1 | s | |
| **7-OH** | - | - | 10.747[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 |

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

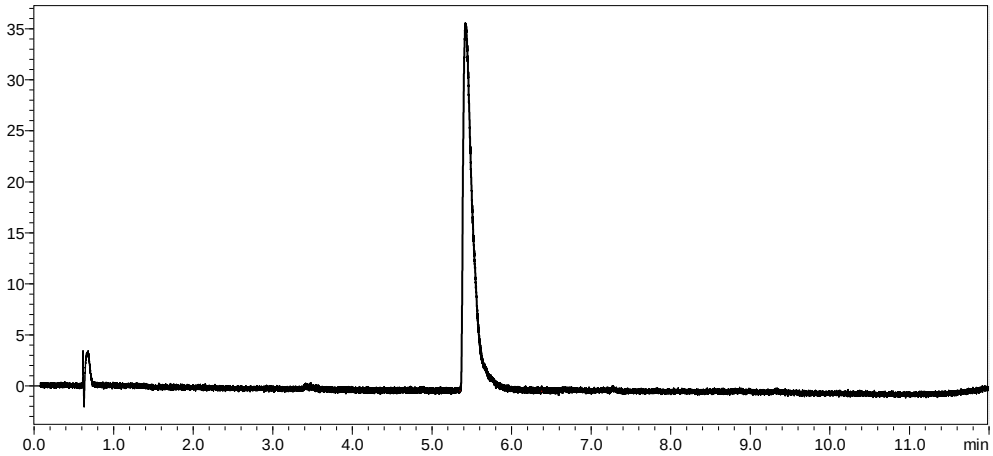



**Figure S10.** HPLC chromatogram of silychristin-19-*O*-sulfate.

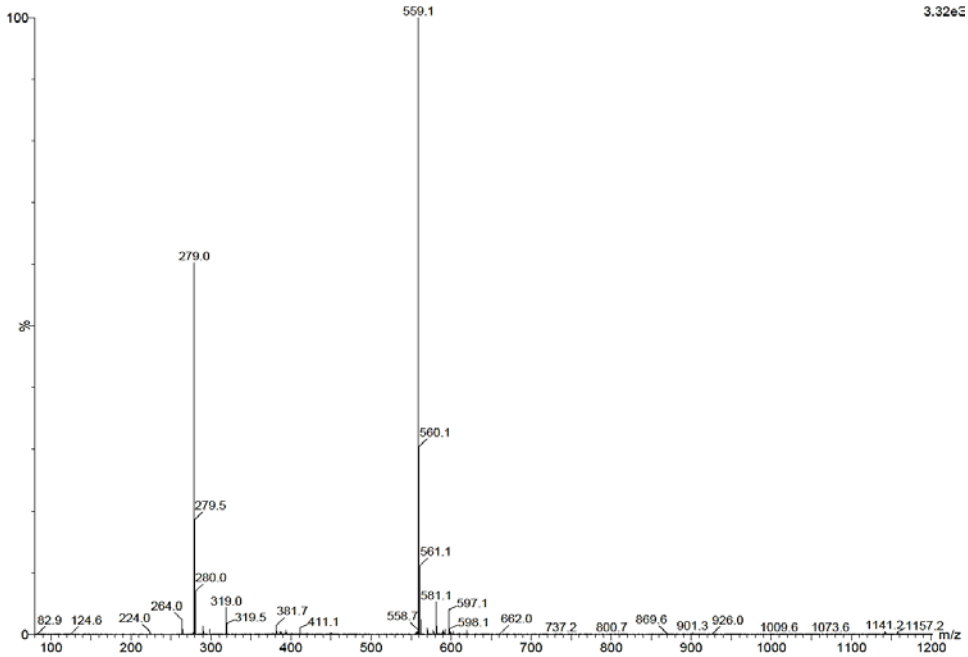
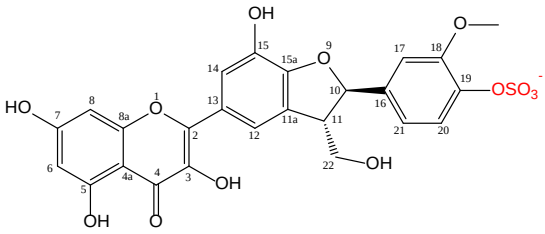



**Figure S11.** ESI-MS− spectrum of 2,3-dehydrosilychristin-19-*O*-sulfate ($[M - H]^-$, *m/z* 559.1; $[M - 2H]^{2-}$, *m/z* 279.0.)

**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 | $\delta_C$ | m. | $\delta_H$ | n(H) | m. | *J* [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 | T | 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** | - | - | 9.599 | 1 | s | |
| **O** | | | | | | |
| **18-OMe** | 55.87 | Q | 3.745 | 3 | s | |
| **22-OH** | - | - | 5.081 | 1 | dd | ΣJ = 10.8 |

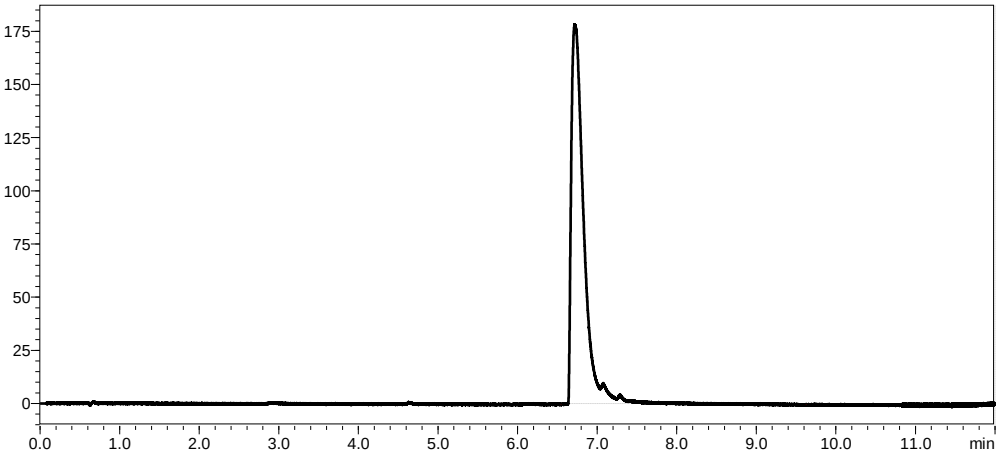



**Figure S12.** HPLC chromatogram of 2,3-dehydrosilychristin-19-*O*-sulfate.

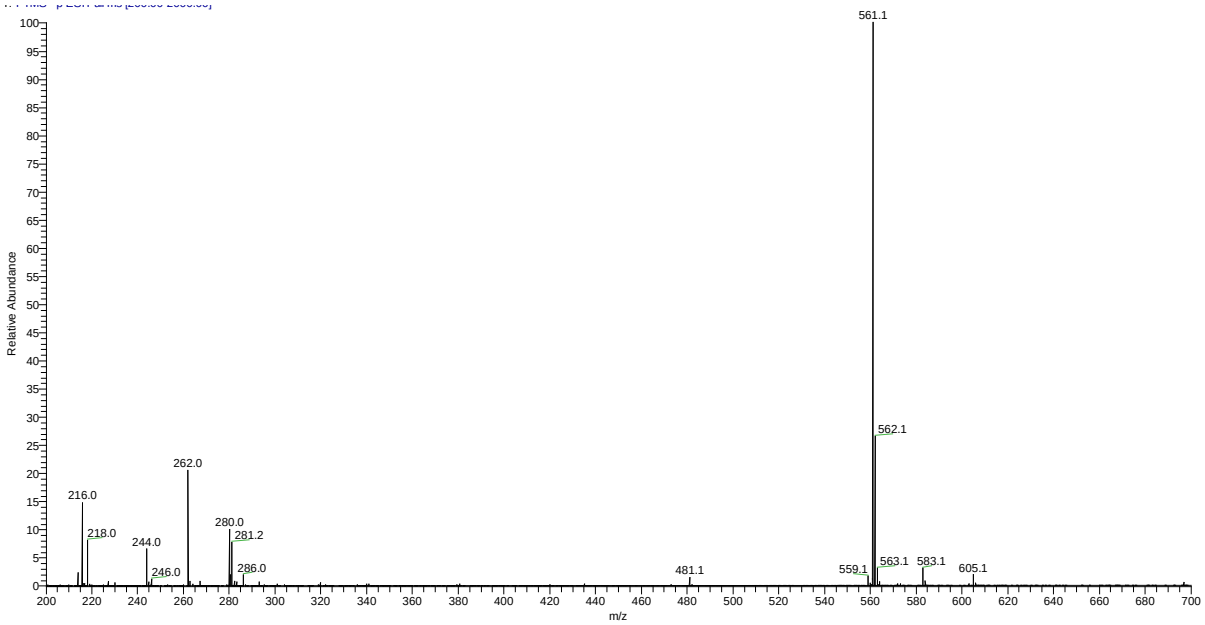
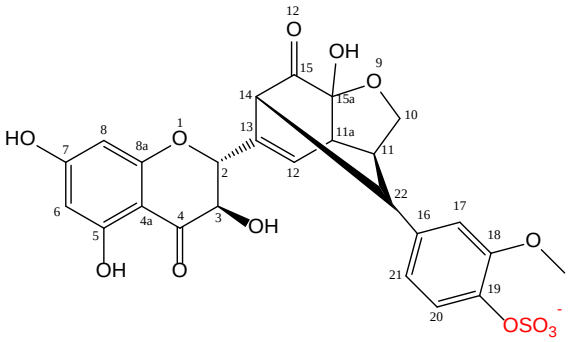



**Figure S13.** ESI-MS− spectrum of silydianin 19-*O*-sulfate.
([M – H]$^-$, *m/z* 561.1)

**Table S5.** $^{13}C$ and $^{1}H$ NMR data of silydianin 19-*O*-sulfate (600.23 MHz for $^{1}H$, 150.95 MHz for $^{13}C$, DMSO-$d_6$, 30 °C)




| Atom | $\delta_C$ | m. | $\delta_H$ | n(H) | m. | *J* [Hz] |
|---|---|---|---|---|---|---|
| **2** | 81.66 | D | 4.835 | 1 | dd | 10.9, 0.7 |
| **3** | 70.82 | D | 4.446 | 1 | d | 10.9 |
| **4** | 196.19 | S | - | 0 | | |
| **4a** | 99.95 | S | - | 0 | | |
| **5** | 163.38 | S | - | 0 | | |
| **6** | 96.53 | D | 5.86[a] | 1 | m | |
| **7** | 167.74 | S | - | 0 | | |
| **8** | 95.34 | D | 5.85[a] | 1 | m | |
| **8a** | 162.07 | S | - | 0 | | |
| **10** | 72.79 | T | 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 |
| **15** | 201.76 | S | - | 0 | | |
| **15a** | 96.81 | S | - | 0 | | |
| **16** | 137.32 | S | - | 0 | | |
| **17** | 112.94 | D | 6.809 | 1 | d | 2.1 |
| **18** | 150.11 | S | - | 0 | | |
| **19** | 141.39 | S | - | 0 | | |
| **20** | 120.58 | D | 7.340 | 1 | d | 8.4 |
| **21** | 119.71 | D | 6.673 | 1 | dd | 8.4, 2.1 |
| **22** | 46.24 | D | 3.356 | 1 | m | ΣJ = 4.6 |
| **3-OH** | - | - | 5.788 | 1 | br s | |
| **5-OH** | - | - | 11.77 | 1 | br s | |
| **7-OH** | - | - | n.d. | | | |
| **15a-OH** | - | - | n.d. | | | |
| **18-OMe** | 55.60 | Q | 3.723 | 3 | s | |

[a] … HSQC readout